CARL T. BERGSTROM • LEE ALAN DUGATKIN

evolution

SECOND EDITION

EVOLUTION

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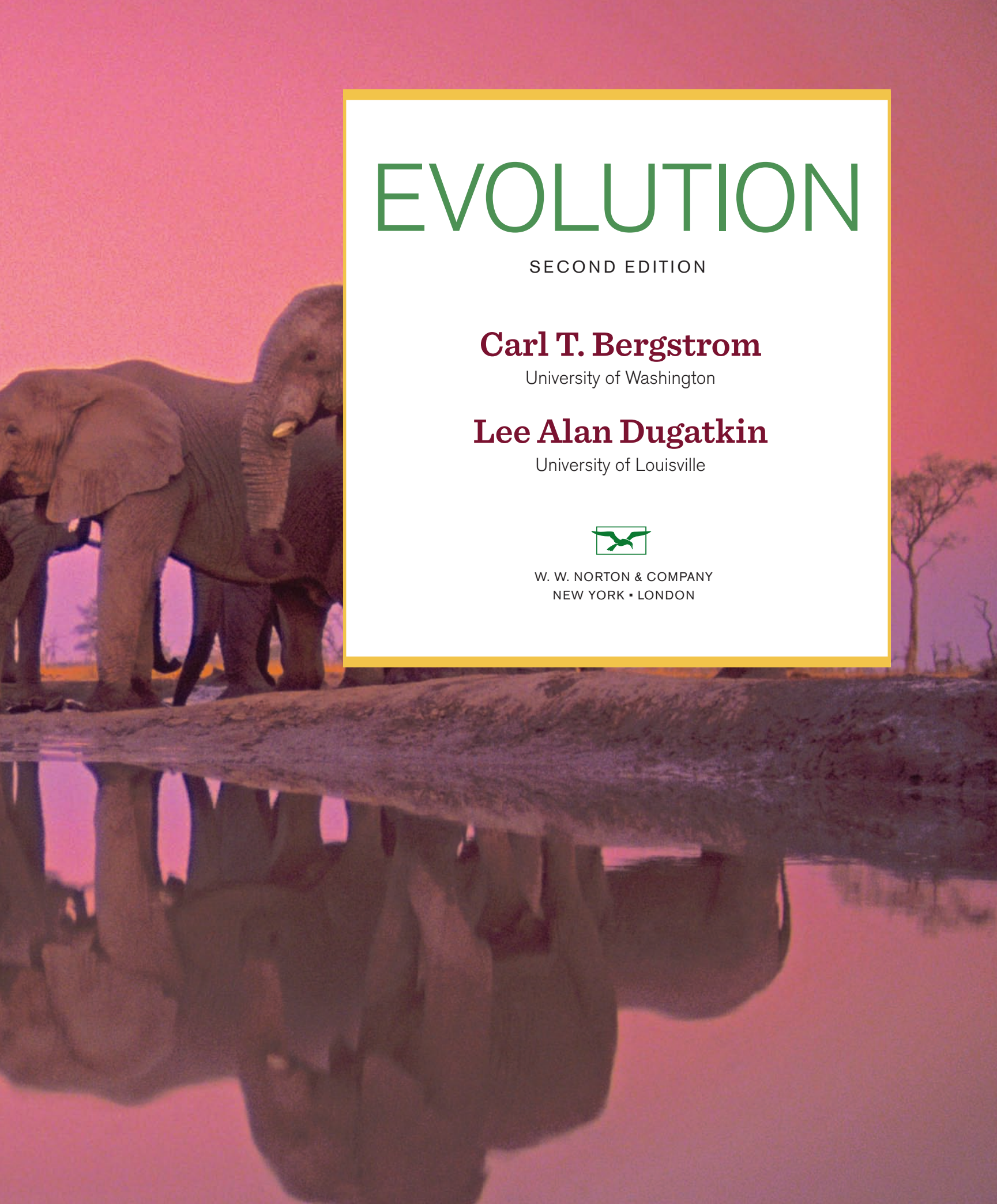

EVOLUTION

SECOND EDITION

Carl T. Bergstrom
University of Washington

Lee Alan Dugatkin
University of Louisville

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PREFACE

What a time it is to be an evolutionary biologist! In the first edition of this book, we wrote that we envy the student taking a class in evolutionary biology today. Recent events only strengthen this sentiment. For example, since the first edition of the book was released, our understanding of human evolutionary history has been upended by findings including definitive evidence of substantial interbreeding between humans and other *Homo* species such as Neanderthals and Denisovans. Or to provide another example, as the final drafts of this edition were being completed, extensive evidence of a new hominin species, *Homo naledi*, was uncovered in a South African cave. We scrambled to tell its remarkable story before the book went to press. These findings, along with other major advances in our understanding of human evolutionary history, stimulated us to expand our coverage of human evolution from a short section in our first edition to an entirely new chapter in this second edition.

Evolutionary biologists continue to collaborate in new and dynamic ways with researchers in many disciplines and bring to such collaboration a diverse set of perspectives—from areas such as phylogenetics, population genetics, the study of adaptation, molecular genetics, and developmental biology, to name just a few. The result is a much deeper understanding of the history and diversity of life on Earth over the past 4 billion years or so. Our job as the authors of this book is to capture the exciting work that has gone into this effort and to present it in a rigorous and engaging fashion.

To achieve this goal, we draw on our dual roles as researchers in and teachers of evolutionary biology. We each run active labs abuzz with the excitement that surrounds the science of evolution. We both lecture about evolution to students at our own universities and to audiences around the world. And we are each enthusiasts about the history of science in general and the history of evolutionary biology in particular. The successful strategies we've developed for communicating with these diverse audiences have informed the tone, emphases, and features in this textbook in a way that we hope will excite the scientific imaginations of students and instructors alike.

We relish the fact that *all science* is about testing hypotheses. Hypothesis-driven science has proved to be the most powerful approach ever devised for understanding the nature of the physical world we live in. No other approach even comes close. We convey this through the abundant use of examples in which evolutionary biologists generate and test hypotheses. In this second edition, we continue the path we took in the first edition and include the newest work from around the globe. Through these examples, students will gain an intimate understanding that evolutionary biology is a continually developing field in which theoretical ideas translate into testable predictions and in which the process of hypothesis testing leads to refinements of theory. Through the lens of current research, students can see how the scientific understanding of evolutionary biology is ever changing and that built into science is a system that allows each assumption to be challenged and refined or even rejected based on a preponderance of evidence.

We understand that it is *stories*, not catalogs of facts, that resonate with students (or anyone else). And so, in each chapter, we make use of the natural human inclination to acquire and process information in narrative form. Within the field of evolutionary biology are fascinating stories on many levels: stories of individual scientists and how they came to their discoveries, stories of how human thought has changed over the centuries, stories of how major evolutionary innovations arose in the history of life, stories of how individual species have changed over millennia through biological evolution or, as in the case of many microbes, how a population can change dramatically in a matter of weeks.

Science is much more than narrative, of course. As in all mature sciences, models play a fundamental role in evolutionary biology today. In this book, we devote considerable attention to simple conceptual models of evolutionary processes. Often, such models can be profitably expressed through the language of mathematics, and one of our principal aims in the text is to help students become comfortable with this approach. One of the most important things that students learn in college-level physics or economics classes is how to formulate questions about the real world in the language of mathematical models and how to answer these questions appropriately using mathematical analysis. We believe strongly that this should be a critical component of a college education in the biological sciences as well. At the same time, we recognize that students enter this course with varying degrees of mathematical preparedness, and so we have placed the more advanced concepts in boxes in an effort to offer instructors maximum flexibility in integrating mathematical models into their course.

So that students will gain a firm understanding of the essential foundations of evolutionary reasoning, we introduce several fundamental components of evolutionary thought in Chapter 1 and emphasize them throughout this textbook. These include:

- **Phylogenetics**. All living things on the planet today—and indeed all life that has ever existed—are linked by a shared evolutionary history that evolutionary biologists represent using phylogenetic trees. Thus, to understand evolutionary relationships, whether between two HIV strains or among the different domains of life, students must learn to think in terms of phylogenetic relationships. We consider it crucial that any textbook on evolution seamlessly integrates phylogenetic thinking throughout, and we have done so here. If students walk away remembering just one thing about this book—though of course we hope they walk away remembering much more—it will be the importance of phylogenetic thinking.
- **Population thinking**. Evolutionary change occurs in populations, but most contemporary biology curricula train students to think at the level of the individual, as one would in a physiology course, for example. In this book, we demonstrate how to think at the population level as well, paying careful attention to the properties of populations: population composition, variation among individuals within and between populations, change in the properties of a population over time, and so forth. This population-level perspective, particularly as it relates to the process of natural selection, permeates this book. Because we know that some students initially struggle to master this type of population-level thinking, we devote considerable space to teaching this skill.
- **Natural selection**. Evolution is often defined as "descent with modification." As a population geneticist (CTB) and a behavioral evolutionary biologist (LAD), we both study the processes responsible for such "modification." We convey the importance of this topic to students by teaching them how the process of natural selection has shaped the diversity of life on this planet and how other processes—most notably genetic drift—have also contributed to the myriad forms of life around us.

Features

This textbook integrates the big themes in evolutionary biology—phylogenetics and population thinking—in a way that is both current and accessible. Extensive, in-depth, current research examples, an emphasis on problem solving, and a stunning art program engage students, helping them understand fundamental concepts and processes. Major features include:

- Extensive coverage of **phylogenetics**, which is introduced in Chapter 1 through the examination of a few engaging examples that demonstrate the power of phylogenetic thinking. Soon after, in Chapter 4, Phylogeny and Evolutionary History, and Chapter 5, Inferring Phylogeny, students are taught how to interpret and then build trees that generate testable hypotheses about evolutionary history and compare the relatedness of living organisms. This strong foundation in phylogenetic reasoning is then integrated into the text and art in virtually every chapter that follows.
- We explore fundamental concepts through the lens of phylogenetics and population thinking and reinforce these concepts using **current research examples**, many of which are drawn from research done in the past decade. From Chapter 3's in-depth examination of Hopi Hoekstra's work on natural selection, phylogeny, cryptic coloration, and the Mc1R and Agouti genes in oldfield mice (*Peromyscus polionotus*), to Chapter 11's coverage of Jack Szostak's work on lipid membranes and reproduction in the earliest cellular life forms, to Chapter 19's story of how genetic evidence of interbreeding between humans and both Neanderthals and Denisovans has radically revised our understanding of our evolutionary history, the excitement of current research is captured throughout.
- Significant coverage of **contemporary topics** such as genomics, evo–devo, molecular evolution, and human evolution, including full chapters on the following subjects: Genome Evolution (Chapter 10), Evolution and Development (Chapter 13), Coevolution (Chapter 18), Human Evolution (Chapter 19), and Evolution and Medicine (Chapter 20).
- An in-depth focus on **a few research studies** in each chapter promotes a more complete understanding of how evolutionary biologists come to understand specific concepts. The examples were carefully chosen to offer a **balance of classic and contemporary studies** that most fully illustrate the concept being discussed.
- A beautiful and information-rich **art program** was carefully developed to promote understanding of key concepts described in the text by both engaging students visually and providing them with just the right amount of detail. The art includes distinctive figures that help students in the following ways:
  1. **Phylogenetic relationships** are made clear through the many phylogenetic trees that appear in virtually every chapter. Many of these trees also include in-figure captions, photographs, and line art that enrich students' understanding of the concept or example.

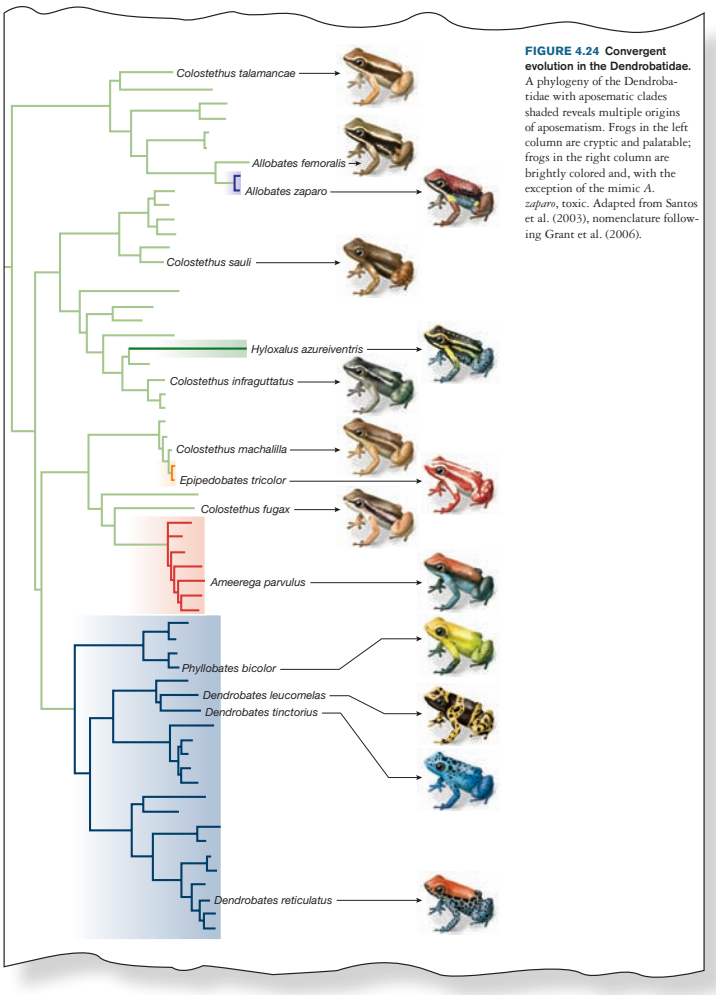

FIGURE 4.24 Convergent evolution in the Dendrobatidae. A phylogeny of the Dendrobatidae with aposematic clades shaded reveals multiple origins of aposematism. Frogs in the left column are cryptic and palatable; frogs in the right column are brightly colored and, with the exception of the mimic *A. zaparo*, toxic. Adapted from Santos et al. (2003), nomenclature following Grant et al. (2006).

2. **Research-style data graphics** are presented much like they appear in the primary literature, but with carefully developed labels and in-figure captions that teach students to interpret and analyze the image or graph visually.

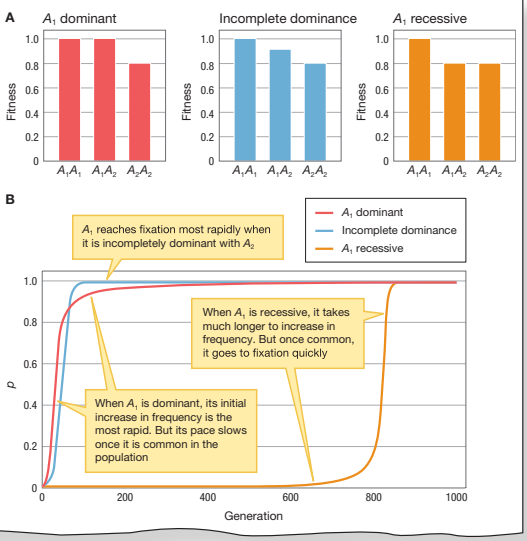

FIGURE 7.13 Directional selection at one locus with two alleles. **(A)** In directional selection, one allele A_1 is favored over another, A_2. This can occur in different ways: A_1 can be dominant (red), A_1 and A_2 can show incomplete dominance (blue), or A_1 can be recessive (orange). **(B)** The trajectories of p, the frequency of the A_1 allele, are illustrated from a starting value of $p = 0.005$.

3. Diagrams of **experimental processes** encourage students to visualize not just the outcome of a research study, but the specifics of how the experiment was constructed so that they can better understand the meaning behind the data.

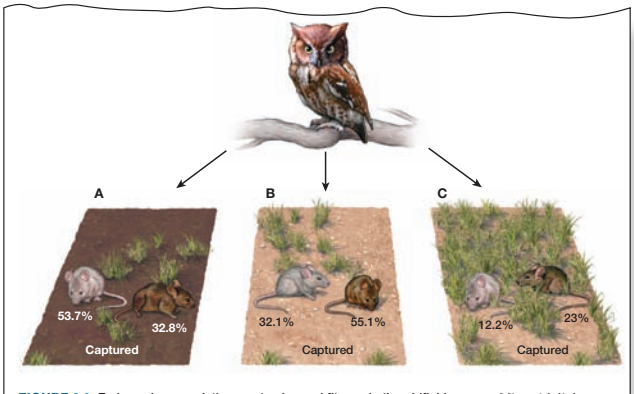

FIGURE 3.9 Early work on predation, coat color, and fitness in the oldfield mouse. Mice with light and dark coats were exposed to owl predators in three different environments: dark background with sparse vegetation **(A)**, light background with sparse vegetation **(B)**, and light background with dense vegetation **(C)**. The identity of the first mouse captured in each trial was recorded. Trials lasted fifteen minutes, and if neither mouse was taken by the owl, the trial ended. The percentages of trials in which mice of a given coat color were the first to be taken by the owl are shown in each panel (percentages in a panel do not sum to 100 because of trials in which neither mouse was taken by the predator). In all cases, owls initially captured a higher percentage of "color-mismatched" mice; namely, those with coat colors that failed to match their environments.

- Clear and accessible coverage of **quantitative methods**, the most difficult of which are in optional boxes. This teaches students how to formulate questions about evolutionary processes and relationships the ways researchers do—in the language of quantitative models.
- High-quality **problem sets** in the end-of-chapter material provide students with extensive practice in formulating and solving problems.

Resources for Instructors

Downloadable Instructor's Resources

These include content for use both in the classroom and online:

- Book art in JPEG and PowerPoint formats.
- Free, customizable Coursepacks, which are accessible directly through instructors' learning management systems and include new adaptive learning modules on interpreting data, phylogenetic trees, and population genetics.
- Test Bank in Examview, Word RTF, and PDF formats.
- Instructor's Manual in PDF format.

For more information and to view samples, go to **wwnorton.com/instructors**.

Test Bank

The Test Bank has been developed using the Norton Assessment Guidelines and provides a quality bank of 1000 items consisting of multiple choice and short answer/essay questions. Each question in the Test Bank is classified by Bloom's taxonomy, learning objective, section, and difficulty, making it easy to construct tests and quizzes that are meaningful and diagnostic.

Instructor's Manual

This helpful online resource for instructors consists of detailed chapter outlines, guides to key readings in the text, and answers to the key concept questions for every chapter. The manual also includes brief guides to accessing and using online simulations, including EvoBeaker.

Coursepacks

At no cost to instructors or students, Norton Coursepacks offer a variety of review activities and assessment materials for instructors who use Blackboard and other learning management systems. With a simple download from our instructor's website, an adopter can bring high-quality digital media into a new or existing online course (with no additional student passwords or logins required). In addition to chapter-based quizzes with art, flashcards, and animations, the Coursepack includes three adaptive InQuizitive modules that develop the core foundational skills students need to do well in the course. The modules, on interpreting data, phylogenetic trees, and population genetics, were written by Christine Andrews, Senior Lecturer at the University of Chicago.

Resources for Students

InQuizitive Learning Modules

InQuizitive is a formative, adaptive quizzing tool that provides a personalized learning experience tailored to each student's learning needs. These free learning modules, accessible directly in the Coursepack, help students hone their understanding outside of class on three key concepts—data interpretation, phylogenetic trees, and population genetics—so that they come to the lectures better prepared. Each module personalizes the quizzing, so students get reinforced practice in the specific areas they need help with most. Instructors can easily review individual and overall class performance data.

Ebook

An affordable and convenient alternative to the print book, the Norton ebook retains the content and design of the print book and allows students to highlight and take notes with ease, print chapters as needed, search the text, and more.

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Creating this textbook has been a labor of love, and there are many people whose extraordinary commitment we'd like to acknowledge. First, we'd like to thank our current editor at Norton, Betsy Twitchell. Without her insightful feedback and careful attention to every aspect of the development and production of the book, you would not be reading these words right now.

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To the reader: Thank you as well! We greatly appreciate your consideration and selection of this book as your introduction to evolutionary biology. We welcome your comments.

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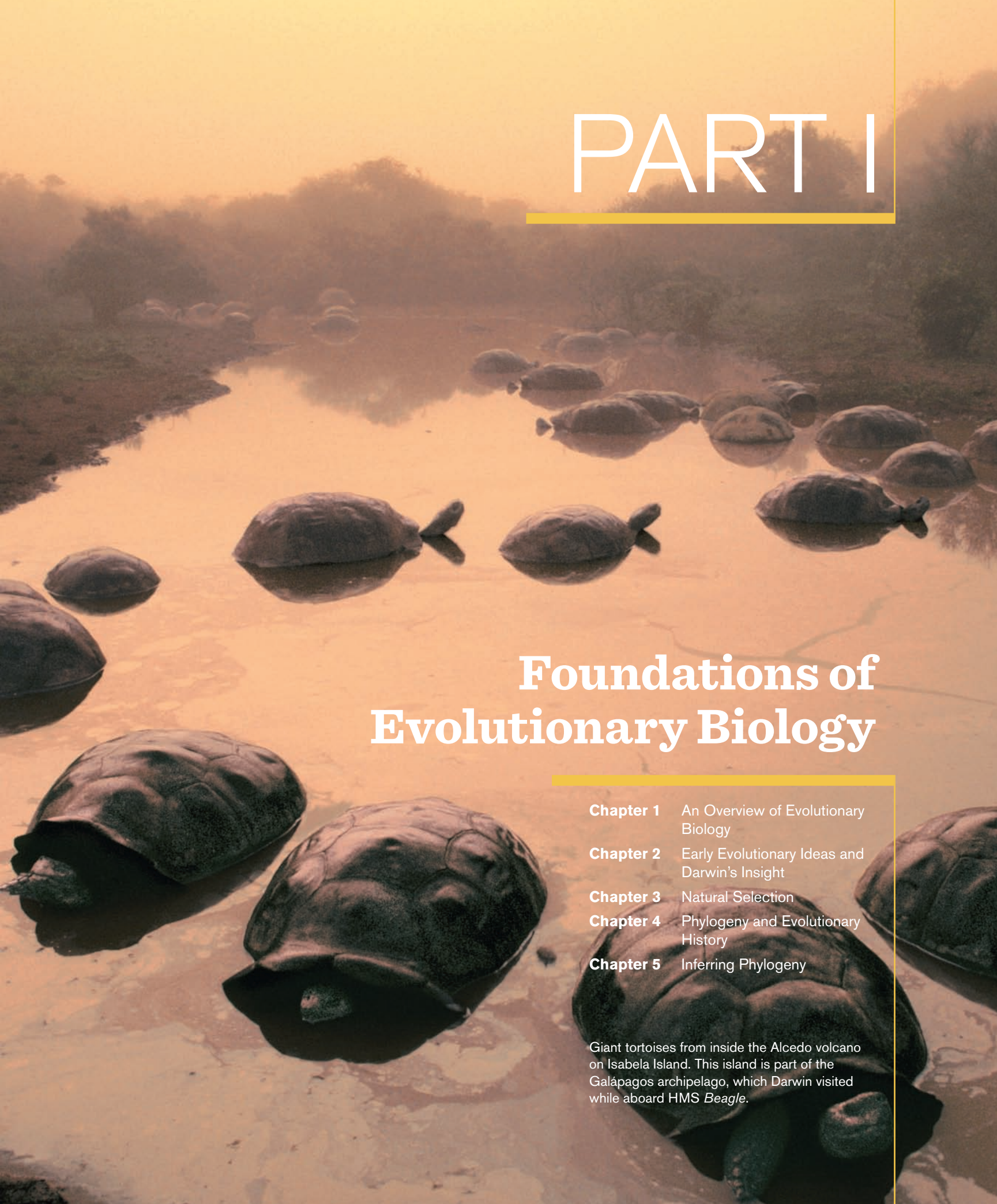
EVOLUTION

SECOND EDITION

PART I

Foundations of Evolutionary Biology



Giant tortoises from inside the Alcedo volcano on Isabela Island. This island is part of the Galápagos archipelago, which Darwin visited while aboard HMS *Beagle*.

1

An Overview of Evolutionary Biology

In his classic book, *The Structure of Scientific Revolutions*, philosopher and historian of science Thomas Kuhn argued that major advances in science are rare, and that true scientific revolutions involve not simply the accumulation of new facts and theories but fundamental changes in the way we think (Kuhn 1962). Once such a revolution takes place, the world is never seen or understood in the same way. When early astronomers and physicists demonstrated that Earth was not at the center of the universe, what Kuhn described as a *paradigm shift* occurred. The very way we thought of Earth and our place in nature fundamentally changed. A similarly dramatic paradigm shift occurred when Charles Darwin laid out his theory of evolution.

In *On the Origin of Species*, published in 1859, Darwin presented two revolutionary ideas. Each had been suggested independently by others before, but never had they been brought together with the conceptual brilliance and the naturalist's eye of Charles Darwin (Chapter 2). First, after decades of observations, collecting data from near and far, reading incessantly, and

◀ The carnivorous dusky pitcher plant (*Nepenthes fusca*) of Borneo traps insects in a liquid reservoir at the bottom of its pitcher.

synthesizing and resynthesizing theories from a number of different disciplines, Darwin recognized that the diversity of life we see around us has descended from previously existing species, which share a common ancestor from further back in time. Second, Darwin realized that the often exquisite fit of species to their environments is primarily a result of **natural selection**, a gradual process in which forms that are better suited to their environments increase in frequency in a population over sufficiently long periods of time. As we will see throughout this book, "sufficiently long" can range from a matter of days to tens of thousands of years, depending on the strength of natural selection and the rate of reproduction of the organisms we are studying. Together, these two ideas proposed by Darwin suggest that the entire organic world—much of everything we see, feel, smell, taste, and touch—is the result of evolutionary changes that have taken place over time.

Once the theory of evolution by natural selection was developed, scientists had at their disposal a natural—as opposed to a supernatural—explanation for the diversity of life on the planet, as well as an explanation for why the vast majority of life-forms that have ever existed are now extinct. More than that, they had a theory that could be used to explain the similarities and differences among all the creatures on Earth and to explain why organisms are usually so well suited to the environments in which they live.

Paradigm shifts have wide-ranging effects, and that was certainly the case for Darwin's theory—so much so that the renowned geneticist Theodosius Dobzhansky wrote, "nothing in biology makes sense except in the light of evolution" (Dobzhansky 1973, p. 125). Without evolutionary theory, biology is composed of a large number of important but disparate subdisciplines. With evolution as its theoretical and conceptual foundation, the biological sciences share a common framework that allows us to understand both the commonalities and differences among living forms; it allows us to make sense of the way that living things function now and to understand how they came to be.

The study of physics is fundamental to understanding our universe, because it allows us to reconstruct the grand story of how the universe came to be as it is, and it lets us understand how the universe operates today. The study of evolution is similarly fundamental in that it allows us to reconstruct the grand story of how all living things came to be and how they (and we) function.

As you will see as you work your way through this book, the characteristics of the organisms you are studying have been shaped by evolutionary processes. Whether you are interested in anatomy, physiology, behavior, molecular biology, genetics, development, medicine, or any other area of biology, a solid understanding of evolution is indispensable.

In this chapter, we will

- Provide a brief introduction to evolution and natural selection, including examples related to (1) artificial selection, (2) antibiotic resistance, (3) conservation biology, and (4) molecular genetics, evolution, and behavior in primates.
- Give an overview of empirical and theoretical approaches to the study of evolution.
- Discuss a more detailed example of the way that empirical and theoretical approaches interact by looking at the evolution of sex ratios.

1.1 A Brief Introduction to Evolution, Natural Selection, and Phylogenetics

The science of evolutionary biology reads like a thrilling detective story in the sense that it unravels a great mystery. Indeed, evolutionary biologists *are* detectives—as are all scientists—but they are much more than that. The study of evolutionary biology allows us not only to infer the relationships among all life that has ever lived and to track the diversity of life across vast stretches of time, but also to test hypotheses through a rigorous combination of observation and experimental manipulations. These observations and experiments may involve examining fossils or contemporary organisms; they may use, among other things, anatomical, physiological, hormonal, molecular genetic, developmental, and behavioral data; and they may involve analyzing data from DNA sequences to population composition (**Figure 1.1**).

At its core, evolutionary biology is the study of the origin, maintenance, and diversity of life on Earth over approximately the past 3.5 billion years. To understand the **evolution** of a species fully, we need to know the ancestral species from which it *descended*, and we need to know what sort of *modifications* have occurred along the way. Darwin referred to this entire process as **descent with modification**.

FIGURE 1.1 Sources of data for testing models of evolution. A few examples of the sources of data that evolutionary biologists use to test their hypotheses: **(A)** data from the fossil record, as shown by this fossil ammonite found in Dorset, England; **(B)** behavioral data, as shown by observing the behavior of gelada baboons in Ethiopia; **(C)** morphological data, as shown by this display of wing color patterns on *Bicyclus anynana* butterfly wings; **(D)** embryological data, as shown by the magnetic resonance imaging of developing mouse embryos between day 9.5 and day 19, when the mouse is born; and **(E)** molecular genetic data, as shown by this DNA sequence film.

To understand the evolution of *Homo sapiens,* for example, we need to understand the primate species from which it descended (as well as other species closely related to this ancestral species) and the changes that occurred over the period in which *H. sapiens* evolved. Because those earlier species are no longer present, we often have to infer their properties by comparing the properties of multiple living species. We use the same reasoning if the species in question is the malaria parasite (*Plasmodium falciparum*) or corn (*Zea mays*). That is, we try to discern the ancestral history of the species in question, and, at the same time, we attempt to track the modifications that have occurred in that species. We aim to understand the process of descent with modification.

One of the most important processes responsible for the modifications that occur over time is natural selection. We will discuss natural selection and other evolutionary processes in greater detail in later chapters. For the time being, we can summarize the process of natural selection as follows. Genetic **mutations**, or changes to the DNA sequence, arise continually and change the **phenotype**—the observable, measurable characteristics—of organisms. These mutations can increase fitness, decrease fitness, or have no effect on fitness, where **fitness** is measured in terms of relative survival rates and reproductive success. Many, perhaps most, mutations will disrupt processes that are already fine-tuned, and thus they will have harmful effects on fitness. By analogy, consider tinkering with a computer program. If you randomly change one line of code, chances are that you will break the program entirely, degrade its performance or, at very best, have no effect on the program's function. But some times you will get lucky—your change may actually improve the program's operation. Genetic mutations are similar. Most are deleterious or neutral, but some mutations turn out to be advantageous in the sense that the individuals who carry them may have more surviving offspring than average. Such genetic changes that improve the fitness of individuals will tend to increase in frequency over time.

The result is evolutionary change by natural selection. The accumulation of advantageous genetic changes, amassed over long periods of time, can produce dramatic effects within a population, even to the extent of producing new species, genera, families, and higher taxonomic orders. Indeed, as we will see many times throughout the course of this book, the process of natural selection is fundamental in what are called the **major transitions** that have taken place over the past 3.5 billion years of life on Earth—the evolution of the prokaryotic cell, the evolution of the eukaryotic cell, the evolution of multicellularity, and so on.

Repeatedly throughout this book, we will examine the power of natural selection in shaping the life that we see around us. We begin with some of the practical applications of understanding evolution via natural selection. Then we examine phylogenetics—how evolutionary history can be inferred using patterns of common descent—to again address an issue of practical application, in this case policies in conservation biology. The examples in this section, as well as all the examples we discuss in this chapter, are meant to illustrate some of the major concepts, methods, and tools that biologists use to understand evolution.

Evolutionary Change and the Food We Eat

The next time you sit down for a meal, take a look at the items on your plate. Whether you're enjoying a home-cooked supper or fast-food takeout, the food you are eating is almost certainly the product of evolutionary change due to intense **selective breeding** over time (Denison et al. 2003; Abbo et al. 2012; Larson et al. 2014) (**Figure 1.2**). Indeed, humans have been selectively breeding grains, such

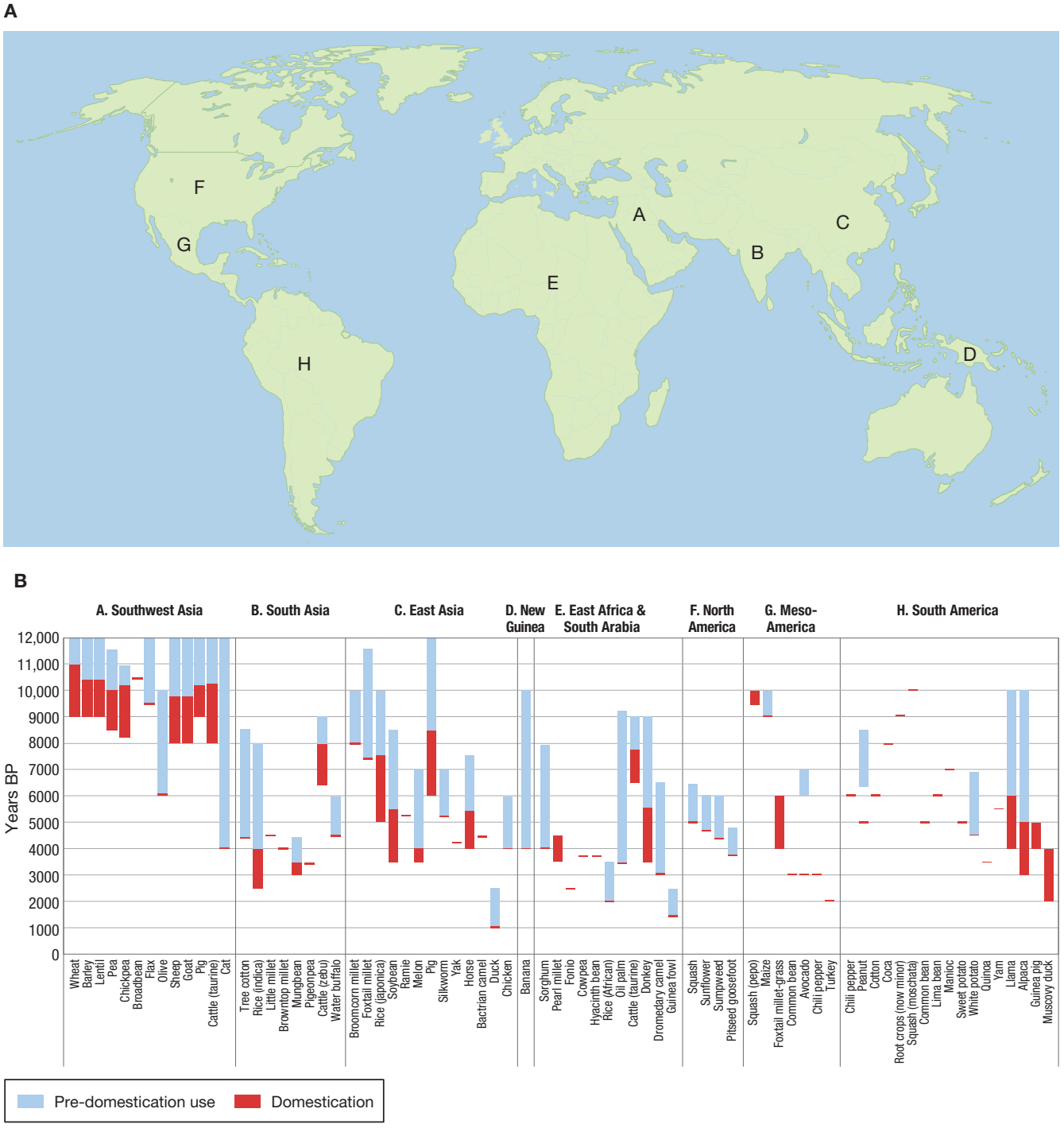


FIGURE 1.2 Domestication of plants and animals around the world. (A) A map showing locations where at least one plant or animal domestication event is thought to have occurred. Labels A–H represent geographic regions seen in panel B. **(B)** A chronology of when and where plants and animals were domesticated. Where possible, extended bars denote the period of pre-domestication use (blue) and the period during which domestication took place (red). Where exact domestication periods are unknown, narrow bars denote the latest possible date of domestication. Adapted from Larson et al. (2014).

as barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*), as well as lentils (*Lens culinaris*) and peas (*Pisum sativum*), for more than 10,000 years (Garrard 1999; Zohary and Hopf 2000; Abbo et al. 2003).

The process of human-directed selective breeding, known as **artificial selection**, is straightforward. In the case of crops, in each generation the best plants—for example, those that are the hardiest, quickest growing, and best tasting—are chosen as the parental stock for the next generation (**Figure 1.3**). If this process is repeated over time, the population of plants increasingly takes on these beneficial characteristics.

Artificial selection by humans is thus a counterpart to natural selection. With natural selection, traits that are associated with increased survival and reproduction increase in frequency. With artificial selection, humans choose which individuals reproduce, and in so doing, we select traits that are in some way beneficial to us. Such selective breeding can produce dramatic results. For example, the productivity of wheat (*Triticum aestivum*), rice (*Oryza sativa*), and corn (*Zea mays*) has doubled since 1930; much of that increase is due to selection for genetic crop strains better adapted to their agricultural environments (Jennings and de Jesus 1968; Ortiz-Monasterio et al. 1997; Duvick and Cassmann 1999). And the same holds true when we look at the selective breeding of animals, which has resulted in increased egg production by chickens and increased milk production by dairy cows (Tixier-Boichard et al. 2012; Mancini et al. 2014).

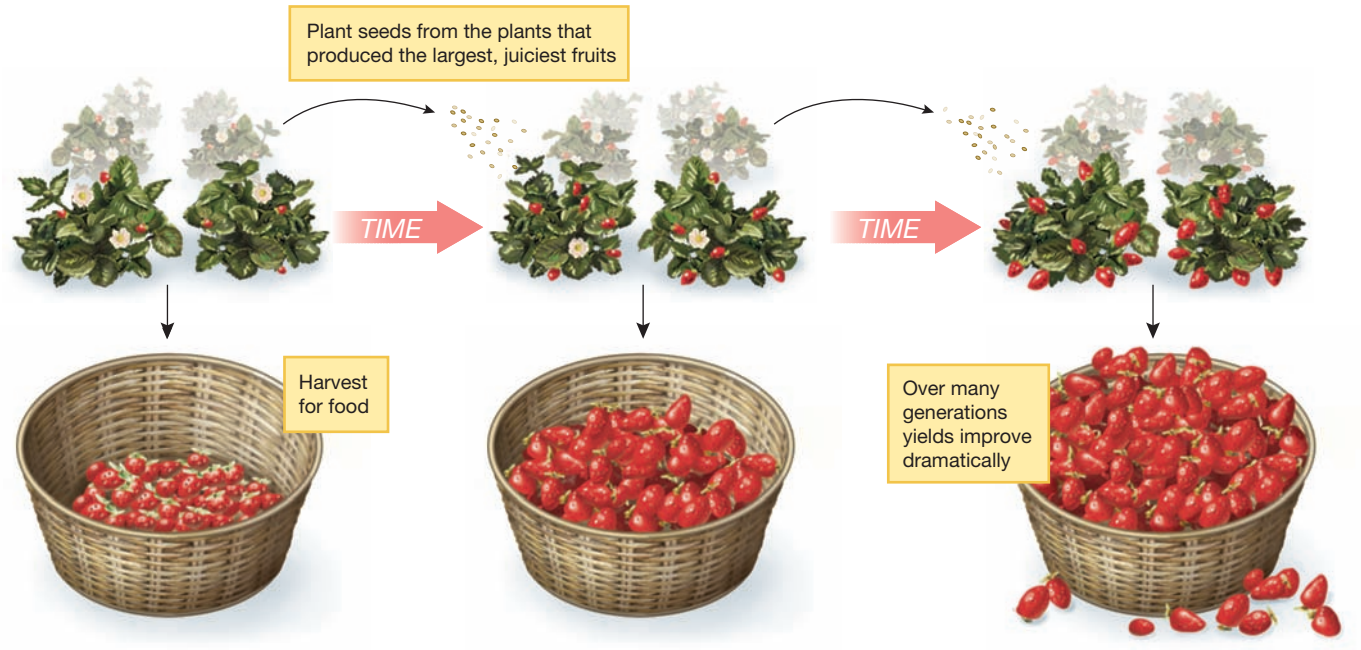


FIGURE 1.3 The process of artificial selection. Darwin used strawberries as an example of artificial selection, writing, "As soon, however, as gardeners picked out individual [strawberry] plants with slightly larger, earlier, or better fruit, and raised seedlings from them, and again picked out the best seedlings and bred from them, then, there appeared (aided by some crossing with distinct species) those many admirable varieties of the strawberry which have been raised during the last thirty or forty years" (Darwin 1859, pp. 41–42).

Even as artificial selection improves the quality and yield of crops and livestock, other evolutionary changes have detrimental effects on the human food supply, as we see with pesticide resistance. Although 10% to 35% of all U.S. crops are still lost to insect damage each year, the development of pesticides was a major breakthrough in reducing crop pests and thereby increasing crop productivity (Pimentel and Lehman 1991; National Research Council 2000). Natural selection, however, will tend to favor crop pests that are most resistant to such pesticides—as occurred when diamondback moths evolved resistance to one of the most frequently used insecticides of the late 1980s—resulting in an "arms race" between pest species that feed on crops and humans determined to get rid of such species (Ceccatti 2009; Furlong et al. 2013). As resistant pests increase in frequency, humans produce ever-stronger insecticides. Because evolutionary change occurs quickly in insects because of their short generation times, humans often lose this particular arms race, and therefore we continually need to develop new pesticides.

Why do we call the evolution of resistance to pesticides natural selection instead of artificial selection, given that humans are the ones producing and distributing the pesticides? The distinction between artificial and natural selection refers not to whether human activity is involved, but rather to whether humans deliberately choose which individuals will reproduce. In the case of increasing grain yields, humans actively select those varieties with higher yield; in the case of increasing pesticide resistance, humans produce the pesticides but do not deliberately choose pesticide-resistant strains of insects for further reproduction. Indeed, what we want—pests easily killed by our pesticides—is just the opposite of what natural selection produces. Desirable or otherwise, evolutionary change due to human activity is sometimes called *anthropogenic evolution* (Carroll et al. 2014).

A problem similar to that of resistance to pesticides unfolds when we look at another product produced by humans: antibiotics.

Evolutionary Change and Pharmaceuticals

One theme that we will return to repeatedly throughout this book is the manner in which research in evolutionary biology can inform our understanding of disease and help us to design more effective responses to the problems associated with disease. For example, the discovery and development of antibiotic drugs for preventing or treating bacterial infections was one of the major medical developments of the twentieth century. But ever since humans first began using antibiotics, medical practitioners have had to deal with bacteria that are resistant to these drugs. The first modern antibiotic, penicillin, was introduced clinically in 1943; within a single year, penicillin resistance was observed, and within 5 years it had become common in a number of bacterial species. Since then, numerous new antibiotics have been developed and introduced to the market, only to lose their effectiveness within a matter of years as bacteria evolved resistance to the drug (Lacey 1973; Piddock et al. 1998; CDC 2007) (**Figures 1.4** and **1.5**). The evolution of **antibiotic resistance** is the result of natural selection and can be understood only in the context of evolutionary biology.

Bacteria reproduce at an astounding rate—in some cases, as frequently as once every 20 minutes. They reach enormous population sizes—a single gram of feces

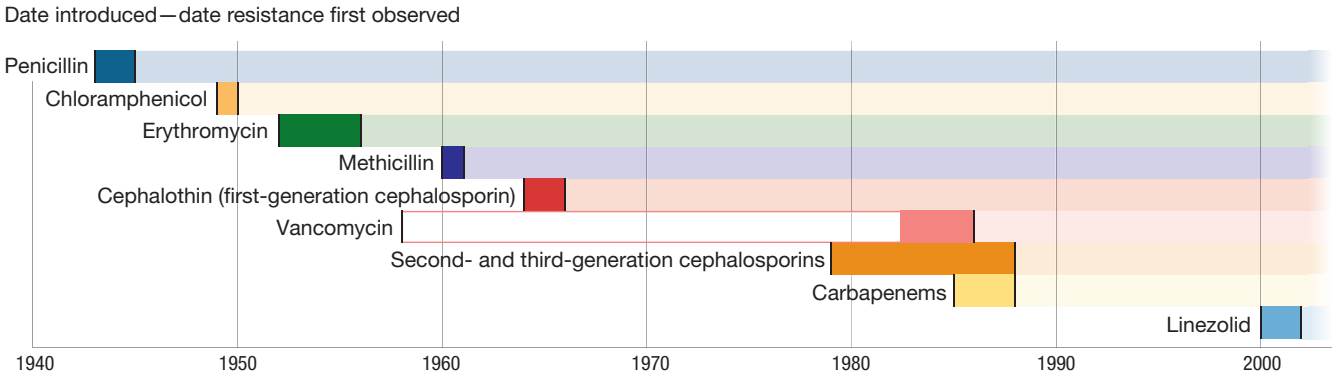


FIGURE 1.4 Bacteria have rapidly evolved resistance to clinical antibiotics. Ever since the first modern antibiotic, penicillin, was introduced in 1943, new antibiotics that come to market quickly lose their effectiveness within a few years because bacteria evolve resistance to the new drugs. Note that vancomycin was first released in 1958; however, it was not widely used until the early 1980s. Adapted from Bergstrom and Feldgarden (2008).

can contain 100 billion bacteria—which offer plentiful opportunities for mutations that provide resistance to arise. Antibiotics impose very strong natural selection for resistant strains. For all of these reasons, bacteria can evolve extremely rapidly, and when they are exposed to antibiotics, this is precisely what they do (Genereux and Bergstrom 2005).

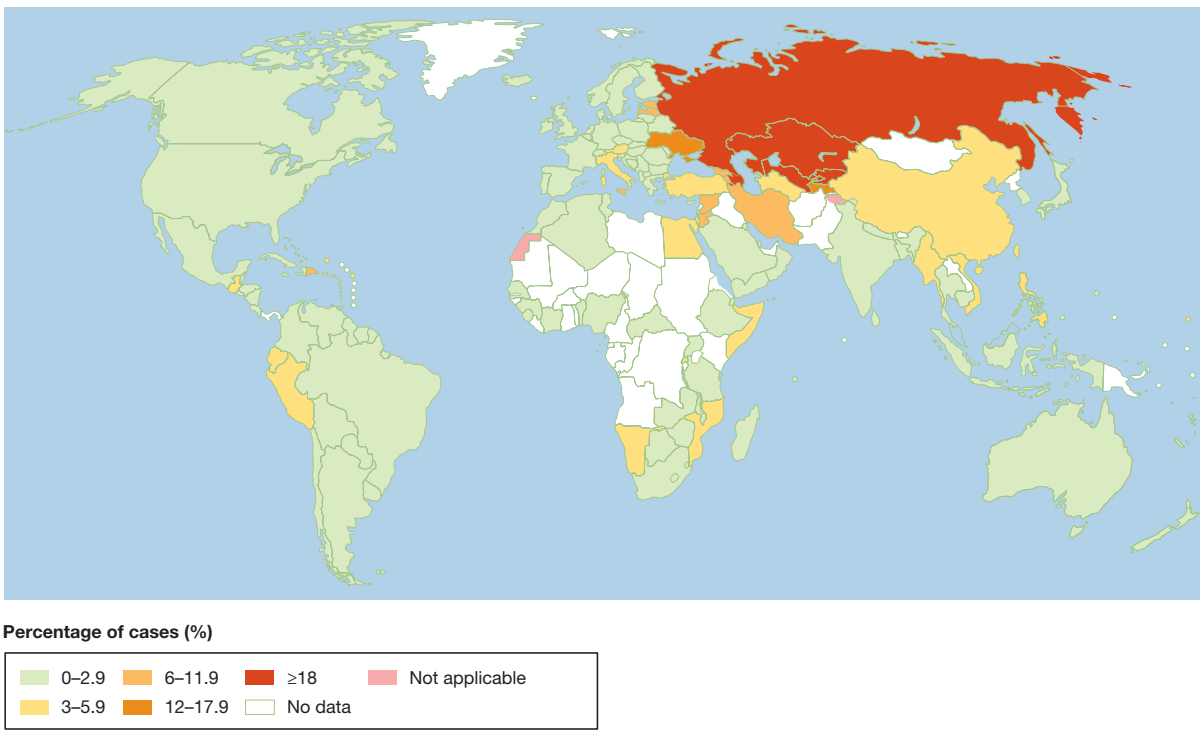


FIGURE 1.5 The rise and spread of drug-resistant tuberculosis. Color indicates the percentage of new cases of tuberculosis in which strains of *Mycobacterium tuberculosis* are resistant to treatments with multiple antibiotic drugs. From World Health Organization (2013).

Imagine that we are watching the evolution of resistance to the antibiotic ciprofloxacin. Among other uses, ciprofloxacin is often prescribed for severe cases of food poisoning by the bacterium *Campylobacter jejuni.* This bacterium is common in the intestines of livestock, where it causes no symptoms, but it can cause acute food poisoning in humans who acquire it by eating contaminated meat. At the start of the process, the gut of a single infected human patient houses millions or even billions of *Campylobacter* cells that are exposed to ciprofloxacin. Early on, the antibiotic may be deadly to these cells. But with vast numbers of bacterial cells exposed to the antibiotic, and with each cell dividing quickly, it is only a matter of time (weeks, months, perhaps years) before a mutation appears that creates a strain of *Campylobacter* cells that are somewhat resistant to our antibiotic. In a patient being treated with ciprofloxacin, this new strain can outcompete the susceptible strain, and the resistant *Campylobacter* strain will eventually become the dominant form. The process then starts anew, and soon another genetic change occurs, producing a strain of *Campylobacter* that is even more resistant to the antibiotic, and that strain quickly takes over. Repeating this process over and over results in a strain of *Campylobacter* that is highly resistant to the antibiotic (**Figure 1.6**).

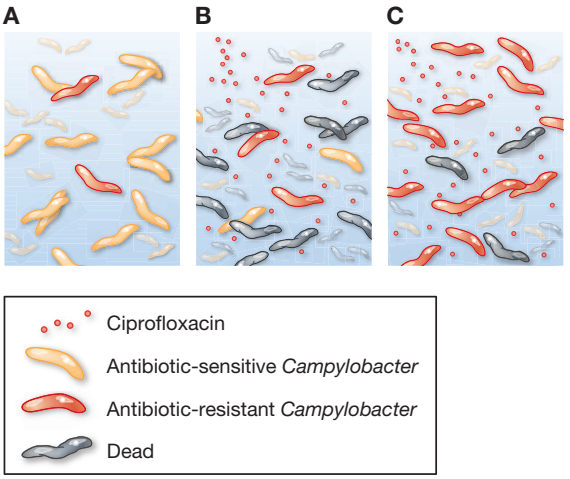


FIGURE 1.6 Ciprofloxacin use selects for resistant *Campylobacter*. (A) Prior to antibiotic treatment, most *Campylobacter* are ciprofloxacin-sensitive (meaning that they cannot grow in the presence of ciprofloxacin), but a few resistant variants may also be produced by mutation. (B) Ciprofloxacin treatment kills or halts the growth of the sensitive strains, but the resistant strains survive. (C) In the presence of ciprofloxacin, resistant *Campylobacter* take over the population.

While *Campylobacter* is rarely life threatening, its consequences are certainly dramatic when considered in aggregate: Antibiotic-resistant strains of *Campylobacter* are estimated to be responsible annually for nearly 500,000 more days of diarrhea in the United States than would occur in the absence of *Campylobacter* (Travers and Barza 2002). Other antibiotic-resistant bacteria such as *Staphylococcus aureus, Enterococcus* species, and *Pseudomonas aeruginosa* pose an even more significant threat (**Figure 1.7**). Today, antibiotic-resistant strains of these and other bacteria are largely responsible for an epidemic of hospital-acquired infections that kill an estimated 90,000 people per year in the United States—more than AIDS, influenza, and breast cancer combined (Bergstrom and Feldgarden 2008).

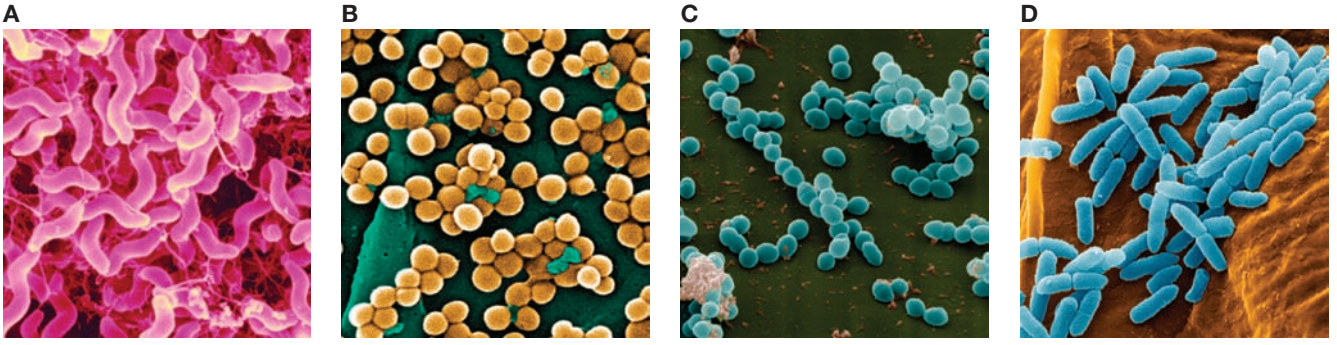


FIGURE 1.7 Antibiotic-resistant bacteria pose serious health problems. (A) *Campylobacter jejuni* is a leading cause of food poisoning in the United States. (B) While *Staphylococcus aureus* is commonly carried on the human body without ill effect, this species can cause severe skin infections and invade surgical wounds. Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for many hospital-acquired infections and, once acquired, is difficult to treat. (C) Vancomycin-resistant strains of the normally harmless gut bacterium *Enterococcus faecalis* are another cause of hospital-acquired infections; mortality is fivefold higher for patients infected by antibiotic-resistant strains rather than antibiotic-sensitive strains. (D) *Pseudomonas aeruginosa* is an opportunistic pathogen that causes hospital-acquired infections and is responsible for chronic lung infections in individuals with cystic fibrosis.

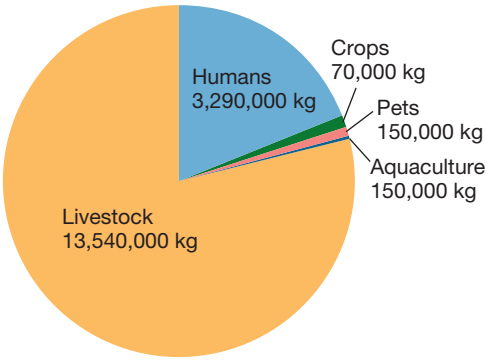


FIGURE 1.8 Antibiotic use in the United States. Less than a fifth of the antibiotics (as measured in kilograms per year) used in the United States are deployed for human use. The majority of antibiotic use occurs in agriculture. Adapted from Hollis and Ahmed (2013).

The study of evolutionary biology allows us to understand how antibiotic resistance evolves in these bacteria; this understanding in turn helps us deal with the health threat that such bacteria pose. In the course of drug development, pharmaceutical companies routinely screen potential new antibiotics by exposing bacteria to a wide range of antibiotic concentrations in an effort to find drugs to which antibiotic resistance does not readily evolve. Physicians often prescribe antibiotics in combination because drug combinations retard the rate at which antibiotic resistance evolves; even if the mutations needed for resistance to one drug should arise, the other drug may kill these bacteria before they can spread. In Europe, the agricultural use of many antibiotics has been banned now that we understand how antibiotic-resistant strains of bacteria can evolve in farm animals and then spread to humans (Cogliani et al. 2011). In the United States, antibiotics are still widely used in agriculture (**Figure 1.8**), but recently the Food and Drug Administration has banned the agricultural use of a few antibiotic classes and encouraged the discontinuation of others (Hollis and Ahmed 2013).

The use of evolutionary models to address questions relevant to disease is not limited to antibiotic resistance. In subsequent chapters, we will see other examples in which ideas and experiments from evolutionary biology have contributed to a fundamental understanding of influenza, sexually transmitted diseases such as AIDS, and many other infectious diseases. Evolutionary biology has likewise contributed to our understanding of chronic ailments such as diabetes and obesity, and even to an understanding of the phenomenon of aging itself. In some instances, such as that of antibiotic resistance, we can use our understanding of natural selection to design and construct models and experiments relevant to the study of disease; in other instances, we will examine how understanding patterns of common descent can achieve the same ends.

Phylogenetic Diversity and Conservation Biology

Evolutionary biologists hypothesize that all living things have descended from a common ancestor, and over eons the descendants of this common ancestor have diversified to yield the myriad forms that we observe in the world today (Chapter 4). We can view all species that live or ever have lived as forming a vast branching tree of relationships known as the **tree of life** (**Figure 1.9**). Such a tree captures the historical relationships among life-forms and is known as a **phylogenetic tree**. Often, each tip of a phylogenetic tree represents a species that is currently living or a species that has gone extinct; branch points represent points of divergence—events associated with the origin of a new lineage—that occurred in the past. This branching pattern of common ancestry and descent is one of the most important conceptual foundations of biology. The tree of life provides us with a map of the history of life, a map that reflects the process of descent with modification that gave rise to all living forms. It connects evolutionary history to the current diversity of life on Earth.

An understanding of the tree of life as the product of evolutionary processes tells us about the history of living things, and it also has immediate practical consequences for the world today. For example, phylogenetic thinking provides new ways to conceptualize the challenges of conserving biodiversity. When we

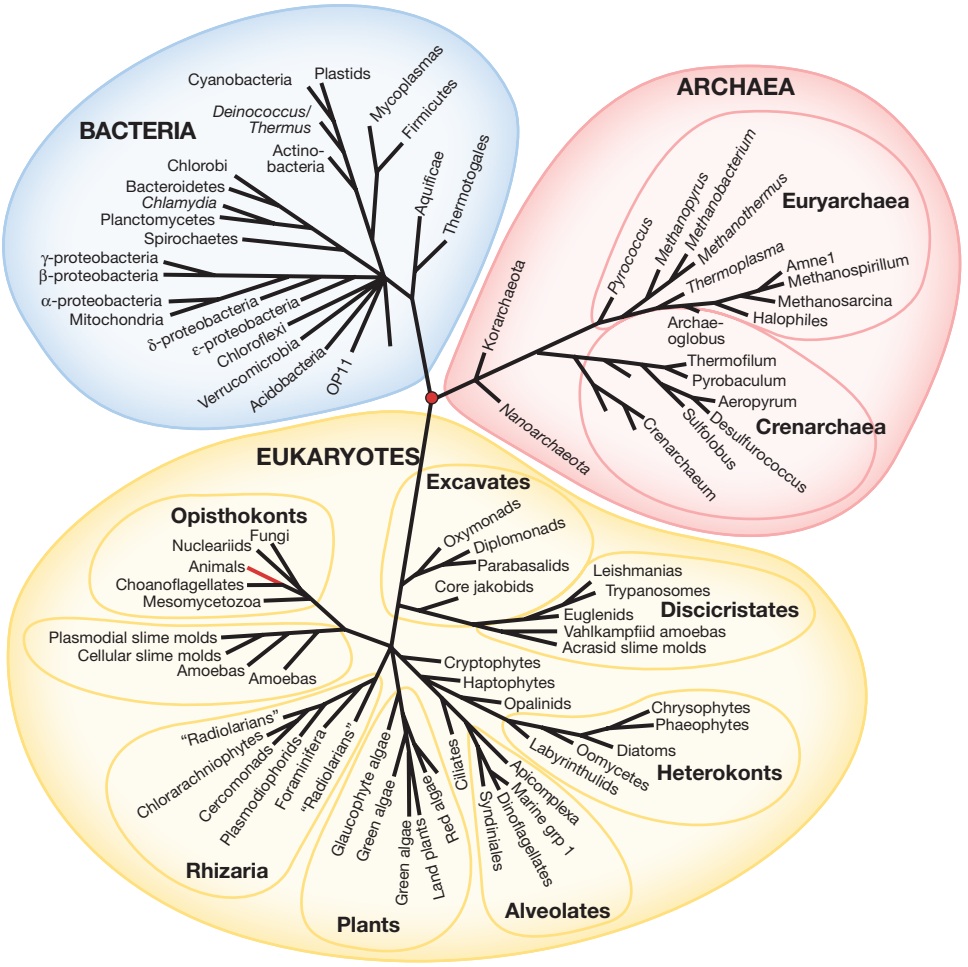


FIGURE 1.9 The tree of life. According to the three-domain model, the tree of life has three main branches: Bacteria, Archaea, and Eukaryote. These three groups are often referred to as domains. In Chapter 12 we will consider a recent alternative model that organizes life into only two domains. Adapted from Baldauf et al. (2004).

think about **extinction**—that is, the loss of species—we typically focus on the ecological consequences: When a species goes extinct, it disappears from a community or ecosystem where formerly it had occurred. But extinction has evolutionary consequences as well. Each time a species or group of species goes extinct, a part of the tree of life is pruned away, and so part of the evolutionary history of life on Earth is lost (**Figure 1.10**).

As we attempt to slow the rate of human-caused extinctions, we often have to make hard choices about which species and which habitats to try to save. Traditionally, conservation biologists have tried to minimize the rate at which species go extinct, because it seems obvious that the best way to preserve biodiversity is to protect as many species as possible. But some conservation biologists are starting to suggest that instead of trying to conserve as many species as possible, we should try to conserve the maximum amount of **phylogenetic diversity**. That is, we should conserve as much as possible of the evolutionary history represented by currently living species (Mace et al. 2003; Winter et al. 2013).

For example, in **Figure 1.11**, the extinction of the three species E, F, and I results in the loss of three twigs (indicated in red) at the tips of the tree, but nothing more. By contrast, extinction of the two species B and C results in the loss of

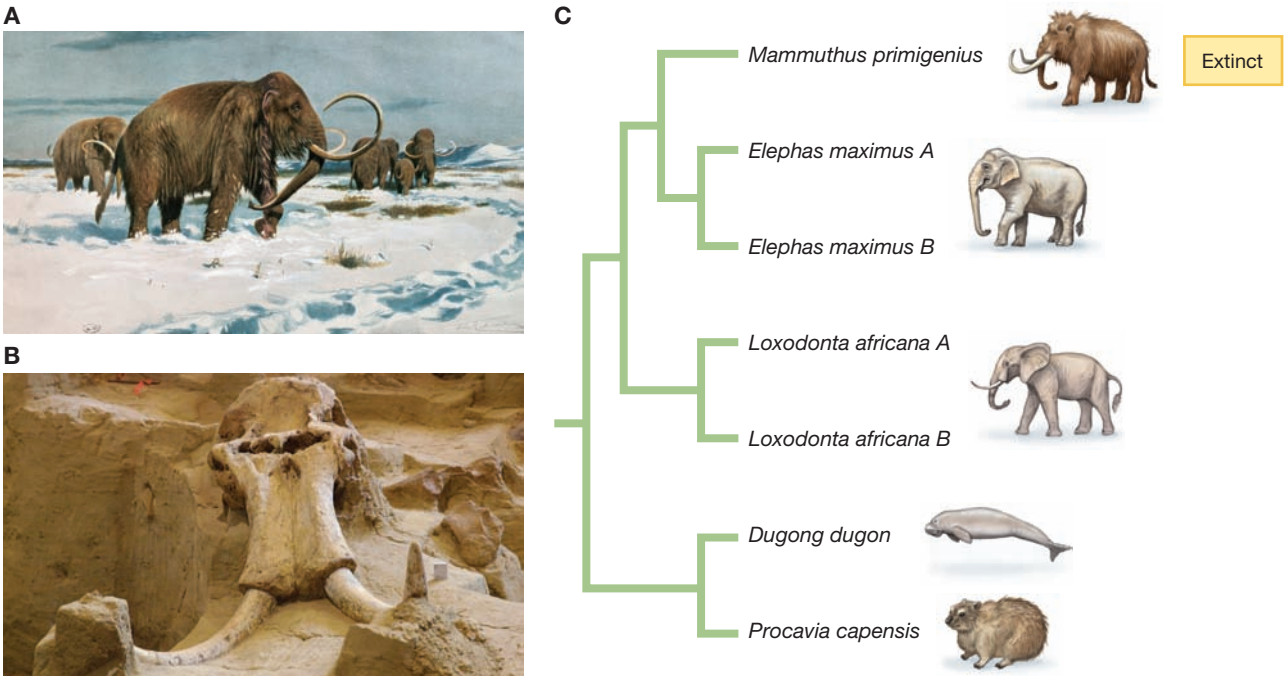


FIGURE 1.10 Woolly mammoth extinction. **(A)** The woolly mammoth (*Mammuthus primigenius*) once roamed northern North America and northern Eurasia. It went extinct approximately 10,000 years ago. **(B)** A fossilized skull of the woolly mammoth. **(C)** A phylogeny of paenungulata mammals. Panel C adapted from Rogaev et al. (2006). Extinction here causes one twig of this tree (the branch leading to *Mammuthus primigenius*) to be pruned.

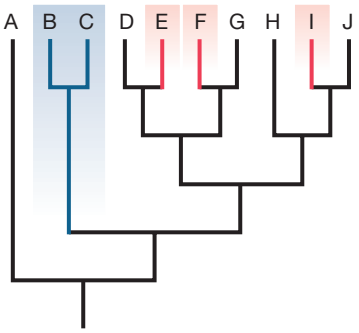


FIGURE 1.11 Extinction and twigs on phylogenetic trees. Assuming that species D, G, H, and J survive regardless, the extinction of species B and C (in blue) prunes this phylogenetic tree more severely than does the extinction of species E, F, and I (in red).

a major branch (indicated in blue) of the phylogenetic tree. If we are interested in conserving phylogenetic diversity, the latter is a greater loss.

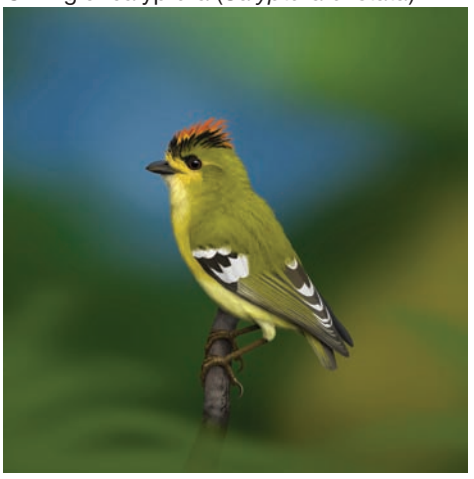
If conservation biologists are interested in preserving phylogenetic diversity, they may need to rethink the way that they prioritize which species to protect. For example, one of the major ways that species are prioritized today is by their categorization on the Red List of Threatened Species, a document published by the International Union for Conservation of Nature (IUCN; http://www.iucnredlist.org). The Red List is designed to draw attention to species most in danger of going extinct. Until recently, however, there was little data available to determine whether probability of extinction bore any relation to phylogenetic diversity; that is, we simply did not know whether targeting threatened species had any effect on phylogenetic diversity.

To address this question, Jose Hidasi-Neto and his colleagues looked at bird species in Brazil, which is home to 18% of the world's bird species. In particular, they focused on bird species that were listed in one of four Red List categories: near threatened, vulnerable, endangered, or critically endangered (**Figure 1.12**). Using these data, they asked whether protecting these species would increase, decrease, or have no effect on phylogenetic diversity compared to protecting a random sample of Brazilian bird species. What they found was that protecting the species listed as near threatened, vulnerable, endangered, or critically endangered was no better than randomly conserving the phylogenetic diversity of Brazilian birds (Hidasi-Neto et al. 2013). This is not to suggest that the Red List is unimportant or that it necessarily should be changed, but rather that if protecting

A Cherry-throated tanager (*Nemosia rourei*)

B Glaucous macaw (*Andoorhynchus glaucus*)

C Kinglet calyptura (*Calyptura cristata*)



D Stresemann's bristlefront (*Merulaxis stressmanni*)

FIGURE 1.12 Endangered birds of Brazil. The cherry-throated tanager (*Nemosia rourei*), glaucous macaw (*Andoorhynchus glaucus*), kinglet calyptura (*Calyptura cristata*), and Stresemann's bristlefront (*Merulaxis stressmanni*) are all listed as critically endangered on the IUCN Red List.

phylogenetic diversity is an aim, using the Red List to allocate resources may not be the optimal strategy.

Appealing as it may sound to base conservation goals on phylogenetic diversity, perhaps our conservation agenda should not focus exclusively on preserving evolutionary history. For example, you might reasonably argue that, rather than focusing on history, it is important to save a population in which evolution is occurring rapidly and new species are being formed. While you might think that such a population would also be a major contributor to phylogenetic diversity, the opposite is often true. Species in areas where rapid diversification is occurring will be relative newcomers—new twigs on the tree of life—and it will be unlikely that all twigs on a major branch of the tree will perish at once. So, if we wish *only* to preserve phylogenetic diversity, we need not be as concerned with areas where rapid **speciation** is occurring (**Figure 1.13**).

The point here is not that one particular evolutionary model is best suited to solve all problems in conservation biology. Rather, the point is that when making decisions regarding biodiversity, conservation biologists could not even address these issues or have this important debate until they started thinking

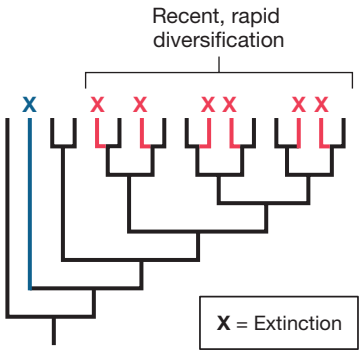


FIGURE 1.13 Hot zones, extinction, and evolutionary history. If we try to preserve evolutionary history, the loss of the single blue species—which represents the only species on its branch of our tree—would produce a deeper cut than the loss of all the red species in a hot zone where speciation is occurring rapidly.

about evolutionary processes and consequences (Nee and May 1997). Thinking in this way also helps us put the current wave of human-caused extinctions into context. As Georgina Mace and her colleagues point out, "The tree of life is currently being pruned by extinction very much more rapidly than it is growing" (Mace et al. 2003, p. 1708). We need to consider all options when combating this alarming trend.

KEYCONCEPT QUESTION

1.1 Can you think of other ways that evolutionary thinking might affect studies in conservation biology?

1.2 Empirical and Theoretical Approaches to the Study of Evolution

We have seen how evolutionary principles can be applied to a variety of subjects, but what *approaches* do evolutionary biologists use in their quest to understand why things are the way they are? Any field of scientific endeavor requires us to generate and test alternative hypotheses. Indeed, the scientific process is all about postulating a series of testable hypotheses, ruling out alternatives, and homing in on the hypotheses that best seem to represent what is happening in nature (Mayr 1982, 1983). In generating and testing hypotheses, evolutionary biologists use a combination of empirical and theoretical approaches.

Empirical Approaches

The majority of this book focuses on empirical research. As we will see, empirical work in evolutionary biology can take many forms, but it almost always falls under one of two categories: observations or manipulations. Observational work entails gathering data to test hypotheses without attempting to manipulate or control the system being studied. Examples include (1) studying the fossil record to test predictions from evolutionary biology, as well as to generate new predictions; (2) inferring evolutionary history from genetic sequences; and (3) recording and measuring behaviors occurring in a natural population of organisms (we will examine all of these in later chapters). Observational studies like these make up a powerful form of scientific research, and they have been used to test a myriad of evolutionary hypotheses.

Another approach is to design controlled manipulative experiments to test a hypothesis. Manipulative experiments allow a scientist directly to assess how changes in one component of a system influence the other components. This allows us to examine not only correlations among data but also causality; that is, what causes what. Ideally, manipulative experiments alter only one variable at a time, so that the investigator can ascertain which changes yield what results.

To examine how empirical studies in evolution work, we will consider two examples: (1) a comparison of the human and chimp genomes, and what this can teach us about primate evolution; and (2) a comparative behavioral study on how breeding system affects testes size in 33 species of primates.

Molecular Genetics and Evolution in Chimps and Humans

More than 100 years ago, Darwin and his colleague Thomas Henry Huxley hypothesized that humans share a common ancestor with the great apes (chimpanzees, gorillas, and orangutans) and gibbons. Their hypothesis was primarily based on data from **comparative anatomy**. Darwin and Huxley made inferences about the evolutionary history of humans by comparing the anatomical similarities and differences observed between humans and other primates in such traits as tooth and jaw shape, bone structure of the hands and feet, mode of locomotion, and brain size and structure (**Figure 1.14**).

If Darwin and Huxley's hypothesis is correct—if the great apes are our closest living relatives—data from modern molecular genetics should corroborate the inferences drawn from comparative anatomy. Indeed, this is the case. Evidence from molecular genetics provides strong support for Darwin and Huxley's hypothesis, with chimpanzees and bonobos (pygmy chimps) as our closest living relatives. Humans and chimps, for example, have very similar genomic structure. They differ by one set of chromosomes: Humans have 23 pairs, and chimps have 24 pairs. When high-resolution pictures are taken of human and chimpanzee chromosomes, researchers can see that human chromosome 2 is the result of a fusion of two chromosomes at some point in human evolutionary history (Yunis and Prakash 1982) (**Figure 1.15**). Subsequent molecular genetic analyses, in which the DNA sequences from chromosome 2 in both chimps and humans were lined up and compared—nucleotide by nucleotide—has shown researchers the exact location where the chromosomal fusion occurred (Fan et al. 2002).

The entire genomes of both the chimpanzee and the human have now been mapped out in great detail. This allows us to make unprecedented molecular genetic comparisons to examine questions of primate evolution (Mikkelsen et al. 2005; Khaitovich et al. 2006). Tarjei Mikkelsen and his colleagues in the Chimpanzee Sequencing and Analysis Consortium mapped out approximately 95% of the chimpanzee genome (from eight chimpanzees) and compared that with the human genome (mapped out from a small set of humans). A whole-genome comparison of DNA nucleotides found that humans and chimps differ by about 1.3%, although comparisons of specific sections of the genomes reveal that the DNA sequences differ more in some areas and less in others (**Figure 1.16**).

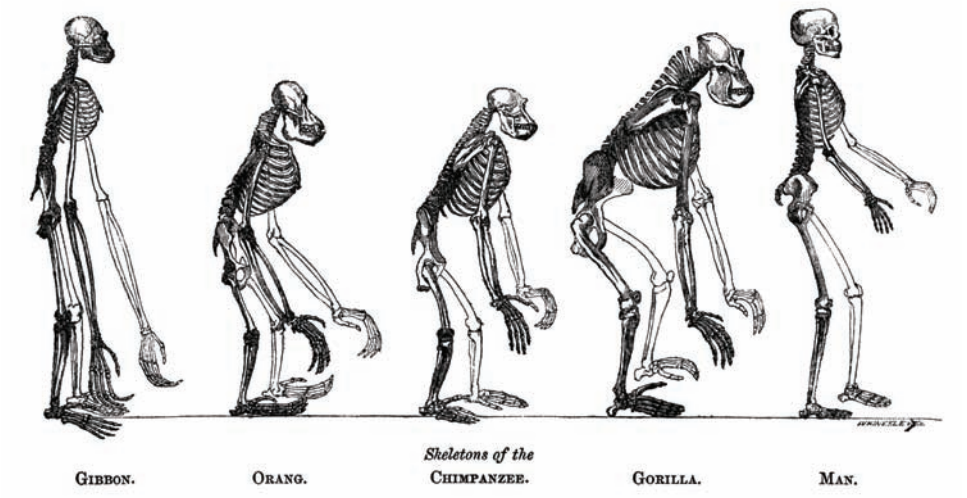


FIGURE 1.14 Huxley, Darwin, and primate evolution. Huxley and Darwin often used anatomical comparisons to infer the evolutionary history of humans and other primates. This example is from Huxley's *Evidence as to Man's Place in Nature* (originally published in 1863).

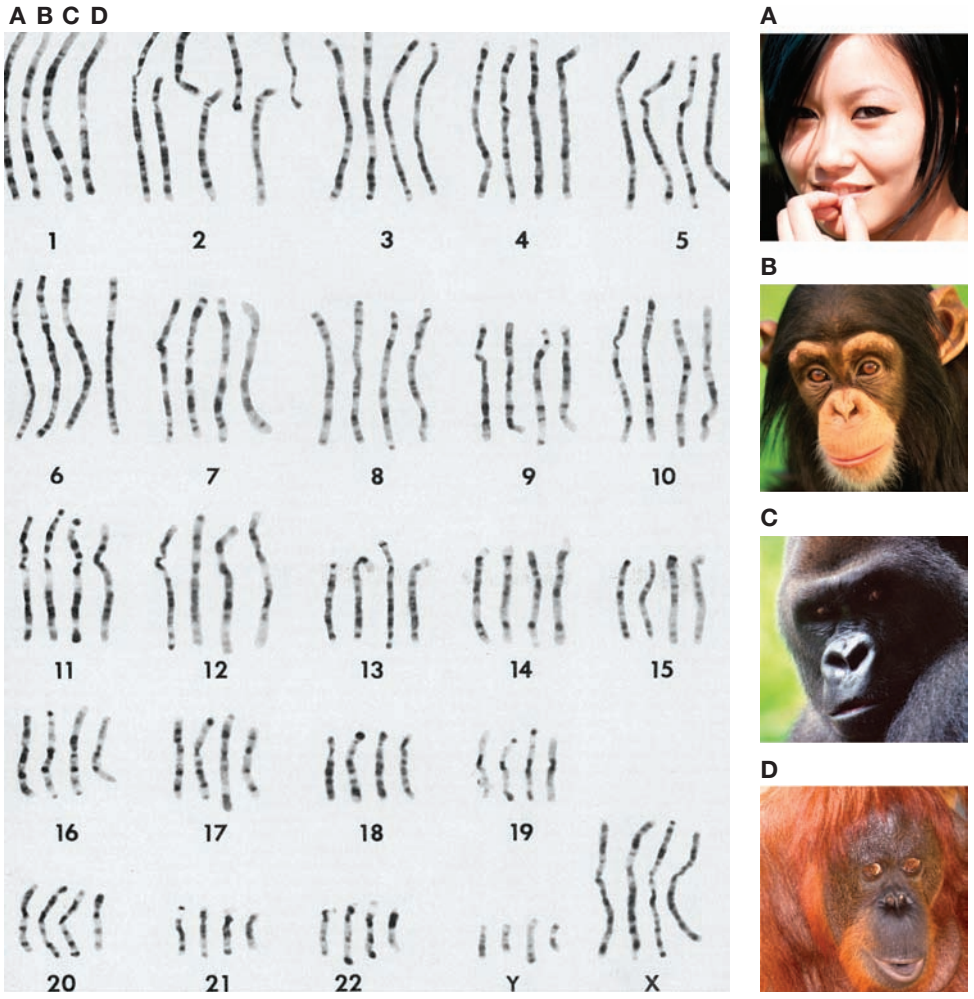


FIGURE 1.15 Primate chromosomes. From left to right for each set of chromosomes: the chromosomes of (**A**) humans, (**B**) chimpanzees, (**C**) gorillas, and (**D**) orangutans. Humans have one fewer pair of chromosomes as a result of the fusion of chromosomes 2p and 2q in chimpanzees (the second and third strands in the chromosome 2 panel).

When Mikkelsen's group compared 13,454 pairs of genes in humans and chimpanzees, they began by calculating how much we would expect the human and chimp genomes to diverge because of the accumulation of **neutral mutations**; that is, genetic changes that have no effect on fitness. This served as a baseline value that accounted for differences between the human and chimp genomes that were not due to natural selection.

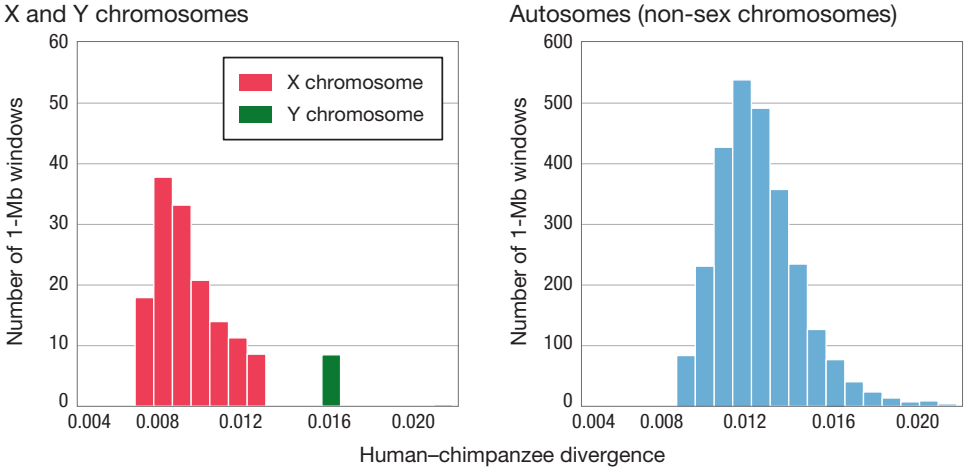


FIGURE 1.16 Human–chimp divergence rates. Human–chimp divergence rates across 1-megabase areas of the human and chimp genomes (1 megabase [Mb] = 1 million base pairs). Divergence is generally low but varies across locations. Adapted from Mikkelsen et al. (2005).

Once these neutral genetic differences were accounted for, Mikkelsen and his colleagues could search for evidence of divergence between chimps and humans that was due to natural selection. To do this, they examined whether some genes changed at higher rates than expected for neutral genes. When they found such genes, Mikkelsen and his team could often correlate these increased rates of divergence with known functions of the genes in question. This type of analysis found evidence for rapid evolutionary changes as a result of natural selection. These included genetic changes in humans associated with increased resistance to a bacterium that causes tuberculosis and a protozoan that causes malaria.

Mikkelsen and his colleagues used the same approach outlined above to compare *clusters of genes* in humans and chimps. That is, they again calculated the rate of divergence between humans and chimps expected due to neutral genetic change, and then they searched for evidence of divergence that is above that rate as evidence for natural selection; however, this time they used this approach for clusters of genes rather than single genes. In addition, they compared the rates at which clusters of genes have been evolving in both humans and chimps to those in other mammals whose genomes have been sequenced. This analysis revealed that natural selection has been acting strongly on both human and chimp genes in gene clusters associated with both survival and reproduction, the two components of fitness. Gene clusters associated with resistance to disease are evolving rapidly, as are gene clusters linked with reproductive traits such as sperm production and production of various proteins during pregnancy. Understanding such evolutionary changes has implications for many medical issues, including maternal health and male infertility.

If chimpanzee and human genomes differ by only about 1.3% at the level of DNA base pairs, then how can we explain the dramatic differences in appearance and behavior between humans and chimps? Because these genomes have only recently been sequenced, and the amount of data cataloging tens of thousands of genes is astronomically large, we are just beginning to address this sort of question. Progress is already evident from many different lines of research. For example, researchers have found that important differences between humans and chimps may stem from the *expression* of genes. To understand the power of **gene expression**—which genes are turned on and off, and the timing of when they are turned on and off—remember that every cell in your body has the same set of genes, but skin cells look, feel, and do very different things than cells in muscles, cells in the liver, and so on. This is because the expression of genes differs among cell types.

The different way in which genes are expressed in humans and chimps may in part explain why chimps and humans look and act so differently, despite limited divergence at the level of DNA base pairs (Khaitovich et al. 2005, 2006). Philip Khaitovich and his colleagues at the Max Planck Institute for Evolutionary Anthropology measured gene expression in 21,000 genes expressed in the heart, liver, and kidney, in both humans and chimps. Using the basic statistical approach that we outlined earlier, they found evidence suggesting that gene expression in the heart, kidney, and liver has not diverged more than what would be expected by chance. In contrast, a much higher divergence rate, and much stronger evidence for natural selection, was found when Khaitovich and his colleagues compared gene expression in the cells of human and chimp testes. Divergence in gene expression in the testes is likely a result of the very different **mating systems**—the way in which reproductive behaviors are structured in a population—seen in humans and chimps (Harcourt et al. 1981; Kappeler and van Schaik 2004).

Khaitovich and his team also examined gene expression in the brains of humans and chimps (Khaitovich et al. 2006; Somel et al. 2013). Here the results were surprising. Given the evolution of language and other cognitively sophisticated traits in humans, we might expect high divergence in gene expression in the brains of humans and chimps (Dorus et al. 2004). Yet, this was not the case. Indeed, divergence in gene expression between the brains of humans and chimps was quite small compared to differences in gene expression in other organs. There is, however, some subtle evidence that natural selection has operated on gene expression in the brain during human evolution. Although the divergence in gene expression in the brains of humans and chimps is low, much of the difference that does exist appears to be due to natural selection on humans, not chimps, suggesting selection for brain function in humans relative to other primates. When Khaitovich and his team compared gene expression in *both* humans and chimps to gene expression in other mammalian species, they found evidence that, although there were relatively few changes in gene expression in the brains of humans versus chimpanzees, the changes that had occurred were large in magnitude and were more often due to changes in the human brain rather than the chimp brain. This result highlights a question that has been central to evolutionary biology since the time of Darwin and which we are just starting to answer using studies in evolutionary genomics: Does major evolutionary change occur as a result of a large number of mutations with modest effects or a small number of mutations that have large effects?

Primate Breeding Systems and Testes Size

While molecular genetic studies can reveal a great deal about the evolutionary process, we need not restrict our analysis of evolutionary questions to this level. Evolutionary processes can be studied at levels far removed from the nucleotides that make up DNA. Indeed, much work on evolution by natural selection has examined behavioral traits—traits that are sometimes (but not always) very difficult to trace back to the action of a particular gene or a set of genes. To see this, let's examine natural selection, mating systems, and testes size in primates.

Primatologists have long noticed an interesting relationship between mating systems and testes size in primates. Gorillas and orangutans have *single-male* breeding systems, in which females mate with only one resident male, but resident males mate with many females. In species with single-male breeding systems, the weight of the testes is relatively low compared to overall body weight. Chimpanzees, in contrast, have a promiscuous *multi-male* breeding system, in which males mate with multiple females, and females mate with multiple males. In chimpanzees, testes weight is comparatively high relative to body weight. Evolutionary biologists have hypothesized that natural selection has favored high testes weight to body weight ratios in multi-male breeding systems. The logic is straightforward: When females mate with multiple males, a male's sperm must compete with the sperm of many other males to fertilize a female. High testes weight, which correlates with high sperm output, should be favored more strongly in these systems than in single-male breeding systems. But there is only so much that can be inferred from a comparison across three species (chimps, orangutans, and gorillas). To test this hypothesis, a comparison across many primate species is needed.

Sandy Harcourt and his colleagues examined the relationship between breeding system and testes weight relative to body weight in 33 species of primates from

18 different genera and 6 different families. The species ranged from a tiny marmoset that weighed about 320 grams to gorillas that weighed upwards of 170 kilograms (Harcourt et al. 1981, 1995; Dziuk 1982; Harcourt 1982). The breeding systems ranged from multi-male systems to single-male systems to monogamous systems, in which both males and females have a single mating partner. Because some of the species in the analysis were closely related (that is, shared a recent common ancestor), the analysis was undertaken at the level of the genus (we will explore the details of how this sort of analysis is done in much greater detail in later chapters).

To test their hypothesis, Harcourt and his team began by graphing the relationship between testes weight and body weight in their 18 genera. From these data, the researchers calculated a line of *best fit*, which shows the overall relationship between testes size and body weight. Not surprisingly, on average, larger-bodied animals have heavier testes. But it is the deviation from this line of best fit that let researchers test their hypothesis about breeding system and testes size. In **Figure 1.17**, we can see that multi-male breeding systems tend to fall above the line of best fit, indicating larger than expected testes weight to body weight ratios, and systems in which females mate with only one male (single-male and monogamous systems) tend to fall below the line of best fit, indicating smaller than expected testes weight to body weight ratios.

The results from this study provide evidence that natural selection more strongly favors large testes relative to body weight when a male's sperm must compete directly with that of other males, as in multi-male breeding systems.

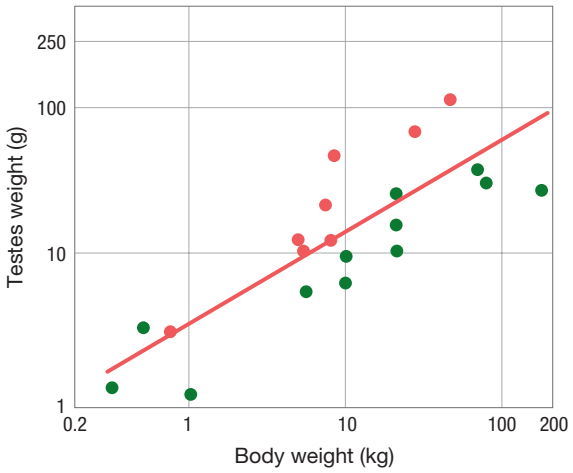


FIGURE 1.17 Breeding system influences the ratio of testes weight to body weight. Testes weight and body weight are plotted for a number of primate species. Red circles indicate multi-male breeding systems. Green circles represent single-male or monogamous mating systems. Points above the line of best fit have greater than expected ratios of testes weight to body weight, while points below the line of best fit have ratios of testes weight to body weight that are less than expected. Adapted from Harcourt et al. (1981).

KEYCONCEPT QUESTION

1.2 What sort of follow-up studies might be done further to test the hypothesis that natural selection more strongly favors large testes relative to body weight in multi-male breeding systems?

The studies described in this section offer just a glimpse of how researchers investigate the evolutionary process and its consequences. There are literally tens of thousands of observational and experimental studies of evolution in the literature. For the time being, however, let us move on to the next tool in the evolutionary biologist's toolbox—theoretical approaches.

Theoretical Approaches

In evolutionary biology, theory plays an important role in shaping and furthering the research agenda of the field. Theoretical biology often, but not always, involves creating mathematical models of biological systems (Godfrey-Smith 2006). In evolutionary biology, as in science more broadly, mathematical models are used for many different purposes. At the most general level, models help us understand how complicated systems work. A good model does this, in part, by making assumptions that allow us to focus on only the critical details of a system, so we can understand how that system operates. Once we do this, we can use our model to make predictions and inferences.

Throughout the sciences, one of the most common uses of models is to make predictions and plan for the future. When we check a weather report, we are relying on a set of models of weather patterns to help us predict what the weather will be like tomorrow and to enable us to make sensible decisions about what to wear and whether to bring along an umbrella. Models from evolutionary biology can be used similarly. For example, when conservation biologists design captive breeding plans for highly endangered species, they use population genetic models (Chapters 7–9) to ensure that they are able to preserve sufficient genetic variation for the species to remain viable.

Another common use of models is to make inferences. Models of processes that we understand in detail help us use observable patterns to infer information that is more difficult to observe directly. When a police officer clocks the speed of a motorist using a radar gun, she is not measuring speed directly. Rather, she is measuring the Doppler shift in radio waves emitted by the gun as they bounce off of the target automobile. The radar gun then uses a simple mathematical model to compute a motorist's speed from the observed Doppler shift. When evolutionary biologists estimate fitness by measuring the change in allele frequencies over time, they are doing something similar: They are using a mathematical model to connect the observable changes in gene frequencies to the less easily observed differences in fitness (we discuss this in more depth in Chapter 7). Similarly, whenever we infer phylogenetic trees from genetic data, we are applying a model of how genetic sequences change over time to observed gene sequences in order to make inferences about evolutionary history (Chapter 5).

For a better sense of how evolutionary biologists use models in their work, let's consider the evolution of the **sex ratio**.

Why Is There an Even Sex Ratio?

In this section, we will use a model to address a simple but far-reaching question: Why do so many species—humans included—exhibit an approximately even sex ratio of one male to one female? Is it a consequence of natural selection? While we are so accustomed to a sex ratio of one male to one female that it may be hard to imagine things any other way, it is not at all obvious that natural selection should favor an even sex ratio. For example, in most species a single male can fertilize numerous females, and often males provide nothing toward the care of the offspring. Why could there not be an excess of females in these species, so that the sex ratio was heavily biased toward females? For mammals, one answer to this question lies in the mechanics of our **chromosomal sex determination**. Females have two X chromosomes. Males have an X and a Y chromosome: During meiosis, these segregate evenly to produce 50% X-bearing sperm and 50% Y-bearing sperm. As a result, roughly half of the fertilized embryos are XX females and half are XY males, producing an even sex ratio in zygotes. But the evolutionary question for us is this: Why has this sort of system evolved instead of some system that produces a different sex ratio? And why do species with other sex determination systems also commonly exhibit sex ratios near 1:1?

Using a simple model of sex ratios, first hypothesized by Darwin and then fully developed by Sir Ronald A. Fisher in 1930, we can examine why natural selection

usually favors an even sex ratio (Fisher 1930). The key to developing a useful model of this type is to find a way to express all of the important features relevant to the problem, while removing as many unimportant details as possible. The challenge and the art of modeling are to determine what features one needs to retain and what details may safely be omitted.

Let us look at what Fisher chose to include in his model and what he chose to omit. He envisioned a sexually reproducing species, but he did not specify the details of its diet, habitat, life span, and so forth. At a first approximation, these are likely irrelevant to the sex ratio problem that he was trying to answer; after all, most species have an even sex ratio irrespective of their diet, habitat, and life span. He then assumed that sex ratio is under genetic control. This is an important assumption: If sex ratio were not under genetic control, it could not evolve by natural selection. Fisher assumed that parents can influence the sex ratio of their offspring, but he could have obtained equivalent results by assuming that individuals determine their own sex. He also assumed that the fitness of a male depends on the frequency of males in the population, and similarly the fitness of a female depends on the frequency of females. And finally, Fisher realized that when parents influence the sex ratio of their offspring, their actions are manifested not in the survival of their offspring, but rather in the reproductive success of their offspring. This is because by altering the sex ratio of their offspring, individuals are not affecting the *number of young* they produce, just the proportion of males versus females. Thus, we cannot measure the benefits in the first generation by directly counting the number of offspring. Instead, we have to measure the benefits in the second generation by counting the number of surviving grandchildren.

Given this imagined population and method for assessing fitness, evolutionary biologist William D. Hamilton clearly summarized Fisher's basic conceptual argument. In Hamilton's words:

1. Suppose male births are less common than female.
2. A newborn male then has better mating prospects than a newborn female, and therefore can expect to have more offspring.
3. Therefore parents genetically disposed to produce males tend to have more than average numbers of grandchildren born to them.
4. Therefore the genes for male-producing tendencies spread, and male births become commoner.
5. As the 1 : 1 sex ratio is approached, the advantage associated with producing males dies away.
6. The same reasoning holds if females are substituted for males throughout. Therefore 1 : 1 is the equilibrium ratio. (Hamilton 1967, p. 477)

This is a purely conceptual way to think about the evolution of an even sex ratio, and the logic Hamilton invokes is powerful. But we can also construct a simple mathematical model to check our intuition. We present such a model in **Box 1.1**.

BOX 1.1 A Mathematical Model of the Sex Ratio

Imagine a population of sexually reproducing organisms. Let us suppose that there are m adult males and f adult females in this population. For simplicity, assume that these individuals live for a single year, reproduce at the end of their lifetimes, and then die. Let N be the number of offspring produced annually in this population. Our model, so far, contains just three variables: m, f, and N.

Regardless of the sex ratio, each offspring in our population has a mother and a father. This may seem obvious, but for our purposes it tells us something important—the total reproductive success of the males in the population must be equal to the total reproductive success of the females in the population. In other words, the total reproduction, N, is shared among f females and m males. On average, each male therefore has N/m offspring and each female has N/f offspring.

Suppose a parent produces offspring such that a fraction k is sons and the remaining fraction, $1 - k$, is daughters. How many grandoffspring will that parent have? On average, that parent will have

$$k\frac{N}{m} + (1-k)\frac{N}{f}$$

grandoffspring per child. The first term in this expression represents the number of grandoffspring produced by male offspring, and the second represents the number of grandoffspring produced by female offspring.

When there are more females than males—that is, when $f > m$—parents with high k values will have more grandchildren than parents with lower k values. Under this condition, natural selection favors parents who produce more males, and the sex ratio moves toward 1:1. Conversely, when $f < m$, parents with low k values will have more grandchildren than parents with higher k values. Now selection favors parents who produce more females, and again, the sex ratio moves toward 1:1. What this model demonstrates is that, as Fisher surmised, natural selection drives the sex ratio to an even 1:1 ratio.

A numerical example helps illustrate the model. Imagine a population with more males than females, such that there are $m = 25$ males and $f = 20$ females, and they produce a total of $N = 100$ offspring. In this case, the average number of offspring produced by a male will be $100/25 = 4$, whereas the average number of offspring produced by a female parent will be $100/20 = 5$. Now suppose that a parent produces half sons and half daughters ($k = 1/2$). The average number of grandoffspring will be $0.5(4) + 0.5(5) = 4.5$ grandoffspring per child produced. Suppose instead that a parent produces all daughters ($k = 0$). Now the average number of offspring per child will be $0(4) + 1(5) = 5$. Thus, in a population with an excess of males, parents will have more grandoffspring when they produce extra daughters. Thus selection favors parents who produce offspring of the under-represented sex.

KEYCONCEPT QUESTION

1.3 Fisher's sex ratio model, as detailed in Box 1.1, predicts a 1 : 1 female : male sex ratio. But this model assumes that the cost to a parent for producing and providing for a female offspring is equal to the cost to a parent for producing and providing for a male offspring. Suppose that this is not the case. Consider a case where each male offspring is twice as expensive to produce and raise to maturity as each female offspring. How would you represent the number of grandoffspring produced by individuals in such a population?

Testing the Sex Ratio Model—A Rapid Change of Sex Ratio

As we discussed, models allow us to simplify a complex reality and thereby make useful predictions about what should happen under specific circumstances. We can then test such models through observational or manipulative experiments. One of the predictions that Fisher's sex ratio model makes is that if the sex ratio should deviate from 1 : 1, natural selection will strongly favor genetic changes that restore an even ratio. Thus, when the sex ratio becomes unbalanced, we expect a rapid return to a 1 : 1 ratio. This prediction was put to the test in a species of butterfly that lives on the adjacent Samoan islands of Upolu and Savaii (Charlat et al. 2007b). In 2001, 99% of the blue moon butterflies (*Hypolimnas bolina*) on Upolu and Savaii were female and only 1% were male (**Figure 1.18A**). This extreme sex ratio bias

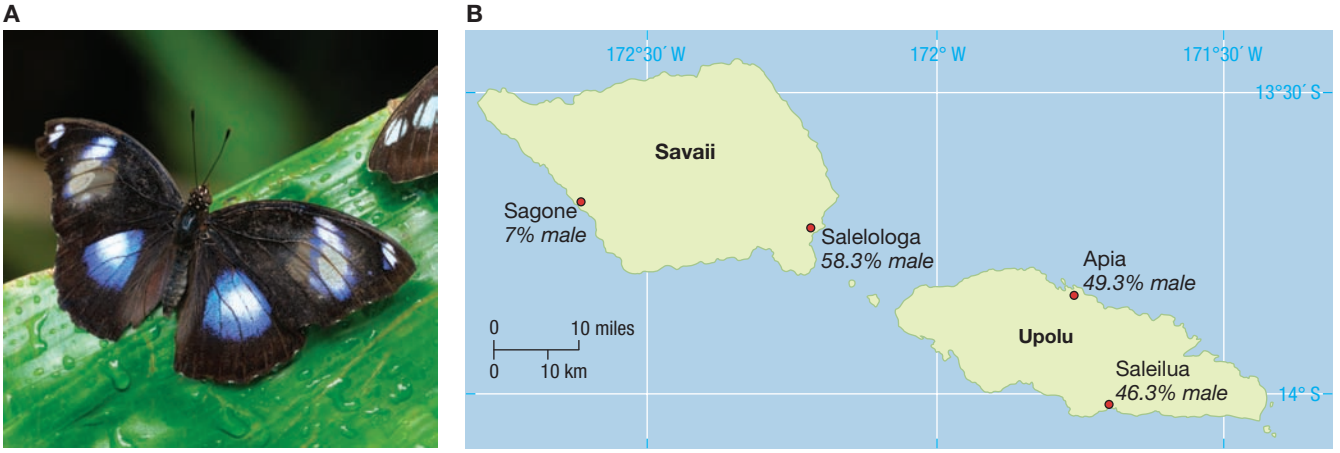


FIGURE 1.18 Sex ratios of butterflies on the Samoa Islands. (A) The blue moon butterfly (*Hypolimnas bolina*) was used to test sex ratio theory on the Samoan islands of Upolu and Savaii. In 2001, 99% of the butterflies on the islands were female. **(B)** By 2006, the sex ratios of the blue moon butterflies had returned to near even except in Sagone.

was the result of male mortality due to a widespread infection by the *Wolbachia* bacterium. This infection has the curious effect of killing most males during larval development, while leaving females unharmed (Charlat et al. 2007a,b).

Fisher's model predicts that if there were to arise a genetic variant of the blue moon butterfly that produced as many males as females, despite infection by *Wolbachia*, this variant would spread rapidly. As this variant spread, the sex ratio would approach 1 : 1. This is exactly what happened on Upolu. Sometime after 2001, such a mutant arose on Upolu (or arrived from another island). By 2006, the sex ratio among *Hypolimnas* butterflies on the island had returned to approximately 1 : 1. Even though female butterflies on the island were still infected with the same variety of *Wolbachia* as in 2001, they now produced as many surviving males as females. On the nearby island of Savaii, sex ratios were returning to 1 : 1 as well. In one population, on the side of Savaii that was nearest to Upolu, males actually outnumbered females among the offspring followed by the experimenters. Only on the far side of the island of Savaii was the sex ratio still strongly female biased. Sex ratio theory predicts a return to 1 : 1 on this far side of the island as well, once migrants capable of generating the 1 : 1 sex ratio arrive and spread there (**Figure 1.18B**).

How do we know that the shift back to an even sex ratio was the result of genetic changes in the butterfly and not in the bacterium? To test this, Charlat and colleagues extracted the *Wolbachia* bacterium from the offspring of Samoan females who produced an even sex ratio (Charlat et al. 2007a). They introduced these bacteria into captive blue moon butterflies from the island of Moorea (near Tahiti). After they were infected by the Samoan *Wolbachia* strain, the Moorean butterflies produced only female offspring. That is, the tendency for Samoan *Wolbachia* to kill male butterfly embryos had not changed. From this, Charlat was able to conclude that evolutionary change had occurred in the Samoan butterflies and not in the Samoan *Wolbachia*, and that this involved the evolution of a gene that allowed the butterfly to suppress the male-killing effect of *Wolbachia*. This drastic change in sex ratio—from 99% female to approximately 50% female in only 5 years, or 10 generations for the butterflies—illustrates both the predictive power of Fisher's model and the speed with which evolution by natural selection can change the characteristics of a population.

KEYCONCEPT QUESTION

1.4 How does the sex ratio study on butterflies demonstrate that sometimes important work in science involves not only a sound theoretical base and good observational and experimental skills but often a bit of serendipity?

Theory and Experiment

In the case of sex ratio evolution, Fisher's mathematical theory was the impetus for many subsequent experiments. Yet, Fisher developed his model in part because so much observational data suggested that the 1 : 1 sex ratio was common in nature, and he wanted to understand why that was. This raises a series of general questions: Is there any natural ordering when it comes to empirical and theoretical approaches? Does theory come before or after empirical work? The answer is, "It depends." Good theory can either precede or postdate data collecting and hypothesis testing. On some occasions, an observation or experiment will suggest to a researcher that a model should be developed. On the other hand, theory can precede, encourage, and facilitate experimental research. Regardless of whether theoretical work precedes or postdates empirical work, a powerful feedback loop typically emerges wherein advances in one area—either theoretical or empirical—lead to advances in the other area.

In this chapter, we have skimmed the surface in terms of understanding how evolution operates. To understand the details of evolutionary biology, however, we need to examine the historical context in which the discipline developed. And so in Chapter 2, we will explore some of the ideas that existed before Darwin revolutionized the study of biology and then proceed to treat Darwin's insights.

SUMMARY

1. Charles Darwin's theory of evolution by natural selection produced a paradigm shift in the life sciences.
2. In *On the Origin of Species*, Darwin presented two revolutionary ideas: (1) the wide diversity of life we see around us has descended from previously existing species, which share common ancestry, and (2) the current forms of these species are primarily a result of natural selection, a process in which forms that are better suited to their environment increase in frequency over time.
3. Evolutionary biologists infer the causes of ancient events and develop and test hypotheses through a combination of observation and experimental manipulations.
4. Artificial selection by humans is the counterpart to natural selection. Humans select which individuals get to reproduce by choosing those that possess traits that are beneficial to us, and as a result the phenotypes of domesticated varieties change over time.
5. Practical applications of evolutionary biology include, but are not limited to, controlling resistance to insecticides and antibiotics, as well as using evolutionary principles to address problems in conservation biology and the medical sciences.
6. All species that have ever lived form a vast branching tree of evolutionary relationships known as the tree of life.
7. Theory plays an important role in shaping and furthering the research agenda in evolutionary biology. Models can be used both to make predictions and to use observable patterns to infer information that is more difficult to observe directly.

KEY TERMS

antibiotic resistance (p. 9)
artificial selection (p. 8)
chromosomal sex determination (p. 22)
comparative anatomy (p. 17)
descent with modification (p. 5)
evolution (p. 5)
extinction (p. 13)
fitness (p. 5)
gene expression (p. 19)
major transitions (p. 6)

mating systems (p. 19)
mutation (p. 6)
natural selection (p. 4)
neutral mutations (p. 18)
phenotype (p. 6)
phylogenetic diversity (p. 13)
phylogenetic tree (p. 12)
selective breeding (p. 6)
sex ratio (p. 22)
speciation (p. 15)
tree of life (p. 12)

REVIEW QUESTIONS

1. What is meant by a paradigm shift?
2. What two basic ideas did Darwin lay out in *On the Origin of Species?*
3. What sort of data do evolutionary biologists collect to test hypotheses?
4. What is artificial selection?
5. Why is antibiotic resistance such a persistent problem?
6. What is phylogenetic diversity?
7. What are the two basic ways to gather empirical evidence to test hypotheses?
8. What are neutral mutations?
9. What is the sex ratio?
10. What is meant by the feedback loop between empirical and theoretical studies?

KEY CONCEPT APPLICATION QUESTIONS

11. In addition to the arms race that we discussed with respect to antibiotic resistance, can you describe another such evolutionary arms race that has practical applications?
12. Can you think of another paradigm shift that has occurred in science in the past 100 years?
13. What do you think was the key point Dobzhansky was trying to make by postulating that "nothing in biology makes sense except in the light of evolution?"
14. How has artificial selection been used to domesticate animals such as dogs?
15. How has work on gene expression opened up powerful new ways to study evolutionary change?
16. Why are mathematical models such as the sex ratio model we discussed so important in evolutionary biology (indeed in all sciences)?

SUGGESTED READINGS

Engelstädter, J., and G. D. D. Hurst. 2009. The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution and Systematics* 40: 127–149. A detailed review of issues we discussed in the *Wolbachia*–blue moon butterfly sex ratio example.

Huxley, T. H. 1863. *Evidence of Man's Place in Nature*. D. Appleton, New York. Huxley—Darwin's colleague—presented evidence for human evolution in this book.

Kuhn, T. 1962. *The Structure of Scientific Revolutions*. University of Chicago Press, Chicago. In this volume, a classic in the philosophy of science, Kuhn outlines the idea of a paradigm shift.

Varki, A., D. H. Geschwind, and E. E. Eichler. 2008. Explaining human uniqueness: genome interactions with environment, behaviour and culture. *Nature Reviews Genetics* 9: 749–763. An interesting discussion of how to understand what molecular genetic comparisons tell us (and don't tell us) about similarities and differences between humans and other primates.

Weiner, J. 1995. *The Beak of the Finch: A Story of Evolution in Our Time*. Vintage Books, New York. A Pulitzer Prize–winning book on Peter and Rosemary Grant's studies of the evolutionary processes that have shaped finches in the Galápagos Islands.

2

Early Evolutionary Ideas and Darwin's Insight

◀ Some of the Galápagos finch species that so fascinated Darwin on his voyage aboard HMS *Beagle*. These museum specimens are arrayed on a copy of Darwin's research journal.

Long before the science of evolutionary biology was born, people contemplated both the origin of life and why it was that organisms often seem so well suited for the environments in which they live. More than two millennia ago, the Greek philosopher Empedocles (ca. 492–432 B.C.E.) proposed that body parts arose independently from the ground, describing organisms

> where many heads grew up without necks, and arms were wandering about naked, bereft of shoulders, and eyes roamed about alone with no foreheads. (Empedocles, Book II, 244, in Fairbanks 1898, p. 189)

These unattached parts, Empedocles continued, then wandered Earth before reassorting, sometimes into monstrous combinations such as creatures with two faces and animals with human heads, and sometimes into the well-proportioned forms that we observe in the animal world. When we read of such theories, we need to be careful not to fall into the trap of judging them based on what we know today. At the time, Empedocles was making

a serious attempt to understand the origin of animals. He *might* have been correct, he just wasn't; but most ideas turn out to be wrong over the long run.

Empedocles' ideas did more than suggest how animal life originated: They also provided an explanation for why organisms seem to be so well adapted to their environments. Empedocles argued that if individuals were assembled from parts that were unable to function together to reproduce, they died off and their types became extinct. Without turning to supernatural intervention, Empedocles proposed a theory that explained not only why we observe an incredible diversity of living forms, but also why the component parts of each species tend to be well suited to one another and to the species' habitats (O'Brien 2012).

Empedocles and his ideas remind us that science has a rich and deep history. Sir Isaac Newton, the great physicist and mathematician, wrote in 1676 that if he had seen farther than others, it was only "by standing on the shoulders of giants." Therein lies the tremendous power of the scientific approach. On the one hand, scholars can build on decades, or even centuries, of previous work without needing to reinvent every step themselves. On the other hand, each of these previous discoveries or theories remains continually open to challenge, revision, and reinterpretation based on new evidence. Like all other great scientific ideas, Darwin's theory of evolution by natural selection did not arise in a vacuum. Instead, the idea of natural selection—as a process in which forms that are better suited to their environment increase in frequency in a population—emerged from a rich philosophical and scientific tradition that came before it.

Given that many theories from this pre-Darwinian tradition have since been discredited, why should a contemporary biologist study these ideas about evolution? Why pause in assessing the view from our time to look back at the figures that came before us?

We study the past to improve our work in the present. We hone our own scientific thinking by following the reasoning that led to both correct and incorrect conclusions, and we come to appreciate the intellectual risks that sparked the theories that we now take for granted. We learn from the work of those that came before us to be flexible in our current thinking. Exploring the debates underlying our assumptions reminds us to question our understanding and to approach contemporary problems from new angles.

And so, before investigating Darwin's theory and the developments in biology that have followed from it, we will examine the ideas about the nature of the biological world that preceded the publication of *On the Origin of Species* in 1859. The first part of this chapter will serve as an introduction to how *pre-Darwinian thinkers* tried to answer the big questions about life and biology, including these:

- What separates science from mythology?
- How should scientists reach conclusions about the natural world?
- How does the natural world change, and over what length of time?
- Why is the world filled with an astonishing diversity of living forms instead of a few basic types?
- Where do species come from?
- Why are organisms well suited to the environments in which they live?

Once we have tackled these questions, in the second part of the chapter we will introduce Darwin's ideas on the evolutionary process.

We will begin by briefly addressing what separates science from mythology, and we will discuss what sorts of explanations scientists can pursue.

FIGURE 2.1 Anaximander (ca. 610–546 B.C.E.). Anaximander proposed a mechanistic view of the Earth and heavens. The philosopher is illustrated in the 1493 history of the world, *The Nuremberg Chronicle*.

2.1 The Nature of Science: Natural versus Supernatural Explanations

Throughout recorded history, every human culture has cultivated a set of creation myths that purport to explain—literally or metaphorically—how the world was created and how it came to be the way that it is. These mythologies address universal questions that stimulate the human imagination and gratify our need for explanations of our place in the world. Prior to the sixth or seventh century B.C.E., these creation myths provided the only answers that humankind had to the grand questions of our existence (Armstrong 2005). This approach to knowledge through mythmaking began to change with the early Greek philosophers.

Methodological Naturalism

The early Greeks, of course, had their own creation myths, but philosophers such as Anaximander (ca. 610–546 B.C.E.) (**Figure 2.1**) were among the first to develop a philosophy of a natural world in which physical laws replaced a supernatural world driven by divine action. They sought to explain the world around them according to fixed laws of nature, rather than by the operation of divine whim.

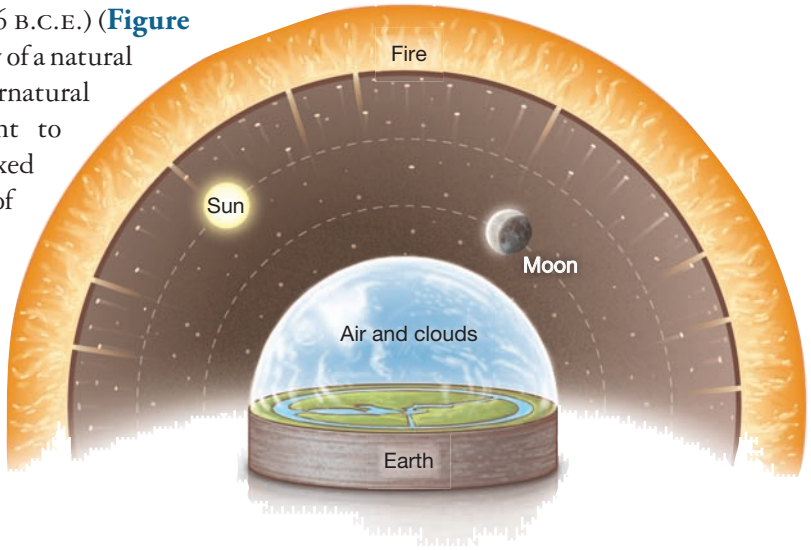


FIGURE 2.2 Anaximander's cosmology. In Anaximander's cosmology, Earth is a disk surrounded by vast wheels on which the Sun and Moon rotate and a dome of fire; stars were explained as light shining from the dome of fire through holes in a firmament.

At a time when heavenly bodies were regarded as divine personages, Anaximander provided a mechanistic rather than divine conception of the Moon, Sun, and stars. He suggested that just like the earthly structures we experience with our senses, the celestial bodies were physical objects (**Figure 2.2**). Earth, he proposed, was a cylindrical disk. The Sun and the Moon rotated around it as if on wagon wheels. Beyond the Sun and the Moon, tiny holes in the firmament let through the light from a vast dome of fire; these pinpoints of light were the stars. Again, it is easy to look back on such ideas and laugh, but that would be a mistake. Anaximander got the details wrong, but given the state of scientific knowledge at the time, this is to be expected. The important thing here is that Anaximander and some of the Greek philosophers who followed him developed explanations based on natural, rather than supernatural, phenomena.

The strategy of trying to explain the world based solely on natural phenomena is fundamental to the scientific method and is at the heart of modern evolutionary biology. It is sometimes called **methodological naturalism**. We call it *naturalism* because of the focus on the natural rather than the supernatural. We use the adjective *methodological* because this strategy provides a method or procedure for seeking scientific explanations of the world. Although philosophers began using methodological naturalism as early as 600 B.C.E., this approach would not be solidified or universally embraced until the eighteenth century (Barzun 2001).

Hypothesis Testing and Logic

Although they were able to make the shift from supernatural to natural explanations, the early Greek philosophers failed to exploit one of the greatest advantages of methodological naturalism: hypothesis testing. If we propose an explanation of a phenomenon based on natural processes, that is, if we develop a **hypothesis**, we can then test this hypothesis because we can observe and often manipulate these processes. By contrast, we have no way to observe, let alone manipulate, the supernatural, and thus we cannot test supernatural explanations. However, the early Greeks formulated hypotheses without refining them through testing. This lack of verification for ideas would begin to change with the great philosopher Aristotle (ca. 384–322 B.C.E.) (**Figure 2.3**).

FIGURE 2.3 Aristotle (ca. 384–322 B.C.E.). The Greek philosopher Aristotle wrote, "We must not accept a general principle from logic only, but must prove its application to each fact; for it is in facts that we must seek general principles, and these must always accord with the facts."

KEYCONCEPT QUESTION

2.1 What does it mean for a hypothesis to be falsifiable?

Unlike those before him, Aristotle recognized the significance of testing one's hypotheses. In his *Natural History of Animals*, Aristotle was clear that "We must not accept a general principle from logic only, but must prove its application to each fact; for it is in facts that we must seek general principles, and these must always accord with the facts" (Aristotle, Book 1, p. 6, cited in Osborn 1894). In other words, principles must agree with the facts. If not, we need to rethink our principles and start over. This sort of approach is well accepted by modern evolutionary biologists, and for this we can thank Aristotle and those who followed in his footsteps. Of course, this approach did not take hold overnight, and even Aristotle did not always follow the practice he preached. In the very same volume where he advocated checking principles against the facts, Aristotle incorrectly asserted that men have more teeth than women. Philosopher Bertrand Russell famously remarked that "Aristotle maintained that women have fewer teeth than men; although he was twice married, it never occurred to him to verify this statement by examining his wives' mouths" (Russell 1952, p. 7).

After Aristotle, one advance in scientific methodology came through the use of logic. Application of logical and mathematical

laws allowed thinkers to move carefully from facts to general principles. In modern evolutionary theory, not only must one gather physical evidence, but also one must formulate and test hypotheses based on such evidence.

Profound as they were, advances in methodological naturalism and logic alone would not prepare the intellectual framework necessary for eventual breakthroughs in evolutionary theory. People also needed to become accustomed to the idea of a world that was both *ancient* and *ever changing*. In the next section, we will examine historical conceptions of the nature of change, of the timescale for such changes, and of the sources of evidence for past changes.

2.2 Time and a Changing World

Darwin's theory of evolution by natural selection explains the form and diversity of living things as the consequence of gradual change over vast periods of time. As we will see in this section, Darwin was not the first to propose this idea, but the notion of change and huge expanses of time arrived late in the history of Western thought. This view was not the dominant one during most of Western history.

The view of the world as unchanging seems counterintuitive to anyone who has watched a storm roll in, a child grow up, or a candle burn. Yet, some Greek philosophers claimed that everything that exists has always existed and will always exist. The material world was permanent, unalterable, and unmoving. Even Aristotle, although he recognized change over small timescales, thought of the world as static and unchanging over longer periods of time. In contrast, Empedocles (**Figure 2.4**) proposed that historically, plant life preceded animal life, and Xenophanes (570–470 B.C.E.) studied fossils in sedimentary rocks in the mountains and concluded that at one time the rocks must have been underwater.

The ideas of both Empedocles and Xenophanes implied that important changes in the biological world had occurred. What sorts of changes had occurred, however, remained contentious for nearly 2000 years. Indeed, until the work of French natural historians Georges-Louis Leclerc, comte de Buffon (1707–1788), and Georges Cuvier (1769–1832) in the eighteenth century, the idea that species had gone extinct was thought of as an absurd challenge to the notion of a flawless Creator.

FIGURE 2.4 Empedocles (ca. 492–432 B.C.E.). Empedocles argued that plant life preceded animal life.

Even if philosophers accept and study the importance of change, a full theory of evolution by natural selection cannot exist without an understanding of the vast expanses of time over which some changes take place. That would not come for almost 2000 years after these early conjectures by the Greeks. Along the way, in the late Middle Ages, the written records of the Bible provided a starting place for estimating the age of Earth. Following similar endeavors by scholars before him, James Ussher (1581–1656), a seventeenth-century Anglican archbishop in Northern Ireland, performed complex calculations based on the Old Testament, and he concluded that the universe had been created on October 23, 4004 B.C.E. Though the precision of the date may sound ludicrous today, Ussher's attempt to date the creation of the world was part of a serious research tradition at the time

(Gould 1991). Famous scientific contemporaries of Ussher made similar attempts; for example, Isaac Newton dated creation at 3998 B.C.E.

At the same time that Archbishop Ussher was making his calculations, a radical shift was taking place in the way that other scholars viewed time and history. Inspired by the vastness of space made clear with the invention of the telescope and the discovery of countless stars beyond those visible to the naked eye, thinkers looked to an equally vast expanse of time.

Scientists began to suggest that both the universe and Earth were much, much older than the thousands of years suggested by a literal interpretation of the Old Testament. In the latter part of the eighteenth century, Buffon used physical laws about the rate at which objects as large as Earth both heat up and cool down to calculate the age of Earth at between 75,000 and 2 to 3 million years (Buffon 1778; Roger 1997). Around the same time, James Hutton (1726–1797), a Scottish geologist, naturalist, and chemist, argued that geological evidence—the way that rock strata were aligned, the processes of erosion and sedimentation, and the fossil data—suggested that the world was inconceivably old (Hutton 1795; Repcheck 2003). Once the idea of a changing world and vast stretches of time became established, the question became this: How can we fully use the power of observation, experimentation, and hypothesis testing to understand change over immense periods of time? To do so, we require explanations that not only appeal to natural processes but also, more specifically, appeal to natural processes that are ongoing and observable or otherwise somehow accessible to us. Historically, the method to do this emerged first in the field of geology and from there migrated to the biological sciences. To see how, we need to examine the work of Scottish geologist Charles Lyell (1797–1875) (**Figure 2.5**).

Building on ideas first proposed by Hutton, Lyell aimed to explain Earth's geological features by appealing to the same geological processes currently observable. He argued that these same processes have operated over very long periods of time in a slow, gradual manner. From this, Lyell came up with the title of his famous book, *Principles of Geology, Being an Attempt to Explain the Former Changes of the Earth's Surface, by Reference to Causes Now in Operation* (Lyell 1830). As we will see shortly, this approach, known as **uniformitarianism**, had a strong influence on Charles Darwin.

Uniformitarianism explained the geological features of Earth in a radically different way than did **catastrophism**, the common theory of the time. According to catastrophism, Earth's major geological features arose through sudden cataclysmic, large-scale events, rather than through slow gradual change. Catastrophism also posited that these cataclysmic events often involve different forces than those that are currently operating.

The shift from catastrophism to uniformitarianism was an important development not only for geology, but also for science as a whole, because science attempts to relate natural processes to observable patterns. In the extreme catastrophic view, these processes are themselves neither observable nor subject to manipulative experiments, and they are not expected to occur again in the future, making it hard—but not impossible—to test hypotheses about how observed patterns have been generated. In the uniformitarian view, all of the processes that have generated the current geological patterns we see around us can themselves be

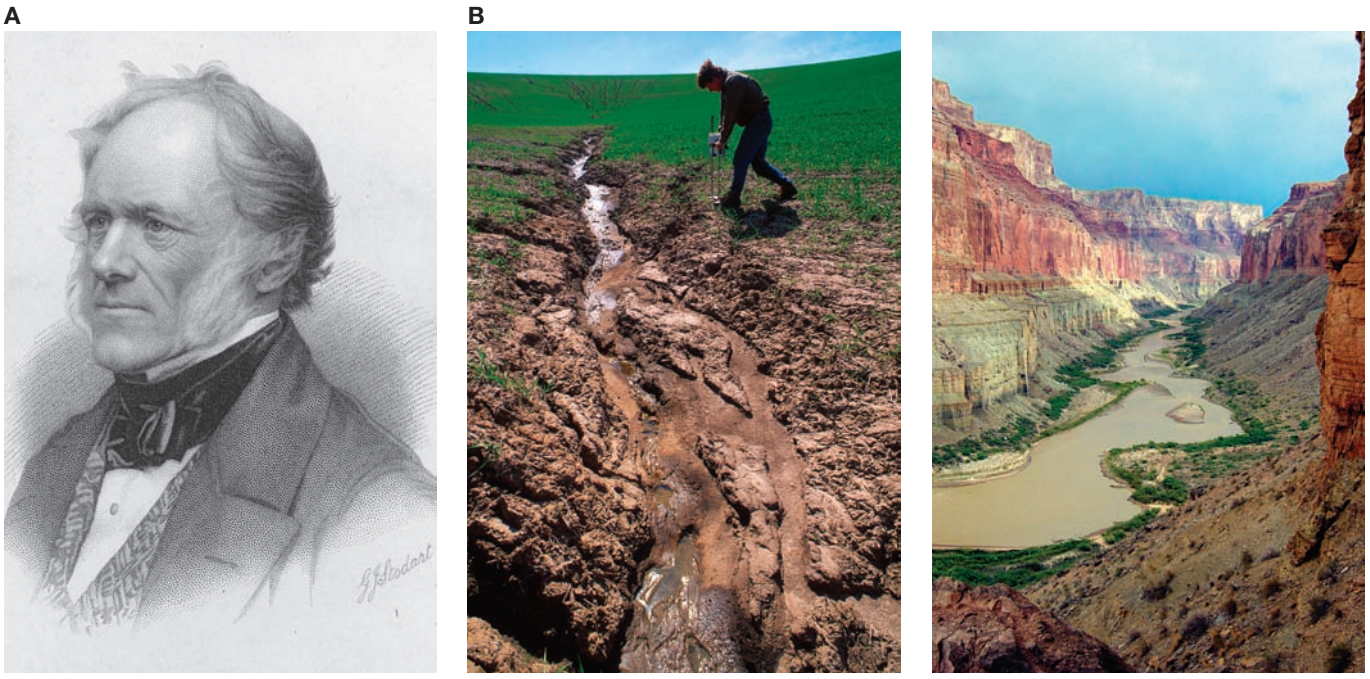


FIGURE 2.5 Charles Lyell (1797–1875) and uniformitarianism. **(A)** Lyell's theory of uniformitarianism helped pave the way for modern evolutionary thinking about the vast expanse of time. **(B)** Uniformitarianism posits that the slow process of erosion (left), when carried out over long stretches of time, can produce massive canyons (right).

observed in operation at present, providing scientists with much more power to test hypotheses.

While Lyell's work related directly to geology, his concept of change over time would also influence evolutionary biology. Darwin read Lyell's *Principles of Geology* while serving as captain's companion and ship's naturalist aboard HMS *Beagle*, and he was profoundly affected by Lyell's ideas (Recker 1990). Prior to publishing *On the Origin of Species*, Darwin wrote three books on geology, each of which drew heavily on Lyell's work on uniformitarian change. And, as we will see later in this chapter, in many ways Darwin's ideas on the gradual changes associated with evolution by natural selection are a sort of biological interpretation of Lyell's uniformitarianist ideas on geological processes. The diversity of life on Earth, Darwin proposed, can be explained by mechanisms that are in operation today, acting over very long periods of time.

By explaining the dramatic features of Earth's geography through uniformitarianism, Lyell conceived the world as changing across enormous expanses of time. As such, by the time Darwin began his work, the approach to scientific inquiry had changed from mythmaking and supernatural explanations to methodological naturalism—a method built on an increasingly sophisticated system of hypothesis testing and reason.

In the next section, when we explore theories of how new species come into existence, we will see that both uniformitarianism and the concept of deep time (vast periods of time) were essential in understanding the origins of the diversity of organisms on Earth.

2.3 The Origins and Diversity of Life

In addition to taking the first steps toward the scientific method and hypothesizing about events from the past, the Greek philosophers also developed a keen appreciation for the study of natural history. Again, Aristotle's contributions were exceptional. Aristotle's books *Physics* and *Natural History of Animals* marked the birth of the field of **natural history**, an enterprise that would be important for the development of any theory of the astonishing diversity of life, whether that theory was evolutionary or not (Schneider 1862).

Aristotle distinguished among 500 species of birds, mammals, and fish, and he wrote entire tracts on the anatomy and movement of animals. He also proposed a taxonomy of nature—a classification system of life—that led from polyps at the lowest level to humans at the pinnacle. This would later be called "the great chain of being," or *scala naturae*. According to this linear classification system, each species occupied a link in a chain of ever-increasing complexity. This concept influenced Western thinkers for more than 2000 years. While this view of nature contributed to the sense of the diversity of life, it was missing two critical concepts that were necessary for the development of evolutionary biology: shared degrees of complexity and the potential to change. On the *scala naturae*, every organism represented a specific and unique link in the chain, and each link represented a different level of complexity, which meant that different organisms could not share comparable degrees of complexity. Likewise, in this view, each specific link on the chain of being would remain forever fixed—precluding the possibility that organisms might change. Both of these misconceptions would have to be overcome before evolutionary biology could emerge as a science.

In addition to cataloging the details of natural history, the ancient Greeks also turned their attention to the problem of how life got started and how all of the diverse living forms around them arose. As we learned at the start of the chapter in our discussion of Empedocles, without the ability directly to observe life arising and diversity being generated, and without a broad conceptual framework for the diversity of the life they saw, the Greeks resorted to speculative accounts of how this process may have occurred. While these speculations represented progress in the sense that they involved natural rather than supernatural explanations, many of the specific mechanisms that the Greeks proposed seem bizarre today. The commonality among almost all of their suggestions is that they relied on **spontaneous generation**—the idea that complex life-forms arise repeatedly, without external stimuli, from nonliving matter, and *heterogenesis*—the idea that parents of one species could produce offspring of a different species.

Ideas on spontaneous generation existed before the Greeks and persisted for more than 2000 years after the Greeks. In Egypt, for example, people thought that frogs were created spontaneously from mud. This is because when the Nile River flooded every year, it transformed dry mudflats into wet mud, and simultaneously, hundreds of frogs appeared. Aristotle wrote extensively about spontaneous generation as a source of life and theorized that when parents thus generated went to reproduce, they formed new species by heterogenesis. Many medieval European farmers believed that mice were generated from moldy grain, and many urban residents believed that sewage created rats (McCartney 1920).

Finally, in 1668, in an early example of a controlled experiment, Francesco Redi (1626–1697), an Italian physician and naturalist, addressed the following question: Are flies spontaneously generated from meat carcasses? It seemed as if

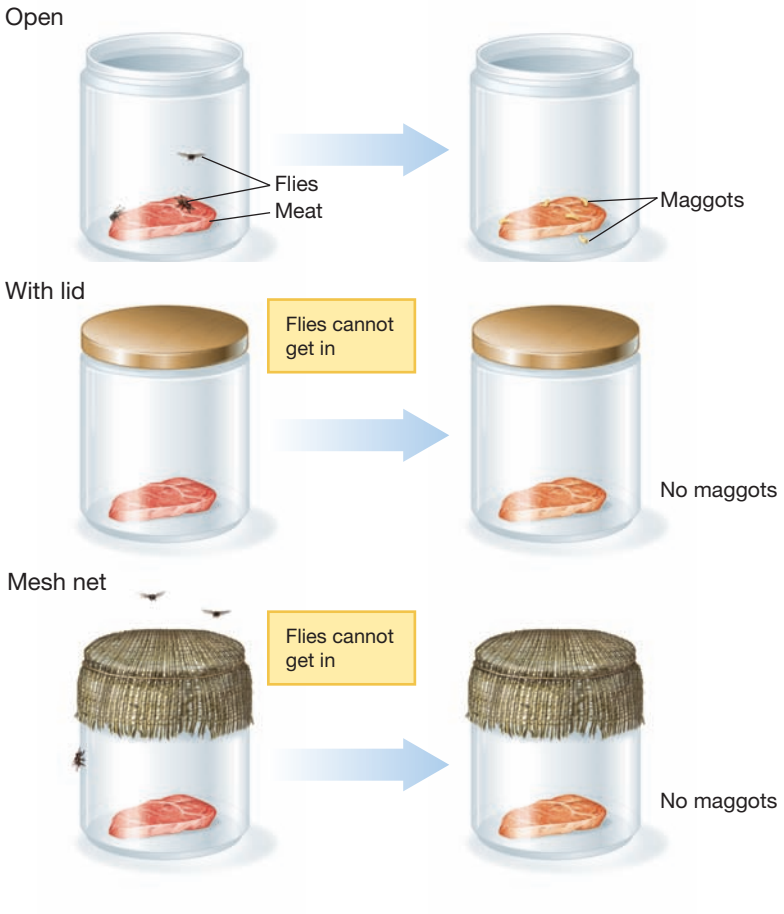


FIGURE 2.6 Redi's experiment. Redi's experiment demonstrated that maggots did not arise through spontaneous generation. Uncovered jars with meat have fly eggs and maggots. When the jars are covered, and flies cannot enter and lay eggs on the meat, no eggs or maggots are found.

they were, because when meat rotted, flies appeared. So, Redi placed raw meat in a series of jars. Covering some jars (for a control group) and leaving other jars uncovered or partially uncovered, Redi determined that flies only arise from the maggot offspring of other flies, and that maggots cannot spontaneously generate from meat (**Figure 2.6**). Redi's experiment prompted his contemporaries to question whether any organism could appear from a nonliving substance. In spite of this experiment, spontaneous generation persisted as a theory, in part because the new technology of the microscope showed organisms such as bacteria and fungi appearing on substances such as spoiled broth without any clear parental source.

The late eighteenth and early nineteenth centuries brought new theories to explain the origins of life and the diversity of species. Erasmus Darwin (1731–1802) (**Figure 2.7**), an English physician, philosopher, and the grandfather of Charles Darwin, was one of the first to propose the idea of evolutionary change in his book *Zoonomia* (Darwin 1796; King-Hele 1998).

Erasmus Darwin argued that all life evolved—although he did not use that word—from what he called a "single living filament" (Darwin 1796). For Erasmus Darwin, this living filament had been modified in endless ways, over millions of years, to produce the life that he saw around him. He also hypothesized that humans had initially walked on four limbs and, even more remarkably, that we had descended from another primate species. This was a radical idea at the time. In addition, Erasmus Darwin understood the **struggle for existence**—the notion that

FIGURE 2.7 Erasmus Darwin (1731–1802). Charles Darwin's grandfather proposed the idea of evolutionary change in his book *Zoonomia*.

FIGURE 2.8 Robert Chambers (1802–1871). Chambers authored the book *Vestiges of the Natural History of Creation*.

organisms are in a constant struggle to obtain resources and to use these resources to produce more offspring than those around them. Despite Erasmus Darwin's insights, he did not develop a full-blown theory of evolution of new species by natural selection for at least two reasons: (1) with a few notable exceptions, he failed to connect the struggle for existence, which he described over and over again, to the evolutionary changes that such a struggle would produce (Krause 1879); and (2) he believed in the widely accepted, but largely incorrect, idea that new traits acquired *during the lifetime of an organism* could be passed down to progeny. We will return to this "inheritance of acquired characteristics" later in our discussion of its most famous proponent, Jean-Baptiste Lamarck.

After Erasmus Darwin, Robert Chambers (1802–1871), a Scottish geologist, writer, and publisher (**Figure 2.8**), presented a more formally developed and widely influential theory on how new species originate in his 1845 book, *Vestiges of the Natural History of Creation* (Chambers 1845).

In the section of his book on what today we would call evolution, Chambers highlighted two critical points: (1) the composition of species has changed over time, and (2) this change was slow, gradual, and unlinked to catastrophes (Mayr 1982). From these ideas, Chambers outlined his *principle of progressive development*, in which he hypothesized that new species arise from old species: "The simplest and most primitive type . . . gave birth to the type next above it . . . and so on to the very highest, the stages of advance being in all cases very small—namely, from one species only to another; so that the phenomenon has always been of a simple and modest character" (Chambers 1845, p. 222).

One aspect of *Vestiges* that often goes unnoticed is that Chambers thought not in terms of individuals so much as **populations**—groups of individuals of the same species that are found within a defined area and, if they are a sexual species, interbreed with one another. Chambers was perhaps the first to recognize that, in the parlance of modern evolutionary biology, populations evolve; individuals do not.

Robert Chambers and his *Vestiges* profoundly influenced a broad range of readers. The book was widely read by scientists and laypeople alike, including a young Abraham Lincoln, who quickly became "a warm advocate of the doctrine" (Herndon and Weik 1893). *Vestiges* would eventually sell an astonishing 100,000 copies (Secord 2000). For all its success, the greatest deficit in Chambers' book was the lack of a theory to explain *why* new species come into being. That is, there was nothing akin to the theory of natural selection that Darwin would propose some 15 years later.

2.4 Organisms Are Well-Suited to Their Environments

While *Vestiges* presented the idea of new species gradually arising from existing species, the book did not explicitly consider the enormous influence of the environment on these slow changes. Any observer of nature will notice the remarkable degree of fit between the structures of organisms and their environments. The mammals of cold climates have thick coats and layers of insulating fat; swimming

animals have shapes that allow them to move efficiently through the water; desert plants have thick waxy cuticles and low surface area that help them avoid water loss. How do we explain this seemingly marvelous fit? Prior to Darwin's work, philosophers and scientists entertained a diverse array of answers to this question.

Paley's Natural Theology

For the English naturalist and theologian William Paley (1743–1805), the fit of diverse species to their environments resulted from the planning of some supernatural deity. In his textbook, *Natural Theology*, Paley discussed the famous metaphor of God as watchmaker (Paley 1802) (**Figure 2.9**). If a single part of the clockwork within a watch were shaped differently or placed elsewhere, he observed, the watch would fail to function. Because living creatures are even more complex than watches, they could not have come to fit their habitats perfectly through chance, Paley argued, just as it is virtually impossible for a fully working watch to come into being simply by chance arrangement of clockwork parts. Organisms, then, must have been intentionally designed by a benevolent deity in order to thrive in their environments.

FIGURE 2.9 William Paley (1743–1805). Paley discussed the exquisite fit of organism to environment by using an analogy in which, just as a watch requires a watchmaker, so too living organisms require a conscious designer.

Years later, Darwin would read and admire Paley's work, particularly his arguments on how the structures of organisms fit the functions they need to serve in order for individuals to survive. As we will see in greater detail in a moment, however, Darwin would disagree with Paley's explanation of the source of these adaptations. Darwin sought to explain adaptation by purely natural, rather than supernatural, causes.

Jean-Baptiste Lamarck and the Inheritance of Acquired Characteristics

With Jean-Baptiste Lamarck (1744–1829), we return fully to methodological naturalism as the explanation for species fitting their environments (**Figure 2.10**). Originally trained as a botanist at the French Jardin du Roi as a student of Buffon, Lamarck eventually became an animal systematist specializing in the study of invertebrates. His long-term studies of such organisms as mussels, which he compared to less complex fossil mussels, no doubt led him to think in terms of increasing complexity occurring in a group of organisms over time.

FIGURE 2.10 Jean-Baptiste Lamarck (1744–1829). Lamarck developed a "transformation" theory for evolutionary change in his *Zoological Philosophy*.

In his 1809 book, *Zoological Philosophy*, Lamarck rejected the idea that new species suddenly appeared after large-scale extinctions resulting from catastrophic events. Instead he proposed that new, more complex species—humans being the most complex—had descended, gradually, from older, less complex species. Because of this, Lamarck is often credited with developing the first truly evolutionary theory for how organisms adapt to their different environments over evolutionary time. Actually, Lamarck outlined two mechanisms for evolutionary change, but here we will focus on his more famous one—the **inheritance of acquired characteristics**.

The idea behind the inheritance of acquired characteristics is that *during the lifetime of an organism*, the habits of the organism bring about changes in its structure, and such structural changes are passed down across generations (Lamarck 1809). Consider Lamarck's description of this process in birds (**Figure 2.11**):

> One may perceive that the bird of the shore, which does not at all like to swim, and which however needs to draw near to the water to find its prey, will be continually exposed to sinking in the mud. Desiring to avoid immersing its body in the liquid [the bird] acquires the habit of stretching and elongating its legs. The result of this for the generations of these birds that continue to live in this manner is that the individuals will find themselves elevated as on stilts, on naked long legs. (Lamarck 1801, cited in Burkhardt 1995, p. 172)

FIGURE 2.11 Lamarck, acquired characteristics, and shorebirds. Lamarck argued that the long legs of shorebirds such as this black-necked stilt (*Himantopus mexicanus*) are the result of birds stretching their legs as far as possible to avoid sinking in the mud. This stretching itself, Lamarck postulated, lengthened the legs of individuals doing the stretching, and their new trait of "longer legs" was then also passed down to offspring.

Lamarck observed that we find long-legged birds in environments in which long legs are beneficial. Rather than crediting a watchmaker deity for this perfect fit, he hypothesized a process of adaptation over time. Lamarck's hypothesis that traits acquired *during* the lifetime of an individual are passed on to its progeny was interesting, reasonable, and based on an idea that was universally accepted by scientists and nonscientists alike. After all, we are all aware of how our habits of life lead to changes in physiology; lifting weights, for example, leads to the development of increased muscle mass and lifting power. In the absence of evidence to the contrary, it is only a short leap from there to suppose that such changes could also be passed on to one's offspring. Today, however, we have plenty of evidence to the contrary. We know that acquired characteristics are not ordinarily inherited, and we now ground our ideas of how traits are passed from generation to generation in the laws of genetics, which were formulated about 100 years after Lamarck (Chapter 6).

Lamarck's legacy, however, is not that he postulated the wrong processes for evolutionary change, but that he proposed a process in the first place, and that he connected it to environmental fit. As we will see, although Darwin did not completely reject the inheritance of acquired characteristics, his ideas on how and why evolutionary changes occur were quite different from those of Lamarck.

KEYCONCEPT QUESTION

2.2 A blacksmith's muscles get larger the more he pounds his metals into shape. Suppose, as is likely the case, that the sons of blacksmiths are on average more muscular than other males their age. Why might this mistakenly lead someone to think that muscle size here is an example of the inheritance of an acquired characteristic? How else could we explain this observation?

Patrick Matthew and Natural Selection

In the history of biology, we hear little about the developments in evolutionary thinking in the 50 years between Lamarck's *Zoological Philosophy* (1809) and Charles Darwin's *On the Origin of Species.* Yet, it was during this period that Patrick Matthew (1790–1874), a Scottish landowner and writer, proposed his own theory

of evolution by natural selection, predating the ideas laid out in *On the Origin of Species* by more than a quarter of a century (Matthew 1831; Mayr 1982; Dempster 1996). In an obscure 1831 work entitled *On Naval Timber and Arboriculture*, Matthew proposed a theory very similar to Darwin's on the interaction between environment and evolutionary change. In the notes at the end of *On Naval Timber and Arboriculture*, in a section only tangentially related to the rest of the book, Matthew outlined his ideas on both evolution and natural selection. He understood the idea that individuals best suited to their environments would be selected over others. The difference between this idea and Lamarck's theory is that Matthew relied on what Darwin would one day call natural selection rather than the inheritance of acquired traits.

Matthew's discussion of environmental fit and natural selection—what he dubbed "the circumstance-adaptive law"—is remarkably similar to what Darwin would discuss almost 30 years later. Matthew, for example, noted,

> The self regulating adaptive disposition of organized life may, in part, be traced to the extreme fecundity of Nature, who . . . has in all the varieties of her offspring, a prolific power much beyond (in many cases a thousandfold) what is necessary to fill up the vacancies caused by senile decay. As the field of existence is limited and pre-occupied, it is only the hardier, more robust, better suited to circumstance, individuals who are able to struggle forward to maturity . . . from the strict ordeal by which Nature tests their adaptation to her standard of perfection and fitness to continue their kind by reproduction, . . . the breed gradually acquiring the very best possible adaptation. (Matthew 1831, pp. 384–385)

Matthew outlines three important evolutionary ideas here: (1) resources are limited, and only so many offspring can survive to the age of reproduction; (2) individuals will differ in terms of traits that allow them to garner such resources; and (3) over time, this will lead to organisms that are well adapted to their environments.

Matthew's name is not readily associated with the theory of evolution by natural selection—despite the fact that on page 22 of the preface to the sixth edition of *The Origin of Species*, Darwin noted that Matthew presented "precisely the same view on the origin of species as that propounded by . . . myself . . . in the present volume." There are many reasons for Matthew's relative obscurity. His ideas were published in a book that no one interested in biological diversity would have been likely to read, and even there his ideas were hidden in his notes and appendix section rather than presented as a unified theory. Moreover, Darwin discussed both natural selection and common descent, while Matthew mentioned only the former. Perhaps most important, Matthew presented scant evidence in support of his ideas. Darwin, in contrast, spent 20 years gathering evidence for evolution by natural selection before publishing *On the Origin of Species*. All of that said, Matthew's work merits more attention than it has garnered.

If we stop and take stock for a moment, what we have seen in this chapter so far is that five major developments preceded and facilitated Darwin's *On the Origin of Species*. These changes involved moving (1) from supernatural explanations to methodological naturalism, (2) from catastrophism to uniformitarianism, (3) from logic and pure reason to observation, testing, and refutation, (4) from an unchanging world to a world in flux, and (5) away from the idea of spontaneous generation to the idea that species come from other closely related species.

2.5 Darwin's Theory

We will begin our exploration of Darwin's contributions with a brief overview of the major ideas that he presented in *On the Origin of Species*. Darwin had two fundamental insights that he referred to as "two great laws" about the process of evolution.

Darwin's Two Fundamental Insights

The first of Darwin's fundamental insights deals with the conditions of existence and the process of natural selection. Here, Darwin hypothesized that the environment—what we might think of in the abstract sense as "nature"—selects on variation in the traits of individual organisms, because some variants are more successful than others at increasing the probability of survival and reproduction.

With this hypothesis, Darwin offered a mechanistic explanation both for how the characteristics of organisms change over time and for why organisms are well suited to their environments. That explanation was, of course, the process that Darwin dubbed *natural selection*. The effect that a given variant of a trait has on survival and ultimately reproductive success depends on the environment in which an organism finds itself. As Darwin noted, once the "conditions of existence" are determined, "natural selection acts by either now adapting the varying parts of each being to its organic and inorganic conditions of life; or by having adapted them during past periods of time" (Darwin 1859, p. 206). Here, when Darwin writes of the conditions of existence, he is referring to the living (biotic) and nonliving environment that sets the stage on which natural selection operates.

The second of Darwin's insights centers on the common ancestry of all living things. Darwin hypothesized that all species have descended from one or a few common ancestors; species that share a recent common ancestor tend to resemble one another in many respects for the very reason that they share recent common ancestry. In short, Darwin hypothesized that new species do not arise through independent acts of creation or spontaneous generation, but rather from preexisting species. This process generates a branching pattern of ancestry relating all life.

These two insights are major themes not only within this chapter, but throughout the textbook, and we will go into much more detail about them in other chapters. For now, we will look at how Darwin arrived at these ideas, at how he collected evidence to support them, and at how he chose to present his challenging conclusions to his nineteenth-century contemporaries.

Publication of *On the Origin of Species*

Darwin begins *On the Origin of Species* as follows: "When on board H.M.S. '*Beagle*,' as naturalist, I was much struck with certain facts in the distribution of the inhabitants of South America, and in the geological relations of the present to the past inhabitants of that continent. These facts . . . seemed to throw some light on the origin of species—that mystery of mysteries" (Darwin 1859, p. 1) (**Figure 2.12**).

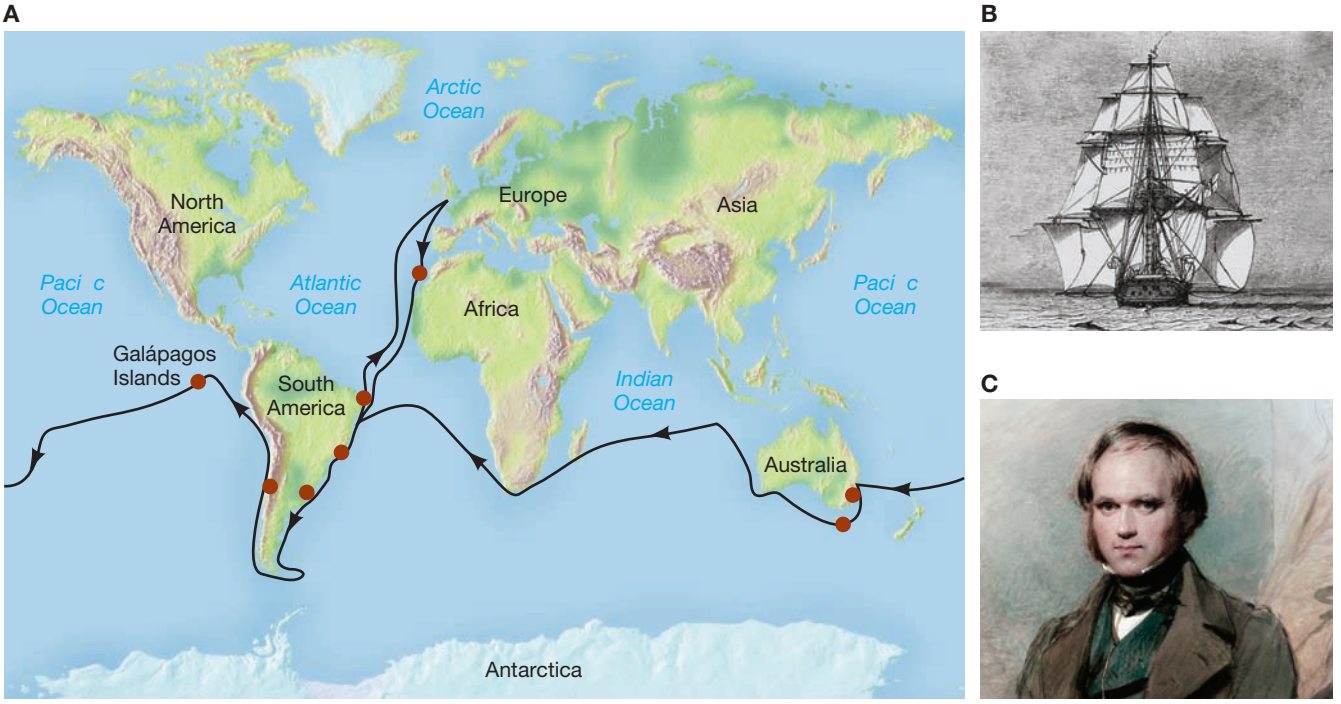


FIGURE 2.12 The voyage of the *Beagle*. **(A)** Map of the voyage of HMS *Beagle*, which began in England. **(B)** HMS *Beagle* was a 10-gun brig of the Royal Navy. **(C)** Portrait of a young Charles Darwin, shortly after returning from his journey aboard the *Beagle*.

As we have seen, some of Darwin's predecessors talked of evolutionary change and even of processes similar to natural selection. Darwin's *On the Origin of Species*, however, was the first to present a *complete theory* of evolution by natural selection and to support that theory with an enormous body of evidence: evidence that included, but was not limited to, his observations of finches, tortoises, coral reefs, and so much more in the Galápagos Islands (**Figure 2.13**).

Twenty-three years separated Darwin's return from his time on HMS *Beagle* and the publication of *On the Origin of Species*. Darwin postponed releasing his work, in part because he knew that his ideas were revolutionary, and he wanted to have the

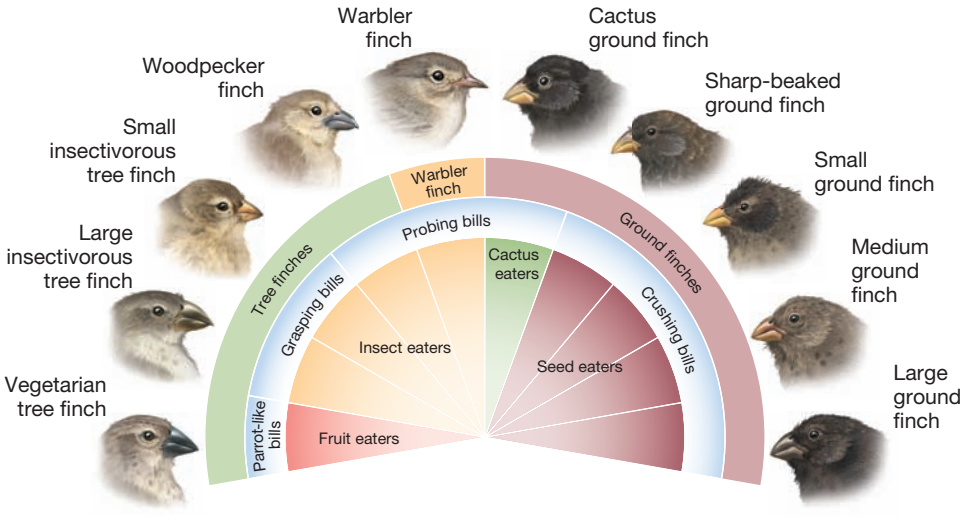


FIGURE 2.13
Darwin's finches. Darwin observed substantial variation in the beak morphologies of the finches across the Galápagos Islands. These observations, along with many others, led Darwin to formalize his ideas on the process of natural selection. Over the years, evolutionary biologists have studied how this variation in morphology maps to differences in food sources and feeding strategies.

FIGURE 2.14 Alfred Russel Wallace (1823–1913). Wallace independently developed a theory of evolution by natural selection very similar to that of Darwin.

strongest possible case before unveiling them to both the scientific world and the general public. But in the end, competition pressured Darwin into publishing. In 1858, as part of an ongoing correspondence with Alfred Russel Wallace (1823–1913), Darwin received a manuscript in which Wallace proposed a theory very similar to his own (**Figure 2.14**).

Wallace was a brilliant natural historian, geographer, and collector; he identified many new species of birds and insects, and his collections can be seen today in natural history museums around the world. Wallace had written a paper in 1855 in which he speculated on the origin of species; there he concluded from the similarity of geographically nearby species that new species must arise from preexisting ones (Wallace 1855). Wallace's concept of how species are formed led him to suggest the hierarchical branching relationship among species that is fundamental to our current understanding of the diversity of life.

It was during a bout with malaria on the Spice Islands, however, as he suffered from fever, that Wallace figured out the mechanism that drives species to change (Raby 2001). As he recollected, "I at once saw that the ever present variability of all living things would furnish that material from which, by the mere weeding out of those less adapted to the actual conditions, the fittest alone would continue the race" (Wallace 1905, pp. 191–192). Darwin would call this process *natural selection*.

When Wallace wrote to Darwin outlining these ideas on evolution, Darwin yielded to pressure from friends and colleagues and publicized his own theories, first in a joint Darwin–Wallace paper that was read to the Linnaean Society in 1858 (with neither Darwin nor Wallace present), and later in longer form as *On the Origin of Species*. Wallace still holds a place in the pantheon of great evolutionary thinkers, but history primarily associates Darwin's name with the theory of evolution by natural selection. In large part this is due to Wallace's professional generosity. While his theory closely resembled Darwin's, Wallace graciously agreed that Darwin deserved the credit. Darwin had worked for decades on developing the theory and had amassed huge amounts of data from many sources to provide evidence for his theory of evolution by natural selection.

In 1859, when Darwin finally published *On the Origin of Species*, he laid out his evidence and his argument carefully, cognizant of the criticism his ideas would draw. But before he could describe either his data or the process involved in generating a new species, Darwin first needed to prepare his reader for what was to come. He did so cautiously, but in a strategically brilliant fashion.

Means of Modification and Pigeon Breeding

The opening chapter of *On the Origin of Species* may strike the modern reader as odd, with Darwin writing:

> It is, therefore, of the highest importance to gain a clear insight into the means of modification. . . . At the commencement of my observations it seemed to me probable that a careful study of domesticated animals and of cultivated plants would offer the best chance of making out this obscure problem. (Darwin 1859, p. 4)

Indeed, Darwin writes about numerous domestication programs, with an emphasis on pigeon breeding (**Figure 2.15**). What most biologists consider the most important book ever written opens not with his grand theory explaining diversity of life on earth, but rather with an extended discussion of how to breed

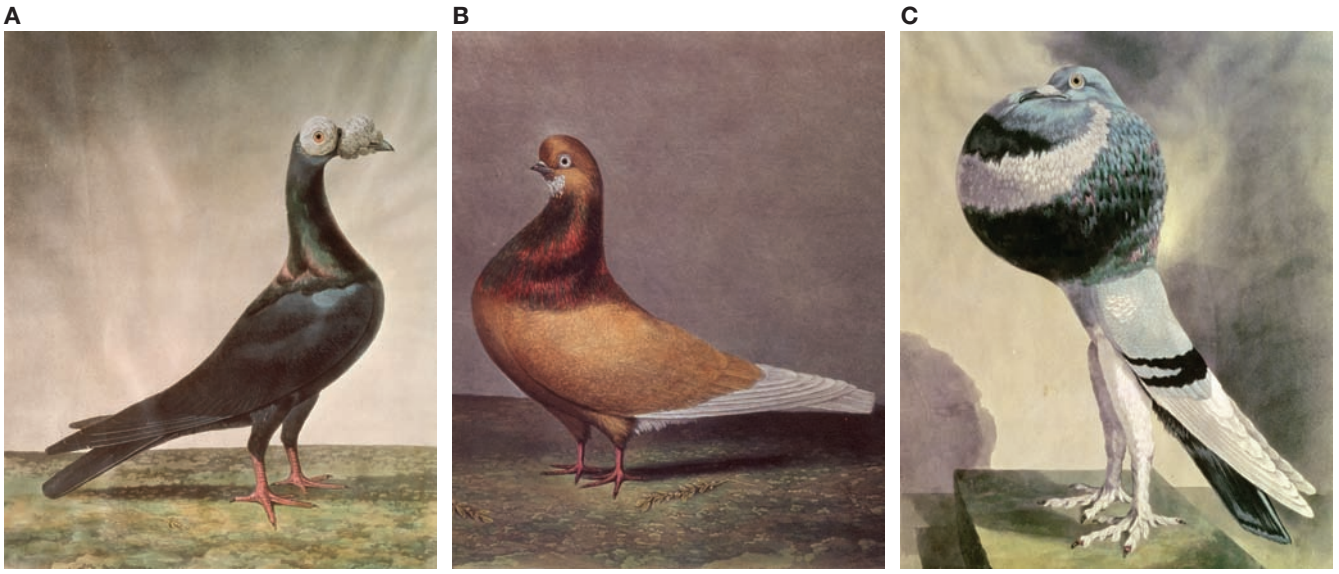


FIGURE 2.15 Pigeon varieties. Darwin used pigeon breeding to explain artificial selection to the readers of *On the Origin of Species*. Here we see three domesticated pigeon varieties: **(A)** the carrier pigeon, **(B)** the beard pigeon, and **(C)** the pouter pigeon.

for bizarre, if beautiful, pigeons. But there was a reason Darwin chose to do this. While this choice of subject matter appears unusual today, pigeon breeding was a popular pastime in Victorian England and would have been comfortingly familiar to Darwin's audience. With this example, Darwin set up an analogy that would help his readers of 1859 relate to the novel ideas in the rest of the book.

Darwin hoped to introduce readers to natural selection by first convincing them that the breeding programs that pigeon fanciers had developed—programs that had led to a wide range of extraordinary variation in pigeon color, flying habits, behavior, and so on—resembled the processes that led to differences within and between species in nature. Here, Darwin aimed first to illustrate the processes by which he thought species changed over time and second to help his readers get beyond their preconceptions of species as eternal and immutable. We address these two aims in turn.

Artificial Selection

In artificial selection, humans systematically breed certain varieties of an organism over others. For thousands of years, humans have been shaping animals and plants by this process. Ever since our ancestors *selected* some varieties of wheat, corn, and rice over others, and systematically planted such seeds, we have engaged in artificial selection. The same process describes our systematic breeding of certain types of dogs and our domesticated livestock. The process that pigeon breeders developed is an example of *artificial selection*, whereas the process leading to the wide variety of traits we see in nature is *natural selection*.

Following Darwin, let us examine how artificial selection works in the context of pigeon breeding. Suppose that like pigeon breeders in Victorian days, we want to produce a variety of pigeon with snow-white plumage. We would begin our artificial selection process by systematically allowing only those individuals in our population

with the whitest plumage to breed. We would then continue this process generation after generation, in each generation sorting the birds based on plumage coloration, and allowing the whitest—those that are closest to the type we want to produce—to breed. *If* offspring resembled their parents in terms of plumage coloration, each generation of offspring would have whiter and whiter feathers. Eventually, we would exhaust all genetic variation for plumage coloration, and, so far as possible, we would have achieved our goal of a snow-white pigeon (**Figure 2.16**).

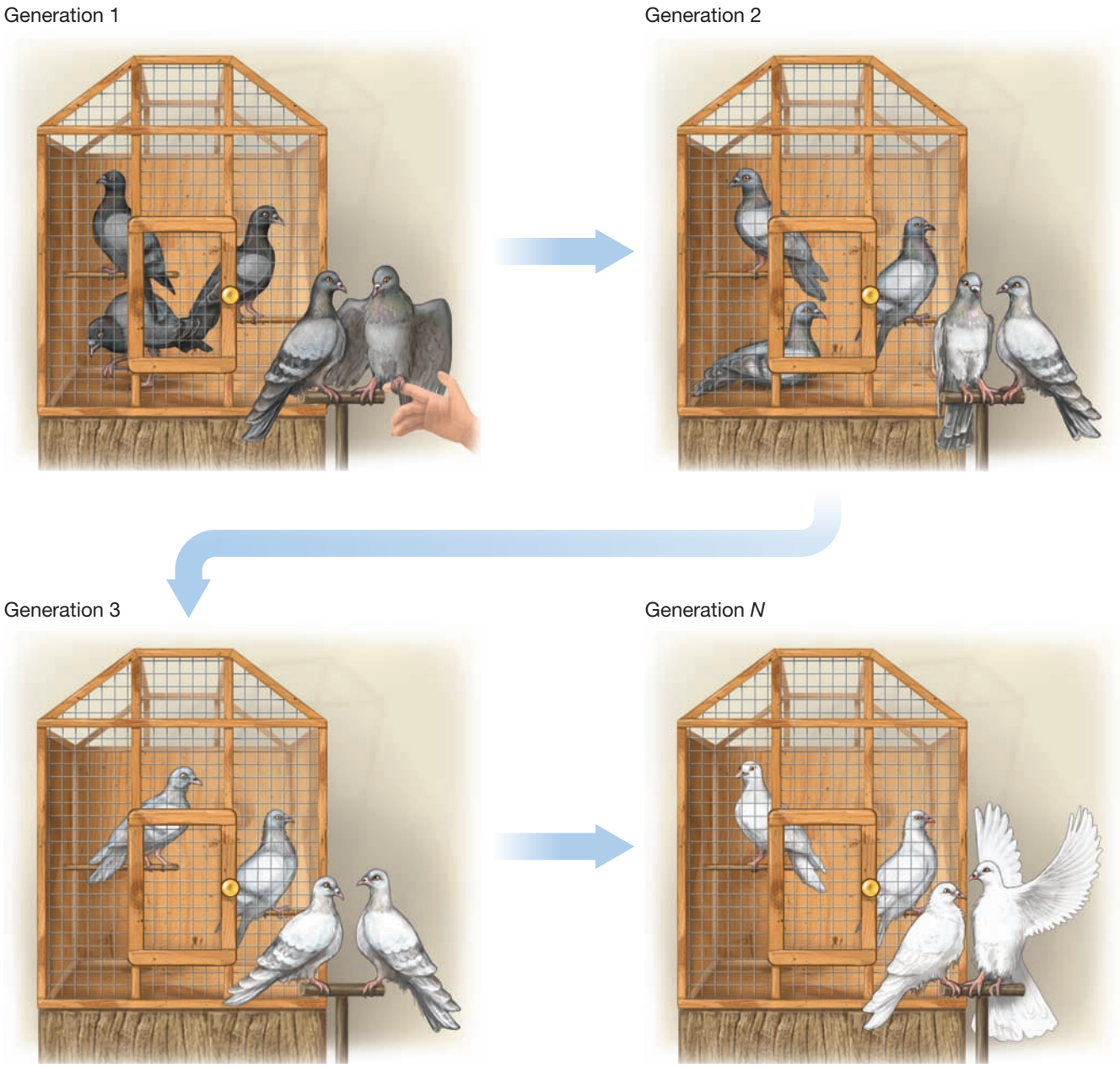


FIGURE 2.16 Artificial selection for white plumage in pigeons. Each generation, a breeder selects the pigeons with the whitest plumage and allows them to breed. Many generations later (generation *N*), at the end of the process, the breeder has a pigeon variety with much whiter plumage than that of the original stock.

KEYCONCEPT QUESTION

2.3 Choose another example of artificial selection and describe a breeding program that would produce the desired aim of the breeder.

Changing Species

While many of Darwin's contemporaries would have accepted the explanation of artificial selection as the mechanism producing new *varieties* of pigeons—pigeons with new colors, new morphological traits, new behaviors, and so on—the claim that this process could generate new *species* was much more controversial, as it implied that it would lead to original and new life-forms, an idea that was still widely unaccepted at the time. Darwin knew this all too well and in Chapter 2 of *On the Origin of Species*, he seems almost obsessed with the definition of a variety versus a species and with the problems in distinguishing between these two categories.

Darwin presents example after example in which one naturalist calls a group of organisms "species 1," while another classifies the same group as a "variety of species 2." In Darwin's eyes, the line between a variety and a species was arbitrary. He conceptualized species as merely "strongly marked and permanent varieties." Conversely, when he saw varieties, he viewed them as "leading to subspecies and then to species," and he often spoke of varieties as "incipient species"—species in the making.

Challenging the distinction between species and varieties was essential to Darwin's overarching argument. Pointing to examples in plant and animal breeding, Darwin could provide extensive evidence that new *varieties* often arise from a single stock through a branching mechanism of descent. Having established that varieties are similar to species, Darwin could then claim that they probably both respond to similar processes, most notably, some process of selection (artificial or natural). As such, he could argue that, like varieties, species change over time, and that new species arise from other species.

To explain how varieties were on the path to becoming new species, Darwin introduced the concept of descent with modification. For example, he hypothesized that if we want to understand how species 2 got to be what it is today, we need to recognize that it *descended* from another species—let's call it species 1—and that over evolutionary time, numerous *modifications* occurred. Darwin argued that these modifications resulted largely from the process he dubbed natural selection, a process analogous to the familiar technique of artificial selection that had been used by breeders for thousands of years.

Once Darwin had walked the reader of *On the Origin of Species* through the process of artificial selection and the concept of species as changing entities similar to varieties, he could move on to the details of the process of natural selection.

2.6 Darwin on Natural Selection

Darwin argued, over and over, that the process of natural selection resembles that of artificial selection. The two important differences between the processes are the *selective agent* and the *traits* being selected. With artificial selection, the selective

agent is the human breeder who chooses which traits to modify and attempts to modify them in a way that is beneficial to the breeder. In the case of natural selection, we can think of nature as the selective agent, though nature is not, in any sense, a conscious agent in the way that humans are.

With respect to what traits are selected, Darwin noted,

> Man can act only on external and visible characters; nature cares nothing for appearances, except in so far as they may be useful to any being. She can act on every internal organ, on every shade of constitutional difference, on the whole machinery of life. (Darwin 1859, p. 83)

That is, the process of natural selection favors any variant of a trait that increases the survival and reproductive success of an individual, even if the difference is not easily detected by a human observer or if the increase in reproductive success is small.

Darwin, Variation, and Examples of Natural Selection

In part by taking Lyell's ideas on uniformitarianism and applying them to biology, Darwin hypothesized that evolution by natural selection is a gradual but powerful process. He argued that the process of natural selection acts on small differences between individuals. If one variety of a trait leads to even a small reproductive advantage compared to other varieties, it will be favored by natural selection. These small differences can translate into much larger changes as they accumulate over evolutionary time.

For example, Darwin asked his reader to imagine the wolf that "preys on various animals, securing some by craft, some by strength, and some by fleetness" (Darwin 1859, p. 90). When prey animals are scarce—and prey are almost always scarce—natural selection acts strongly in such wolf populations. Wolves that possess the traits that best suit them for hunting (speed, stealth, and so on) tend to survive longer and produce more offspring. These offspring in turn are likely to possess the traits that benefited their parents in the first place. The repetition of this process for generation after generation produces wolves that are very efficient hunters. "Slow though the process of selection may be," noted Darwin, the eventual outcome is a more effective wolf predator.

Darwin applied similar arguments to many other examples in nature. Among these, he discussed the process of natural selection on plants that rely on insects as their pollinators. Darwin saw this case as more complicated than the case of the wolves, because insects often eat most of the plant's pollen. He argued that natural selection might nonetheless favor plant traits that foster more efficient insect pollination, because only a small amount of pollen is needed by the plant for fertilization (**Figure 2.17**). Darwin explained:

> . . . as pollen is formed for the sole object of fertilisation, its destruction appears a simple loss to the plant; yet if a little pollen were carried . . . by the pollen-devouring insects from flower to flower, and a cross thus effected, although nine-tenths of the pollen were destroyed, it might still be a great gain to the plant; and those individuals which produced more and more pollen, and had larger and larger anthers, would be selected. (Darwin 1859, p. 92)

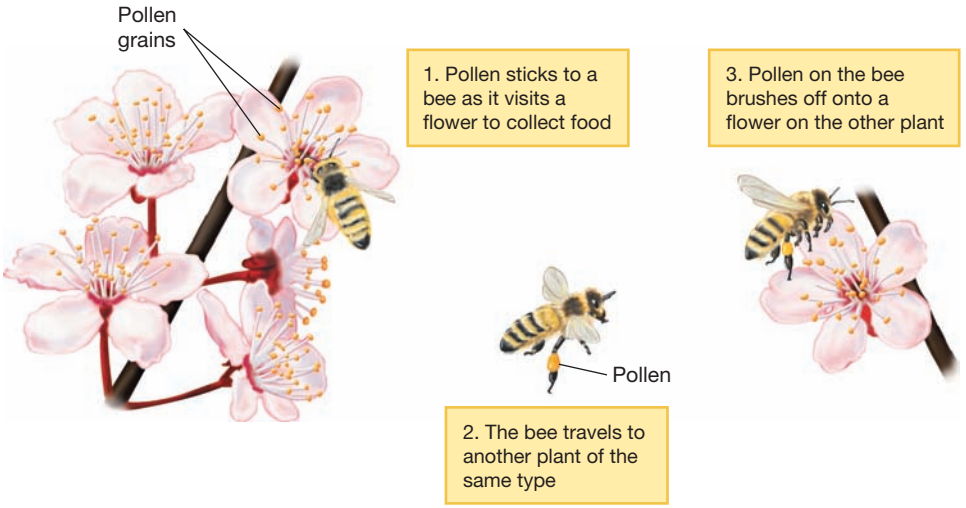


FIGURE 2.17 Plants and their pollinators. Darwin discussed the relationship between plants and the insects that cross-fertilized them as an example of how natural selection operates. Insects, such as the bee seen here, may eat some of the pollen produced by a plant, but if they move enough pollen from plant to plant, their actions may be in the plant's reproductive interests as well.

Once we see traits in terms of their effect on overall *reproductive success*—as Darwin did for wolves, insect-pollinated plants, and myriad other examples—the concept of natural selection becomes a powerful tool for understanding the world around us.

The Power of Natural Selection

Darwin's own writings demonstrate that he attributed enormous power to the process of natural selection. He ends the introductory chapter of *On the Origin of Species* by claiming, "I am convinced that natural selection has been the most important, but not the exclusive, means of modification" (Darwin 1859, p. 6). Darwin lays out his position in even more detail for the reader in a later passage:

> It may be said that natural selection is daily and hourly scrutinising, throughout the world, every variation, even the slightest; rejecting that which is bad, preserving and adding up all that is good; silently and insensibly working, whenever and wherever opportunity offers, at the improvement of each organic being in relation to its organic and inorganic conditions of life. We see nothing of these slow changes in progress, until the hand of time has marked the long lapse of ages. (Darwin 1859, p. 84)

This is a very powerful statement. For Darwin, the process of natural selection operated 24 hours a day, every day, everywhere, over vast periods of time. Only a process of such magnitude could have shaped all the life that we see around us and, for that matter, all life that has ever lived. As long as offspring resemble their parents with respect to a given trait, any differences in reproductive success associated with varieties of a given trait will be acted on by natural selection. This includes differences so slight that even the most thorough and patient human investigator might struggle to detect them.

An analogy might help here: The process of natural selection acts as an editor, removing what is not as well suited to its environment by increasing the frequency of what is better suited. Changes take place constantly, but usually they will not manifest in measurable differences until the passing of eons. In later chapters, we

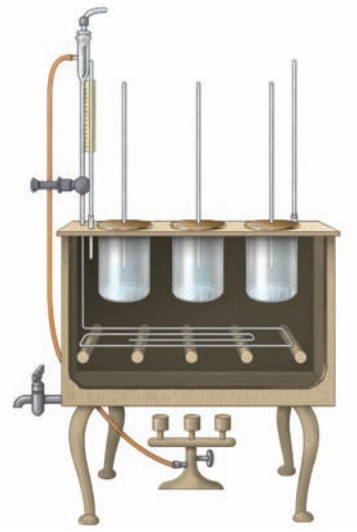


FIGURE 2.18 Experimental evolution, circa 1880. The device that William Dallinger used to examine evolutionary change in temperature tolerance in protozoa over the course of his 7-year experiment. Adapted from Dallinger (1887).

will see that Darwin underestimated the potential rate of evolutionary change in some cases. Under certain conditions the effects of the process of natural selection—particularly selection operating in species that reproduce very quickly—can be detected and measured in a span of years or even less.

Even in Darwin's day, researchers found that they could observe evolutionary change on human timescales. From 1880 through 1886, clergyman, microscopist, and Royal Society member William Dallinger conducted a 7-year **experimental evolution** study in which he tracked changes in temperature tolerance in communities of three protozoan species in which cells reproduced on average every 4 minutes (Dallinger 1887; Haas 2000).

Dallinger, encouraged by Darwin, who wrote to Dallinger that his work will "no doubt . . . be extremely curious and valuable," began by placing large populations of his protozoan communities in an experimental device he built (**Figure 2.18**) and setting the temperature at 16°C. Over time, he gradually raised the temperature. Each time he did so, many cells died, unable to survive at the higher temperature. But some cells, those with the highest thermal tolerance, survived. After the experiment had been going on for 7 years, the cells in Dallinger's experimental device survived at temperatures in excess of 66°C. This adaptation to high temperatures came at a cost—cells that could survive at 66°C died when exposed to the 16°C in which their ancestors flourished.

Malthus and the Scope of Selection

Before his readers could accept the potency of evolutionary change, Darwin needed them to reconsider their beliefs about survival in the natural world. To do this, Darwin used an analogy. Just as selective breeders must discard numerous individuals bearing undesirable traits in order for artificial selection to work, "nature" must "discard" numerous individuals in order for natural selection to be effective. While it may seem obvious to us, in Darwin's time this concept ran against the prevailing notion of an orderly, efficient, and harmonious operation of nature.

To persuade his readers that his mechanism of natural selection could shape the natural world, Darwin first had to prove to them that nature was sufficiently "wasteful" for selection to operate. That is, he needed to demonstrate to his readers that many individuals did not survive to the age of reproduction, and of those that did, only a fraction actually reproduced. To do this, Darwin drew on the ideas of Thomas Robert Malthus (1766–1834), an English political economist and demographer.

Malthus noticed that human population, unless kept in check by war, famine, disease, or other causes, grows geometrically in time (Malthus 1798). He contrasted the geometric growth of unconstrained human populations with the growth of food production, which he believed could increase at best arithmetically (**Figure 2.19**). As a result, Malthus argued that humans would inevitably outstrip the available resources necessary to sustain themselves, and that population growth would inevitably be checked by famine, war, disease, or other forces.

Darwin recognized that Malthus' argument applies to animal and plant populations as well as to human populations. For animal and plant populations in nature, food supply is usually not increasing at all, yet the power of reproduction would lead to a geometric increase in population size if growth were not checked by a struggle for existence. The difference between the potential growth and the maximum size allowed by the food supply denotes the number of individuals lost in the struggle

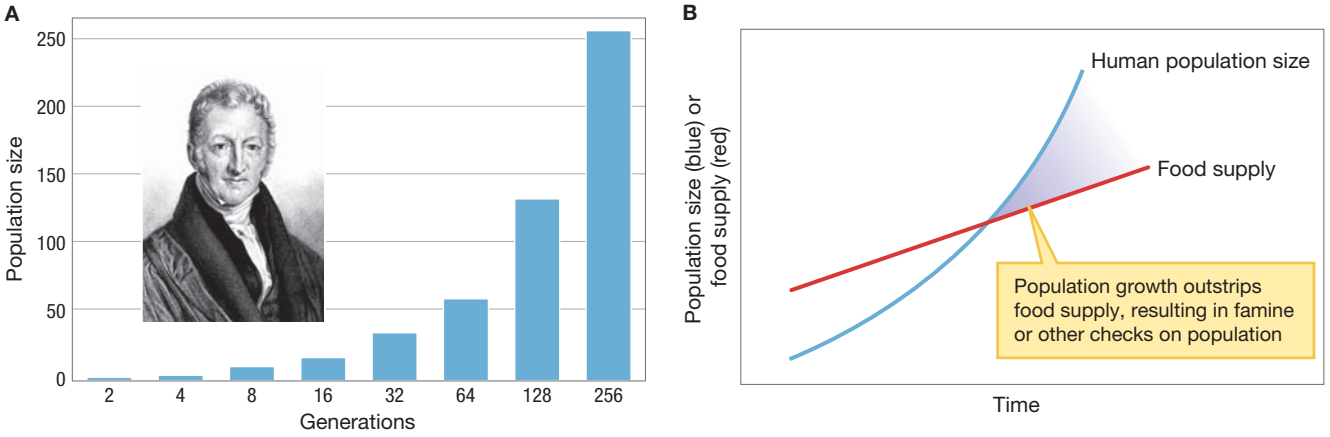


FIGURE 2.19 Malthus and population growth. Thomas Malthus argued that humans would outstrip the available resources necessary to sustain themselves, leading to population growth that would be checked by famine, war, and disease. Malthus' writings were influential in helping Darwin develop his ideas on natural selection. **(A)** Geometric population growth is shown in this graph. If each mother produces two replacements for herself, a single mother at time 0 gives rise to 2 additional mothers after a single generation. There will then be 4 mothers after 2 generations, 8 after 3 generations, 16 after 4 generations, and so forth. **(B)** Malthus argued that the human population was geometrically increasing (blue curve) and thus would inevitably outstrip its food supply (red curve), which he believed to be arithmetically increasing.

for existence, and thus it represents the opportunity that natural selection has to sort based on even the smallest differences in form (**Figure 2.20**). Darwin neatly summarized this as follows:

> As many more individuals of each species are born than can possibly survive; and as, consequently, there is a frequently recurring struggle for existence, it follows that any being, if it vary however slightly in any manner profitable to itself, under the complex and sometimes varying conditions of life, will have a better chance of surviving, and thus be *naturally selected*. (Darwin 1859, p. 5)

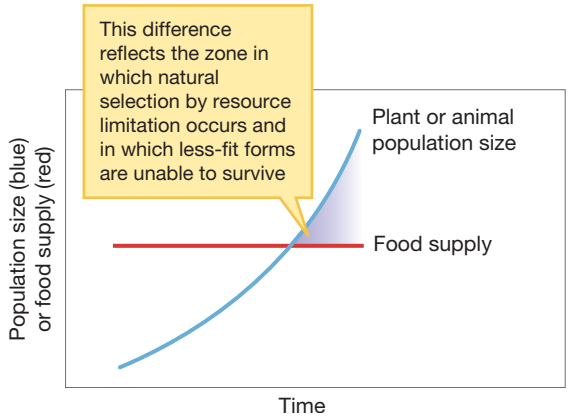


FIGURE 2.20 Darwin, Malthus, and natural selection. Darwin adapted Malthus' argument to natural populations of plants and animals. The food supply curve (red) is flatter here than in Figure 2.19. In that figure, the food supply curve also increased as a result of human innovations in food production.

Transformational and Variational Processes of Evolution

Darwin's mechanism of evolutionary change differed radically from previous concepts of evolution. Before Darwin, scientists had envisioned change as a **transformational process**, in which the properties of an ensemble change because every member of the ensemble itself changes. For example, a mountain range becomes less rugged and more rounded over geological timescales because *each* individual peak itself becomes more rounded.

Lamarck's theory of evolution was a transformational theory. According to Lamarck, the properties of a lineage of organisms shift over time because of changes that each member undergoes during its lifetime and then passes along to its descendants. By contrast, Darwin's theory of evolutionary change was a variational one. In a **variational process** of evolution, the properties of an ensemble change, not because the individual elements change, but rather because of the action of some process *sorting* on preexisting variation within the ensemble (Levins and Lewontin 1987). For Darwin's theory, that sorting process was the process of natural selection.

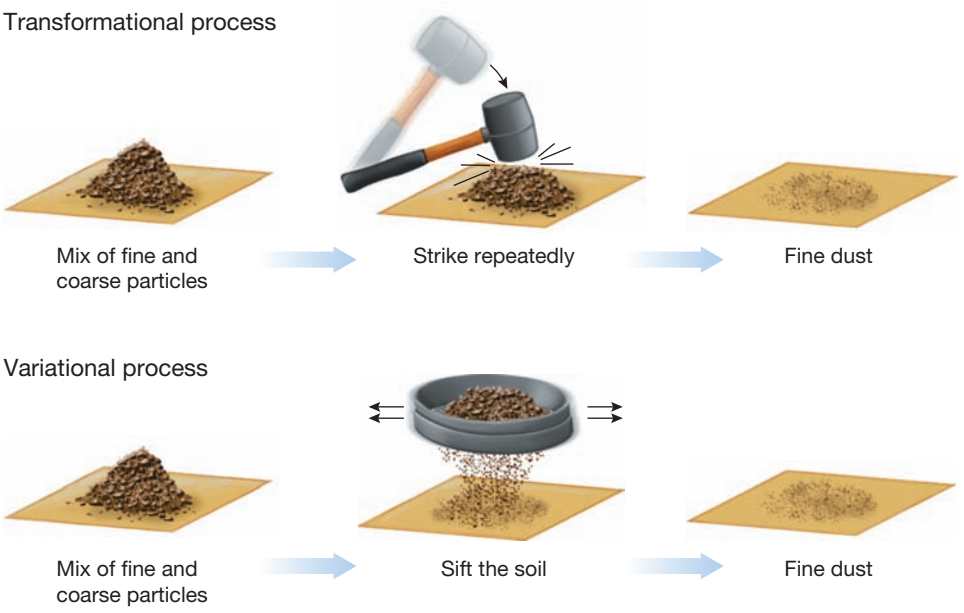


FIGURE 2.21 Different processes of change. In a transformational process, the ensemble changes because each individual member changes. In a variational process, the ensemble changes because something sorts among the variants in the original ensemble. In this example, crushing the soil particles is a transformational process—the ensemble shifts toward smaller particles because the individual particles are reduced in size. Sifting the soil is a variational process—the ensemble shifts toward smaller particles because the larger particles are sorted out.

To see how such a sorting process operates, imagine sifting a bucket of soil with particles ranging in size from fine sand to small pebbles. After sifting, the soil particles remaining in the sifter will be considerably larger on average than those in the original soil mixture. This is not because of any change on the part of individual particles—no transformation in the size of soil particles has occurred—but rather it is because the sifter has sorted the members of the ensemble according to their characteristics (**Figure 2.21**).

This kind of sorting process is what takes place when we use artificial selection to change the characteristics of a breed of animals or plants. And just as a pigeon breeder sorts on variation when selecting breeding pairs so as to produce a snow-white pigeon, the conditions of existence sort on variation within the members of species. Natural selection favors those variants that survive and outreproduce other variants, while passing on their characteristics to their offspring.

To arrive at his theory of evolution by natural selection, Darwin needed not only to establish that the process of natural selection involves "wasteful" deaths within populations but also to dispel the belief in an eternally unchanging world, as discussed earlier in this chapter. To arrive at a specifically *variational* theory of evolution, Darwin also had to reject the existing conception of nature that viewed any variation as aberrant and unimportant, and instead place variation itself in the forefront, as an absolute necessity for a sorting process without which variational evolutionary change cannot occur.

KEYCONCEPT QUESTION

2.4 Robert bought a small iPod that held a small fraction of his full CD collection. It seemed like too much trouble to select his favorite CDs, so he simply picked 50 of his discs at random and put them on the iPod. Each month, he deleted any of the albums that he didn't listen to over the past month; he added new ones, again selected randomly, in their place. At first, Robert thought the music on his iPod was so-so, but after a year, he thought the music it contained was really great. Is this a transformational or variational process of evolution? Explain.

2.7 Darwin on Common Ancestry

Thus far in the chapter, we have concentrated on the details of Darwin's first insight, the process of natural selection. We now turn to the second of Darwin's revolutionary insights, his answer to the question: Where do species come from? Darwin correctly recognized that all living creatures derive from one or a few common ancestors, and that new species are formed when populations of a preexisting species diverge from one another.

The Tree of Life

In *On the Origin of Species*, Darwin explained that just as artificial selection can create multiple new varieties from a single domesticated variety, natural selection can, over time, generate multiple new species from a single ancestral species. Indeed, Darwin conjectured that the vast diversity of species that we see throughout the world has arisen from precisely this process.

Darwin's explanation suggests that all living things are linked by a pattern of descent dramatically different from that implied by either special creation—the idea that each species was created in its current form by a supernatural deity—or Lamarck's theory of evolution (**Figure 2.22**). While these latter explanations envision species as a set of independent organisms, Darwin's theory links species according to their historical pattern of descent.

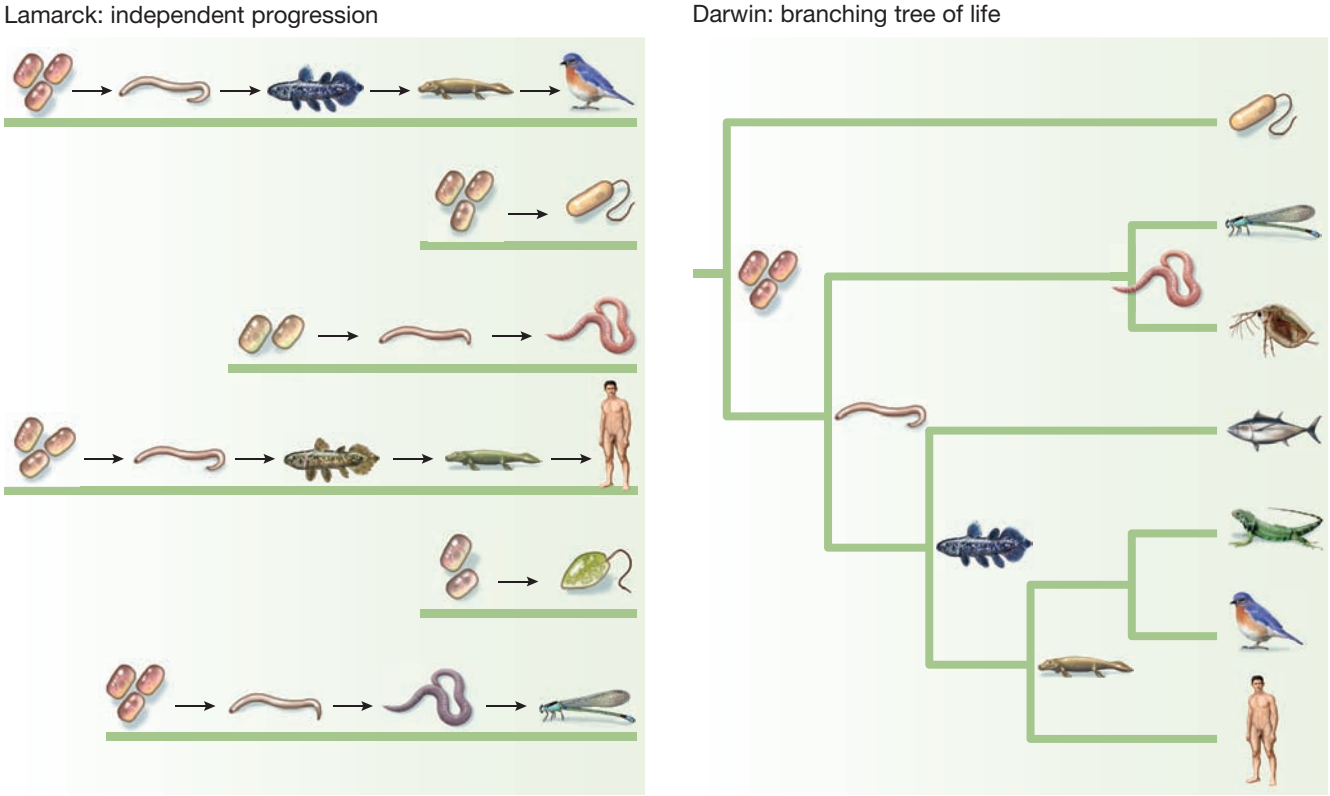


FIGURE 2.22 Darwin's theory versus Lamarck's theory. In Lamarck's theory, species evolve independently and in parallel; in Darwin's theory, species are descended one from another to form a branching tree of life.

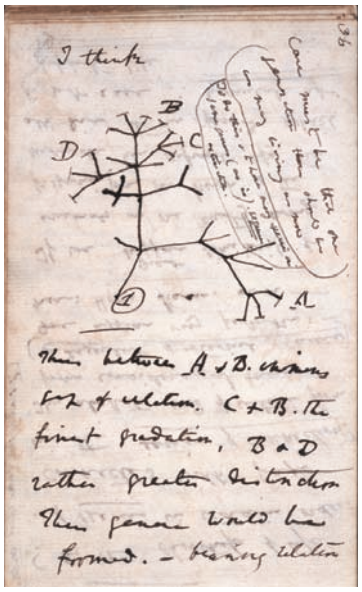


FIGURE 2.23 An early phylogenetic tree from Darwin. One of Darwin's first sketches of the branching relationships among species.

Darwin described the branching historical relationships among all living things using the metaphor of a tree of life (**Figure 2.23**). His eloquent depiction of the tree of life requires us to look at a lengthy quote, but this quotation is worth reproducing because of the profound implications of the tree of life metaphor:

> The affinities of all the beings of the same class have sometimes been represented by a great tree. I believe this simile largely speaks the truth. The green and budding twigs may represent existing species; and those produced during each former year may represent the long succession of extinct species. . . . The limbs divided into great branches, and these into lesser and lesser branches, were themselves once, when the tree was small, budding twigs. . . . Of the many twigs which flourished when the tree was a mere bush, only two or three, now grown into great branches, yet survive and bear all the other branches; so with the species which lived during long-past geological periods, very few now have living and modified descendants. From the first growth of the tree, many a limb and branch has decayed and dropped off; and these lost branches of various sizes may represent those whole orders, families, and genera which have now no living representatives, and which are known to us only from having been found in a fossil state. . . . As buds give rise by growth to fresh buds, and these, if vigorous, branch out and overtop on all sides many a feebler branch, so by generation I believe it has been with the great Tree of Life, which fills with its dead and broken branches the crust of the earth, and covers the surface with its ever branching and beautiful ramifications. (Darwin 1859, pp. 129–130)

Darwin recognized the enormous importance of the branching relationships among species in this tree of life as a model for both life's history and the patterns of life's diversity. He chose to include only a single figure in *On the Origin of Species*, and this figure serves to illustrate the branching historical relationships among all living things (**Figure 2.24**). Today, we refer to this type of figure as a phylogenetic tree.

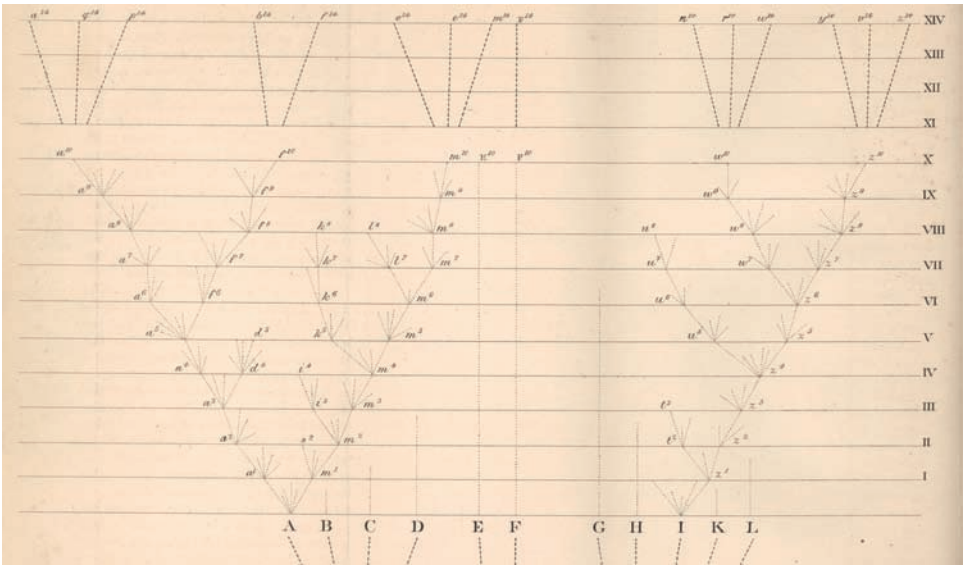


FIGURE 2.24 A phylogenetic tree from *On the Origin of Species*. Darwin included this diagram as the sole figure in *On the Origin of Species*. It illustrates the pattern of branching relationships among a number of initial populations (A–L) over vast periods of time (time moves forward as one moves up the vertical axis, from I to XIV).

Groups within Groups

A major point supporting the hypothesis of common ancestry with branching descent is that it explains hierarchical patterns of similarity that are observed in nature. By hierarchical patterns of similarity, we mean something like this: Different species of squirrels resemble each other more than they resemble any species of deer. And different species of deer resemble each other more than they resemble any species of squirrel. That is, species of squirrels *cluster* together because of their similarity to one another, and species of deer *cluster* together. At a different hierarchical level, species of squirrels and deer are more similar to one another than either is to a species of frog. And so, at this hierarchical level, species of squirrels and deer cluster together (as mammals), and species of frogs, toads, and salamanders cluster together (as amphibians). Finally, squirrels, deer, frogs, and toads are all more similar to one another (as vertebrates) than they are to species of octopus or squid (invertebrates).

In *On the Origin of Species*, Darwin argues that branching descent explains this hierarchical patterning seen in nature, writing that "the forms of life throughout the universe become divided into groups subordinate to groups" (Darwin 1859, p. 59). Neither special creation nor a theory such as Lamarck's can explain these groupings and subgroupings of organisms. But a process of branches dividing and subdividing naturally gives rise to a hierarchical structure of relationships—varieties nested within species within genera (a *genus*, the singular of genera, is a taxonomic group, intermediate in scale between species and families), and so on up to kingdoms. Indeed, the modern field of **systematics**—the naming and classification of organisms—is based on the conceptual foundation of this hierarchical branching structure. As we will see in further detail in Chapter 4, evolutionary systematists aim to classify organisms into hierarchically arrayed groups, or *clades*, of organisms that have descended from a common ancestor (**Figure 2.25**).

Darwin's view of common descent provides an explanation not only for the hierarchy of organisms now studied by systematists but also for the clustering of species: "No naturalist pretends that all the species of a genus are equally distinct

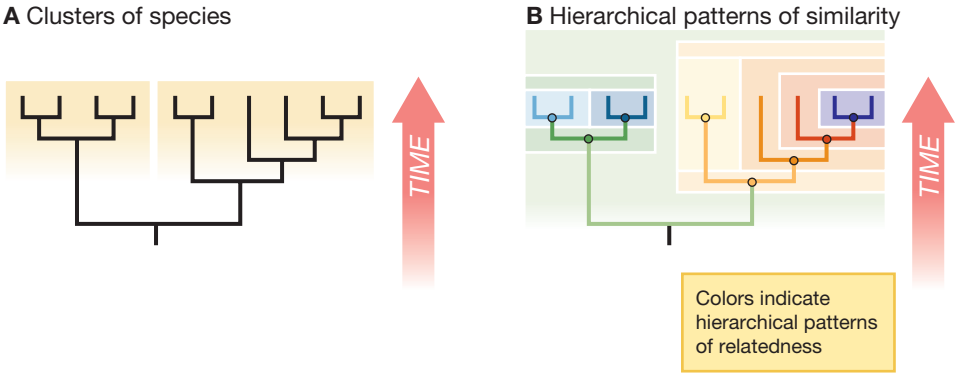


FIGURE 2.25 Branching descent, clustering, and hierarchy. Darwin's view of branching descent explains both the clustering of species in terms of similar form **(A)** and the hierarchical patterns of similarity **(B)** that we can discern when studying groups of species. In panel B, some of the different clades are shown in different colors, with the node representing the common ancestor of that entire clade in the clade's characteristic color.

from each other," Darwin told the reader of *On the Origin of Species* (Darwin 1859, p. 57). That is, we expect to see clusters at many levels, including that of the genus. Darwin reasoned that this clustering arose as a result of common ancestry. Groups of closely related species share common characteristics, in large part because they share a recent common ancestor.

Common Descent and Biogeography

Both Wallace and Darwin traveled extensively across the globe, and in doing so, both were struck by the strong patterns that they observed in the geographic distribution of nature's diversity. In his 1855 paper that preceded Darwin's *On the Origin of Species* by 4 years, Wallace noted that living species tend to be similar to other species that are geographically nearby, and that species from the fossil record tend to be most similar to species that lived around the same time. In other words, species that closely resemble one another tend to be closely clustered in time and space, and from this observation Wallace proposed that "Every species has come into existence coincident both in space and time with a pre-existing closely allied species" (Wallace 1855, p. 186).

Wallace recognized that this pattern of descent—new species coming into existence from previous species—implies the branching system of phylogenetic relationships that we have described in detail earlier in this section. Like Darwin, Wallace proposed a tree metaphor in which groupings of species form a "complicated branching of the lines of affinity, as intricate as the twigs of a gnarled oak or the vascular system of the human body" (Wallace 1855, p. 187).

Darwin came to similar conclusions about the causes for groupings of species based on similar evidence. In *On the Origin of Species*, Darwin notes that similarities in "conditions of existence"—climate and physical conditions, for example—are insufficient to explain the geographic clustering of similar, closely related species. Instead, he thought that geographic features seemed to play an important role. He described the following pattern: Species separated by major geographic barriers to migration—mountain ranges, deserts, or large bodies of water—tend to be dissimilar even when the climate and physical conditions are similar on each side of the divide. Adjacent species that are not separated by geographic barriers tend to be similar to one another despite major differences in climate and habitat.

Darwin found some examples that seemed to violate the tenet that species separated by major geographic barriers to migration tended to be dissimilar, and he wanted to understand why. For example, while on the *Beagle* Darwin took note of how similar plants on mainland South America were to those on nearby islands. But the ocean separated the mainland and islands, and plants can't swim. The ocean, then, should be a major geographic barrier, and plants on the mainland and island should not be all that similar. The solution, Darwin posited, was that while the ocean *can be* a major geographic barrier to plant dispersal, *in this case* it was not, because seeds could survive in salt water and be transported by ocean currents to islands. Darwin even ran a series of experiments in which he tested whether seeds soaked in salt water survived to germinate, and found that they did (Darwin 1855a,b, 1857). Darwin also hypothesized that bivalves from the South American mainland might be transported to the islands when adhering to the mud-soaked

feet of ducks, and evidence he had gathered from other friends suggested they might (Darwin 1882). Species separated by true geographic barriers to migration do tend to be dissimilar, but Darwin discovered that one must be very careful about what constitutes a true geographic barrier.

These geographic correlations supported Darwin's theory that each species arises only a single time in a single place, by descent with modification from a closely related species. Darwin then proposed the grandest uniformitarian extrapolation in the history of science. From these patterns he observed among groups of related species, Darwin hypothesized that in fact all living things have descended, with modification, from one or a few common ancestors. If so, all living things—plants, protozoa, humans, birds, insects, and every other life-form—share a common origin. In the next few chapters, we will explore the overwhelming weight of evidence that has since accumulated in support of Darwin's conclusion. But first, we will consider some of the problems with his theory of descent with modification that troubled Darwin in his lifetime.

2.8 Problems with Darwin's Theory

In science, no grand theory is without its problems, especially in its early stages. The important issue is whether scientists acknowledge such problems and generates new hypotheses or simply ignores any inconsistencies. In *On the Origin of Species*, Darwin was not afraid to discuss many of the problems associated with his theory of evolution by natural selection.

Here we briefly touch on three of the major challenges that Darwin faced, and we provide pointers to where we will discuss some of these problems in greater detail in later chapters. Although not all of these challenges were resolved within Darwin's lifetime, today we have a good understanding of how to account for each of them. In Chapters 6 and 7, we will also show how another challenge Darwin faced—understanding how inheritance operated—was finally resolved.

Problem 1: Accounting for Complex Structures with Multiple Intricate Parts

Darwin generally portrayed natural selection as a slow process acting on very small differences between individuals. It is relatively straightforward to see how this process could lead to gradual adjustments in the thickness of an otter's fur or the length of a badger's forelimb. But how might natural selection operate as a genuinely creative process? How might it generate complex structures such as the eye, the mammary gland, or the instincts needed to construct the hexagonal cells of a honeycomb (**Figure 2.26**)?

Darwin's critics seized on this issue. If natural selection operates by gradual increments, they reasoned, the eye must be preceded by a quarter of an eye, then half of an eye, and so forth—and what good is half of an eye? These critics argued that

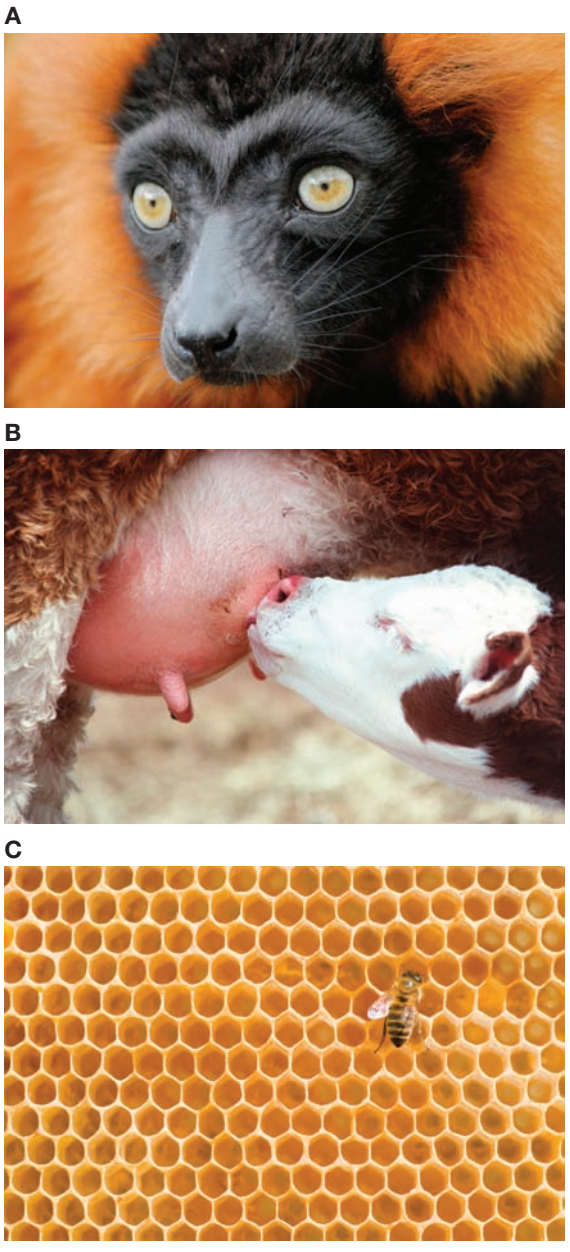


FIGURE 2.26 Complex traits. One of the challenges that Darwin faced was to explain how natural selection could create complex traits such as **(A)** the vertebrate eye, **(B)** the mammary gland, or **(C)** the ability to construct the hexagonal cells of a honeycomb.

complex traits would have no selective value until fully formed, and thus natural selection would not favor the intermediate steps necessary along the way. Darwin responded to this challenge with confidence; we will explore his explanation in depth in Chapter 3.

Problem 2: Explaining Traits and Organs of Seemingly Little Importance

At the opposite extreme, Darwin wondered how his theory could explain traits that appear to lack any biological function. If a trait does not contribute to survival and/or reproductive success, it will not be favored by natural selection, and yet it seemed as though such traits existed. Snakes have "limb buds" that appear to have no function, ruminants have incisor teeth that never break through their gums, and so on. How can these things be explained? We explore the answers in Chapter 4 (where we treat vestigial traits) and Chapter 8 (where we consider the neutral theory of evolution).

Problem 3: Why Does Variation Persist in the Face of Natural Selection?

As we saw earlier in this chapter, Darwin's theory relied on a variational process of evolution rather than a transformational one. This posed a problem: In order for natural selection to operate, it must have variation to sort on—but the action of natural selection itself reduces the amount of variation in a population as less-fit variants are eliminated from that population. Thus, the fire of natural selection threatens to consume the variation that fuels it. How can we explain the persistence of variation? Why doesn't evolution just come to a halt as variation is exhausted?

Adding to the scope of the problem, when Darwin wrote *On the Origin of Species*, biologists did not understand the basic principles of heredity. Mendel's laws were not known to Darwin; instead, like most of his contemporaries, Darwin envisioned inheritance as a blending of the hereditary elements from each parent. Such a blending process also consumes variation. In Chapters 6 and 7, we will explore the sources of new variation, and in Chapter 9, we will see how scientists in the early part of the twentieth century reconciled the process of inheritance with Darwin's ideas about natural selection.

2.9 The Reaction to Darwin and Early History of the Modern Synthesis

While various religious leaders challenged almost all of the major conclusions that Darwin presented in *On the Origin of Species*, the scientific community exhibited a more mixed reaction (Mayr 1982). Early on, for example, British scientists almost universally embraced Darwin's ideas on common ancestry, but many were unconvinced that the primary force generating evolutionary change was natural selection. That is, they accepted that evolutionary change, rather than special acts of creation, explained the world that we see around us, but they rejected the idea that the primary force generating evolutionary change was natural selection. A few British naturalists, including Alfred Russel Wallace, Henry

Walter Bates (1825–1892), and Joseph Dalton Hooker (1817–1911), thought that natural selection was important in driving evolutionary change, but many early evolutionary biologists disagreed (Glick 1974).

In the 1880s, experimental work—primarily that of German geneticist and evolutionary biologist August Weismann (1834–1914), who demonstrated that traits acquired during the lifetime of an organism could not be inherited—dealt a death blow to previous theories of Lamarckian inheritance. Scientists were left with only two possible mechanisms of evolution. The processes were either natural selection acting in a slow and methodological way on small genetic differences or **saltationism**; that is, "evolution via large, sudden changes from the existing norm" (Mayr 1982).

In his now-famous experiments of the 1850s and 1860s, Augustinian monk, plant breeder, and biologist Gregor Mendel (1822–1884) found that inherited factors that form the basis of traits come from both parents. His work on pea plants demonstrated that each parent plant has two copies of each gene, and that the two gene copies separate with equal probability into gametes (eggs, sperm, pollen, and so on). In Chapter 6, we will discuss Mendel's experiments in more detail.

Mendel's results remained virtually unnoticed until 1900, when three scientists (Hugo de Vries, Carl Correns, and Eric von Tschermak) independently rediscovered his work and made it available to the scientific world. Biologists began to explore how natural selection might operate when inherited material operated as Mendel suggested.

At that time, evolutionary biologists fell into one of two camps. On one side were the Mendelians, who viewed evolution as a saltational process. These scientists primarily worked in the lab, were trained more as physical than as biological scientists, and thought that the continuous variation in so many traits seen in nature was not primarily genetic in origin. This was because the Mendelian camp's original interpretation of Mendel's work allowed for discrete variation—for example, tall versus short—but not continuous variation in traits. In the other camp were the biometricians, including the English geneticist and statistician Karl Pearson (1857–1936). The biometricians were impressed by the amount of continuous variation—that is, extremely fine gradations of difference—that they saw all around them and thought natural selection was a slow, gradual process.

The differences between the Mendelians and the biometricians began to dissolve with experimental work in the 1930s and 1940s in what came to be called the **modern synthesis**, or the **evolutionary synthesis**. This synthesis included experimental work in genetics demonstrating that:

- Genes are passed on from parents to offspring in an intact form, even if they are not expressed in the offspring's phenotype. That is, genes are particulate: they don't "blend" with other genes.
- One source of genetic variation is mutation.
- Genetic variants that generate large and small phenotypic differences are not qualitatively different from one another—the effects of large differences may be more pronounced, but genetic variation is generated and inherited in similar ways in both cases.
- Not all genetic mutations are harmful, so positive changes can accrue over time—either slowly or in some cases more rapidly.

- Sexual reproduction is an important contributor to the production of massive amounts of genetic variation.
- Some traits are the result of the interaction of numerous genes, while some genes can affect more than one trait, helping to explain the evolution of complex traits without necessarily assuming some saltational (that is, large and sudden) change.
- Many (but not all) changes in the genotype affect the phenotype. Variation in the phenotype is the raw material for natural selection.

We discuss each of these points in more depth in later chapters, but for now, what we wish to emphasize is that this work demonstrated that there was no conflict between what was being found in the new, burgeoning field of genetics and Darwin's idea that evolutionary change was primarily a slow process, driven by natural selection. Another crucial ingredient of the modern synthesis was the work of mathematical population geneticists such as Sir Ronald A. Fisher (1890–1962), Sewall Wright (1889–1988), and J. B. S. Haldane (1892–1964), who developed mathematically sophisticated models of how evolutionary processes lead to changes in gene frequencies and how changes in gene frequencies map onto changes in the phenotypes of organisms (Chapters 7–9).

The modern synthesis represented the collected efforts of systematists, geneticists, paleontologists, population biologists, population geneticists, and naturalists. Although often associated with the publication of British biologist Julian Huxley's (1887–1975) book, *Evolution: The Modern Synthesis*, this synthesis was not so much an event per se, but the result of a gradual accumulation of information that melded together to shape biology at the time (Huxley 1942). In addition to the work listed earlier, this synthesis involved a combination of theoretical models and experimental manipulations, like that of German-American evolutionary biologist and ornithologist Ernst Mayr's (1904–2005) pathbreaking work on the process of speciation and its relationship to systematics (classifying organisms) (Mayr 1942). In essence, the evolutionary approach provided a framework for understanding both the fit of organisms to their environment and the diversity and history of life. We will discuss the major findings of the evolutionary synthesis in many subsequent chapters.

We have seen that midway into the nineteenth century, thinkers began to develop mechanistic, rather than supernatural, explanations for the world around them, and science as a whole began to center on experimentation, data gathering, and hypothesis testing. Theories in geology had created a sense of deep time and gradual, versus catastrophic, changes. Robert Chambers and others had suggested that new species might arise from existing species, Jean-Baptiste Lamarck had hypothesized that there were generational adaptations to environmental needs, and Patrick Matthew had presented a preliminary theory of natural selection. It was in this context that Charles Darwin developed his ideas. Having laid out both the basic elements of Darwin's theory and the problems facing that theory, we are now in a good position to examine the components of evolutionary change in subsequent chapters.

SUMMARY

1. Critical changes that set the stage for Darwin and Wallace to come up with their ideas on evolutionary change and natural selection included the shift from supernatural to natural explanations, the move from catastrophism to uniformitarianism, the use of logic and pure reason, the acceptance that the world—both the biotic and abiotic worlds—was constantly changing, and the rejection of the idea that life formed by spontaneous generation.
2. Scientists sought mechanistic rather than supernatural explanations for the features of the physical world; they valued experimentation, data gathering, and hypothesis testing.
3. Lyell's ideas in geology created a sense of deep time, Robert Chambers and others proposed that new species arose from existing species, Jean-Baptiste Lamarck hypothesized generational adaptations to environmental needs, and Patrick Matthew presented a preliminary theory of natural selection.
4. Darwin prepared his readers for his revolutionary ideas on natural selection by introducing them to the artificial selection programs that breeders had long used.
5. Darwin's ideas on natural selection put variation at the forefront of evolutionary change. In this way, they differed dramatically from the transformational evolutionary changes that Lamarck had suggested at the start of the nineteenth century.
6. Darwin had two great insights: (1) natural selection occurs because populations are variable and because some individuals are more successful than others at surviving and reproducing in their environment, and (2) all species have descended from one or a few common ancestors; species that share a recent common ancestor tend to resemble one another in many respects for the very reason that they share recent common ancestry.

KEY TERMS

catastrophism (p. 34)
evolutionary synthesis (p. 59)
experimental evolution (p. 50)
hypothesis (p. 32)
inheritance of acquired characteristics (p. 39)
methodological naturalism (p. 32)
modern synthesis (p. 59)
natural history (p. 36)
population (p. 38)
saltationism (p. 59)
spontaneous generation (p. 36)
struggle for existence (p. 37)
systematics (p. 55)
transformational process (p. 51)
uniformitarianism (p. 34)
variational process (p. 51)

REVIEW QUESTIONS

1. What is methodological naturalism? Why is it an important foundation for science?
2. How did the discovery of fossils by the ancient Greeks help lead to the view that the world changes over time?
3. How did Lyell's uniformitarianism help set the stage for Darwin's ideas on evolution by natural selection?
4. In the Middle Ages, what did people believe about the age of Earth? What evidence led to this conclusion?

5. Define spontaneous generation. Why did early observations of bacteria and fungi using microscopes delay the abandonment of the idea of spontaneous generation?
6. What do evolutionary biologists mean by the inheritance of acquired characteristics?
7. What are Darwin's "two great laws"?
8. What are the two most important differences between artificial selection and natural selection?
9. What did Wallace conclude from the observation that "Every species has come into existence coincident in both space and time with a pre-existing closely allied species"?
10. Within the context of evolutionary biology, what is the difference between transformational and variational processes?
11. Explain why the linear hierarchy of Aristotle's *scala naturae* is incompatible with Darwin's phylogenetic view of biological diversity.

KEY CONCEPT APPLICATION QUESTIONS

12. Why do you think the discovery that species go extinct was important for the development of evolutionary ideas?
13. Sarah bought herself a cheap turntable and a stack of her favorite records on vinyl. Unfortunately, each time she played a record, the poor-quality phonographic needle scratched and wore down the record, so that after a year, her music collection didn't sound nearly as good as when she first bought it. Is this a transformational or variational process of evolution? Explain.
14. It is well known that many lizard species have evolved the ability to detach their tails as a mechanism of escaping from the grasp of predators. In his *Natural History*, Pliny the Elder (23–79 C.E.) spins a similar tale about beavers (Healy 1991). He reports that beavers castrated themselves in order to escape hunters who pursued them for their testicles, which could be used to produce an analgesic medication (Book 8, Chapter 47). Borrowing from Pliny, the Roman author Claudius Aelianus (ca. 175–ca. 235 C.E.) describes this behavior in detail in his encyclopedic series *On the Nature of Animals* (Johnson 1997). When pursued by hunters, he writes, the beaver "puts down its head and with its teeth cuts off its testicles and throws them in their path, as a prudent man who, falling into the hands of robbers, sacrifices all that he is carrying, to save his life, and forfeits his possessions by way of ransom." Of course, beavers do not actually do anything of the sort. Explain why Darwin would have considered it reasonable that lizards should drop their tails, but implausible that beavers should self-castrate even to spare their own lives.
15. Many British readers in the 1850s were familiar with the sorts of breeding programs that were used to produce dog varieties, and Victorian Englishmen and Englishwomen were fascinated with pigeon breeding. Given this, why was it such a brilliant strategy for Darwin to open *On the Origin of Species* with a discussion of artificial selection?

SUGGESTED READINGS

Burkhardt, R. W., ed. 1984. *The Zoological Philosophy of J.-B. Lamarck*. University of Chicago Press, Chicago. An edited volume on the work of Lamarck, with special reference to his theories of transformation.

Costa, J. T. 2014. *Wallace, Darwin, and the Origin of Species*. Harvard University Press, Cambridge, Mass. An interesting read on both Darwin and Wallace's ideas on evolution.

Darwin, C. 1859. *On the Origin of Species*. John Murray, London. Here Darwin lays out his grand theory of descent with modification.

Glick, T. F., ed. 1988. *The Comparative Reception of Darwinism*. University of Chicago Press, Chicago. An excellent discussion of cross-cultural reactions to Darwin's ideas.

Malthus, T. 1798. *An Essay on the Principle of Population, As It Affects the Future Improvement of Society*. J. Johnson, London. This text gives insight into why overpopulation and competition play a role in Darwin's theory of natural selection.

Mayr, E. 1991. *One Long Argument*. Harvard University Press, Cambridge, Mass. A short but wonderful book on Darwin and evolutionary biology.

3

Natural Selection

◀ A katydid (*Tettigoniidae* species) beautifully matches the leaf on which it sits in Borneo.

Droughts can devastate human populations—crops fail, people lack drinking water, livestock starve. But the sudden, dramatic changes to environment that droughts create can also provide unique opportunities to test hypotheses generated in the natural sciences, including evolutionary biology. Let us take a look at an example.

Southern California is accustomed to fluctuations in rainfall because of El Niño cycles, but from 2000 to 2004 the area was hit by a severe drought—even by Southern California standards. The droughts were so intense that the governor of California declared a state of emergency each year from 2000 to 2004. The drought hit animals hard. But animals are mobile, and they have the ability to respond with flexible behaviors. They can search out cooler, wetter refuges, for example. Plants can't.

One species hit hard by this California drought was the mustard plant, *Brassica rapa*. In *B. rapa*, the growing season normally runs through late spring, until rainfall tapers off. But the 2000–2004 drought dramatically shortened the growing season in Southern California, in particular by reducing the amount of rainfall toward the end of the usual growing season.

So, what does evolutionary theory predict the response to intense drought should be in plants such as *B. rapa*?

Evolutionary theory predicts that in such scenarios, natural selection should favor plants that flower earlier in their abbreviated growing seasons (Inouye 2008; Miller-Rushing and Primack 2008). It predicts this shift in flowering time because such a strategy should increase the reproductive success of plants that flower early compared to that of plants that flower later. Steve Franks and his colleagues put this theory to the test using an ingenious experimental approach (Franks et al. 2007; Franks and Weis 2008, 2009; Franks 2011).

Franks and his colleagues wanted to test the hypothesis that postdrought *B. rapa* plants flowered earlier than predrought *B. rapa* plants of the same regional populations. It sounds simple enough in principle, but how could they do this? Obtaining postdrought plants was easy enough—the researchers simply went out to the field in late 2004 and collected them. But all plants from predrought years were long gone—how could they compare the flowering times of postdrought plants to those of plants present before 2000 but long since gone?

The researchers' solution to the problem of how to compare predrought and postdrought populations tells us something about the importance of long-term studies and the collection of specimens in evolution and ecology. To gain a deep understanding of their system, Franks and his team had studied this population of *B. rapa* for many years, and they had collected seeds in 1997, just a few years before the drought (Franks et al. 2008). Because they had this foresight, they could *directly* compare predrought and postdrought seed stocks. But first they had to surmount one hurdle: The 1997 seeds were older than the 2004 seeds, and seed age might influence other aspects of the plants' physiology. To control for differences in age of the 1997 and 2004 seeds, they grew adult plants from each seed stock and crossed those plants. In this way they obtained a supply of fresh seeds from 1997 parents and a separate supply of fresh seeds from 2004 parents. They then grew seeds under similar conditions and tested whether natural selection had affected flowering times as they predicted. They found that plants derived from the seeds of the 2004 parents flowered earlier, on average, than plants derived from the seeds of the 1997 parents (**Figure 3.1**). As predicted, flowering times had shortened from 1997 to 2004 as a result of natural selection imposed by the drought.

The process of natural selection has played an essential role in driving the endless modifications that lead to the biological diversity of the living world. We have discussed this process in general terms, but we are now ready for a more detailed exploration of natural selection. We are also ready to move from Darwin's discoveries to the specific manifestation of his theory in contemporary evolutionary biology.

In this chapter, we will examine the following questions:

- What are the components of natural selection?
- What is an adaptation, and how do we study adaptations?
- How can natural selection be examined in the wild and in the laboratory?
- How do complex traits originate?
- Why are there constraints on natural selection, and what are these constraints?

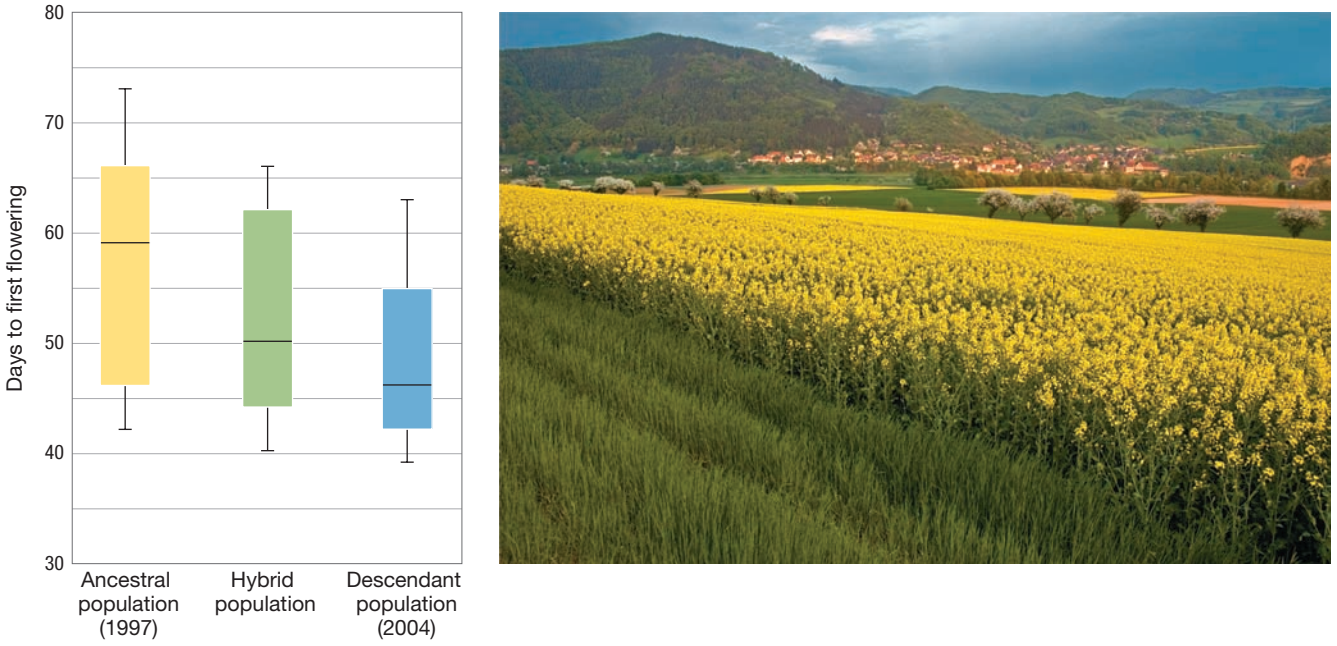


FIGURE 3.1 A prolonged drought alters flowering time. Descendant populations of *Brassica rapa* from after the intense 2000–2004 drought flowered much earlier in the season than those from predrought (ancestral) populations. Hybrids—crosses between the ancestral and descendant populations—show intermediate values. Here the data are represented as *box and whisker plots*: In each, the central line represents median flowering time, and the shaded areas denote the 25th to 75th percentiles. Adapted from Franks et al. (2007).

3.1 The Components of Natural Selection

People tend to assume that important ideas must be complex, complicated, and difficult to comprehend—because of the very fact that they are considered important. This is not necessarily true. Natural selection, the primary process responsible for generating the exceptional diversity and complexity of all living forms, is in fact, conceptually, a very simple idea.

Natural selection is the inevitable consequence of three conditions (**Figure 3.2**):

1. **Variation**. Individuals in a population differ from one another.
2. **Inheritance**. Some of these differences are transmitted from parent to offspring.
3. **Differential reproductive success**. Individuals with certain traits are more successful than others at surviving and reproducing in their environment.

We will explore variation, inheritance, and differential reproductive success in detail later in this section, but before we do, let's examine why each is necessary and how together they lead to evolution by natural selection. In so doing, we should keep four points in mind.

First, mutation is one of the major sources generating the variation on which natural selection acts. While some mutations may be favored by natural selection,

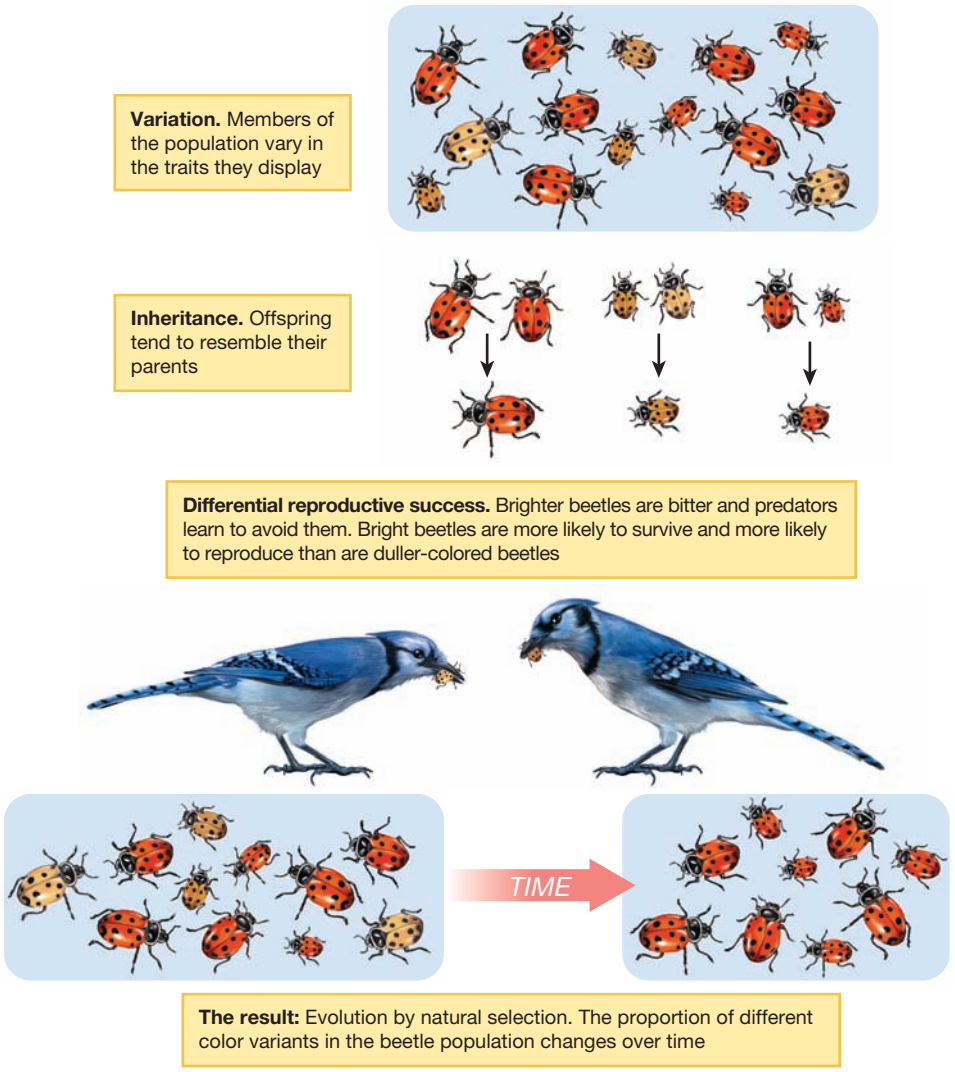


FIGURE 3.2 The three components of natural selection. Evolution by natural selection occurs when there is variation, inheritance, and differential reproductive success among individuals in a population.

mutations *occur* at random with respect to the needs of the organism, independently of whether or not they would be favored by natural selection. We explore this point in greater depth in Chapter 6.

Second, when evolutionary biologists study the process of natural selection, they typically focus on how some *trait* of interest changes or remains constant over time. Researchers can study many different kinds of traits. They often examine a physical characteristic of an organism; for example, the color of a bird's plumage, the shape of a mammal's tooth, or the structure of a plant's flower. Other times, researchers study behavioral traits, such as the elaborate dance of a lyrebird or the predator-avoidance behavior of the sea slug *Tritonia*. Sometimes the trait will simply be a genetic character: Which sequence of some particular gene does an individual have or how many chromosomes does a species of grass have? Irrespective of the type of trait, most studies of natural selection begin by specifying which trait or traits are to be considered.

Third, natural selection is a process by which the characteristics of a population—not those of an individual—change over time. When we study natural selection,

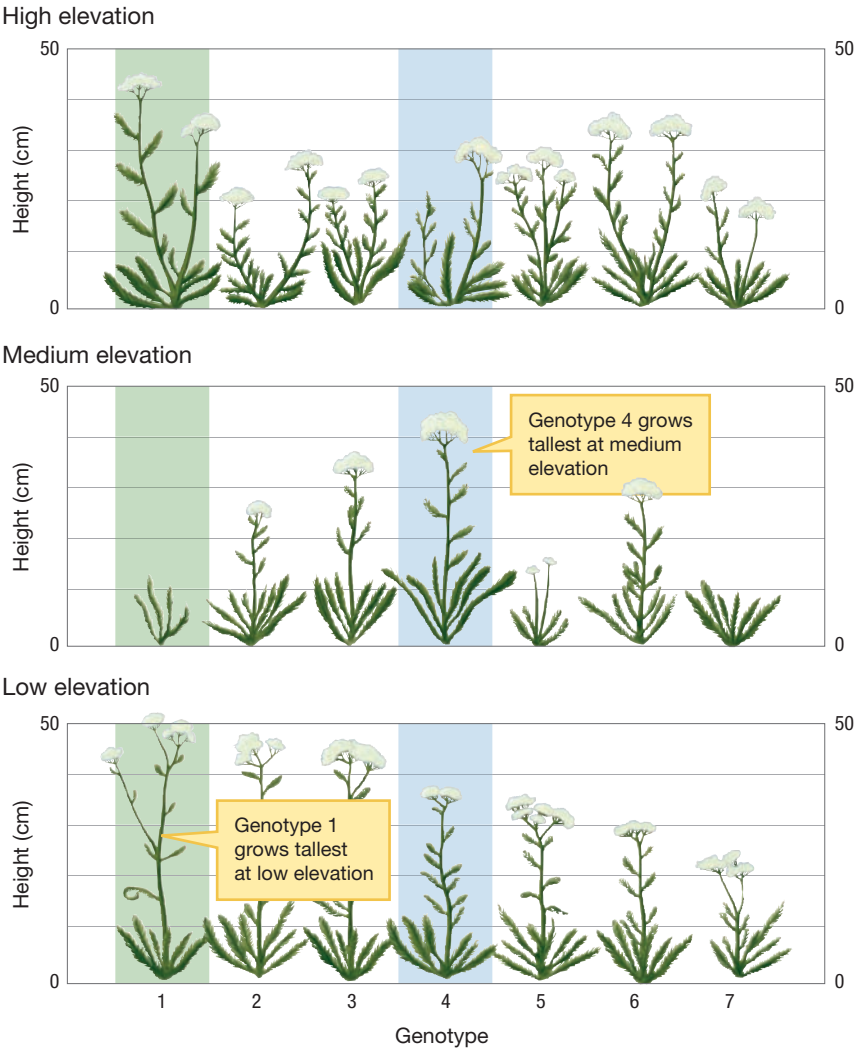


FIGURE 3.3 Phenotype depends on the effects of both genotype and environment. Here we see how the height of a yarrow plant (*Achillea millefolium*) depends on its genotype and the altitude at which it is raised, as shown by populations of yarrow plants grown in gardens at three sites that were at different altitudes: high, medium, and low elevation. For example, the green screen behind the plants of genotype 1 shows that these plants grow tall at high and low elevations but are short at medium elevation. The blue screen behind the plants of genotype 4 shows that these plants respond very differently to elevation. This genotype grows tallest at medium elevation and shorter at high and low elevations. Adapted from Clausen et al. (1940, 1948).

we will typically do so with reference to one or more specified populations of individuals. Thus in the study of natural selection, traits are usually the object of explanation, and populations are the level of analysis.

Fourth, natural selection does not directly sort on genotypic differences, but rather it sorts on phenotypic differences—the expression of genotypes—among the individuals in a population. Thus, to understand natural selection, we have to understand how the interplay between genotype and environment determines the phenotype. The key here is that a gene by itself does not code for a trait, but rather a gene codes for a trait *in the context of a particular set of environmental conditions*. For example, **Figure 3.3** illustrates the way that elevation and genotype interact to determine the height of individuals in

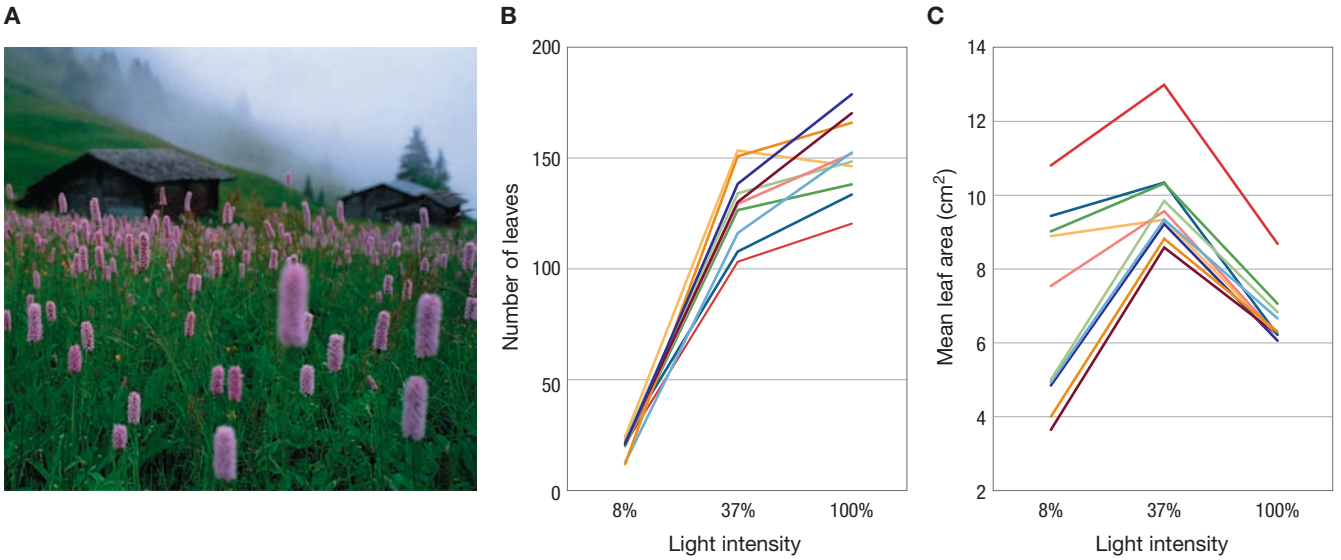


FIGURE 3.4 Norm of reaction curves. In the weedy annual plant *Persicaria maculosa* **(A)**, the total number of leaves **(B)** and the mean leaf area **(C)** depend on the light intensity—ranging from full shade to full direct sunlight—that the plant experiences. Each curve for one specific genotype is called a norm of reaction. Here we see the norms of reaction for 10 different genotypes (each a different color), under light intensities of 8%, 37%, and 100% of available sunlight. Thus, the genotypes do not code for a fixed number of leaves or a fixed average leaf size, but rather for a number and size of leaves that depend on the intensity of light to which the plant is exposed. Panels B and C adapted from Sultan and Bazzaz (1993).

different populations of a yarrow plant (*Achillea millefolium*). In most cases, a genotype does not lead to the production of a single phenotype, but rather produces what we call a **norm of reaction**. Each column in Figure 3.3 gives us the information we need to construct a norm of reaction for one particular genotype. For example, the column with green shading shows how the heights of plants of genotype 1 depend on the elevations at which they are grown. Genotype 1 doesn't just produce "tall" or "short" plants. Rather, genotype 1 specifies the norm of reaction "tall at low and high elevations, short at medium elevation." Norms of reaction are often represented as functions or curves, as illustrated in **Figure 3.4**. Each genotype is represented by a single curve, showing how expression of a genotype depends on the environmental conditions. Environmental conditions are shown on the *x* axis, and phenotypes are shown on the *y* axis. Such norms of reaction can be quite complex, with a given genotype producing different phenotypes across an environmental gradient, such as an altitudinal gradient.

Natural Selection and Coat Color in the Oldfield Mouse

With these points in mind, let's now work through an example of how evolutionary biologists study the process of natural selection. We will focus on an elegant set of studies by Hopi Hoekstra and her colleagues that examines natural selection on coat color in populations of the oldfield mouse, *Peromyscus polionotus*. This species of small mouse, native to the American Southeast, suffers considerable mortality from predators that hunt visually, such as owls.

Throughout most of its range, *P. polionotus* individuals are uniformly dark in coloration. But on Santa Rosa Island off the Gulf coast of northern Florida, and along the nearby beaches and barrier islands, these mice often have a much lighter coat color. In this subsection, we will evaluate a number of experiments designed to test the hypothesis that natural selection favors a match between coat color and environmental background, favoring light coat color in the coastal dune populations that live on light sand and dark coat color in inland populations that live in more vegetated environments (**Figure 3.5**).

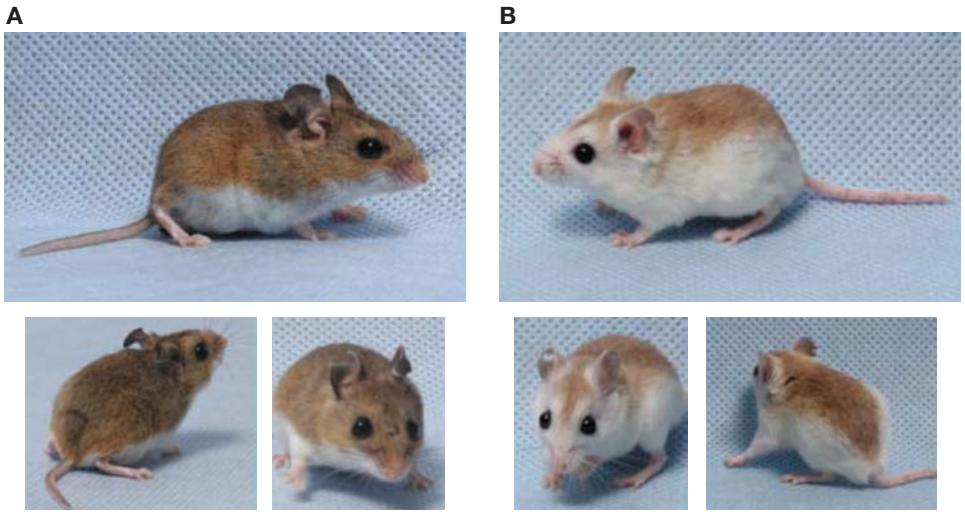


FIGURE 3.5 Coat color variation in mice. Two color variants of *Peromyscus polionotus*: **(A)** the darker inland form, and **(B)** the lighter beach-dwelling form.

Now that we have specified our trait of interest—coat color—and our populations of interest—dune and inland populations—we can study the process of natural selection by examining variation, heritability, and fitness in the oldfield mouse.

Variation

As we learned in the previous chapter, natural selection is a variational process, in which the properties of the members of a population change over time as a consequence of a sorting process. Thus, natural selection requires as raw material some *variation* in the trait under investigation. Without variation in a population, there is nothing for natural selection to select. If, for example, all mice had identically colored coats, natural selection with respect to coat color could not occur.

For a readily observable trait such as coat color, we can easily determine whether the first condition for natural selection—the presence of variation—is satisfied. Hoekstra and her colleagues observed considerable phenotypic variation in coat color *within* populations (Mullen et al. 2009), and they also uncovered substantial genetic variation at the *Mc1R* (melanocortin-1 receptor) locus associated with coat color. The variation in coat coloration is even more striking *between* populations, as illustrated in **Figure 3.6**. Although we do not currently see this wide a range of variation within any given population, the between-population variation present gives us a sense of the possible range of genetic variation in this species.

Heredity

Phenotypes result from the interplay of genes and environment. Thus, variation in phenotype can arise through variation in genes alone, variation in environment alone, or through a combination of both. In principle then, variation in coat color could result from genetic differences, from environmental differences such as differences in diets or in exposure to sunlight, or from some combination of these factors. Although almost any trait we might study shows both environmental and genetic variation, natural selection can operate only if there is a genetic component to variation.

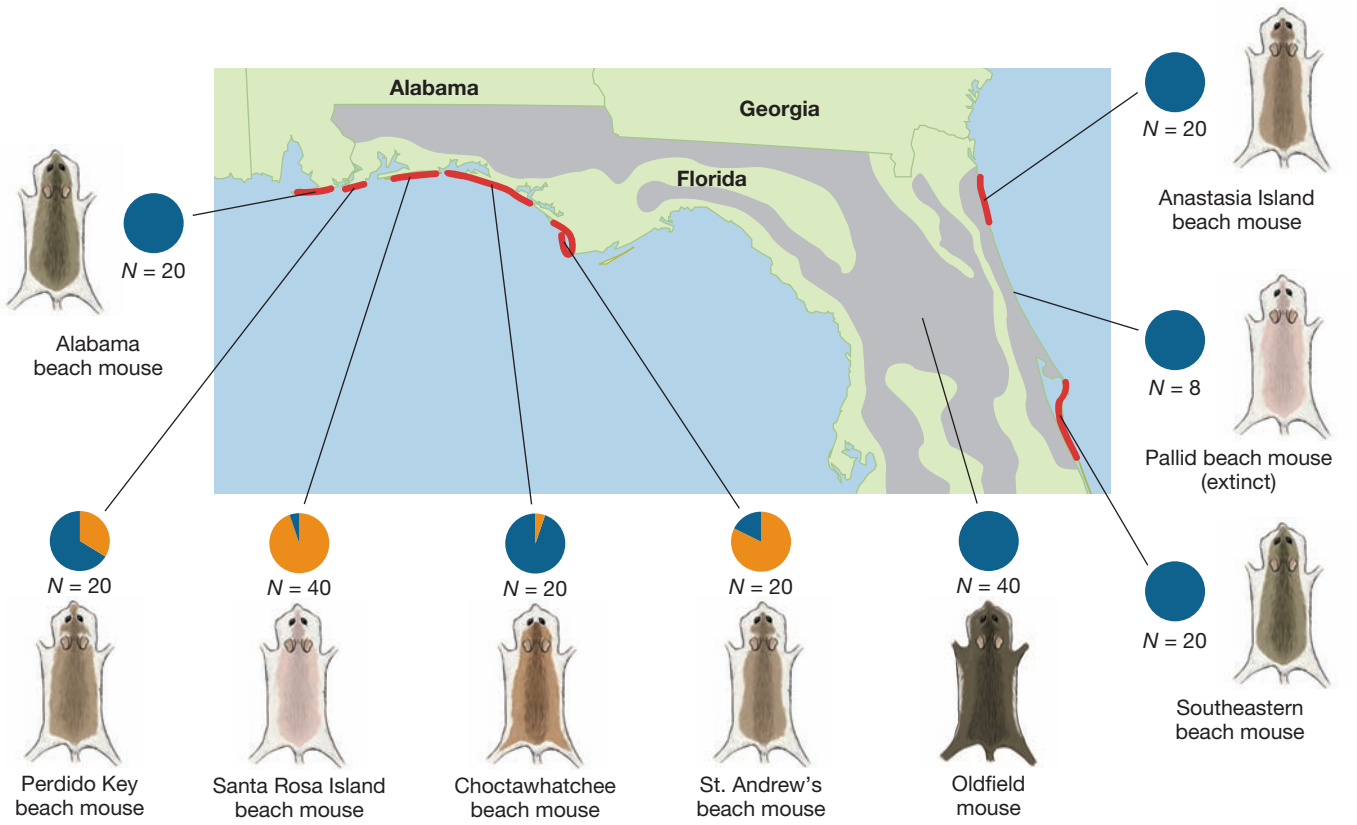


FIGURE 3.6 Variation in coat color and genotypes at the Mc1R locus. *Peromyscus polionotus* exhibits extensive coat color variation across localities in Florida. Red areas indicate the distribution of beach populations; gray areas denote the distribution of inland populations. Characteristic phenotypes for each population are indicated by the coat coloration sketches, but coat color varies within populations as well. The pie charts indicate that the Perdido Key, Santa Rosa Island, Choctawhatchee, and St. Andrew's beach mouse populations had more than a single variant of the *Mc1R* locus associated with coat coloration. All populations shown here are considered part of a single species—*Peromyscus polionotus*. Adapted from Hoekstra et al. (2006) by permission of AAAS.

As we mentioned previously, at the time that he wrote *On the Origin of Species*, Charles Darwin knew almost nothing of the mechanistic biology behind the hereditary factors that we now call genes, but the resemblance between parents and offspring was critical for Darwin, because the process of natural selection requires *inheritance*. Without inheritance, any fitness differences among the varieties of a trait would not result in different frequencies of the trait varieties in the next generation. In the *P. polionotus* example, selection requires inheritance to alter coat color in our mouse population. To see why, imagine that dark-colored mice produce five offspring on average, and light-colored mice produce 10 offspring on average. If the offspring don't resemble their parents with respect to coat color, the dark parents will be no more likely to produce dark offspring than will the light parents, and vice versa. Any consequences of differing reproductive success between coat colors are lost once the parents produce new offspring.

What does it take for trait variants to be inherited? Usually, inheritance in biological evolution occurs when some of the variation in the trait of interest

arises from *genetic* variation. Most traits that vary do so, at least in part, because of underlying genetic variation. Consequently, almost all traits in natural populations meet the prerequisite for inheritance (Darwin 1868; Endler 1986; Clark and Ehlinger 1987; Mousseau et al. 1999). Indeed, numerous studies from evolutionary biology, population genetics, and animal behavior suggest that many of the traits that in principle could be acted on by natural selection—be they morphological or behavioral—are at least partially inherited from parents by their offspring (Mousseau and Roff 1987; Price and Schulter 1991; Weigensberg and Roff 1996; Hoffmann 1999).

How can evolutionary biologists show that variation in a trait is inherited? The most direct way is to identify the gene or genes responsible for this variation. In the case of the oldfield mouse, Hoekstra and her colleagues have identified several genes that are responsible for much of the coat color variation in *P. polionotus* (Hoekstra et al. 2006; Steiner et al. 2007). We will consider two of these genes here.

The first of these genes is the melanocortin-1 receptor gene (*Mc1R*), which produces a protein known to influence coat color in many species of mammals, as well as plumage color in many species of birds. *Mc1R* functions as a critical part of a genetic switch that controls the type of pigment that is created and incorporated into hair or feathers (Kronforst et al. 2012). Depending on the environment and the interaction with other genes, this one gene switches back and forth between producing a dark pigment, known as *eumelanin*, or a light yellow pigment, known as *phaeomelanin* (Barsh 1996). When a protein called alpha melanocyte-stimulating hormone (α-MSH) is present, it binds to the Mc1R transmembrane receptor, initiating a signaling pathway that triggers the production of eumelanin. When the Mc1R receptor is not bound by α-MSH, phaeomelanin is produced instead (**Figure 3.7A, B**).

Hoekstra and her colleagues have documented a single mutation in the *Mc1R* gene in many of the beach populations of *P. polionotus* that dwell along the Gulf coast of Florida, where oldfield mice have light coat color (Hoekstra et al. 2006). This mutation changes the amino acid sequence of the Mc1R protein, reducing the ability of that protein to bind α-MSH. The consequence is *reduced* eumelanin production, resulting in a lighter coat color (**Figure 3.7C**). Phylogenetic analysis suggests that this mutation occurred before islands were colonized by beach mouse populations (Domingues et al. 2012) (**Figure 3.8**).

A mutation in the *Mc1R* gene is not the only way that lighter coat color can be produced. The second major gene involved in coat color is called *Agouti*. This gene's product is a protein called the agouti signaling protein (ASP). ASP competes with α-MSH to bind to the Mc1R receptor; when it does so, it blocks the eumelanin pathway and the cell instead produces phaeomelanin (**Figure 3.7D**). Hoekstra and her colleagues found that beach mice typically carry a recently evolved form of the *Agouti* allele that contributes to their lighter coat color (Hoekstra et al. 2006).

Hoekstra and her colleagues measured the expression level of *Agouti* using quantitative polymerase chain reaction (qPCR), a technique that allows researchers to determine not only the presence of an allele in a tissue sample but also the level of expression—that is, the concentration of messenger RNA molecules for

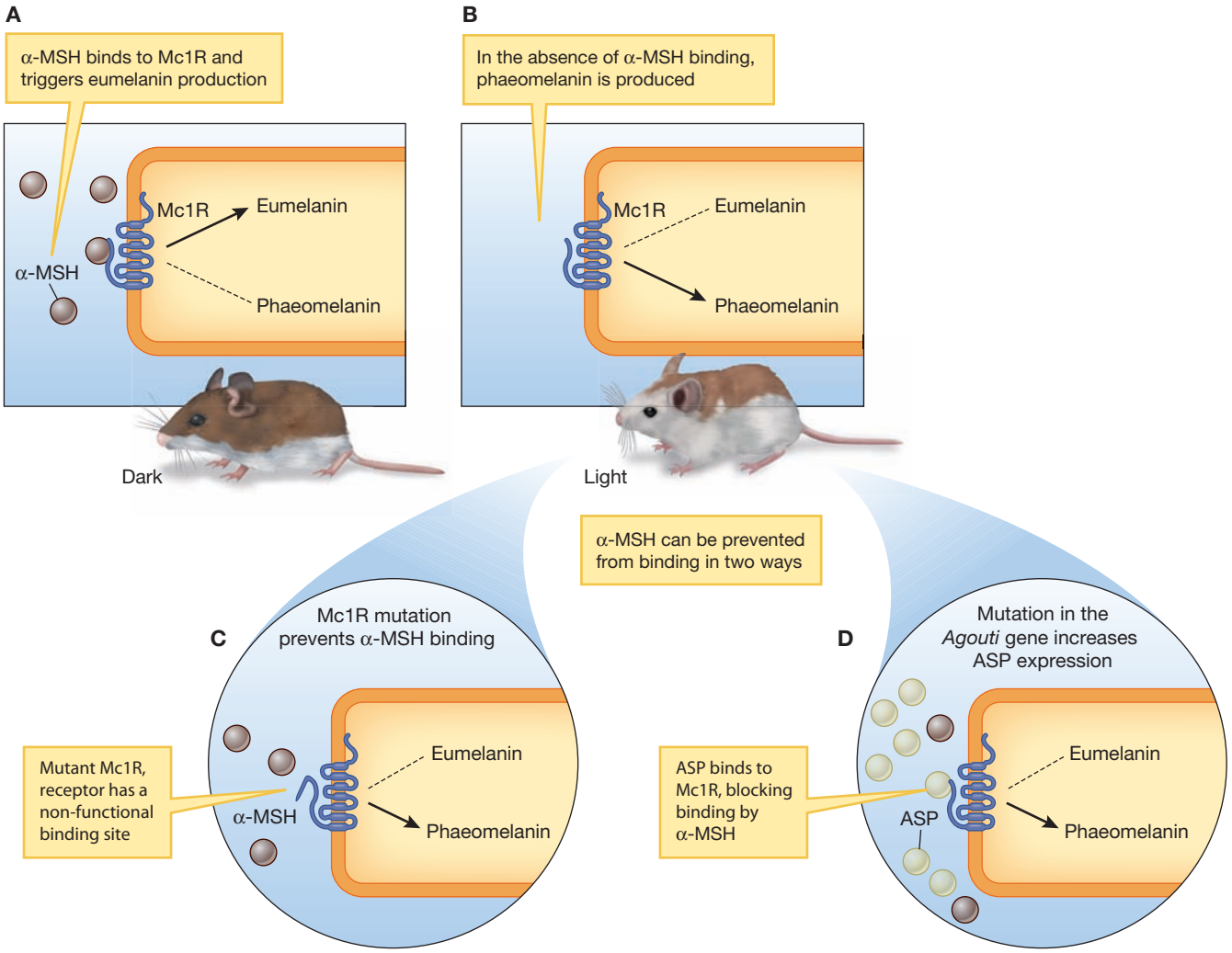


FIGURE 3.7 Genetics of coat color determination in mice. The protein Mc1R acts as a genetic switch, determining whether dark eumelanin or light phaeomelanin is produced. **(A)** When the Mc1R receptor binds α-MSH, it triggers eumelanin production. **(B)** When α-MSH is absent, phaeomelanin is produced instead. Two different mutations prevent α-MSH from binding to the Mc1R receptor: **(C)** A mutation to the Mc1R receptor results in a nonfunctional binding site, and **(D)** a mutation in the regulatory region of the *Agouti* gene increases the expression of a protein known as agouti signaling protein (ASP). This protein competes with α-MSH for the Mc1R binding site and thus inhibits eumelanin production.

the allele—in that tissue. They found that, in the mice with the *Agouti* mutation that generates light coat color, the *Agouti* gene was more highly expressed. This presumably leads to a greater concentration of ASP, leading to a lighter coat. Hoekstra and her team have also used what is known as "next generation sequencing" to identify the specific regions of the *Agouti* gene responsible for light coloration on different parts of the body in oldfield mice and in deer mice (*Peromyscus maniculatus*) that inhabit the Sand Hills of Nebraska (Manceau et al. 2011; Linnen et al. 2013).

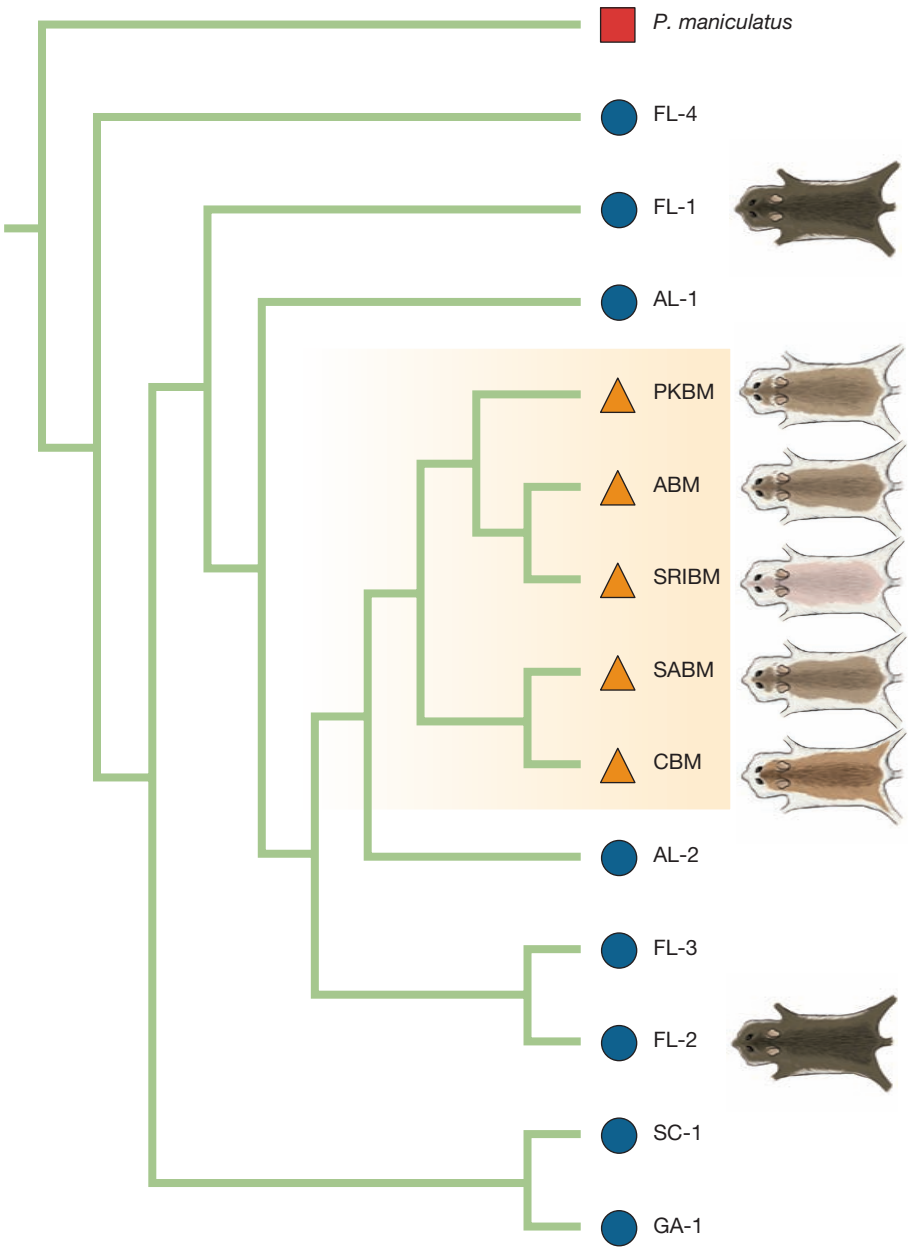


FIGURE 3.8 Phylogeny of oldfield mouse populations. Genomic data from the *Mc1R* locus, as well as from other areas in the genome of oldfield mice, allows researchers to reconstruct colonization events for beach mice. All beach populations form a single clade (shaded in orange) and share a derived "light-colored" *Mc1R* allele. Subsequent analysis found that colonization of beaches took place in a single event approximately 3000 years ago and that the "light-colored" *Mc1R* allele originated before the colonization event. Beach populations are shown as triangles. Inland populations are shown as circles. *P. maniculatus* is the closely related deer mouse, *Peromyscus maniculatus*. ABM, Alabama beach mice; PKBM, Perdido Key beach mice; SRIBM, Santa Rosa Island beach mice; CBM, Choctawhatchee beach mice; SABM, St. Andrews beach mice. From Domingues et al. (2012).

Genetic variation alone, however, is not sufficient to allow the process of natural selection to operate. The genetic variation must also correlate with differential reproductive success: genetic variation must have *fitness consequences*.

Fitness Consequences

While the term *fitness* has the everyday implication of something that is well matched—or *fit*—to its circumstances of life, the formal definition in evolutionary biology pertains to reproductive success. The *fitness* of a trait or allele is defined as the expected reproductive success of an individual who has that trait or allele *relative* to other members of the population. So, when we speak of fitness here, we are referring to the *differential effect* of the trait on the expected reproductive success of an individual relative to other individuals in its population (Fisher 1958; Williams 1966; Clutton-Brock 1988; Reeve and Sherman 1993). In many instances, it will be apparent that a trait has an effect on fitness; in the case of the mouse *P. polionotus*, we will see in a moment that coat color influences survival. The reason is straightforward. Coat color influences the visibility of mice against their background. Mice that stand out against their background are more readily captured by predators; less visible mice are more likely to survive and reproduce.

To see the fitness effect of coat color, let us first examine a 1974 experiment by G. C. Kaufman in which pairs of mice, one with a dark coat and one with a light coat, were released into a large cage with an owl present (Kaufman 1974). For each environmental background—dark soil with sparse vegetation, light soil with sparse vegetation, and light soil with dense vegetation—Kaufman recorded the coat color of the mouse that the owl captured first. As can be seen in **Figure 3.9**, this experiment demonstrates a selective advantage to mice with coats that match the color of their background environment. Those mice are more likely to escape predators and thus to survive long enough to reproduce.

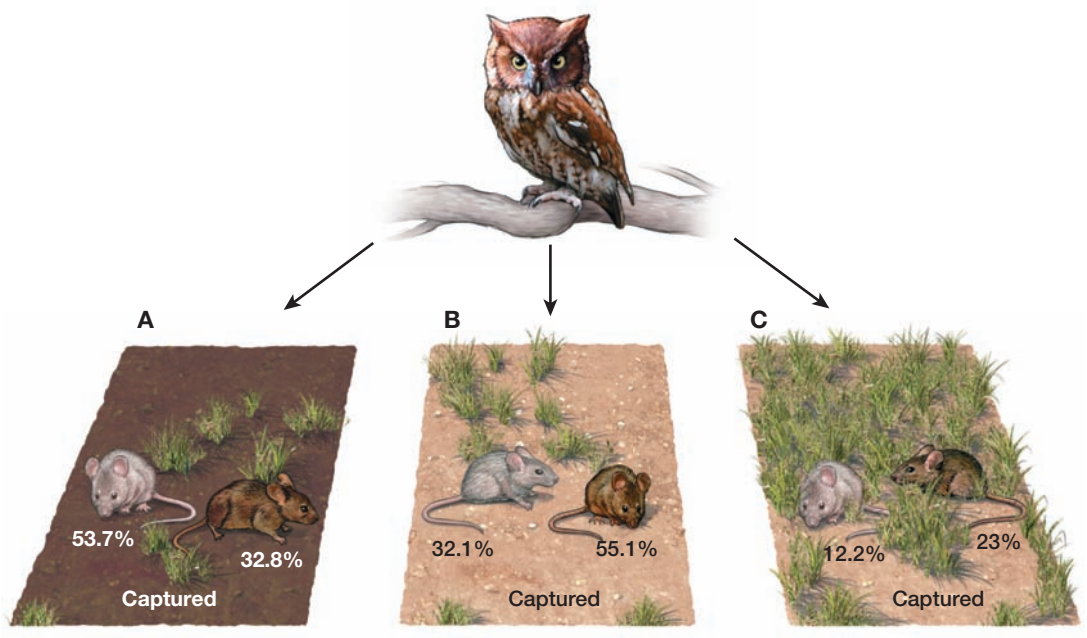
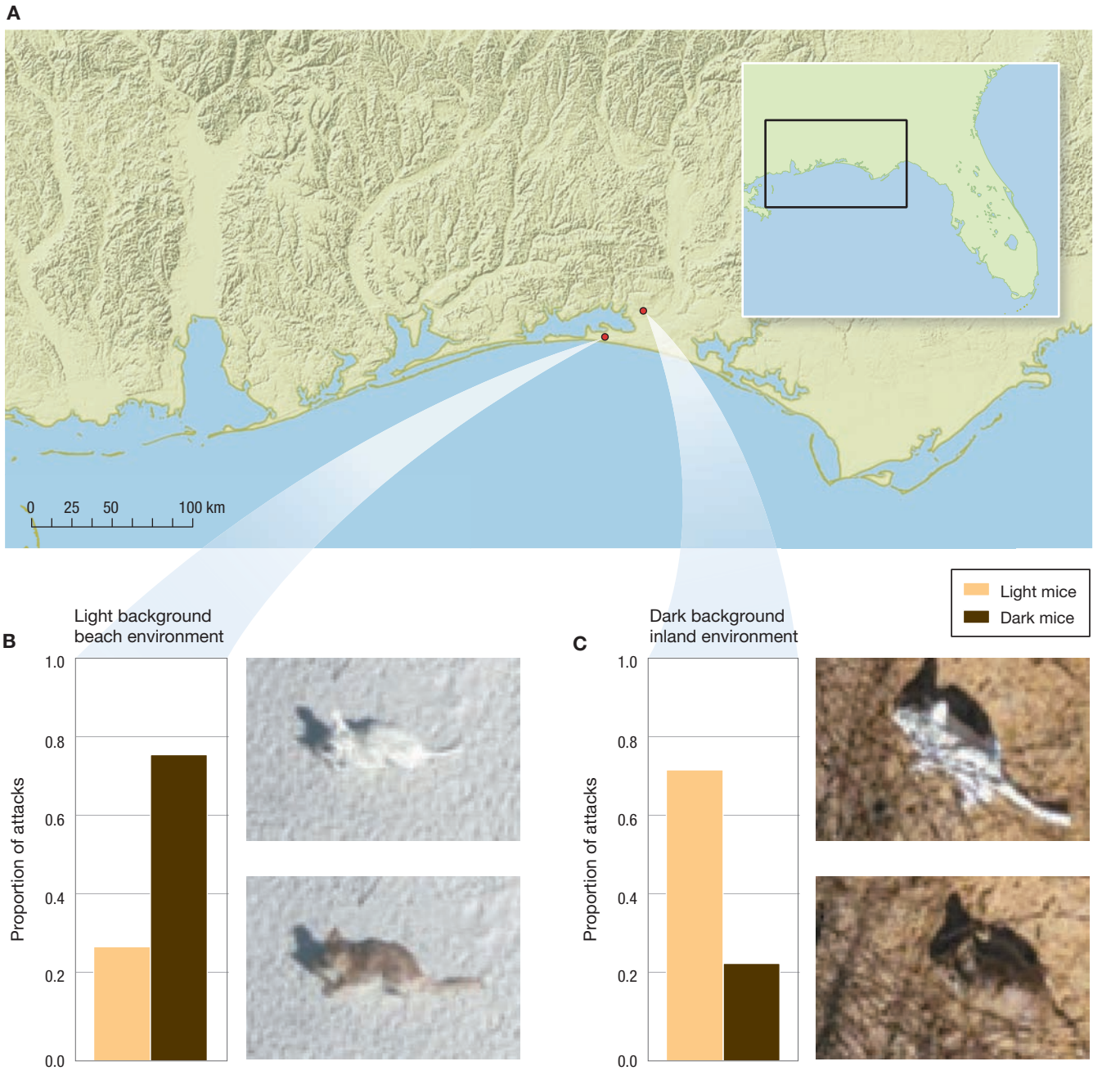


FIGURE 3.9 Early work on predation, coat color, and fitness in the oldfield mouse. Mice with light and dark coats were exposed to owl predators in three different environments: dark background with sparse vegetation **(A)**, light background with sparse vegetation **(B)**, and light background with dense vegetation **(C)**. The identity of the first mouse captured in each trial was recorded. Trials lasted fifteen minutes, and if neither mouse was taken by the owl, the trial ended. The percentages of trials in which mice of a given coat color were the first to be taken by the owl are shown in each panel (percentages in a panel do not sum to 100 because of trials in which neither mouse was taken by the predator). In all cases, owls initially captured a higher percentage of "color-mismatched" mice; namely, those with coat colors that failed to match their environments.



Many years later, in a follow-up to the Kaufman experiment, Hopi Hoekstra and her colleagues constructed silicone models that they painted to mimic either the dark- or light-coated oldfield mice, and they placed 125 models of each type in the natural environment of light sandy beaches or darker inland habitats (Vignieri et al. 2010). By using silicone models, Hoekstra and her team were able to remove a possible confounding variable that was present in the Kaufman experiment. In that earlier experiment, it is possible that different colored mice behaved differently, and that behavioral differences were responsible for differences in survival. Using silicone models eliminates this possibility. Attacks by predators could then easily be detected by looking at the presence or absence of the silicone models over time, as well as marks from teeth, talons, or beaks on models that were not removed from a site by predators. They found strong evidence for a fitness advantage to mice that matched the color of their environment (**Figure 3.10**).

FIGURE 3.10 Predation, coat color, and fitness in the oldfield mouse using plastic models in the field. Hoekstra and colleagues placed light and dark silicone mouse models in light and dark environments to test predation rates. **(A)** The experimental sites: a light beach environment and a dark inland environment. **(B)** Proportion of attacks against light and dark mice in the light environment. **(C)** Proportion of attacks against light and dark mice in the dark environment. Adapted from Vignieri et al. (2010).

It is important to understand that small differences in fitness can translate into large changes in allele frequencies over time. For example, suppose that individual mice whose coat colors matched their environments produced just 1% more offspring per generation than those whose coat colors did not. Mathematical models show that over evolutionary time, this small difference could result in a population composed completely of individuals matching their backgrounds (we delve more into these mathematical models in Chapter 7). In a basic model with a few simple assumptions, the frequency of a gene associated with 1% more offspring per generation would double every 70 generations. In a population of 10,000 individuals, this gene could easily increase from a single copy to a frequency of 100% in a few thousand generations: a blink of the eye on an evolutionary timescale.

Based on the oldfield mouse studies, natural selection appears to operate very strongly in the oldfield mouse populations. Indeed, we say that coat color in the oldfield mouse example is an adaptation. Let us now examine adaptations in greater detail.

KEYCONCEPT QUESTION

3.1 Thus far we have focused on genes as the means by which information is transferred across generations. This is only one way that such a transfer of information can occur. Cultural transmission is another. Examples of culturally transmitted information including farming practices, musical tunes, fashions in clothing, and architectural techniques. Could some analog of natural selection operate when culture is the means by which information is transferred from one generation to another?

3.2 Adaptations

In Chapter 2, we discussed early theories that tried to explain the remarkable match between the structure of organisms and the environments they inhabit. Now that we understand how the process of natural selection shapes the traits of organisms, we will use the word *adaptation* to describe the results of this process.

Defining Adaptation

The word *adaptation* has been defined in many ways over the years, so we need to be specific in our own use of this term (Williams 1966; Mayr 1982; Sober 1987; Mitchell and Valone 1990; Reeve and Sherman 1993; Barrett and Hoekstra 2011). An **adaptation** refers to an inherited trait that makes an organism more fit in its abiotic (nonliving) and biotic (living) environment, and that has arisen as a result of the direct action of natural selection for its primary function.

KEYCONCEPT QUESTION

3.2 Explain why hooves would be considered adaptations but horseshoes would not.

Adaptations and Fit to Environment

Adaptations help organisms deal with both the abiotic and biotic aspects of their environment. Consider a saguaro cactus in the Sonoran Desert. The waxy coating

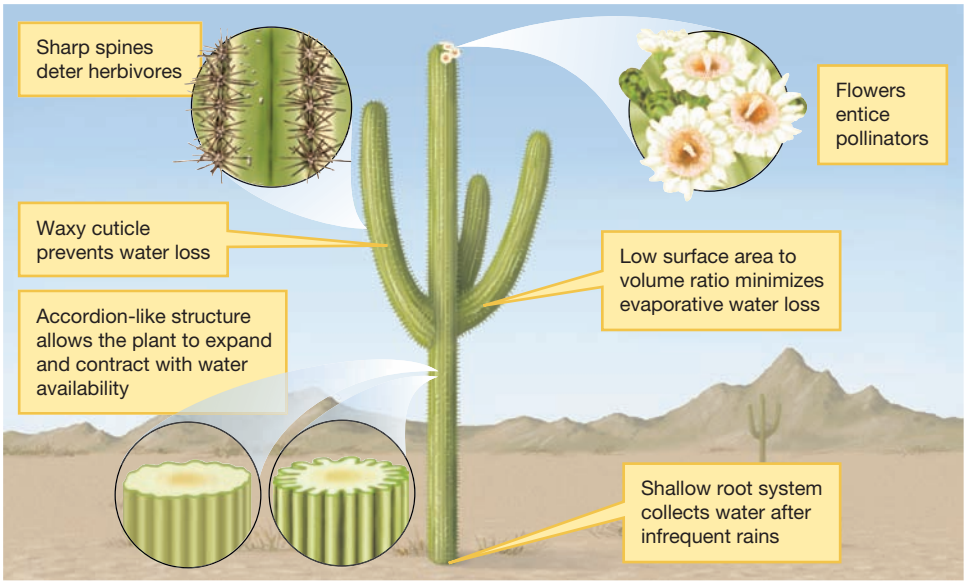


FIGURE 3.11 Adaptations of a cactus. A saguaro cactus exhibits adaptations to its abiotic environment (waxy stem coating, shallow root system, low surface area to volume ratio) and to its biotic environment (spines to keep away herbivores, flowers to attract pollinators).

on its surface, its shallow root system, and its low surface area to volume ratio are adaptations to its abiotic environment: They help it gather and retain water and survive the high temperatures and often low humidity to which it is exposed. Its spines, meanwhile, are an adaptation to its biotic environment, in that they serve to protect the valuable water stored inside from herbivores that might otherwise rip open and consume the plant (**Figure 3.11**).

To be considered an adaptation, a trait must have been shaped by natural selection *to serve the same primary function or functions that make it beneficial today* (Sober 1984). Picture a bird soaring gracefully through the air. It is hard to watch such a wonder of nature without thinking of how wonderfully suited feathers are for flying. And it is tempting to assume that because the primary function of feathers today is related to flight, the primary function of feathers has *always* been their effect on flight. But this need not be the case. A trait may serve one function today, but it may have evolved under different selection conditions and served a different function in the past. Such traits are called **exaptations** (Simpson 1953; Bock 1959; Gould and Vrba 1982). We will treat exaptations, and return to the case of feathers, in detail in Section 3.5.

The term *adaptation* has a long history in the field of evolutionary biology, and it has been used in different ways by different people. If we restrict our definition of an adaptation to a trait that is shaped by natural selection for the same primary function that makes it beneficial today, then we can generate testable hypotheses about how natural selection produces adaptations. Evolutionary biologists can do just this, both in the field and in the laboratory, although at times this is a difficult and very time-consuming process. In the next section, we examine how such studies are designed, what hypotheses they test, and how the data collected have helped biologists understand the process of natural selection.

3.3 Natural Selection in the Field

Natural selection acts on the entire spectrum of traits present in an organism, including molecular, morphological, behavioral, and physiological traits. The manner in which natural selection acts can be tracked in wild populations, with long-term studies being most effective at doing this. In this section, we will examine two long-term field studies on natural selection: one on a behavioral/physiological trait (life history strategy) and another on a morphological trait (wing length).

Predation and Natural Selection in Guppies

A species' **life history strategy** refers to the schedule and manner of investment in survivorship and reproduction over the lifetime of an individual. Life history traits include the timing of sexual maturity, the timing of aging or senescence (Chapter 20), the number and size of offspring, and whether an organism reproduces repeatedly over the course of its lifetime or just once during its lifetime. A beautifully documented example of studying life history and natural selection in the field comes from decades of work on life history strategies in the guppy *Poecilia reticulata* (Houde 1997; Magurran 2005).

In many of the streams of the northern mountains of Trinidad and Tobago, guppy populations can be found both upstream and downstream of a series of waterfalls (Seghers 1973; Houde 1997; Magurran 2005). Upstream and downstream sites in a stream may only be separated by a very small geographic distance (a few hundred feet in some instances), but the waterfalls act as a physical barrier to guppies and their aquatic predators alike. Upstream of such waterfalls, guppies typically face only mild predation pressure from one small species of fish, *Rivulus hartii*. Downstream of the waterfalls, however, populations of guppies are often under severe predation pressure from voracious predators such as the pike cichlid (*Crenicichla alta*).

Because upstream and downstream populations face different predation pressures, evolutionary biologists have hypothesized that natural selection should favor different suites of traits across these populations. Indeed, this turns out to be the case, and between-population comparisons in guppies have found differences in color, antipredator behavior, and numerous life history traits, including the number of offspring born in each clutch, the size of offspring at birth, the age at reproduction, and the timing of senescence (Endler 1995; Reznick 1996; Houde 1997; Magurran 2005). Let us examine some of these in more detail.

David Reznick and his colleagues found that guppies from downstream, high-predation sites mature faster than fish from upstream, low-predation sites (Reznick 1996). Females from downstream sites also produce more broods (clutches of offspring) than their counterparts in upstream sites, and broods from downstream females contain many small fry (newborn fish), while broods from upstream females tend to contain larger but fewer fry (**Figure 3.12**). Why? That is, why should differences in predation lead to such differences across our guppy populations?

To understand why these guppy populations have diverged, let us examine the different selective conditions at downstream and upstream sites. At upstream sites, the small fish *Rivulus hartii* is the only aquatic predator that guppies face. If females produce offspring that start off relatively large and can quickly grow past a certain size

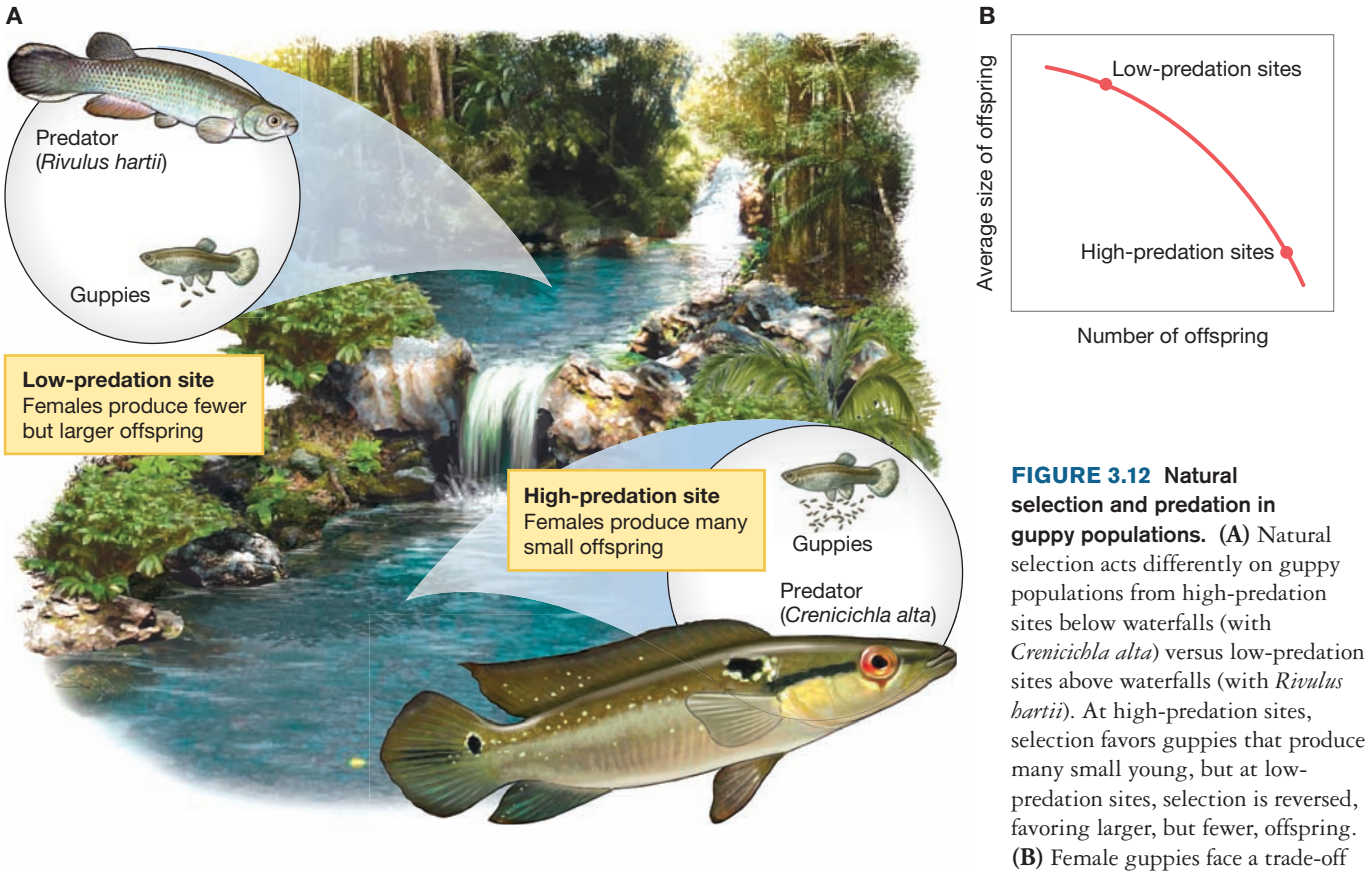


FIGURE 3.12 Natural selection and predation in guppy populations. **(A)** Natural selection acts differently on guppy populations from high-predation sites below waterfalls (with *Crenicichla alta*) versus low-predation sites above waterfalls (with *Rivulus hartii*). At high-predation sites, selection favors guppies that produce many small young, but at low-predation sites, selection is reversed, favoring larger, but fewer, offspring. **(B)** Female guppies face a trade-off (red curve) between the number of offspring they can produce and the size of those offspring at birth. The optimal point along the trade-off curve illustrated depends on the predation pressures that the offspring experience.

threshold, such offspring will be safe from predation by *R. hartii*. So, females face a **trade-off**: Larger offspring may survive with higher probabilities, but because such offspring require more resources during their in utero development than do smaller offspring, fewer larger offspring can be produced (Figure 3.12).

At high-predation sites, guppy predators are much larger; they can eat a guppy no matter how large it gets. At such sites, natural selection should favor producing many smaller fry. That is, because a predator can eat a guppy fry no matter how big it is, natural selection should now favor females that produce as many fry as possible, rather than producing larger but fewer fry, because such females will have higher reproductive success. This pattern is precisely what we see when we study reproduction in downstream females (Reznick 1996).

In the guppy system, evolutionary biologists can do more than infer adaptation by observing life history differences. In the mountain streams of Trinidad and Tobago, biologists can *experimentally manipulate* natural selection on guppy populations, make specific predictions about the changes that should occur, and test these predictions.

David Reznick, John Endler, and their colleagues experimentally manipulated predation pressure in wild guppy populations by transplanting a group of 100 male and 100 female guppies from a high-predation, downstream site into a low-predation, upstream site, and they cordoned off the transplanted guppies so they could track the populations over time (**Figure 3.13**). *If* it is correct that producing larger but fewer offspring at upstream sites is an adaptation to predation pressure there, then given sufficient genetic variation for offspring size, we would expect that over many generations, natural selection will favor the descendants of those

FIGURE 3.13 Guppy transplant experiment. Reznick and his colleagues transplanted guppies from high-predation sites below a waterfall to low-predation sites above a waterfall to test whether descendants of transplanted individuals evolved adaptations to their new selective conditions.

fish transplanted from high-predation sites who produce larger but fewer offspring than their recent ancestors (Reznick et al. 1990).

When Reznick and his team sampled the descendants of the transplanted populations 5 and 12 years after the original transplant, they found that the descendant population had evolved in the predicted direction, with females producing larger but fewer offspring than their ancestors from a high-predation site (Reznick et al. 1990). The researchers then brought guppies from the area of the transplant into the laboratory and found that the new life history strategy was inherited. Guppies from the descendant population born and raised in the laboratory displayed the same life history strategies in the lab as in the field, suggesting that the differences in life history were not solely caused by environmental differences. Thus, experimental manipulation of natural selection led to evolutionary changes in life history strategy, just as predicted.

Natural selection has also operated on various aspects of guppy behavior (Endler 1995; Reznick 1996; Houde 1997; Magurran 2005). One suite of behaviors that has been studied extensively in natural populations of guppies is their antipredator activities (Seghers 1973; Magurran et al. 1995; Magurran 2005). Depending on whether they evolved in populations with heavy or light predation pressure, natural selection has produced a different suite of antipredator behaviors in guppies.

Because swimming in large, tight groups provides more protection from predators than swimming in smaller, looser aggregations, we might expect that guppies from high-predation sites would shoal in larger, tighter groups than guppies from low-predation sites. Data collected from natural populations confirm this prediction (Magurran and Seghers 1991).

As with the work on reproductive allocation, evolutionary biologists can do more than correlate behavior with selective conditions. We can conduct manipulative experiments to see whether and on what timescale changes in selective conditions lead to changes in behavior. In the early 1990s, Anne Magurran and her colleagues learned of a unique opportunity to examine a "natural experiment" on natural selection and the evolution of antipredator behavior in guppies (Magurran et al. 1992; Sievers et al. 2012). Back in 1957, C. P. Haskins, one of the original researchers of guppy population biology, transferred 200 guppies from a high-predation site in the Arima River to a low-predation site in the Turure River; the latter site had been previously unoccupied by guppies. Magurran realized that Haskins' manipulations of several decades before created an opportunity to examine the consequences of natural selection on antipredator behavior. If natural selection shapes antipredator responses, then the lack of predation pressure in the Turure should have led to selection for weakened antipredator behavior in guppy descendants. Magurran and her colleagues sampled numerous sites in the Turure River (Magurran et al. 1992; Shaw et al. 1992). Genetic analysis suggested that the high-predation fish transferred from the Arima River back in 1957 had indeed spread all throughout the previously guppy-free site in the Turure River. More to the point, released from the predation pressure of their former habitat, the descendants of the Arima River fish at the Turure had evolved shoaling and other anti-predator behaviors that were more similar to those of guppies at low-predation sites than they were to the behaviors of their ancestors from the dangerous sites in the Arima River.

In addition to nicely illustrating how we study the evolution of behavior and life history, the guppy example reveals the rapidity with which natural selection can operate. We know from geological evidence that upstream and downstream guppy populations have been separated from one another for less than 10,000 years, yet largely as a result of differences in predation pressure, natural selection has produced significant differences in behavior and life history in guppy populations over this fairly brief evolutionary time period (Endler 1995). Indeed, Magurran and Reznick's transfer experiments demonstrate that natural selection can act even faster than that on antipredator behavior in wild populations of vertebrates—in this case, on the timescale of years to decades.

Roadkill and Natural Selection on Wing Length in Swallows

Environmental disturbance by humans can create persistent and strong new forms of natural selection. For example, in the United States, 80 million birds die each year as a result of roadkill—a fatal collision with a vehicle (Erickson et al. 2005). Roadkill of birds has been occurring for decades and may have strongly selected for birds who avoid such collisions. Charles and Mary Brown examined this possibility in a study of colonial cliff swallows (*Petrochelidon pyrrhonota*) (Brown and Brown 2013). Cliff swallows often form colonies under bridges and in other areas near highways, making them an ideal species in which to examine whether

FIGURE 3.14 Cliff swallow colonies. A cliff swallow colony under a bridge. Individuals from such colonies have been studied to examine whether selection has favored certain traits in response to mortality caused by roadkill.

selection has favored certain traits in response to mortality caused by roadkill (**Figure 3.14**).

The Browns have been studying populations of cliff swallows in Nebraska since 1982, and they have detailed census records on these populations each year. As they traveled between colonies over the years, they also collected data on roadkills involving swallows. Over the 30-plus years they have collected such data, there has been a significant decrease in the number of swallow roadkill. Why? Has selection favored certain traits that reduce roadkill of swallows? Or is there perhaps a simpler explanation? The Browns wanted to know, and they began a systematic analysis of possible explanations.

The Browns first checked the population size of the cliff swallow colonies they were studying. After all, if population size was decreasing, then a decrease in roadkill would be expected, but this need not have anything to do with natural selection related to roadkill. In fact, the data show that cliff swallow population size has increased since the Browns' studies began in 1982. They next rechecked the routes they had taken to each colony every year, and for the most part they were the same year after year. So the drop in roadkill they found wasn't due to their sampling different routes. What's more, traffic on these routes increased during the course of their study, so the decrease in roadkill wasn't just the result of fewer cars on the road for swallows to crash into. The Browns also examined the possibility that roadkills decreased because of an increase in scavengers who removed the dead swallows before the Browns could find them. The data didn't support this hypothesis because, at least for avian scavengers, scavenger population sizes stayed constant over the course of the Browns' study. Because each of these alternatives was ruled out, it became more likely that the decrease in roadkill was the result of natural selection favoring some swallow trait that reduced mortality. But what trait?

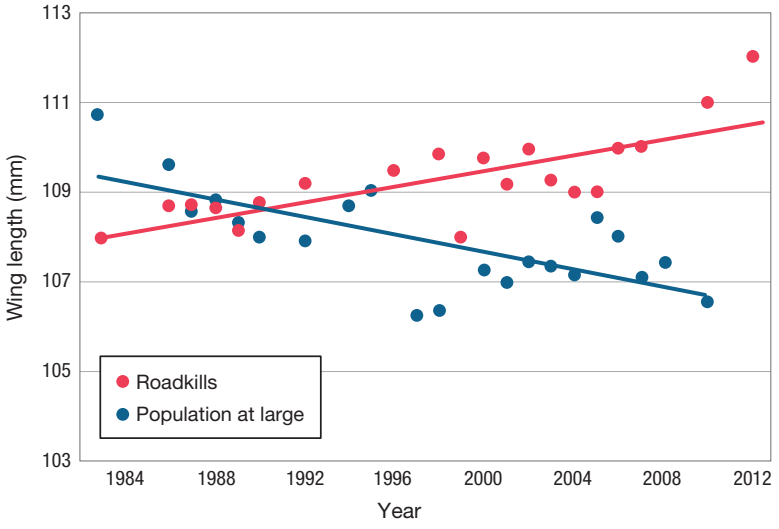


FIGURE 3.15 Roadkill, natural selection, and wing length in swallows. Over the course of three decades, the mean wing length of swallows that died via roadkill increased, while the mean wing length of swallows in colonies decreased. The increasing divergence between lines suggests that natural selection was favoring birds with shorter wings. Adapted from Brown and Brown (2013).

The first clue the Browns had for answering this question came from the fact that the wing length of birds that died via roadkill was significantly longer than the wing length in populations they censused. A more detailed analysis found that the average wing length of swallows in the populations at large had *decreased* over the course of their three-decade study, while the average wing length of swallows that died via roadkill had increased over the same period (**Figure 3.15**). Wing length matters because long wing length reduces the vertical take-off ability in birds; that is, the ability to get into the air quickly. Because swallows often sit on the road eating food, reduced vertical take-off ability will lead to increased collisions with cars. Natural selection thus appears to favor shorter wings because they allow swallows to survive with higher probability in the face of oncoming vehicles.

3.4 Natural Selection in the Laboratory

Thus far, we have considered several examples of how evolutionary biologists generate and test hypotheses on natural selection in the wild. Biologists can do the same when it comes to natural selection in the laboratory. Before we investigate how, let us pause for a moment to take a flight of fancy. Imagine that you are an evolutionary biologist, but not an ordinary one. Suppose that you have a set of powers that you could use in the service of your research. Imagine that you can

- Watch as tens of thousands of generations of evolution take place before your eyes.
- Manipulate the physical environment to control nutrient availability, temperature, spatial structure, and other features, and manipulate the biotic environment, adding or removing competitors, predators, and parasites.
- Create multiple parallel universes with the same starting conditions in which to watch evolution unfold in replicate worlds.
- Move organisms around in a "time machine" so that they can interact with—and compete against—their ancestors or their descendants.
- Go back in time to rerun evolution from any point, under the same or different environmental conditions.
- Easily measure both allele frequencies and fitnesses to accuracies of 0.1% or smaller.

If you could do all of these things, how would you study the process and consequences of evolution? What questions would you ask, and what experiments would you do?

Lenski's Long-Term Evolution Experiment

As far-fetched a fantasy as this may seem, researchers can indeed do all of this and more when they study microbial evolution in the laboratory. One of the most striking examples has been provided by Richard Lenski and his colleagues, who have been tracking evolutionary change for more than 60,000 generations in the bacterium *Escherichia coli* (Le Gac et al. 2012; Wiser et al. 2013). Let us examine Lenski's experimental system in some detail and see how it allows him to perform the seemingly superhuman manipulations enumerated earlier and to test fundamental ideas in evolutionary biology.

Lenski's study species, *E. coli*, reproduces rapidly, dividing at rates upward of once per hour under favorable environmental conditions. As a result, Lenski and his colleagues have been able to observe evolution occurring in real time, and they have been able to monitor more than 60,000 generations of bacterial evolution. To put this number into perspective, Lenski's bacterial evolution experiment now encompasses more generations than there have been in the entire history of our species, *Homo sapiens*.

Starting with a genetically homogeneous strain of *E. coli* bacteria, Lenski created 12 parallel experimental lines—the original colonists of 12 parallel "universes"—differing only by an unselected **marker gene** that allowed researchers to keep track

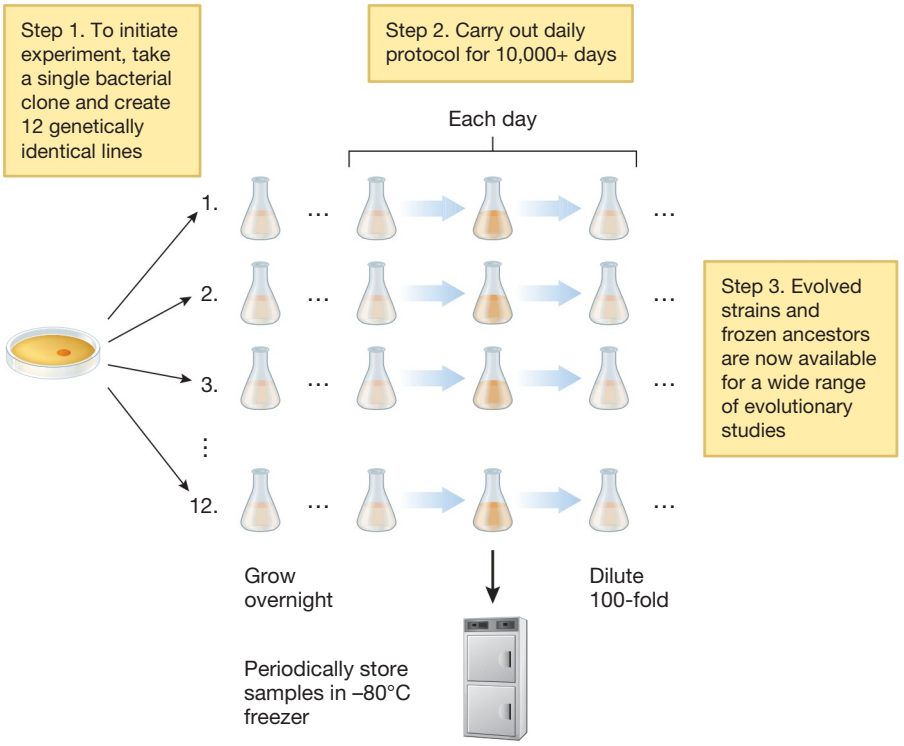


FIGURE 3.16 Lenski's experimental evolution system. The basic protocol for the Lenski *E. coli* experiment. Each day, Lenski and his team transferred cells from the 12 lines into fresh growth medium. These cells went through six to seven generations of replication overnight, and the next day the process started anew. Periodically, they froze a sample of the cells from each line in a −80°C freezer. This open-ended system allows for a large number of potential experiments.

of which experimental line was which. All 12 lines were kept in identical experimental conditions, but the 12 lines were never mixed with one another (**Figure 3.16**). Instead, every day, Lenski and his team transferred cells from each of the 12 lines into fresh growth medium. Overnight, these cells went through six to seven generations of replication, and the next day the process started anew. Periodically, Lenski froze a sample of the cells from each line in a –80°C freezer. This freezer served as his "time machine": Researchers could thaw those cells at any point and could let them compete with their descendants. They could even use them to "start over" and could thus replicate the experiment from any point in time.

Evolutionary Change: Predictability and Quirks

So what can you do with an experimental system like this? We only know about one history of life: the one that actually took place on Earth and of which we are a living part. One question that has always fascinated evolutionary biologists is, what if you could "run the evolutionary process over again"? Would the same phenotypes evolve the second time around? Or would we see something completely different? And if the same phenotypes did evolve, would the same underlying genetic changes be responsible or would natural selection find a different genetic path to a similar phenotypic outcome?

Lenski and his colleague Michael Travisano set out to address this question by comparing what happened in the 12 replicate *E. coli* lines—the 12 parallel runs of evolutionary history—in their experiment (Lenski and Travisano 1994). To do so, they looked at a trait that evolved rapidly early in their experiment: the physical size of the individual *E. coli* cells. These cells could be thawed at any time and allowed to compete against their descendants in order to see whether the descendants had increased in fitness or whether they had merely changed in phenotype (**Box 3.1**). As **Figure 3.17A** illustrates, the average cell volume increased substantially over the first 2000 to 3000 generations of the experiment.

In the course of their experiment, the researchers removed a sample of *E. coli* cells after every 500 generations and then stored them in a freezer. **Figure 3.17B** reveals that the fitness of *E. coli* cells did indeed increase over the course of the experiment. Only 500 generations into the experiment, natural selection had already increased the fitness of the evolved strains relative to their ancestors, and this fitness difference continued to accumulate as the experiment progressed and more generations elapsed.

Figure 3.17 shows results from just one of the 12 lines, and in this line, cell size increased and fitness increased with it. Was this outcome a quirk of fate? What would happen if we were to replay the tape? Would cell size increase again? Lenski and Travisano were able to test this question directly by looking at the other 11 lines, each of which was an independent evolutionary run (Lenski and Travisano 1994). They found that in these lines, as in the first line, cell size invariably increased, and fitness of the cells increased relative to ancestral cells (**Figure 3.18**).

Phenotypically, the populations evolved in a similar fashion. Cell size always increased. But notice that despite starting with genetically identical cells and subjecting them to identical environments, cell size increased more in some lineages than in others. Natural selection operated in a similar direction in each case, but it appears not to have taken an identical path. Likewise, fitness increased in every one of the 12 lines, but some of the lines seem to have found better paths than others, and there was considerable variation in fitness between the lines after 10,000 generations. Lenski and Travisano's results highlight the fact that evolution by natural selection is in some aspects a predictable, repeatable process—and yet it is also one in which random events, such as which mutations occur or the order in which they occur, can play a significant role in shaping the course of history.

Over the past 25 years, Lenski and his colleagues have studied numerous additional traits in these 12 bacterial lines, and in doing so, they have tested a number of evolutionary hypotheses. In the next subsection, we will look at a thermal adaptation experiment that Lenski and colleagues used to test another important question in evolutionary biology: What are the constraints on what natural selection can achieve? Why are organisms not perfectly adapted to all environmental conditions?

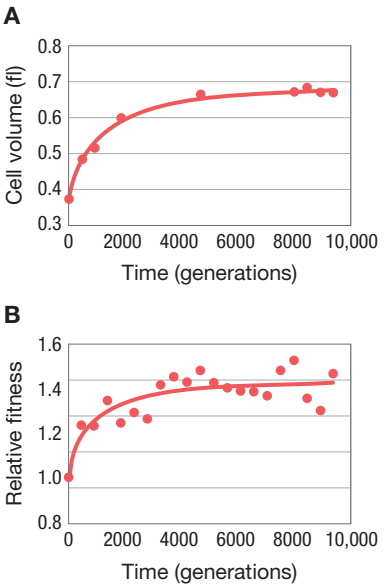


FIGURE 3.17 Cell size and fitness in one *E. coli* line. **(A)** Change in average cell volume (fl. = femtoliter) in one of Lenski's 12 long-term lines. **(B)** Change in fitness for the same line, relative to its ancestor. Fitness values greater than one indicate higher fitness than the ancestor. From Lenski and Travisano (1994).

Thermal Adaptation and Antagonistic Pleiotropy in *E. coli*

Let a bacterial population evolve for a few hundred generations under any particular set of laboratory conditions, and fitness under those conditions will tend to increase significantly. For example, *E. coli* is a gut bacterium that is commonly exposed to a temperature of 37°C within its hosts. Yet Lenski and his team found that *E. coli* lines grown at a steady temperature of 37°C evolved higher fitnesses at that temperature over the course of their experiment. What is going on here? Why should fitness have increased in this experiment? After all, before Lenski ever began his experiments, *E. coli* had already undergone many billions of generations of adaptive evolution in which they might have evolved higher fitness at 37°C. Why hadn't they already done so?

One possibility is that there are trade-offs between an organism's ability to perform under one set of environmental conditions and its ability to perform under another. Perhaps *E. coli* cells are not optimized for growth at 37°C because, unlike the controlled temperature conditions they experienced in Lenski's laboratory, they normally experience other temperatures as well—and adaptations that increase fitness at 37°C may decrease fitness at those other temperatures. To address this hypothesis, Lenski and his colleagues asked whether evolutionary changes that increase growth rate at one specific temperature will be associated with a reduction in growth rates at other temperatures (Huey and Hertz 1984; Palaima 2007).

The growth rates of *E. coli* cells from generations 2000, 5000, 10,000, 15,000, and 20,000 were each compared to the original population of cells, and

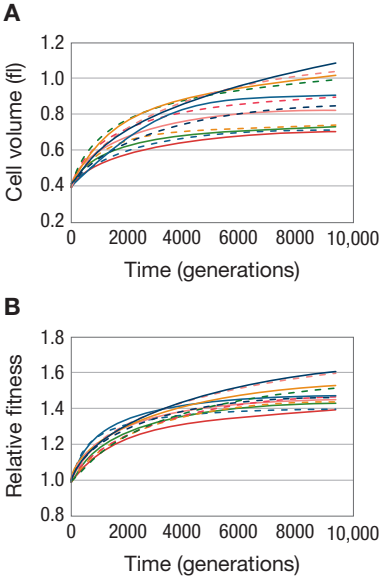


FIGURE 3.18 Cell size and fitness in 12 *E. coli* lines. Change in **(A)** cell volume (fl. = femtoliter) and **(B)** relative fitness in each of the 12 lines. Fitness values greater than one indicate higher fitness than the ancestor. From Lenski and Travisano (1994).

BOX 3.1 Measuring Allele Frequencies and Fitnesses in *E. coli*

Studying natural selection in the wild can be hard, partially because of the challenges of measuring allele frequencies and fitness differences in a wild population of mobile animals such as salmon or sandpipers. When evolution is studied in the laboratory using microbial organisms, these measurements are substantially easier to perform. Researchers studying bacterial evolution in the laboratory commonly work with genetically labeled strains of bacteria. One of the most straightforward approaches to labeling is the so-called $Ara^{+/-}$ marker system. This system uses genetic markers within the *ara* operon that have no selective consequences. The strains, however, can be distinguished easily: Ara^- strains form red colonies and Ara^+ strains form white colonies when grown on tetrazolium–arabinose agar. To measure the relative frequencies of two different strains, a researcher can simply spread a diluted solution containing *E. coli* cells from the population of interest, allow the cells to grow into visible colonies, and count the number of colonies of each color. Other marker systems include alternative color markers and differences in antibiotic resistance or sensitivity that a researcher can use to screen the colonies and thus distinguish the genotypes.

Measuring fitness differences is only slightly more complicated. To measure the fitness of a strain of *E. coli* relative to some other strain (for example, its ancestor), we grow each strain separately in a flask, then mix together samples from each flask, dilute, and plate as described earlier. This allows us to measure the frequency of each strain before they begin to compete. We then grow the strains together in the same flask for some period of time, often 1 day. After this period of growth, we again dilute and plate the bacterial cells, then count colonies (**Figure 3.19**). From any shift in the frequencies of the two strains relative to our initial sample, we can estimate the fitness difference between the two strains. By using the same basic approach, but with automated single-cell sorting techniques replacing the process of plating and counting colonies, researchers have been able to measure differences in fitness as small as 0.1%.

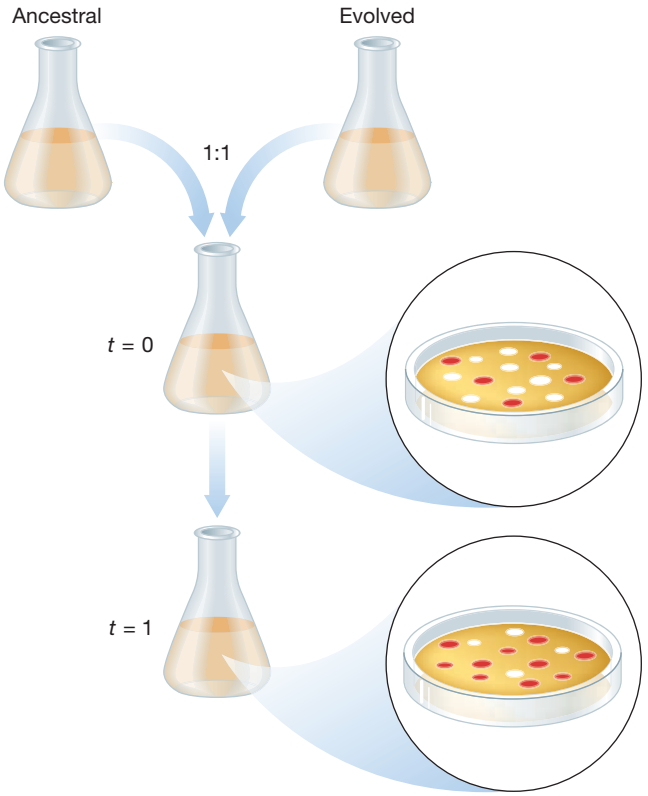


FIGURE 3.19 Measuring bacterial genotype frequency and fitness in the laboratory. Ancestral and descendant populations are competed against each other, and fitness is assayed using the neutral Ara^+ (white) and Ara^- (red) markers to count colonies. Adapted from Elena and Lenski (2003).

this comparison of growth rates was repeated across an array of temperatures from 20°C to 42°C in all 12 of Lenski's *E. coli* universes (Cooper et al. 2001) (**Figure 3.20**). After 20,000 generations in an environment where the temperature was 37°C, natural selection led to an increase in growth rate at that temperature. Moreover, the optimal temperature for growth shifted from approximately 40°C to near 37°C. Lenski and his team also found an evolutionary change toward *lower* growth rates at both extremes of the temperature range—20°C and 42°C—in the majority of populations that evolved optimal performance at 37°C (Cooper et al. 2001; Bennett and Lenski 2007).

Why did this happen? Why did evolving an optimal performance at 37°C lead to suboptimal results at the other temperatures (20°C and 42°C)? One

possibility is a nonselective explanation: Perhaps after growing for 20,000 generations at 37°C, Lenski's lines had accumulated mutations that reduced their ability to grow at 20°C or 42°C. Because the bacteria were never exposed to those temperatures, natural selection would not have acted against such mutations. But Cooper and his colleagues were able to find evidence against this hypothesis in a clever way. Among their 12 lines, 3 lines evolved to become so-called *mutator* strains, with vastly higher mutation rates than those observed in the other 9 lines. If the decline in performance at 20°C and 42°C had been due to the accumulation of unselected mutations, Cooper and his team reasoned, the decline in performance should be greater in the mutator strains, because these strains accumulated far more mutations. But they found no such difference. Simple mutation accumulation seems an unlikely explanation for the fitness decline at the extreme temperatures.

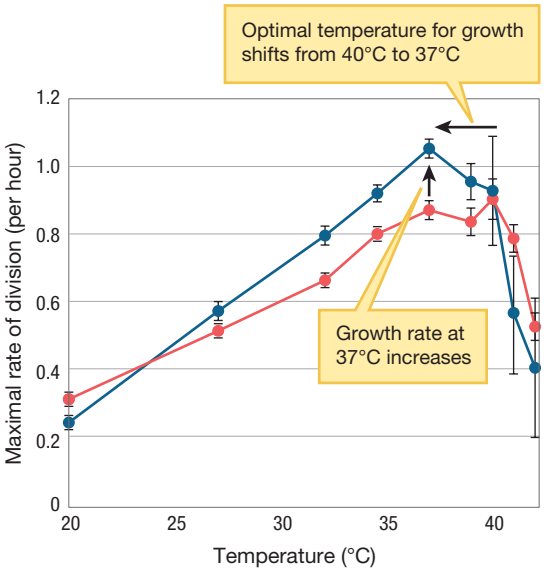


FIGURE 3.20 Thermal adaptation in *E. coli*. The red line represents ancestral population, and the blue line represents the population after 20,000 generations at 37°C. Adapted from Cooper et al. (2001).

Instead, Lenski and his colleagues suggest that their results are best explained by a phenomenon known as antagonistic pleiotropy. The **antagonistic pleiotropy** hypothesis proposes that the same gene (or genes) that codes for beneficial effects—here, rapid growth at 37°C—also codes for deleterious effects in other contexts; in this case, poor performance at 20°C and 42°C (**Figure 3.21**). When genes, such as those hypothesized here, affect more than one characteristic, they are referred to as **pleiotropic genes**. And because we are testing whether such pleiotropic genes have a negative effect in one context but a positive effect in another, we refer to this as *antagonistic* pleiotropy. Thus, antagonistic pleiotropy results in a trade-off between fitness under one set of conditions and fitness under another set of conditions.

One prediction from the antagonistic pleiotropy hypothesis is that the negative components to fitness—in this case, poor performance at 20°C to 42°C—should build up quickly and early in the tested populations because variation in response to temperature will be high at the start of the process, and hence selection for optimal performance will be most powerful. The experimental results provide support for the antagonistic pleiotropy hypothesis because suboptimal performance at extreme temperatures evolved fairly quickly in their populations, with most selection occurring in the first 5000 of the 20,000 generations of Lenski's laboratory populations of *E. coli.*

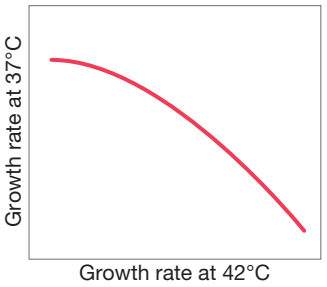


FIGURE 3.21 Antagonistic pleiotropy. The antagonistic pleiotropy hypothesis predicts a trade-off between two characteristics. Shown here is a hypothetical trade-off between growth rates at 37°C and 42°C.

KEYCONCEPT QUESTION

3.3 How might the antagonistic pleiotropy hypothesis be related to diseases that are often associated with old age (for example, Alzheimer's disease)?

3.5 Origin of Complex Traits

Ever since Darwin published *On the Origin of Species*, evolutionary biologists have been fascinated by the problem of how natural selection can produce the exquisite match between organism and environment that we often observe, and how even in the absence of foresight, natural selection can create complex traits with many interdependent components.

How, for example, can we explain the exquisite complexity and detail of the human eye? How can we explain the production of milk in mammals and the associated nursing behaviors that make it such a valuable strategy for parental care? And how do we account for the coupling of wing geometry and variable wing angle that allows a dragonfly to produce the high-lift wing-tip vortices that confer its remarkable flight abilities (Thomas et al. 2004)?

In this section, we will examine two possible explanations for the evolution of such complex traits. The first explanation centers on the idea that each intermediate step on the way toward the evolution of complex traits was itself adaptive and served a function similar to the modern-day function. The second explanation—co-option of a trait to serve a new purpose—posits that intermediate stages of complex traits were functional and selected, but they did not serve the same function in the past as they do today.

Intermediate Stages with Function Similar to Modern Function

When looking at an organ as complex as the eye, we are struck by the extraordinary complexity of a trait that requires so many intricate parts, all of which must work together. How could such a complex trait ever evolve in the first place? Darwin raised this issue in *On the Origin of Species*:

> To suppose that the eye, with all its inimitable contrivances for adjusting the focus to different distances, for admitting different amounts of light, and for the correction of spherical and chromatic aberration, could have been formed by natural selection, seems, I freely confess, absurd in the highest possible degree. (Darwin 1859, p. 186)

But Darwin argued that natural selection was responsible for the complexity we see in eyes, and that the evolution of the eye occurred through small successive changes, each of which provided a benefit compared to the last version of the eye. The very next sentence of Darwin's quote reads,

> Yet reason tells me, that if numerous gradations from a perfect and complex eye to one very imperfect and simple, each grade being useful to its possessor, can be shown to exist; if further, the eye does vary ever so slightly, and the variations be inherited, which is certainly the case; and if any variation or modification in the organ be ever useful to an animal under changing conditions of life, then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, can hardly be considered real. (Darwin 1859, pp. 186–187)

Evolutionary biologists L. V. Salvini-Plawen and Ernst Mayr have expanded on Darwin's hypothesis, laying out a series of intermediate stages that represent one plausible sequence by which the eye evolved in gradual steps (Salvini-Plawen and Mayr 1977). Because eyes are made of soft tissue that does not fossilize well, Salvini-Plawen and Mayr used currently living species to show examples of the sorts of eye morphologies that may have been present in ancestral forms, and they found that indeed current forms can be arranged into a series of steps, each only slightly more complex than the previous, which would lead from a simple light-sensing pigment spot to a focusing eye with a lens. The aim was not to reconstruct the exact sequence by which eye evolution did occur; in fact, there is no single answer to this question

because the lensed eye evolved in parallel in several different lineages (**Figure 3.22**). Rather, this work was meant to illustrate that the focusing eye, elaborate as it may seem, could have evolved in gradual steps, each of which was fully functional and each of which improved on the visual acuity of its predecessor.

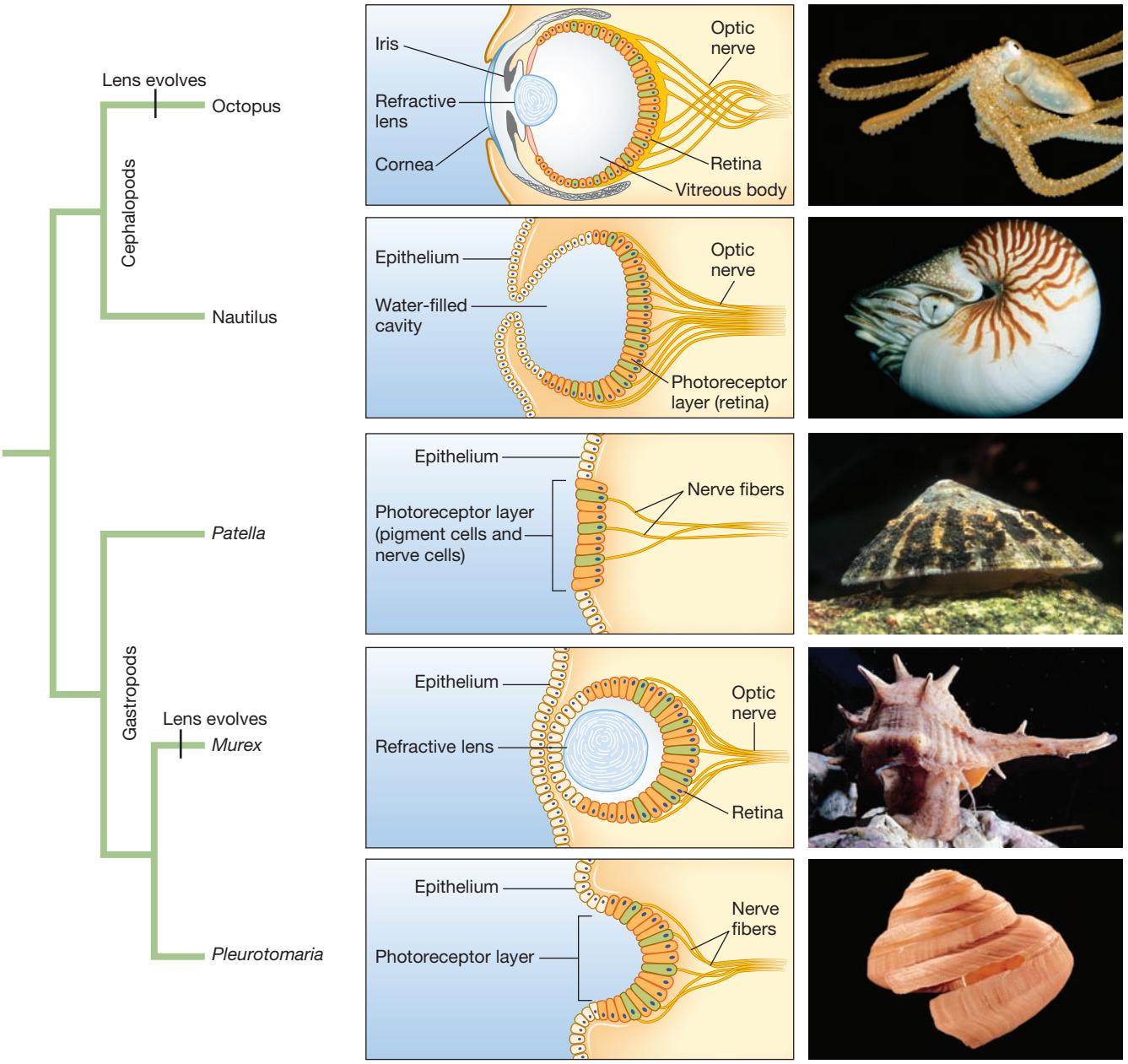


FIGURE 3.22 The evolutionary history of the mollusk eye. Taking a phylogenetic perspective on eye morphology in the mollusks, we see that complex eyes with a lens evolved independently in the cephalopods and in the gastropods (Oakley and Pankey 2008). From top to bottom: The octopus eye uses a lens to focus light on the retina, much as does the vertebrate eye. The nautilus eye functions like a pinhole camera, casting a sharp image on the retina at the expense of a loss in brightness due to its small aperture. The limpet *Patella* has only a light-sensitive patch that can distinguish between light and dark. The predatory snail *Murex* uses a simple lens to focus incoming light. The snail *Pleurotomaria* has an indented eye cup that can detect the direction of a light source. Phylogeny is inspired by Oakley and Pankey (2008) and informed by Ponder and Lindberg (1997).

But is this feasible? Is there enough time for this to have happened? Dan-Erik Nilsson and Susanne Pelger used computer simulations to explore how long it might take to evolve a focusing eye from a simple light-sensitive patch (Nilsson and Pelger 1994). They assumed that individual mutations had only small phenotypic effects, and they made conservative assumptions about the rate at which natural selection would proceed under these circumstances. They found that the focusing eye could have evolved in fewer than half a million years—a very short time compared to the 550 million years since the first simple eyes occurred in the fossil record.

Darwin's intuitions seem correct. Complex focusing eyes have evolved by natural selection, and they have done so independently along several lineages on the tree of life. Each of these lineages may have proceeded along a different path, but along each path, every small step could have been functional in itself and could have improved on the visual system that preceded it.

Novel Structures and Exaptations

As we mentioned earlier in this chapter, some traits were originally selected for one function but were later *co-opted* to serve a different, selectively advantageous function. Such traits are called *exaptations* (Gould and Vrba 1982; Gould 2002).

As an example, consider the bizarre "helmet" structure that is found in all species of treehopper insects but in no other species (Moczek 2011; Prud'homme et al. 2011) (**Figure 3.23**). Today, the helmet functions to camouflage treehoppers by mimicking the seeds, thorns, and other structures found in their environment.

FIGURE 3.23 Elaborate helmet morphology of treehoppers. Species of neotropical treehopper insects (Membracidae) exhibit an elaborate diversity of helmet structures. From Prud'homme et al. (2011).

But how did this novel, complex structure arise to begin with in the treehopper lineage? Did it arise de novo, that is, from scratch, or is it an example of how an existing developmental pathway can be coopted for a new use?

One clue came when Benjamin Prud'homme and his team studied the development of the helmet and found that it forms from paired buds that emerge on the first segment of the treehopper thorax. Other than the helmet structure, the only other appendages known to bud off the first thoracic segment of insects are wings. While modern insects have wings on only the second and third sections of their thorax, an ancient group of extinct insect species developed small wings on the first thoracic segment as well (**Figure 3.24**). Along the lineage leading to modern insects, the development of wings on the first thoracic segment was suppressed.

Prud'homme's group wondered whether the developmental pathway that once led to wings on the first thoracic segment (but was then suppressed) could have been co-opted by treehoppers to produce their distinctive helmet structures. They found several pieces of evidence supporting their hypothesis. From an anatomical perspective, helmets are built in a way reminiscent of the way wings are built; for example, the hinges that connect wings to other body parts and the hinges connecting the helmet to other body parts are structurally very similar. Moreover, some of the same genes—in particular the *sex comb reduced*, or *Scr*, gene—are involved both in wing development and in helmet development (**Figure 3.25**). The helmet in treehoppers is an exaptation: The original trait "wings on the first thoracic segment" was suppressed, but subsequently this developmental pathway was co-opted in the treehopper lineage for use in helmet production.

Exaptations play an important role in the evolution of complex traits. Any time a structure, behavior, or characteristic adopts a new function over evolutionary

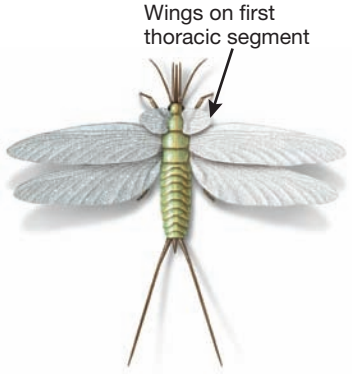


FIGURE 3.24 Primitive wing development in an extinct insect. In *Stenodyctya lobata*, an extinct species of insect, small wings developed on the first thoracic segment (arrow). Modern insects only have wings on the second and third segments of the thorax. From Moczek (2011).

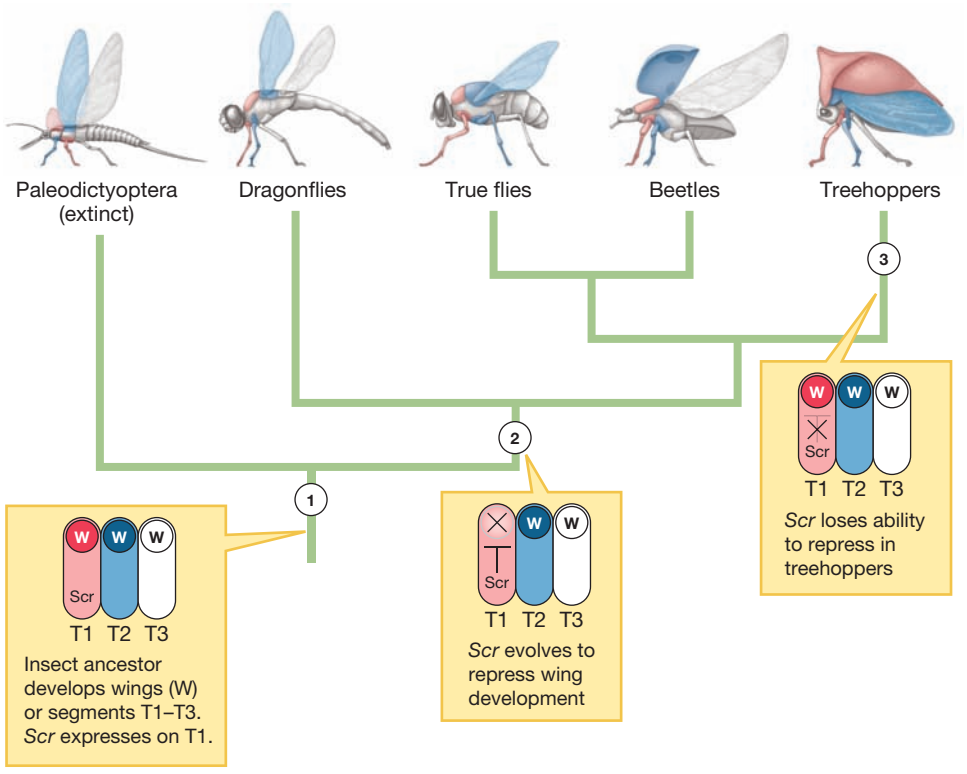


FIGURE 3.25 Development of wings and helmets. A phylogenetic view of *Scr* expression and its role in wing and helmet development on the insect thorax. T1 = thoracic section 1 (in pink), T2 = thoracic section 2 (in blue), T3 = thoracic section 3 (in white). Initially, *Scr* had no effect on wing development in any section of the thorax (1). A likely scenario is that in the ancestral insect at the base of the tree, *Scr* was expressed only in segment T1. At first, *Scr* did not repress wing development on T1. Along the lineage leading to modern insects (2), the developmental pathway evolved so that *Scr* suppressed wing growth on T1. Finally, on the lineage leading to treehoppers (3), *Scr* lost its ability to suppress appendage growth on T1, and in this group the developmental pathway was co-opted to produce helmets. Adapted from Prud'homme et al. (2011).

time, this is an exaptation. Gross morphological structures rarely arise de novo, but instead derive from modifications to previously existing structures. The same can be said of molecular structures, as we will see in the next subsection. As a result, most complex traits will have extensive evolutionary histories over which they have undergone multiple changes in function, and thus such traits will represent a "layering of adaptations and exaptations" (Thanukos 2009).

Although the term *exaptation* was not introduced until 1982 by Stephen Jay Gould and Elizabeth Vrba, Darwin was aware of this phenomenon in *On the Origin of Species*, in which he wrote, "The sutures in the skulls of young mammals have been advanced as a beautiful adaptation for aiding parturition, and no doubt they facilitate, or may be indispensable for this act" (Darwin 1859, p. 197). In this passage, Darwin described cranial sutures, the fibrous connective tissue joining the bones that make up the skull. Because the bones of the skull are not yet fused at birth and because the sutures are relatively elastic, the skull is able to deform somewhat as it passes through the birth canal during parturition (the process of giving birth). While cranial sutures may serve to aid the process of live birth in modern times (particularly in humans, where cranium diameter is a major constraint on size at birth), this need not have been the original function of sutures. Indeed, it was *not* the original function, Darwin argued. He immediately followed the above statement with "sutures occur in the skulls of young birds and reptiles, which have only to escape from a broken egg," (Darwin 1859, p. 197). Cranial sutures could not have evolved to aid the birth process in mammals, as they predated the evolution of mammalian reproduction (**Figure 3.26**).

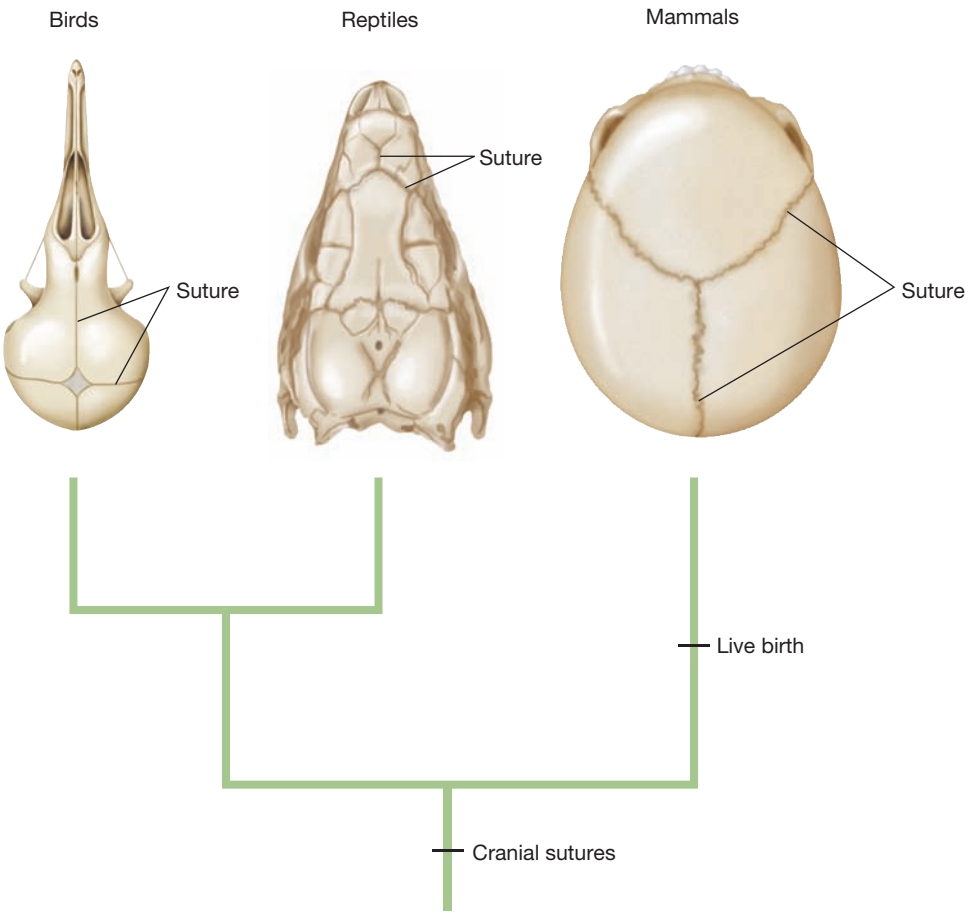


FIGURE 3.26 Darwin realized that cranial sutures evolved before live birth. Darwin used phylogenetic reasoning to conclude that skull sutures did not originally evolve to facilitate parturition. Because cranial sutures are present in birds, reptiles, and mammals alike, Darwin reasoned that they evolved prior to the evolutionary split between birds and reptiles and mammals, as shown. Because live birth arose after this evolutionary split, cranial sutures predated live birth and thus could not have initially evolved for the purpose of facilitating passage through the mammalian birth canal.

The original function of cranial sutures was probably to allow the rigid protective cranium to expand with a growing brain, and indeed this function is retained (Yu et al. 2004). Only subsequent to the original function, once live birth evolved, were sutures *co-opted* to facilitate passage through the birth canal (Darwin 1859). Despite Darwin's usage of the word *adaptation* in his original description, in modern terminology, these sutures are exaptations *with respect to aiding the mammalian birth process*.

Let's consider another complex trait—feathers in modern-day birds—as an additional example of an exaptation. Because feathers play such a prominent role in bird flight, and because they seem so exquisitely suited for that function, we may be tempted to assume that feathers have *always* been selected only in relation to their effect on flight.

But again, as with Darwin's example of skull sutures, phylogenetic evidence is useful for separating adaptation from exaptation (**Figure 3.27**). Paleontological discoveries from northeastern China have revealed that featherlike structures were widespread in a substantial subgroup of the bipedal *theropod dinosaurs*, which did not use these structures for flight. These dinosaurs ultimately gave rise to modern birds (Ji et al. 1998; Xu et al. 2001, 2009, 2010). Moreover, structural studies strongly suggest a single evolutionary origin of feathers. From this, we can deduce that the origin of feathers predates the evolution of wings and flight.

In light of the phylogenetic evidence that feathers evolved prior to flight, it would be a mistake to conclude that feathers originally evolved as an adaptation for flying. Natural selection cannot look ahead to fashion a structure that only later will become useful. Biologists Richard Prum and Alan Brush offer an appealing analogy: They say that, in light of the phylogenetic evidence, "Concluding that feathers evolved for flight is like maintaining that digits evolved for playing the piano" (Prum and Brush 2002, p. 286).

So, what might have been the original function(s) of feathers? Over the years, researchers have proposed a number of possibilities, including (1) retaining heat, (2) shielding from sunlight, (3) signaling, (4) facilitating tactile sensation, as whiskers do, (5) prey capture, (6) defense, (7) waterproofing, and (8) brooding eggs (Prum and Brush 2002; Zelenitsky et al. 2012).

Let's just look at one of these functions—thermoregulation—as an example (Evart 1921; Bock 1969; Ostrom 1974). Feathers, especially the contour feathers that are already seen in the earliest known bird *Archaeopteryx*, help control thermoregulation, both because feather down is itself an insulator and because the air space between feathers acts to insulate animals against temperature change (Ostrom 1974). This early thermoregulatory function also appears to have been very important in the evolution of wings in insects (Kingsolver and Koehl 1985).

Of course, thermoregulation is not mutually exclusive with the other proposed functions. In any event, given currently available evidence, there is little prospect for distinguishing among these alternatives in identifying the original selective function or functions.

Using the arguments we developed earlier, we can say that the basic structure of feathers is, in part, an exaptation with respect to bird flight. That does not mean that feathers, once selected for their initial function, were not subsequently shaped by natural selection because of the fitness effects associated with flight in birds. Rather, once selected for thermoregulation or other purposes, any changes to feathers that also made them more beneficial for early flight would likely have been selected.

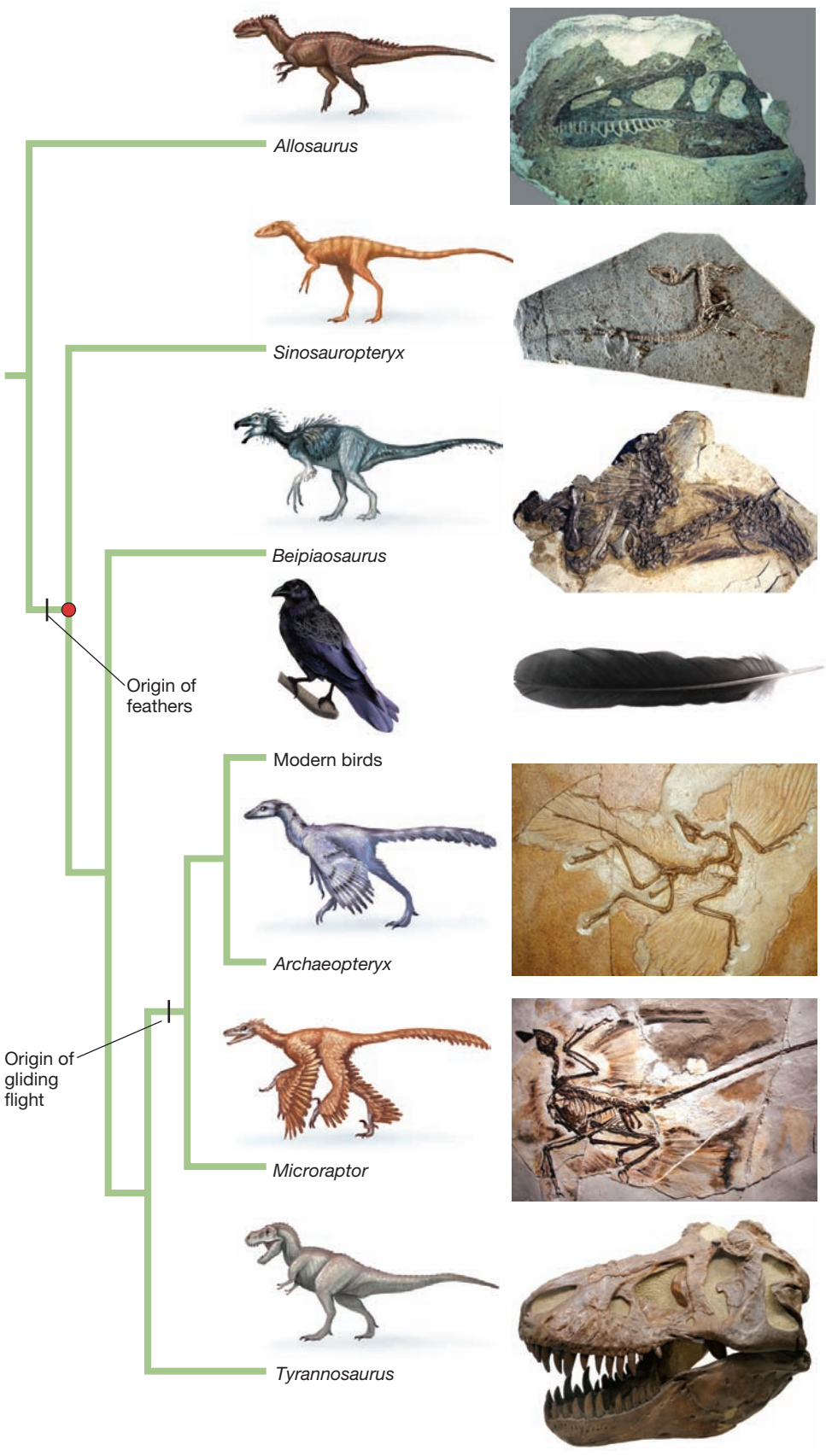


FIGURE 3.27 The evolutionary origin of feathers. Phylogenetic reasoning suggests that feathers did not originally evolve for flight. Feathers likely arose in a lineage of theropod dinosaurs. The common ancestor of these feathered dinosaurs (including birds) is marked with a solid red circle. This species had neither wings nor the ability to fly. Therefore, feathers must have initially evolved for some other purpose. Gliding and flight subsequently evolved in the lineage leading to *Microraptor*, *Archaeopteryx*, and modern birds; at this stage, feathers were co-opted to facilitate flight.

Notice that when a trait switches function, the organism need not lose the original function. Sometimes the trait can serve both purposes. Skull sutures facilitate brain growth and aid parturition. Feathers can serve both to insulate the bird and to facilitate flight.

Next, we will consider two examples of how novelty arises at the molecular level.

Novelty at the Molecular Level

Whether at the morphological level or at the level of individual molecules, the process of evolution is ever tinkering with extant structures. One way that new molecular functions can arise is through the process of **gene sharing**, in which a protein that serves one function in one part of the body is recruited to perform a new and different function in a second location.

There is no better illustration of the breadth and diversity of gene sharing than the lens crystallin proteins. Lens crystallins are structural proteins that form the transparent lens of the eye. While some lens crystallins are used only in the lens, many are dual-function proteins that are also used as enzymes elsewhere in the body. **Table 3.1** lists a number of the lens crystallins that also function as enzymes.

The process of **gene duplication** provides another evolutionary pathway by which a protein can switch functions without loss of the original function. In a gene duplication event, an extra copy of a functional gene is formed. Once an organism has two copies of the gene, one of the two gene copies might change to a new function, while the other can remain unchanged and thus preserve the original function. We conclude this subsection with one such example.

One particularly complex suite of traits is the lock-and-key mechanism of many hormone–receptor pairs, with their exquisite specificity (**Figure 3.28**). These hormone–receptor pairs pose a chicken-and-egg problem: How could a signaling protein possibly evolve to match a receptor that has not yet arisen; or, conversely, how could a receptor evolve to accept a signal that does not yet exist?

TABLE 3.1

Examples of Gene Sharing: Lens Crystallins with Separate Enzymatic Functions

Crystallin	Species	Enzyme
δ	Birds and reptiles	Argininosuccinate lyase
ε	Birds and crocodiles	Lactate dehydrogenase D4
τ	Lamprey, fish, reptiles, and birds	α-Enolase
λ	Rabbit	Hydroxyacyl-CoA dehydrogenase
ζ	Guinea pig	Alcohol dehydrogenase

Adapted from Piatigorsky and Wistow (1989).

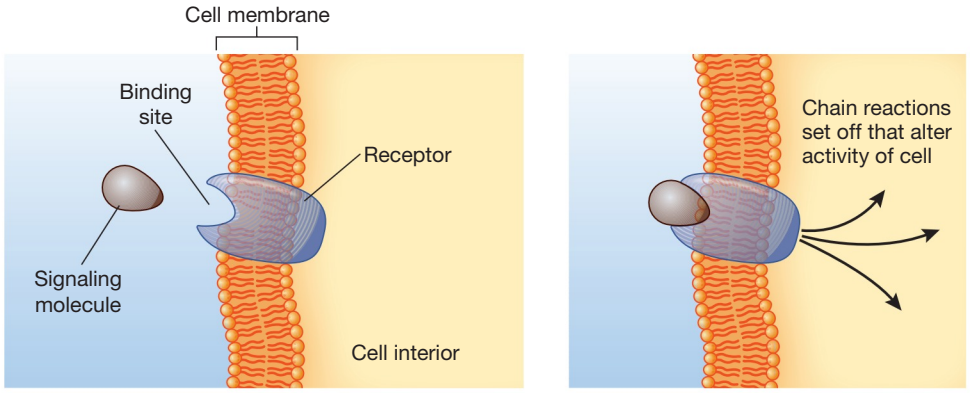


FIGURE 3.28 Lock-and-key systems. The lock-and-key mechanism of many hormone–receptor pairs.

Jamie Bridgham and her colleagues worked out a detailed answer to this question for one such lock-and-key pair: the mineralocorticoid receptor (let's call it the *M receptor*) and the steroid hormone called *aldosterone*, which triggers this receptor (Bridgham et al. 2006, 2009). The M receptor, which is involved in controlling the electrolyte balance within cells, arose in a gene duplication event from an ancestral glucocorticoid receptor.

But how did this gene duplication lead to a novel and highly specific aldosterone–M receptor pair? Again, a phylogenetic approach was the key to unraveling this mystery. By sequencing the mineralocorticoid receptor genes from a wide range of vertebrates, Bridgham's team was able to infer the genetic sequence of the ancestral receptor that was duplicated to produce both the M and modern glucocorticoid receptors.

Bridgham and her colleagues found that the ancestral receptor binds not only cortisol (a glucocorticoid hormone) but also aldosterone. This is surprising because it means that the ancestral receptor could bind a hormone that didn't exist when the ancestral receptor was in place—aldosterone evolved much later. But cortisol was already in existence at the time of the ancestral receptor. Evolutionary biologists have hypothesized that after the gene duplication, a pair of mutations altered the shape of what is now the glucocorticoid receptor, so that it retained its ability to bind cortisol but would no longer bind aldosterone. At the time, aldosterone wasn't present yet, but over millions of years, genetic changes in biosynthetic pathways (associated with cytochrome P-450) by chance eventually led to the production of aldosterone. Because aldosterone could now trigger the M receptor without interfering with the glucocorticoid receptor, there was a new signal–receptor pair that could be used independently to regulate other cellular processes. Now we know which came first in this chicken-and-egg problem. The ability of the receptor to bind aldosterone preceded the evolution of aldosterone itself (**Figure 3.29**).

KEYCONCEPT QUESTION

3.4 Counter the following argument: "Exaptations are common; therefore, natural selection is not nearly as important as many biologists have claimed."

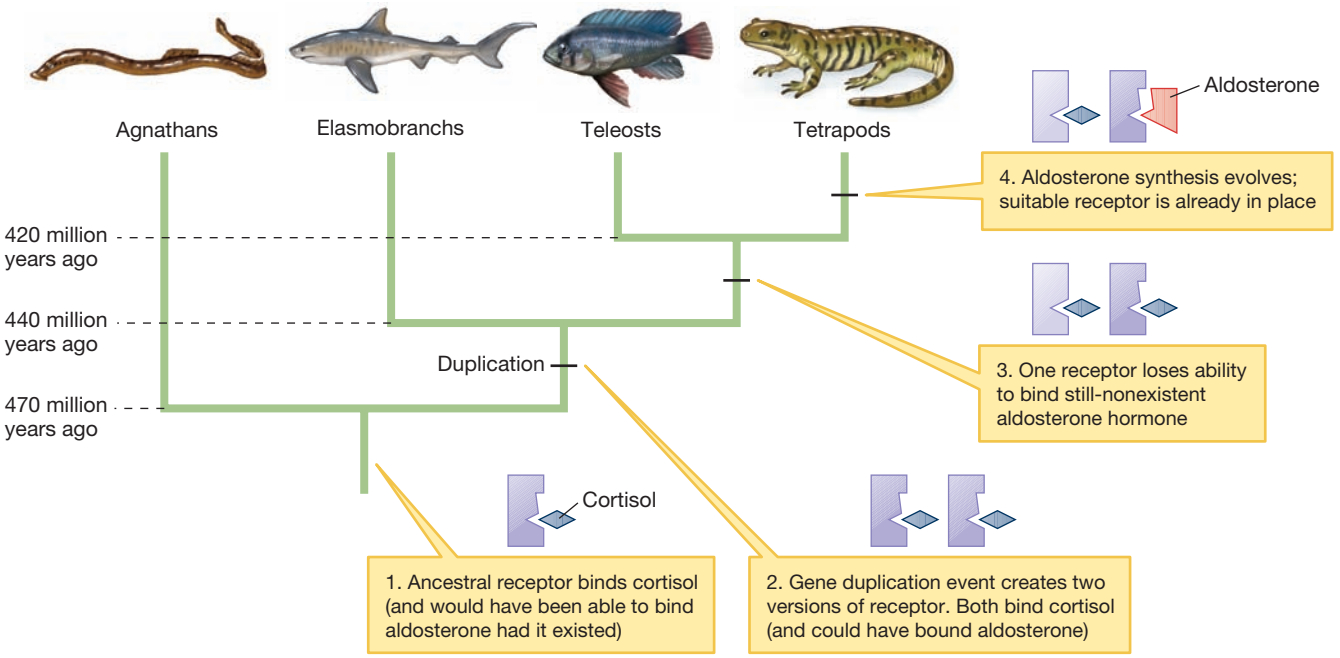


FIGURE 3.29 Gene duplication and the evolution of the aldosterone receptor. Neither the aldosterone hormone nor the aldosterone receptor were present in the vertebrate lineage 470 million years ago. (1) A single glucocorticoid receptor bound cortisol—and would have bound aldosterone, had it been present. (2) About 450 million years ago, a gene duplication created a second copy of the glucocorticoid receptor. (3) Subsequently, genetic changes to one of these receptor copies shifted its structure so that it would not be able to bind aldosterone. The other retained aldosterone binding ability. (4) In the tetrapods, when aldosterone synthesis arose, a receptor was already in place that could bind aldosterone. Because the structure of the other glucocorticoid receptor had changed so that it could bind cortisol but not aldosterone, that pathway was not disrupted by the advent of aldosterone synthesis. Adapted from Bridgham et al. (2006).

3.6 Constraints on What Natural Selection Can Achieve

In our efforts to understand the process of natural selection, it is critical to recognize the limitations on what natural selection can achieve. In the short term, there may be limits on the genetic variation available for natural selection to operate on (Futuyma 2010). Evolutionary biologist J. B. S. Haldane captured this point in *The Causes of Evolution*:

> A selector of sufficient knowledge and power might perhaps obtain from the genes at present available in the human species a race combining an average intellect equal to that of a Shakespeare with the stature of [heavyweight boxer Primo] Carnera. But he could not produce a race of angels. For the moral character or for the wings he would have to await or produce suitable mutations. (Haldane 1932/1990, p. 60)

This sort of constraint on what natural selection can achieve has been examined experimentally many times by evolutionary biologists, including in another set of *E. coli* experiments conducted by Lenski and his team. They found that under certain conditions, the rate of adaptation in *E. coli* was proportional to the supply of new variation available (de Visser et al. 1999).

Even if there is variation in a given characteristic, selection may be unable to act on that characteristic if the genes involved have effects on other characteristics that are also under selection. Another short-term constraint on natural selection is that gene flow into a local population can limit the degree of local adaptation; that is, a peripheral population may be unable to adapt to its local environmental circumstances because of continual gene flow from a larger population that faces different selective conditions.

In the long term (assuming nonextinction), these limitations may be overcome. Even in small populations, mutations that overcome some constraint may *eventually* become available; it may simply be a matter of waiting long enough. Correlated characteristics may become uncoupled once the appropriate mutations arise, removing the constraints associated with pleiotropy. Reproductive isolating mechanisms can reduce or eliminate gene flow into the peripheral population and thus allow local adaptation. This does not, however, mean that natural selection is free of any constraints. Rather, even in the long term, there are a number of limitations to what natural selection can achieve. First we will look at some of these limitations, then we will look at how, in some cases, they may be overcome.

Physical Constraints

From a spider's web, with its minuscule weight and exceptional tensile strength, to an owl's fringed feather edges that muffle any sound from its wings as they cut through the air, natural selection has fashioned countless material marvels. Nonetheless, natural selection is limited in what it can do. It operates on physical structures in the material world, and as such it is constrained by the same physical and mechanical laws that limit the realm of possibility for human engineers.

Compare the placement of the eyes in an ostrich to that in an owl (**Figure 3.30**). The ostrich—which must remain vigilant against predators—has eyes that are set on either side of the head, allowing a nearly 360° field of view, but affording almost no stereoscopic vision because the field of each eye scarcely overlaps with

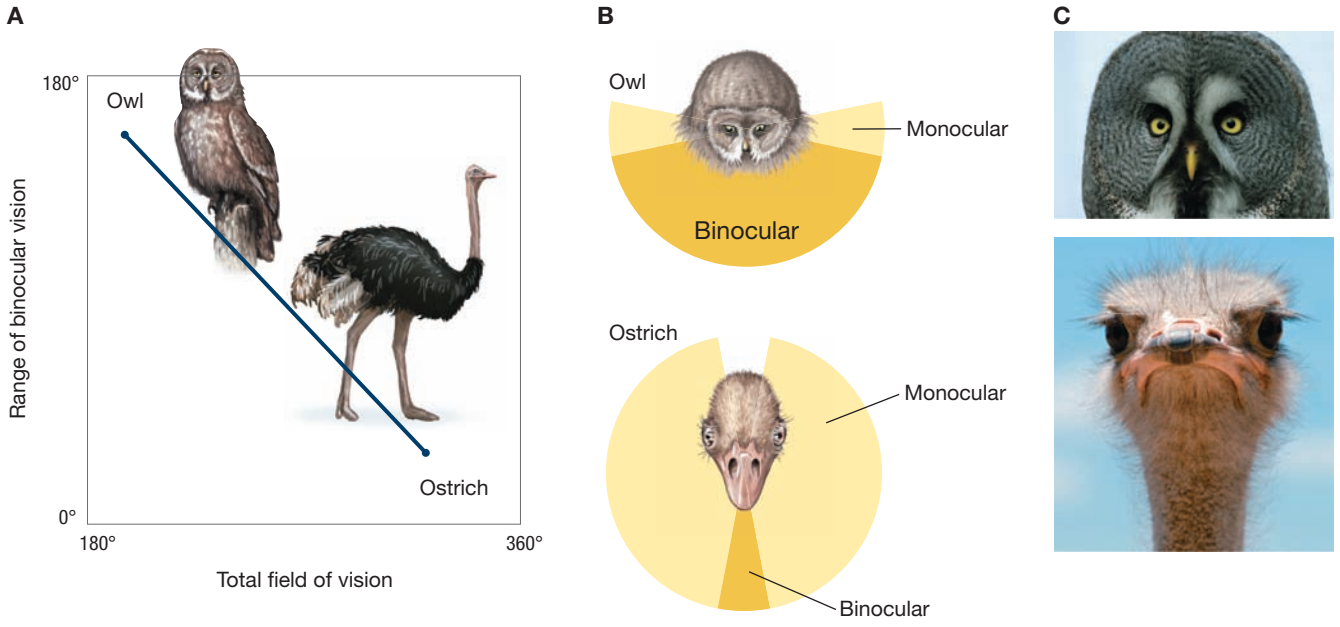


FIGURE 3.30 Trade-offs in binocular vision. **(A)** Birds face a trade-off between the total field of vision (*x* axis) and the range of binocular vision (*y* axis). Because of the different challenges they face, the ostrich and the owl have evolved to different points along this trade-off curve. **(B)** The position of the eyes determines where along the trade-off curve a species falls. The eyes of an owl are positioned side by side in the front of the head, limiting the field of view to about 180°, but with the benefit of binocular vision across this field. The eyes of an ostrich are set on opposite sides of its head, yielding a nearly 360° field of view. **(C)** Great gray owl and ostrich.

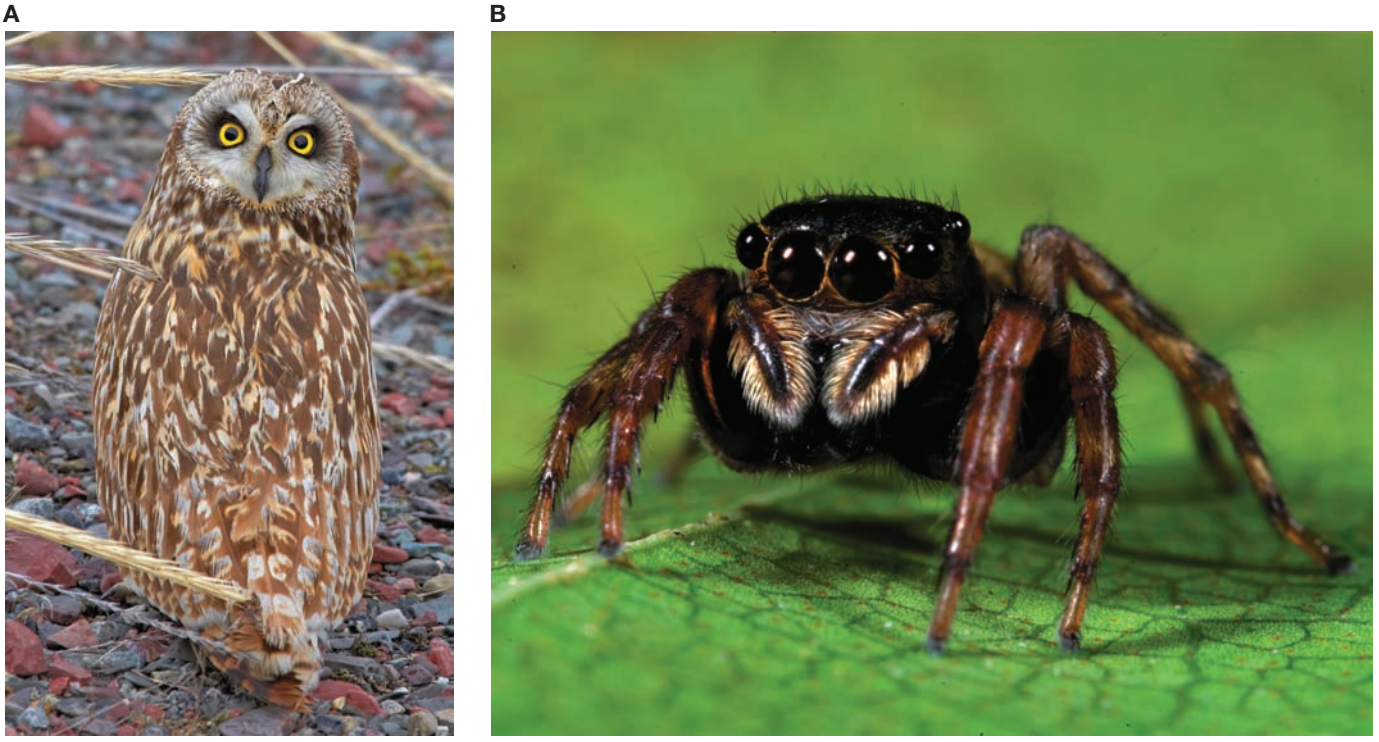


FIGURE 3.31 Overcoming constraints. **(A)** A partial solution to the limited field of view: Owls can turn their heads nearly 180° to look behind themselves, as shown by this short-eared owl. **(B)** A different solution: The jumping spider has eight eyes, allowing both stereoscopic forward vision for visual hunting and a 360° field of view.

that of the other. The owl—a visual predator—has eyes that are set on the front of the head, allowing a fully stereoscopic view of its environment, including prey species, but presenting a much more limited field of view than that enjoyed by the ostrich.

The ostrich and the owl represent two extreme manifestations of the response to the constraint that a two-eyed organism can have a 360° field of view or binocular vision across most of the visual field, but it *cannot* have both. For their part, owls have evolved a *partial* solution to this constraint: An owl can turn its neck nearly 180° over its back without shifting its perch (**Figure 3.31A**). Spiders go a step further. They have eight eyes, allowing them to see in 360° and at the same time to enjoy a binocular (or even multiocular) forward view for visual hunting (**Figure 3.31B**).

Other simple physical constraints become apparent when we look at the sizes and shapes of animals (Thompson 1917; Haldane 1928; Gould 1974). Why are there no insects that are the size of wolves? Why don't single-celled swimmers have the same streamlined shape that we see in dolphins, tuna, or penguins? Why are there no elephant-sized creatures with spindly spiderlike legs?

The answer to each of these questions lies in the constraints that the laws of physics place on the form and structure of living organisms. As an example, let us consider in detail the last of these questions—why are there no elephant-sized creatures with spindly spiderlike legs? When we look at Salvador Dali's sculpture, *Space Elephant*, our intuition about the world tells us that this creature is absurd

FIGURE 3.32 Art and the violation of physical constraints. In his sculpture, *Space Elephant*, Salvador Dali depicts an elephant with long, thin legs, as he did in his famous 1946 painting, *The Temptation of Saint Anthony*, which showed four elephants with long, spindly, fragile legs. Such thin legs would never support a flesh-and-blood creature of elephant-like size.

(**Figure 3.32**). Why? We know that, at least for elephant-sized creatures made of flesh and blood, legs like that would be too fragile to support the immense bulk of the body held high above.

Indeed, if we look at leg size (diameter) relative to body mass, we see that mammals, from the tiny pygmy shrew to the massive African elephant, conform to a tightly defined relationship between body mass and leg diameter. **Figure 3.33** plots the diameter of the femur against total body mass for different species of mammals (Alexander et al. 1979). All of the mammals measured lie along a tight line across a millionfold difference in body mass. Why is this? Why has natural selection not chosen *some* solutions *somewhere* off this line? Is it an accident of history or is there some physical constraint that shapes the relation between body mass and femur diameter?

All else being equal, organisms with longer, thinner legs will be faster and lighter. So, perhaps we should not be surprised that there are no organisms with small bodies and thick legs. But why don't we see the converse—organisms with large bodies and thin legs as illustrated by Dali? We can find the answer in the simple scaling laws of support structures, as illustrated in **Figure 3.34**. Looking at an ensemble of similarly shaped organisms, notice first that body mass increases with the third power of size (for example, measured as body length or height): mass $\sim$ size^3. But the strength (that is, the ability to resist compressional stress) of a supporting structure is proportional to its cross-sectional area, which scales with the second power of size: cross-sectional area $\sim$ size^2.

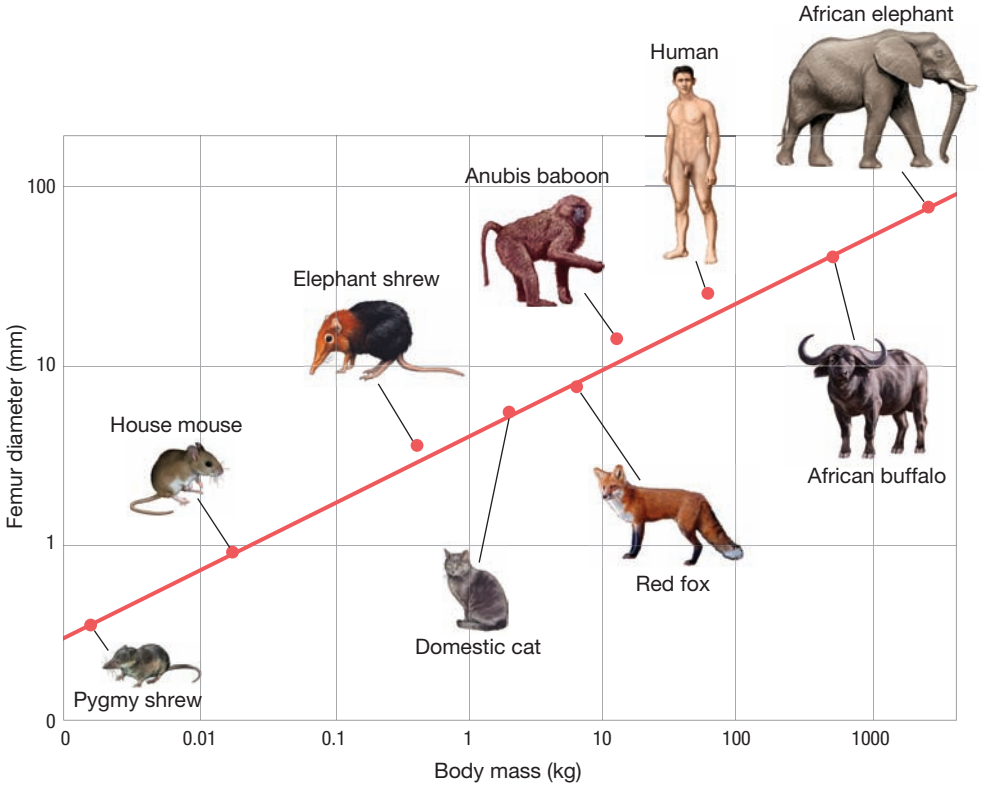


FIGURE 3.33 Femur size and body mass. Femur diameter exhibits a tight relationship with body mass for mammals ranging in size from the 3-gram pygmy shrew to the 5000-kilogram elephant. Both the *x* and *y* axes are plotted on a logarithmic scale. Adapted from Alexander et al. (1979).

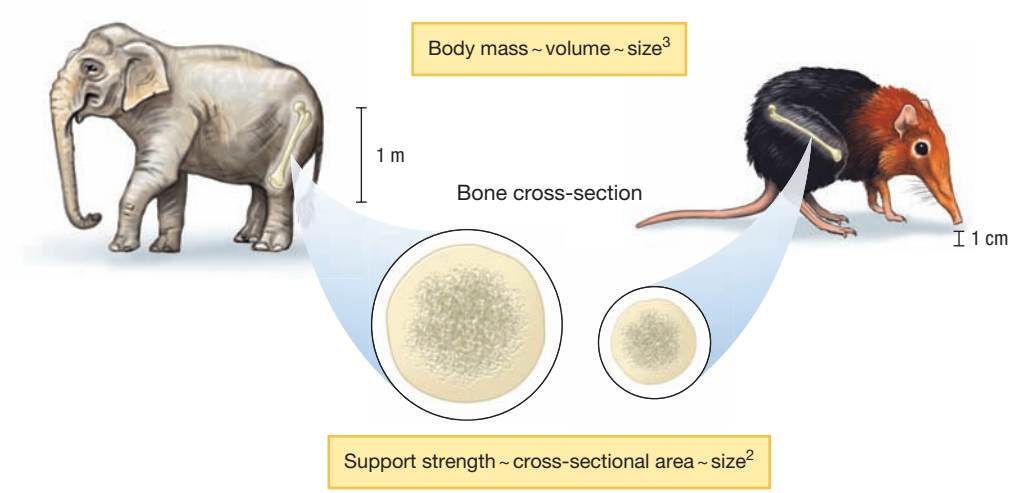


FIGURE 3.34 Elephants require proportionally thicker legs. Body mass scales with the third power of size, but support strength scales with the second power of size. As a result, larger animals such as elephants require proportionally thicker legs than small animals such as elephant shrews. This physical scaling relationship underlies the pattern illustrated in Figure 3.33.

Because of this scaling relationship, legs must get proportionally thicker, relative to size, as an animal gets larger. Thus, it is not that we cannot have creatures with the relative proportions of Dali's elephant; it is merely impossible to have elephant-sized creatures of these proportions. The harvestman arachnids (sometimes called daddy longlegs) and *Pholcus* spiders provide examples of how, at tiny size scales, natural selection can produce creatures with a limb geometry akin to that of Dali's elephant (**Figure 3.35**).

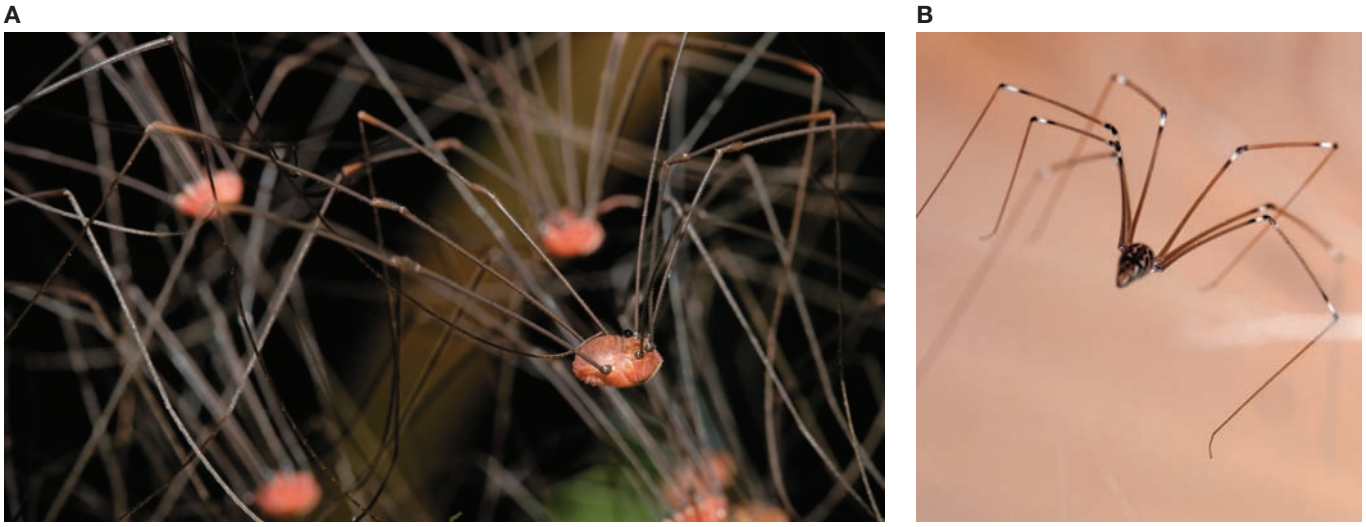


FIGURE 3.35 Harvestman and cellar spider. Arachnids show us that the relative dimensions of Dali's elephant—a large body on long, tiny legs—are not impossible in and of themselves. The problem is having these dimensions at the size of an elephant. **(A)** The harvestman (order Opiliones) is not a true spider. **(B)** The cellar spider (*Pholcus* sp.) is a true spider.

Selection, no matter how strong, is hard pressed to overcome the sort of physical constraints we have discussed. We see this in striking fashion with thoroughbred racehorses, which for centuries have been bred for the extreme speed that comes from having long, thin limbs. There has been sufficient genetic variation to allow breeders successfully to change the leg geometry of these horses—but at the cost of breeding horses that do not stand up particularly well in the real world. Thoroughbred horses suffer an extraordinary rate of limb fractures and other musculoskeletal injuries, and lameness afflicts a high proportion of racehorses. Epidemiological studies from several U.S. states indicate that in a single race, a horse has a greater than 0.1% chance of dying because of catastrophic musculoskeletal injury (Stover 2003).

Evolutionary Arms Races

Another important reason why organisms are not perfectly adapted to their surroundings is that their surroundings do not present a stationary "target" to which natural selection can optimize their phenotype. The abiotic environment changes over geological timescales: Ice ages come and go, oxygen concentrations rise and fall, continents shift, and temperatures fluctuate. Natural selection may produce organisms with adaptations to many of these slow changes, but there are faster changes in the abiotic environment as well. Conditions vary from season to season; on a slightly longer timescale, some years are drier or wetter, hotter or colder than others. But even more important evolutionarily are the changes in the biotic environment. Much of what is significant about an organism's environment is provided by other organisms, *who themselves are evolving by natural selection as well.* It is to this topic that we now turn.

Let us look at a couple of examples in which evolutionary change in one species can affect selective conditions for a second species—a phenomenon known as **coevolution** (Chapter 18). As a case in point, why are almost all organisms—ourselves included—vulnerable to infectious diseases? Why haven't we evolved better defenses against pathogens? We will explore this question in further detail in Chapter 20, but let us now briefly consider just one of the major reasons: We have not evolved impenetrable defenses against pathogens because our pathogens are evolving, too. As a pathogen's *hosts* evolve to deter or fight off infection more effectively, natural selection on the pathogen population intensifies, favoring variants that are able to elude the host's defenses.

The simultaneous action of natural selection on each side of the host–pathogen interaction is known as an **evolutionary arms race**, analogous to the bilateral weapons buildup that characterized the Cold War between the United States and the Soviet Union. Each side is continually selected for new weapons or new defenses that enable it to hold its own against the other.

We see a similar evolutionary arms race in the interaction between predators and prey. Prey are selected to become increasingly effective at escaping their predators; their predators in turn are selected to become increasingly good at capturing these ever-more-elusive prey. The prey is not always able to escape, and the predator is not always able to capture its mark because they are locked into a *coevolutionary* struggle. We will explore the coevolutionary process in detail in Chapter 18.

Natural Selection Lacks Foresight

A third reason why organisms are not perfectly adapted to their environments is that the process of natural selection lacks foresight. Natural selection has no way of anticipating the future beyond reacting to the past and the present, nor can it plan ahead by multiple steps. Selection favors changes that are immediately beneficial, not changes that may be useful at some time in the future. Thus, if a new structure is to arise by natural selection alone, every step along the way must be favored.

To get a sense of just how difficult it can be to evolve major new structures by incremental changes, consider the following challenge. Suppose that we play a game in which we are given an old jalopy and a warehouse full of auto parts. Our goal is to convert the jalopy into a sleek and powerful race car—but there is a catch. Each time we swap even a single part on the car, the rules state that the car has to be in running condition. Worse yet, after each swap, we have to be able to drive the car around a racetrack in faster lap time than it could achieve prior to the swap. This certainly restricts our options for how we do the work. We cannot, for example, strip the entire car down and change the whole transmission or the whole engine in one major overhaul. Instead, we have to find a path of gradual changes, switching single bolts and single belts and single pistons one by one, always improving the lap times, and eventually producing the race car.

Natural selection has to do something similar as body plans change and new structures evolve. Those evolutionary changes that arise by natural selection tend to make the organism more fit than it was before the changes took hold. And, of course, natural selection doesn't have intentionality; it does not have a goal or target "in mind." We could even say that, in our metaphor of the race car, the player doesn't know what the parts are or what they do. The player simply tinkers with the car, making little changes, keeping those that make the car faster, discarding those that do not.

Despite these difficulties, this problem is not insurmountable. There may be a sequence of single part swaps that enables the car to go from jalopy to race car, always reducing the lap times. This may require that some parts of the car change functions. For example, rather than fashioning a spoiler from scratch, we might build it out of another part of the car. Perhaps we might convert the lid of the trunk into a spoiler. Why not? Race cars don't need a trunk for carrying luggage. Another possibility is that we might add new parts to the jalopy before removing old ones. We could add disc brakes before removing the current drum system. We could even add parts that we would later remove entirely; we could add structural supports to carry the car through some of the intermediate stages, and then remove them later to reduce weight.

Natural selection can take analogous paths on the way to evolving new structures. And, of course, natural selection is not the only evolutionary process operating; as we will see in later chapters, mechanisms including genetic drift, genetic hitchhiking, and many other processes also play important roles in determining the direction of evolutionary change. Thus, new structures can arise from a combination of selective and nonselective processes.

We have seen how the process of natural selection requires three components—variation, heritability, and fitness differentials. When a trait has been under

natural selection for a specific function in a specific population, and that trait serves the same primary function or functions today as it did in the past, we call it an adaptation. Adaptations can be studied both in the wild, as we saw with oldfield mice, guppies, and cliff swallows; and in the laboratory, as we discovered in our discussion of cell size and temperature sensitivity in *E. coli*. Through the use of studies that have ranged from the scale of the molecule to the whole organism, we have also explored various ways that the evolutionary process can lead to complex traits, such as the vertebrate eye and the aldosterone–M receptor pairing both through classic step-by-step adaptation for a specific function and through exaptation. We have also seen that constraints limit the power of selection.

We now shift our emphasis from natural selection and the adaptations it produces to phylogeny and common descent in Chapters 4 and 5.

SUMMARY

1. Evolution by natural selection is the inevitable consequence of three simple conditions: variation, inheritance, and differential reproductive success.
2. Natural selection does not act directly on genotypes: It operates on phenotypic differences among the individuals in a population.
3. Evolution by natural selection is a process by which the characteristics of a population—not those of an individual—change over time.
4. The fitness of a trait or gene is defined as the expected reproductive success of an individual with that trait or gene *relative* to the reproductive success of other members of the population.
5. An adaptation is an inherited trait that makes an organism more fit in its abiotic and biotic environments and which has arisen because of the direct action of natural selection for its primary function. An exaptation is a trait that serves one purpose today but served a different function in the past.
6. Evolutionary processes can be observed and manipulated in real time in the field and in the laboratory.
7. The process of natural selection operates on physical structures in the material world, and as such is constrained by the same physical and mechanical laws that limit the realm of possibility for human engineers.
8. The process of natural selection has no way of anticipating the future, nor can it plan ahead. Selection favors changes that are immediately beneficial, not changes that may be useful some time in the future.

KEY TERMS

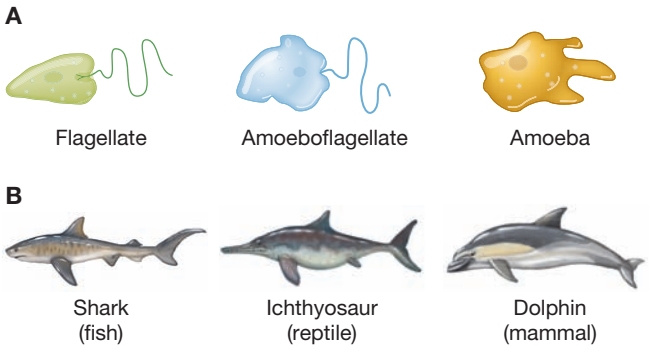
adaptation (p. 78)
antagonistic pleiotropy (p. 89)
coevolution (p. 104)
differential reproductive success (p. 67)
evolutionary arms race (p. 104)
exaptation (p. 79)
gene duplication (p. 97)
gene sharing (p. 97)
inheritance (p. 67)
life history strategy (p. 80)
marker gene (p. 85)
norm of reaction (p. 70)
pleiotropic genes (p. 89)
trade-off (p. 81)
variation (p. 67)

REVIEW QUESTIONS

1. What are the three conditions necessary for natural selection to occur? Explain why each is necessary for evolution by natural selection.
2. What is a norm of reaction?
3. Define the term *fitness* as used by evolutionary biologists.
4. What trade-off led to differences in guppy life history between high- and low-predation sites?
5. Explain how Lenski and Travisano's experiment with replicate lines of *E. coli* revealed limits to how predictable evolution by natural selection is.
6. Explain why a lack of variation can constrain evolution by natural selection.
7. Give an example of an evolutionary arms race.
8. Describe two different pathways by which complex traits can arise through natural selection.
9. Figure 3.3 shows how the heights of yarrow plants depend on genotype and environment. Redraw the data from this figure for genotypes 1–4 as a set of norm of reaction curves, analogous to those shown in Figure 3.4.

KEY CONCEPT APPLICATION QUESTIONS

10. A norm of reaction maps the way that genes are expressed in different environments. Distinguish this from the Lamarckian idea of the "inheritance of acquired characteristics" that we discussed in Chapter 2.
11. How has experimental evolution—along the lines of the *E. coli* experiment we discussed—revolutionized the sorts of questions evolutionary biologists can now test?
12. Jacques Monod said that evolution operates like a "tinkerer." What do you think he meant by this?
13. Explain how it can be true that natural selection acts on *phenotypes*, but the result of natural selection is often measured in terms of changes to *gene* frequencies?
14. As shown in the illustration that follows, unicellular swimmers (**A**) lack the streamlined form of large swimming vertebrates (**B**). Why do unicellular swimming organisms have a very different body shape than that of swimming vertebrates?



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4

Phylogeny and Evolutionary History

◀ A branching quiver tree (*Aloe dichotoma*) in Namibia's NamibRand Nature Reserve.

The world is filled with a bewildering diversity of forms, and nowhere is this more true than in the biological domain (**Figure 4.1**). To make sense of the world with all of its variation, we categorize the objects in it—but this is a difficult endeavor in its own right. What is the best way to break up the infinite variety out there in the world into a set of discrete categories? The Argentine writer Jorge Luis Borges describes one fanciful approach, as taken in a fictional Chinese encyclopedia known as the *Celestial Emporium of Benevolent Knowledge*:

> In its distant pages it is written that animals are divided into (a) those that belong to the emperor; (b) embalmed ones; (c) those that are trained; (d) suckling pigs; (e) mermaids; (f) fabulous ones; (g) stray dogs; (h) those that are included in this classification; (i) those that tremble as if they were mad; (j) innumerable ones; (k) those drawn with a very fine camel's-hair brush; (l) et cetera; (m) those that have just broken the flower vase; (n) those that at a distance resemble flies. (Borges 1964)

FIGURE 4.1 An artist's view of biodiversity. A detail of Henri Rousseau's painting *Exotic Landscape* (1910; left) and his painting *The Merry Jesters* (1906; right).

To most of us, this classification scheme seems strange and disorienting—and that was exactly Borges' intent. But what is the "right" way to divide up the diversity of living things?

Evolutionary biology provides an answer to this question. A bit of history shows how. The basic *Linnaean taxonomy* and resulting system of scientific names that biologists have used for nearly three centuries did *not* derive from evolutionary thinking. The taxonomic system was developed by Carolus Linnaeus (1707–1778), a Swedish botanist, zoologist, and physician who wrote *Systema Naturae*. This taxonomy has proved so very useful because of Linnaeus' insight that organisms can be arranged in a hierarchical classification. Linnaeus recognized that not only can we assign species or subspecies to groups of highly similar organisms, but we can also array these groups of similar species into larger groups of moderately similar organisms, and these larger groups can in turn be categorized into yet larger groups of somewhat similar organisms, and so forth, until we have accounted for all living things. It was a remarkable insight, but Linnaeus came to this realization without having a theoretical basis for *why* these hierarchical patterns of similarity should exist. As we discussed in Chapter 2, Darwin provided the answer for why these patterns are seen. He recognized that an evolutionary process of branching descent with modification would generate nested hierarchies of similarity as the natural results of phylogenetic history. Not only did Darwin's idea of a branching pattern of descent with modification provide a theoretical foundation for the hierarchical patterns Linnaeus suggested, but also Darwin's approach led to changes in the classification of many species, genera, and families.

German biologist Willi Hennig (1913–1976) eventually revisited the problem of taxonomy using Darwin's ideas and, in doing so, established the modern

approach to classification (Zuckerkandl and Pauling 1962; Hennig 1966). The title of Hennig's classic 1966 book—*Phylogenetic Systematics*—is instructive because it emphasizes that in addition to documenting evolutionary history, phylogenetic trees can help us classify, or *systematize*, the world we see around us. We could classify organisms in many ways; for example, by how large they are, by where they live, or by their morphology. But in **phylogenetic systematics**, we classify organisms according to their evolutionary histories—and phylogenetic trees are our way of representing these evolutionary relationships.

Our goal in this chapter is to introduce the central role of phylogenetic thinking within evolutionary biology. In so doing, we will address the following questions:

- How do we read and interpret a phylogenetic tree?
- How do phylogenetic trees help us make sense of—and classify—the diversity of life?
- How do phylogenetic trees help us understand the evolutionary origin of similarities among species and differences between species?
- How do we map traits onto phylogenetic trees to generate and test hypotheses about evolutionary events?

4.1 Phylogenies Reflect Evolutionary History

One of the principal aims of modern evolutionary biologists is to reconstruct and understand patterns of common descent and to use knowledge about the patterns of descent to understand the evolutionary events that have transpired throughout the history of life on Earth. This is the study of **phylogeny**—the branching relationships of populations as they give rise to multiple descendant populations over evolutionary time.

On a grand scale, the study of phylogeny allows us to reconstruct the tree of life—the historical relationships that connect all living things—and to understand the major events in evolutionary history. On a narrower scale, we may be interested in understanding the history of descent and the relationships among genera within a family of organisms, among species within a genus, or even among populations of a single species (**Figure 4.2**). Doing so requires taking a historical perspective and probing for evidence of common ancestry and for information that sheds light on how various species are related to one another (**Box 4.1**).

The study of phylogeny rests on our observations of characters displayed by organisms. **Characters** can be any observable characteristics of organisms; for example, they may be anatomical features, developmental or embryological processes, behavioral patterns, or genetic sequences. Coat color, for example, is a character. **Traits,** or character states, are the specific values of a character. "Brown coat" and "white coat" are possible traits for the coat color character. Until the major advances in molecular genetics that occurred in the 1970s, almost all characters used in the study of phylogeny were morphological or anatomical—bone length, tooth shape, and so on. With the advent of molecular genetics, actual DNA sequences are now the most common characters used to reconstruct phylogenies of extant organisms.

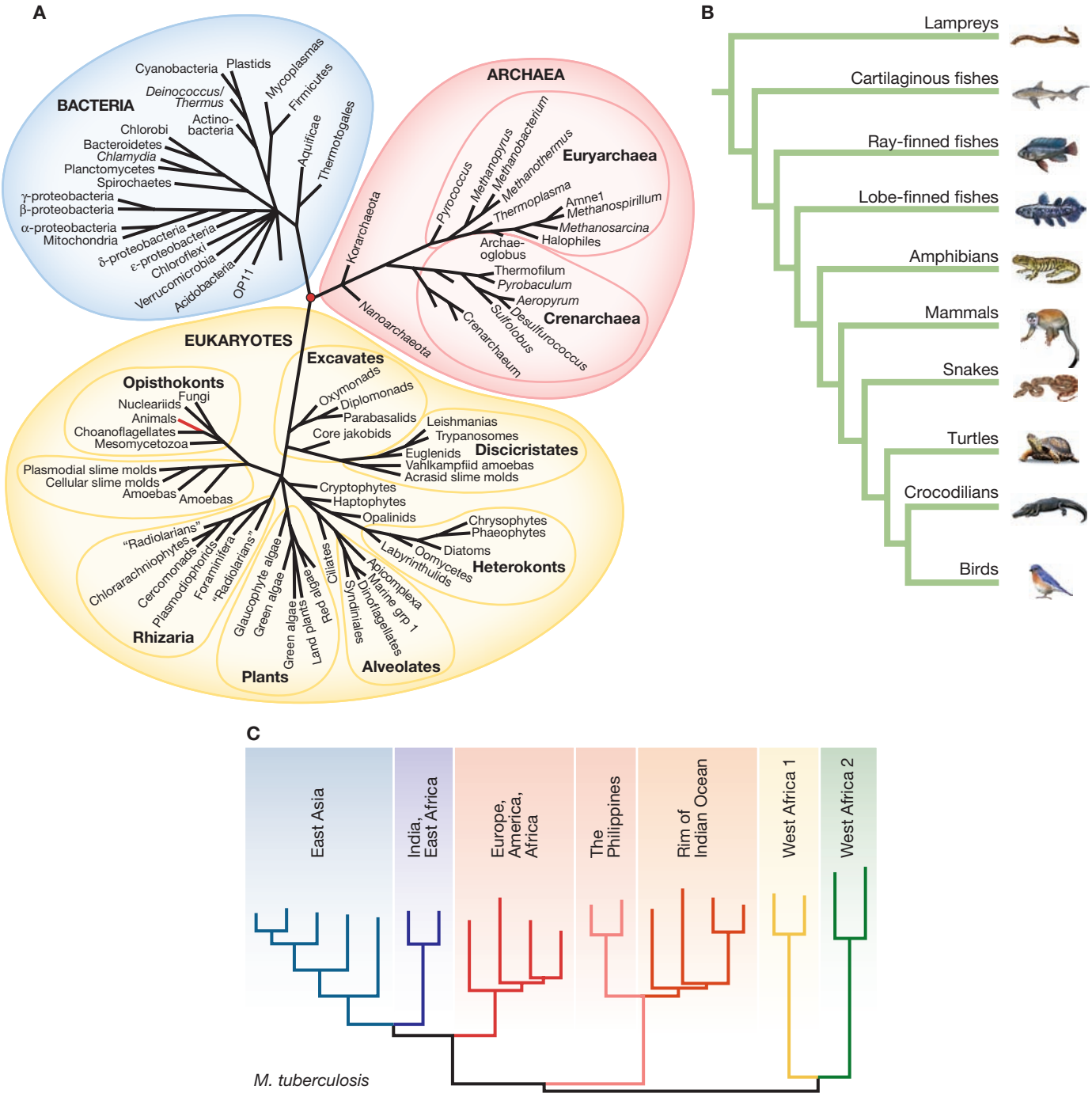


FIGURE 4.2 Phylogenies at different scales. **(A)** The tree of life represents the historical relationships among all living things. The entire animal kingdom is contained in the tiny highlighted branch on the left side of the Eukaryotes. Adapted from Baldauf et al. (2004). **(B)** A phylogeny of vertebrates. Adapted from the Center for North American Herpetology (2010). **(C)** A phylogeny of *Mycobacterium tuberculosis* isolates from human patients, with geographic origins indicated. Adapted from Comas et al. (2010).

KEYCONCEPT QUESTION

4.1 Based on Figure 4.2A, why do you think it has been harder to develop drugs to control fungal infections than it has been to develop drugs to control bacterial infections?

BOX 4.1 What Is the Difference between a Pedigree and a Phylogeny?

If you have ever studied your own family history, you may have come across diagrams known as *family trees* or *pedigrees.* An example is shown in **Figure 4.3**.

In some ways, pedigrees may seem very much like phylogenies. Both represent patterns of ancestry using treelike branching diagrams. But there are important distinctions. A pedigree tells us about the ancestry of *individuals*, whereas most phylogenies tell us the ancestry of *populations*. Thus, the *nodes* in a pedigree represent individuals, while the nodes in a phylogeny typically represent populations. Moreover, because every individual of a sexual species has two parents, each node in a pedigree has two immediate ancestors (mother and father) and can leave any number of immediate descendants. By contrast, in a conventional phylogeny, we assume populations *split* in two, but never recombine. Thus, in a phylogeny, each node has a single direct ancestor and two direct descendants (if any). As a result, a pedigree tends to expand as one looks backward in time: two parents, four grandparents, eight great-grandparents, and so forth. By contrast, a phylogeny expands as we move forward in time. Both are often drawn in a fanlike shape, broad at the top and narrow below; by convention, time typically runs downward in a pedigree and upward in a phylogeny.

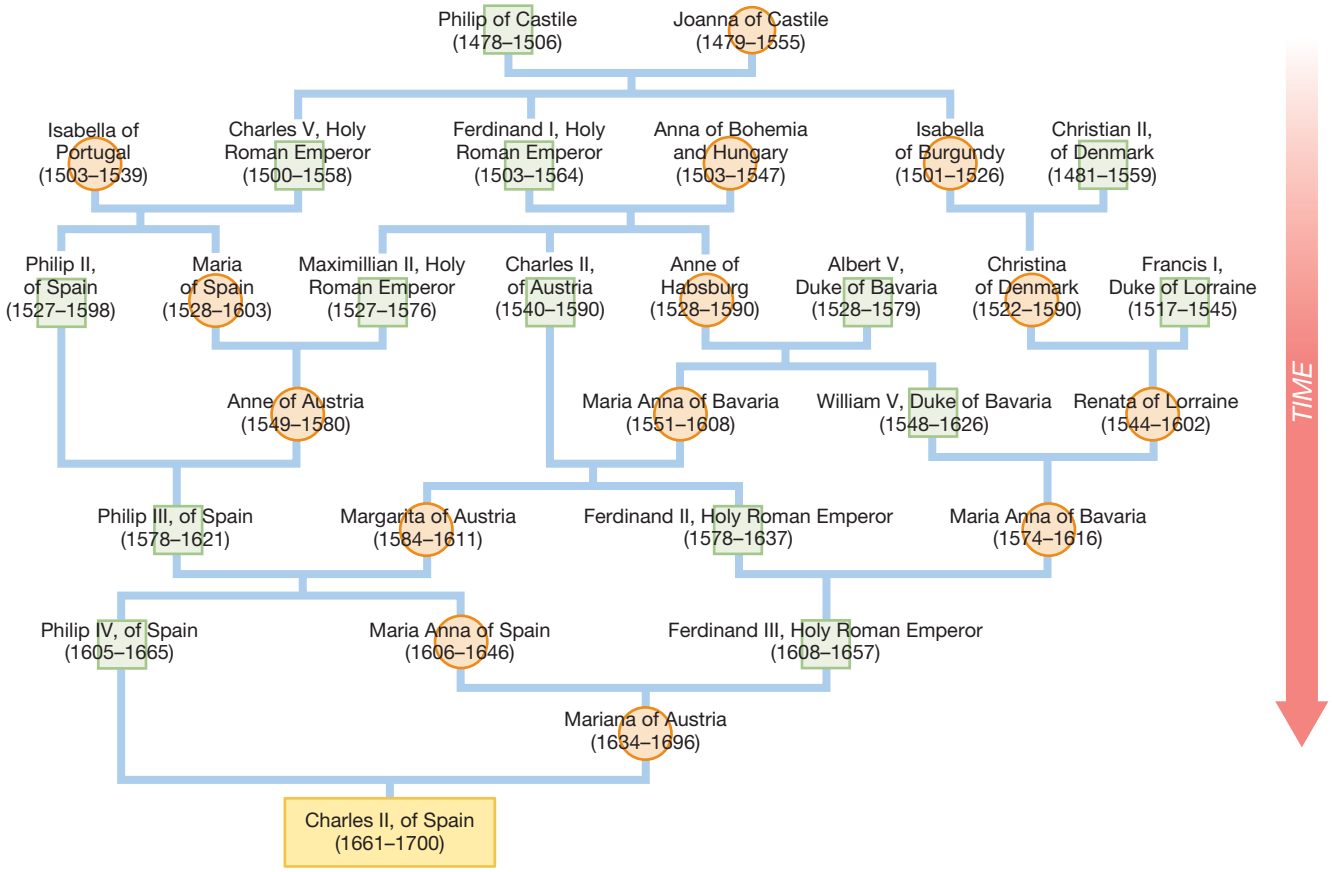


FIGURE 4.3 Pedigrees. The ancestry of King Charles II of Spain. Males are indicated as green squares, and females are indicated as orange circles. This pedigree shows an exceptional degree of inbreeding—mating among close relatives—which was doubtless responsible for the severe genetic disorders that crippled Charles II, the last of the Spanish Habsburgs (Alvarez et al. 2009). Adapted from Wikimedia Commons (2006).

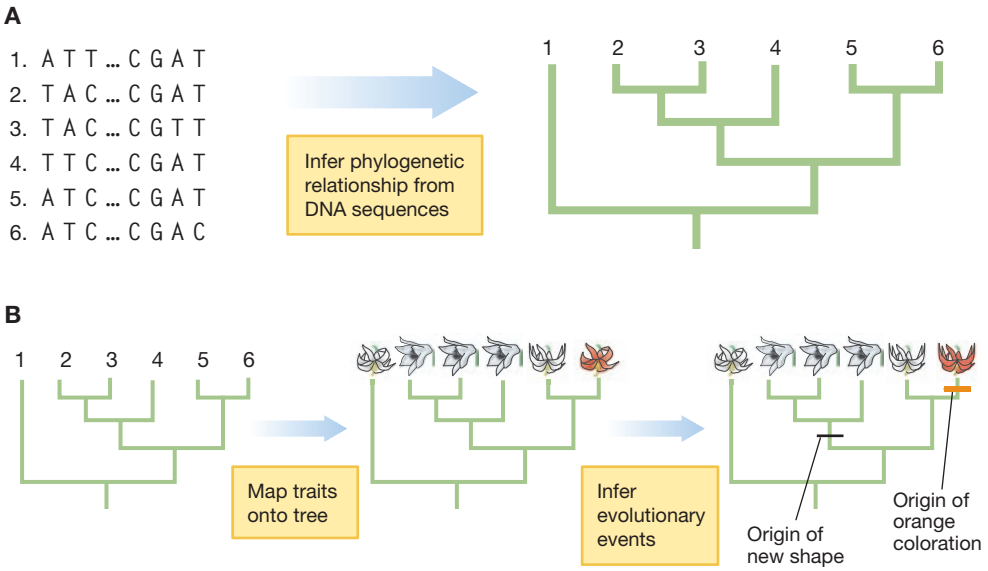


FIGURE 4.4 Characters and trees. We use traits both to reconstruct phylogenetic trees and to generate hypotheses about the timing of events in evolutionary history. **(A)** One set of characters—here genetic sequence data—is used to infer a phylogenetic tree for the species of interest. **(B)** A second set of characters, here flower color and morphology, are mapped onto the tree, helping us to reconstruct evolutionary events. The origin of the orange flower coloration is indicated by the orange horizontal bar. The origin of the novel flower shape is indicated by the black horizontal bar.

Traits are critical in the study of phylogeny for two reasons: (1) We use observations of traits to infer the patterns of ancestry and descent among populations. We then represent these patterns in graphical form as a phylogenetic tree. (2) By mapping additional traits onto a phylogeny we have already created, we can study the sequence and timing of evolutionary events (**Figure 4.4**).

Both the process of reconstructing trees and the process of mapping evolutionary events onto preexisting trees generate hypotheses. A phylogenetic tree is a hypothesis about evolutionary relationships. The location and order of evolutionary events on a tree is likewise a hypothesis about the way that evolutionary history has unfolded. As with any scientific hypothesis, these hypotheses are tested and are subject to refinement or refutation. When new evidence is obtained, we test our current phylogenetic trees, or our current inferences about evolutionary events, against this new evidence to see whether our previous hypotheses are consistent with the new findings. If they are, the phylogenetic trees that we have constructed remain our working hypotheses; if they aren't, we reevaluate and modify the trees given our new evidence. All of science operates in this fashion, and the study of phylogeny, while focused on past events, is no different.

In most instances, we cannot replicate the historical conditions or events in which we are interested, but we can look at how different past scenarios make different predictions about current observations. We can test these predictions by looking at new data and seeing which of the past scenarios best explains these new observations. While we can uncover new data simply by looking in new places, as does a paleontologist who uncovers a new fossil, we often obtain new data through the use of new technologies.

One of the most striking examples of this comes from Darwin's predictions regarding the patterns of phylogenetic relatedness across the tree of life. Darwin inferred the patterns of common ancestry without a mechanistic understanding of genes, DNA, or heredity. His hypothesis about past events—the patterns of common ancestry of all living things—made a strong prediction that later became testable. Once DNA was identified as the carrier of hereditary genetic information and the revolution in molecular genetics allowed researchers to read off this information by DNA sequencing, scientists had a vast body of new data with

which to test Darwin's hypotheses about ancestry. If Darwin's theory of descent with modification is correct, patterns of DNA sequence similarity should reflect the patterns of common ancestry that have been inferred from other evidence, such as morphological characters, fossil evidence, and phylogeography. We would not expect such patterns of DNA sequence similarity under hypotheses of special creation or independent parallel formation of lineages followed by inheritance of acquired characteristics, as Lamarck proposed (Chapter 2). It has been a major triumph for evolutionary biology that the enormously rich data about genetic sequences, although entirely unknown to Darwin, strongly support the patterns of common ancestry he proposed.

4.2 Reading Phylogenetic Trees

Before going further, let us explore how to read a phylogenetic tree. The trees in Figure 4.5 shows the pattern of evolutionary relationships among the vertebrates. In these phylogenies, each branch tip represents a group of related organisms, or a **taxon**. These phylogenies shows the relationships among such taxa (the plural of taxon) as birds, crocodilians, and mammals. Figure 4.5 shows two different ways of conveying exactly the same information: In Figure 4.5A, the phylogeny is drawn in *tree format*, as a set of nested rectangular brackets; in Figure 4.5B, the same phylogeny is illustrated in a slanting structure known as *ladder format* (Novick and Catley

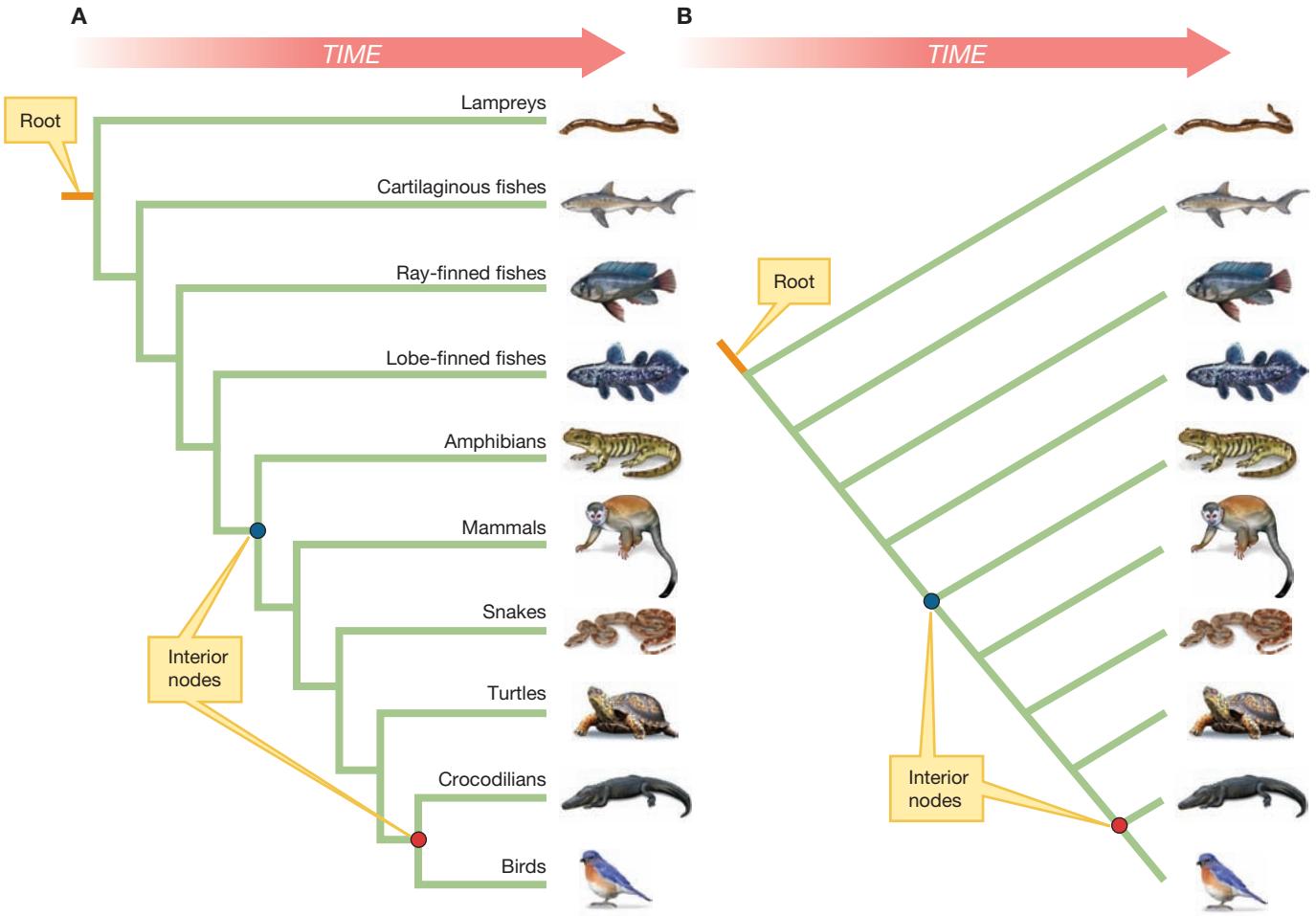


FIGURE 4.5 Two equivalent ways of drawing a phylogeny. The two phylogenies of the vertebrates shown each illustrate exactly the same information. The phylogeny on the left **(A)** is sometimes referred to as a *tree* representation, whereas that on the right **(B)** is termed a *ladder* representation. In each, time flows from left to right, so that the branch tips at the right represent current groups, whereas the *interior nodes* (nodes on the inner section of the tree) represent ancestral populations. For example, the red dot indicates the common ancestor of birds and crocodilians, whereas the blue dot indicates the common ancestor of all tetrapods. The orange line segment is the root of the tree, the ancestral lineage from which all other lineages on the tree are derived. Adapted from the Center for North American Herpetology (2010).

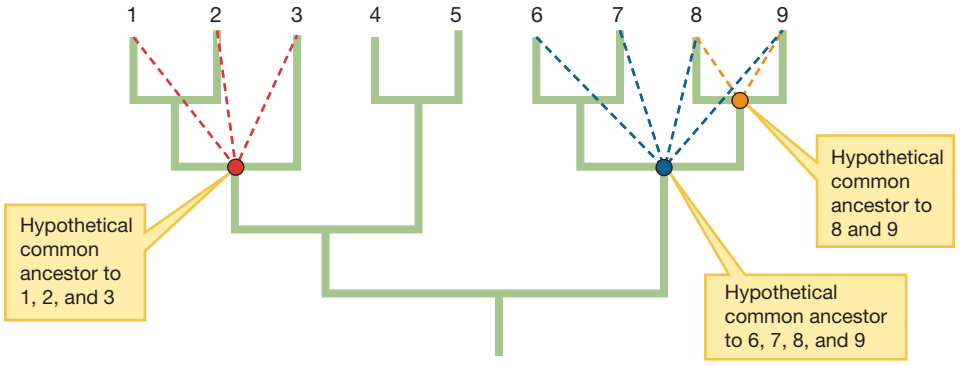


FIGURE 4.6 Interior nodes represent common ancestors. Finding the common ancestor for a group involves tracing backward in time. Follow the dashed lines to see the common ancestors of different groups in this phylogeny.

2007, 2013). These two ways of drawing a phylogeny are entirely interchangeable, and typically a phylogeny will be represented using one (but not both) of these equivalent approaches. Similarly, orientation of the tree does not matter: Phylogenetic trees can be drawn with the root at the left and the branch tips at the right as in Figure 4.5A or, equivalently, with the root at the bottom and the branch tips at the top as in **Figure 4.6**. It makes no difference to the meaning of the tree. Trees can even be drawn with the root at the right and the tips at the left or with the root at the top and the tips at the bottom, although we seldom see these orientations in practice.

The branch points where the tree splits are called **nodes**. These represent common ancestors to the species (or, more generally, taxa) that come after the splitting or branching point. All branch tips arising from a given node are descendants of the common ancestor at that node. For example, in Figure 4.5 the red dot highlights the node representing the common ancestor of birds and crocodilians, and the blue dot indicates the common ancestor of all tetrapods. To find the most recent common ancestor of two or more species, then, we can simply trace backward along the tree until the branches leading to these species converge. Figure 4.6 illustrates this idea. At the base of the tree, indicated in orange in Figure 4.5, we see the **root**—the common ancestors to all the species on the tree.

It is important to recognize that each interior node in a phylogenetic tree represents a population that existed at some time in the past, rather than a present-day population. Thus, the common ancestor of the tetrapods was not identical to any currently living tetrapod. Rather, evolutionary change has occurred along each and every branch leading from this ancestor to the species we observe in the world around us today.

One thing that can be confusing about phylogenetic trees is that any given set of evolutionary relationships can be depicted in multiple ways. As an example, in **Figure 4.7**, notice that you can flip or "rotate" any node on a phylogenetic tree—for example, reversing the position of the green cube and the orange pyramid—without changing the evolutionary relationships that the tree represents. If the tree indicates that any two species A and B are more closely related to each other than to a third species C before a rotation, it will indicate that they are more closely related to each other after a rotation as well.

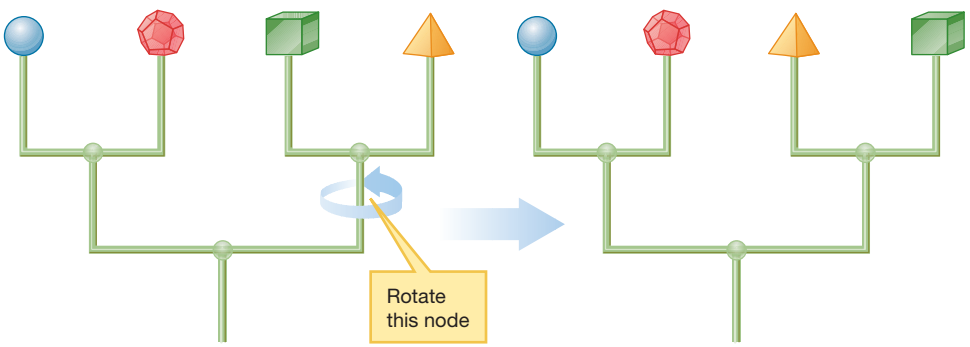


FIGURE 4.7 Rotating around any node leaves a phylogeny unchanged. Imagine that a phylogenetic tree was constructed of balls for nodes and sticks for branches. One could rotate any node 180° in space without changing the structure of the tree itself. The tree may look different, but notice that the relationships between nodes remain unaltered by the rotation.

As a result, there are a number of different ways that we can draw the very same phylogenetic tree, as **Figure 4.8** illustrates. In panel i, we see a phylogenetic tree for four species: 1, 2, 3, and 4. As previously described, however, we can rotate any node—or any combination of nodes—without altering the evolutionary relationships that the tree depicts. Panels ii, iii, iv, and v show four equivalent trees generated from the rotations in panel i.

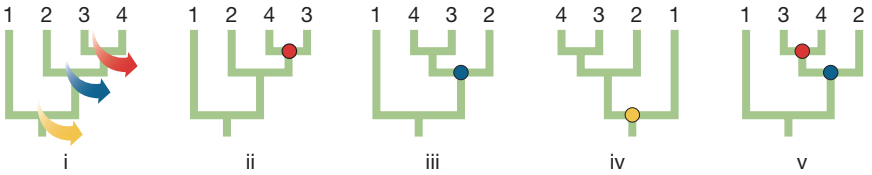


FIGURE 4.8 Rotating phylogenetic trees. One can rotate any node—or any combination of nodes—in a phylogeny without changing the structure of the tree. Thus, all five trees in this row are identical from a phylogenetic perspective. The colored dots indicate the nodes that were rotated to get from panel i to the present tree in each case.

From this equivalence of trees, we can see that the relative positions from left to right of the branch tips do not tell us anything about how closely related two species are. What matters is the distance to the most recent common ancestor. In panel v, for example, species 1 is immediately adjacent to species 3, whereas species 2 is more distant, left-to-right, from species 3. Yet, as we can see by tracing back along the tree to the most recent common ancestor, species 3 is more closely related to species 2 than to species 1.

Clades and Monophyletic Groups

As we mentioned, phylogenetic trees are hypotheses. **Figure 4.9** shows two competing hypotheses for the evolutionary relationships among the mammalian groups of placentals (for example, elephants), marsupials (for example, kangaroos), and monotremes (for example, egg layers such as platypuses). Each phylogeny shows the relationships among these three groups of mammals, along with squamate reptiles as an *outgroup*—a taxon that is related to the groups of interest but which branched off earlier in evolutionary history. Figure 4.9A illustrates the hypothesis favored by a majority of systematists. Here, marsupials and placentals are **sister taxa**—taxa derived from the same node—and they are more distantly related to

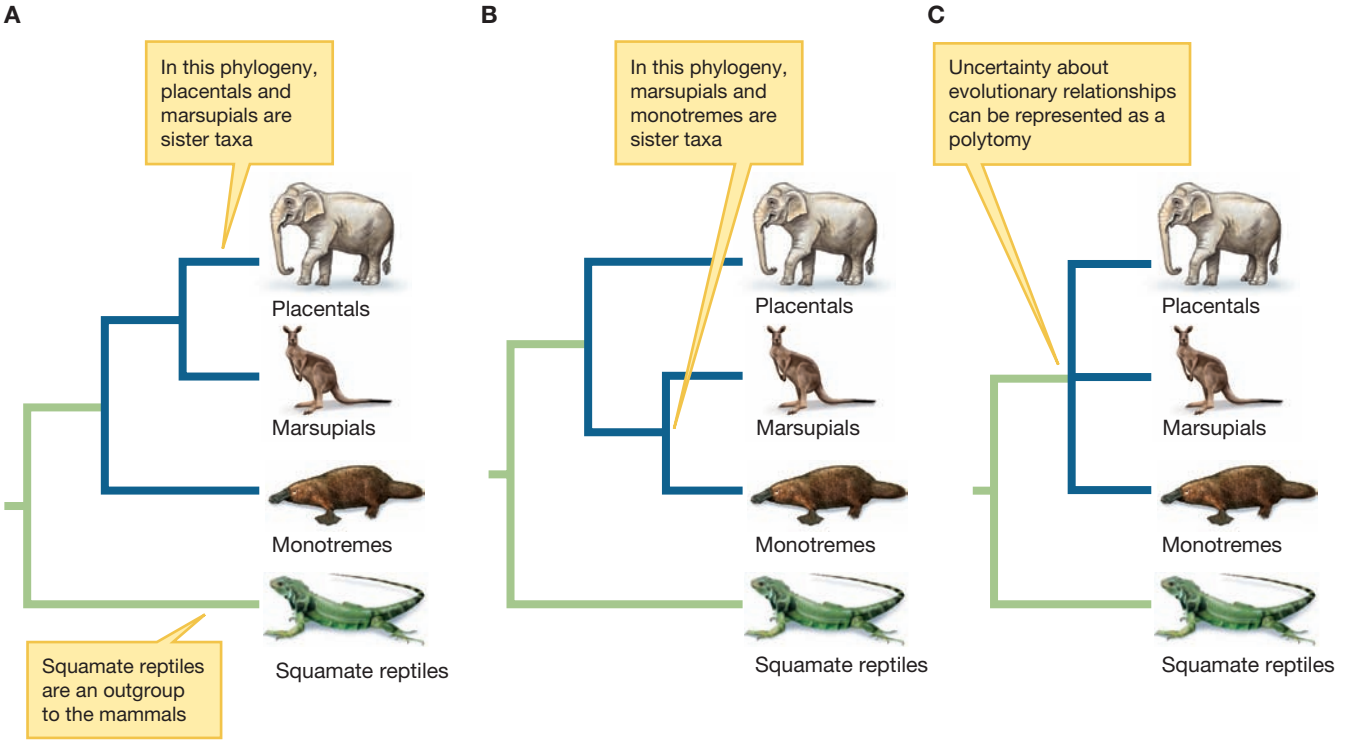


FIGURE 4.9 Polytomies represent uncertainty about phylogenetic relationships. Two competing hypotheses for the evolutionary relationships among mammalian groups: **(A)** Marsupials and placentals may be sister groups or **(B)** marsupials and monotremes may be sister groups. **(C)** We can capture the uncertainty about the relationship among placentals, marsupials, and monotremes by representing the groups as a polytomy. Adapted from Meyer and Zardoya (2003).

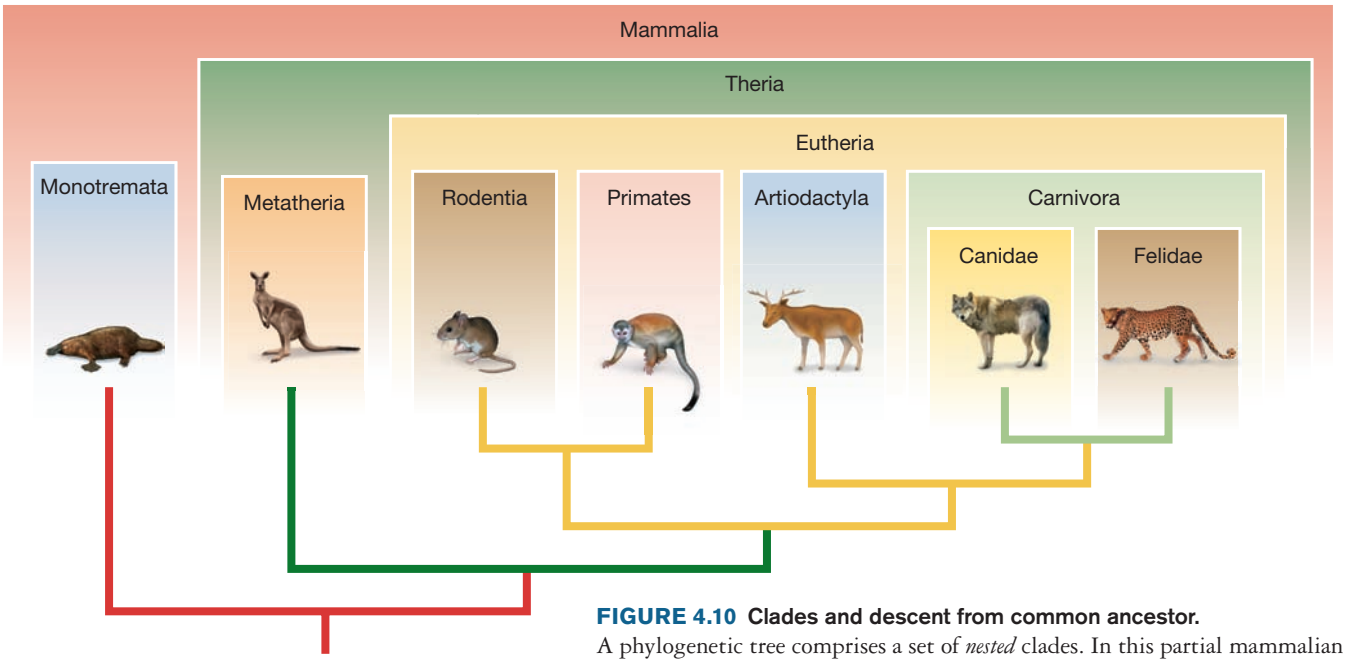


FIGURE 4.10 Clades and descent from common ancestor. A phylogenetic tree comprises a set of *nested* clades. In this partial mammalian phylogeny, different colors represent different clades, with the red clade Mammalia being the most encompassing of the clades here. The dark green clade Theria, and the yellow clade Eutheria are nested within clade Mammalia, as are the other smaller clades such as Monotremata, Metatheria, etc. Thus, a given species is a member of multiple clades at multiple levels. Adapted from Springer et al. (2004).

monotremes than they are to each other. Figure 4.9B shows an alternative phylogeny in which marsupials and monotremes are sister groups and are more distantly related to placentals. In cases where the relationships among three or more groups are unresolved, we can communicate the uncertainty as a **polytomy**—a node with more than two branches arising from it (Figure 4.9C).

A key concept in phylogenetic taxonomy is that we can use a phylogenetic tree to tell us what constitutes "natural" groupings of organisms. Here, the principal idea is that the natural groupings, which we call clades, are monophyletic groups (Baldauf 2003). A **monophyletic group** is defined as a taxonomic group consisting of all descendants of the group's most common ancestor and no other members. A **clade**, then, always consists of a group of species that share a single recent common ancestor. All species that descended from this ancestor are in the clade, and, furthermore, all species not descended from this ancestor are *not* members of that clade. **Figure 4.10** illustrates a partial phylogeny of the mammals, made up of a number of clades from small (Canidae, Felidae) to large (Mammalia). This figure shows how clades are

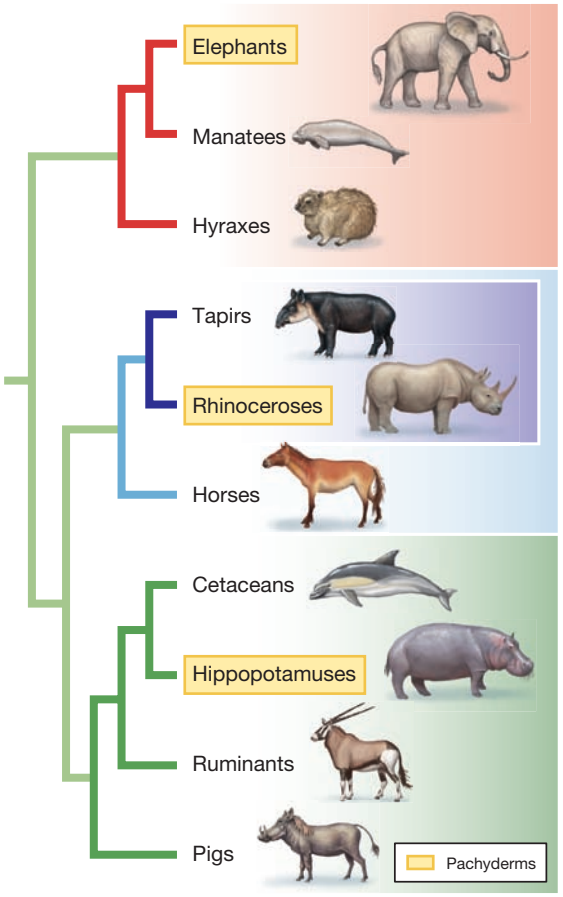


FIGURE 4.11 Pachyderms as a polyphyletic group. A partial phylogenetic tree of the mammals shows examples of monophyletic groups. Elephants, manatees, and hyraxes form one monophyletic group (in red); tapirs and rhinoceroses form another (in purple); and tapirs, rhinoceroses, and horses form a third (in blue). However, *pachyderms*—elephants, rhinoceroses, and hippopotamuses—are not a monophyletic group. Adapted from Murphy et al. (2001).

nested hierarchically just as in Linnaean taxonomy: The clades Canidae and Felidae are nested within the clade Carnivora, which itself is part of the clade Eutheria, which is part of the clade Mammalia.

To understand better the concept of a monophyletic group, let us look at how a group can fail to be monophyletic. **Figure 4.11** is another partial phylogeny of the mammals. In this figure, we see numerous monophyletic groups. For example, the group "elephants, manatees, and hyraxes" is one such monophyletic group; the group "tapirs and rhinoceroses" is another; and "tapirs, rhinoceroses, and horses" is yet a third.

But the group of organisms known as the *pachyderms*—elephants, rhinoceroses, and hippopotamuses—is not a monophyletic group because it includes neither the common ancestor of its members, shown at the root of our tree, nor all descendants of that common ancestor. A disjointed group such as pachyderms is called a **polyphyletic group**. Because polyphyletic groups do not represent proper evolutionary clades, groups such as pachyderms are no longer used in modern systematics.

There is another, perhaps more subtle way that a group can fail to be monophyletic. A **paraphyletic group** is one that contains the group's most common ancestor but not all of its descendants. We turn to yet another tree to illustrate this point. In **Figure 4.12** we revisit our phylogenetic tree of the vertebrates.

Here again we see numerous monophyletic groups; for example, the tetrapod vertebrates are the monophyletic group that includes birds, crocodilians, turtles, snakes, mammals, and amphibians. The group fish—lampreys, cartilaginous fishes, ray-finned fishes, and lobe-finned fishes—might seem to be another natural group. Of these taxa, fish share a common ancestor that we would also classify as a fish. But not all descendants of that common ancestor are fish; after all, its descendants also include all of the tetrapod vertebrates, none of which we would call fish. Thus, fish are a paraphyletic grouping.

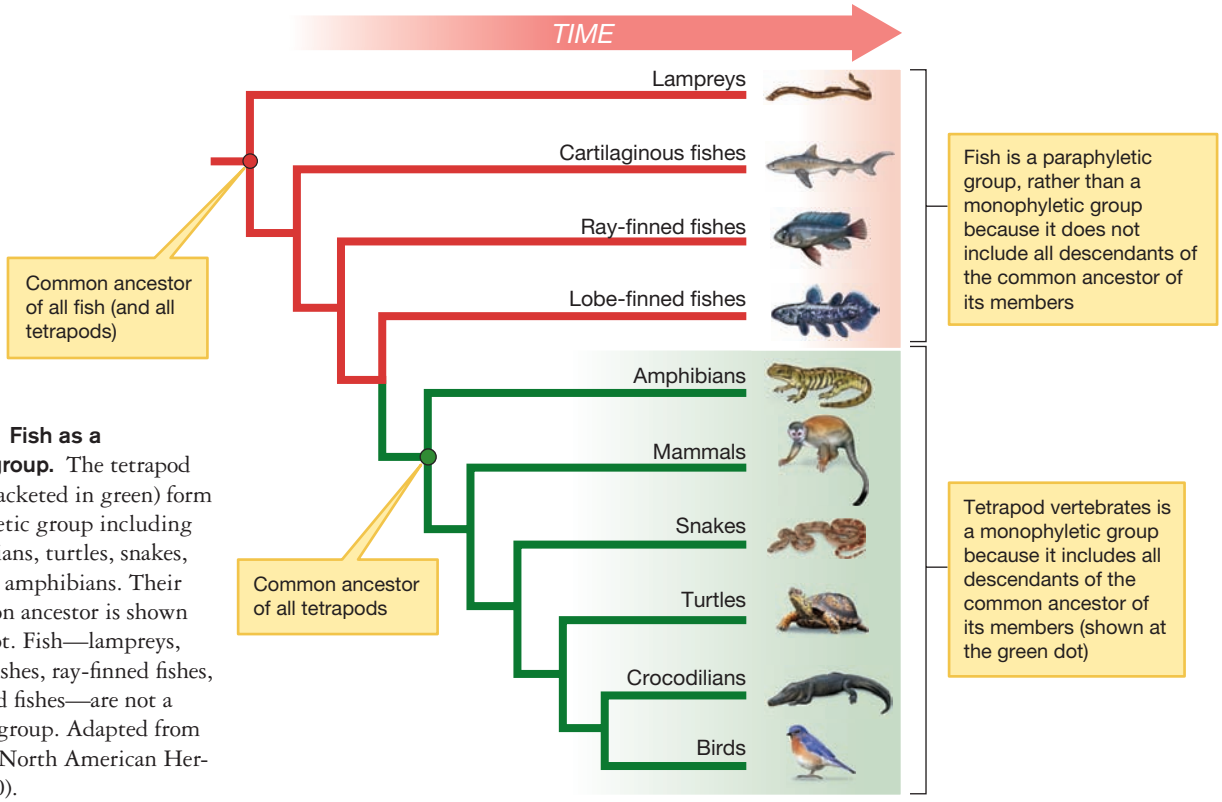


FIGURE 4.12 Fish as a paraphyletic group. The tetrapod vertebrates (bracketed in green) form one monophyletic group including birds, crocodilians, turtles, snakes, mammals, and amphibians. Their unique common ancestor is shown at the green dot. Fish—lampreys, cartilaginous fishes, ray-finned fishes, and lobe-finned fishes—are not a monophyletic group. Adapted from the Center for North American Herpetology (2010).

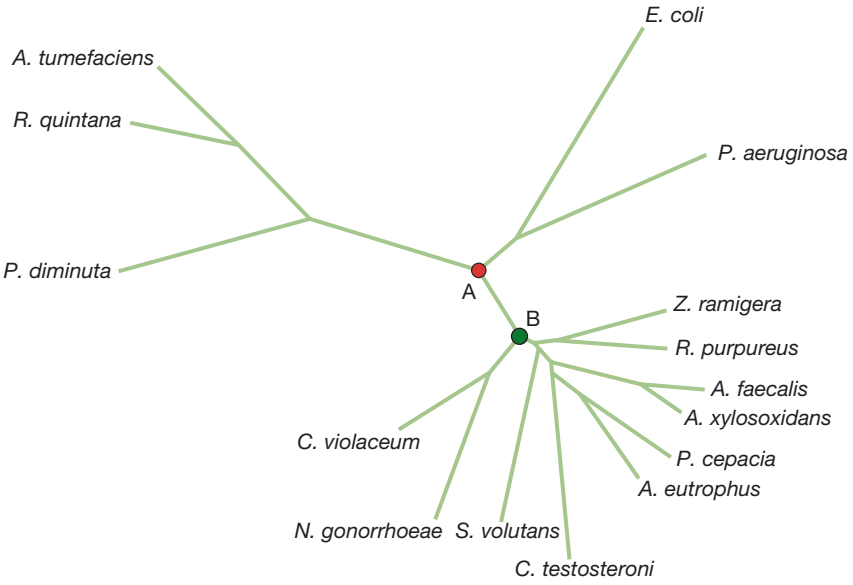


FIGURE 4.13 Unrooted tree of proteobacteria. An unrooted tree illustrates the evolutionary relationships among the proteobacteria, a large group of bacteria including human-associated species such as *Escherichia coli* and nitrogen-fixing species such as *Agrobacterium tumefaciens*. Because the tree is unrooted, it does not indicate whether, for example, interior node A represents a more recent or less recent population than that represented by interior node B. Adapted from Shin et al. (1993).

KEYCONCEPT QUESTION

4.2 Based on the tree in Figure 4.12, explain why reptiles are not a monophyletic group.

Rooted Trees and Unrooted Trees

Thus far, all of the trees we have looked at have been what are called **rooted trees**. On a rooted tree, the common lineage from which all the species on the tree are derived is indicated at the base of the tree. As a result, direction in a rooted tree indicates the passage of time. We see the arrow of time indicated explicitly in Figure 4.12. In that figure, as we move from left to right, we are moving forward in time from the past toward the present.

Most algorithms for inferring phylogenetic trees from character data or DNA sequences generate **unrooted trees**. One such tree is illustrated in **Figure 4.13**. In contrast to rooted trees, unrooted trees do not fully indicate the direction of time. Branch tips represent more recent species than those represented by *interior nodes* (nodes on the inner section of the tree). But given two interior nodes on an unrooted tree, we cannot say, based on the tree topology alone, that one node represents a more recent population than the other. Going from an unrooted tree to a rooted tree—that is, assigning a root to a tree—requires additional information. We will discuss this process in Chapter 5.

Given the rooted/unrooted distinction, what exactly is the relation between an unrooted tree and a corresponding rooted tree or trees? In fact, every unrooted tree corresponds to a set of rooted trees. **Figure 4.14** illustrates an unrooted tree and several—although not all—of the corresponding rooted trees.

In principle, we can "root" an unrooted tree at different points on the tree. Imagine picking up the unrooted tree of Figure 4.14 at point A, and pulling this point down until it becomes the root. Doing so, we are left with the rooted tree labeled A in the lower panel of the figure. If instead we pick up the unrooted tree at point B and pull that point down, we are left with rooted tree B in the lower panel of the figure. Similarly, if we pick up the unrooted tree at point C, we arrive at the third rooted tree, labeled C in the figure.

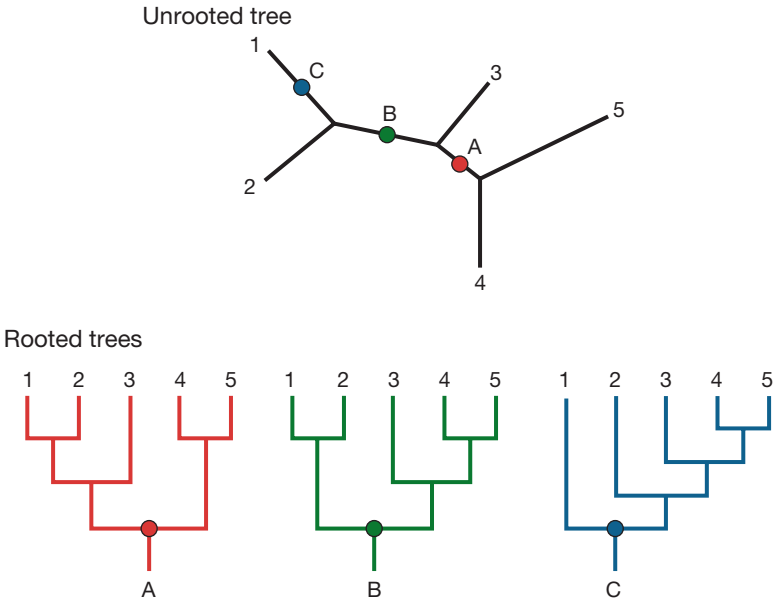


FIGURE 4.14 Rooted trees from unrooted trees. An unrooted tree and three corresponding rooted trees. Each rooted tree is rooted around the labeled point on the unrooted tree.

In general, we can root an unrooted tree around any of its branches. Thus, if an unrooted tree has k branches, there will be k corresponding rooted trees. Of course, assuming our unrooted tree itself is correct, only one of these rooted trees will be correct in the sense that it accurately reflects the historical sequence of branching events.

It is important to realize that where we decide to root the tree influences which clades we hypothesize to be monophyletic. For example, in rooted tree A in Figure 4.14, species 1, 2, and 3 form a monophyletic group. But in trees B and C, which correspond to the same unrooted tree, species 1, 2, and 3 form a paraphyletic group.

Branch Lengths

Many trees, such as the primate phylogeny shown in **Figure 4.15A**, are shown with all of the branch tips aligned. Such trees are intended to convey only the pattern of relationships among the various species displayed. But sometimes we will see trees drawn with branches of different lengths, as for the primate lentiviruses shown in **Figure 4.15B**. In this case, the branch lengths represent the amount of evolutionary change—measured as the actual or estimated number of changes in DNA sequence or other characters used to make the tree—that has occurred along a given branch. In Figure 4.15B, for example, we see that more sequence change has occurred along the branch leading to HIV-2/B than along the branch leading to HIV-2/A, indicating a faster rate of evolution in the HIV-2/B clade.

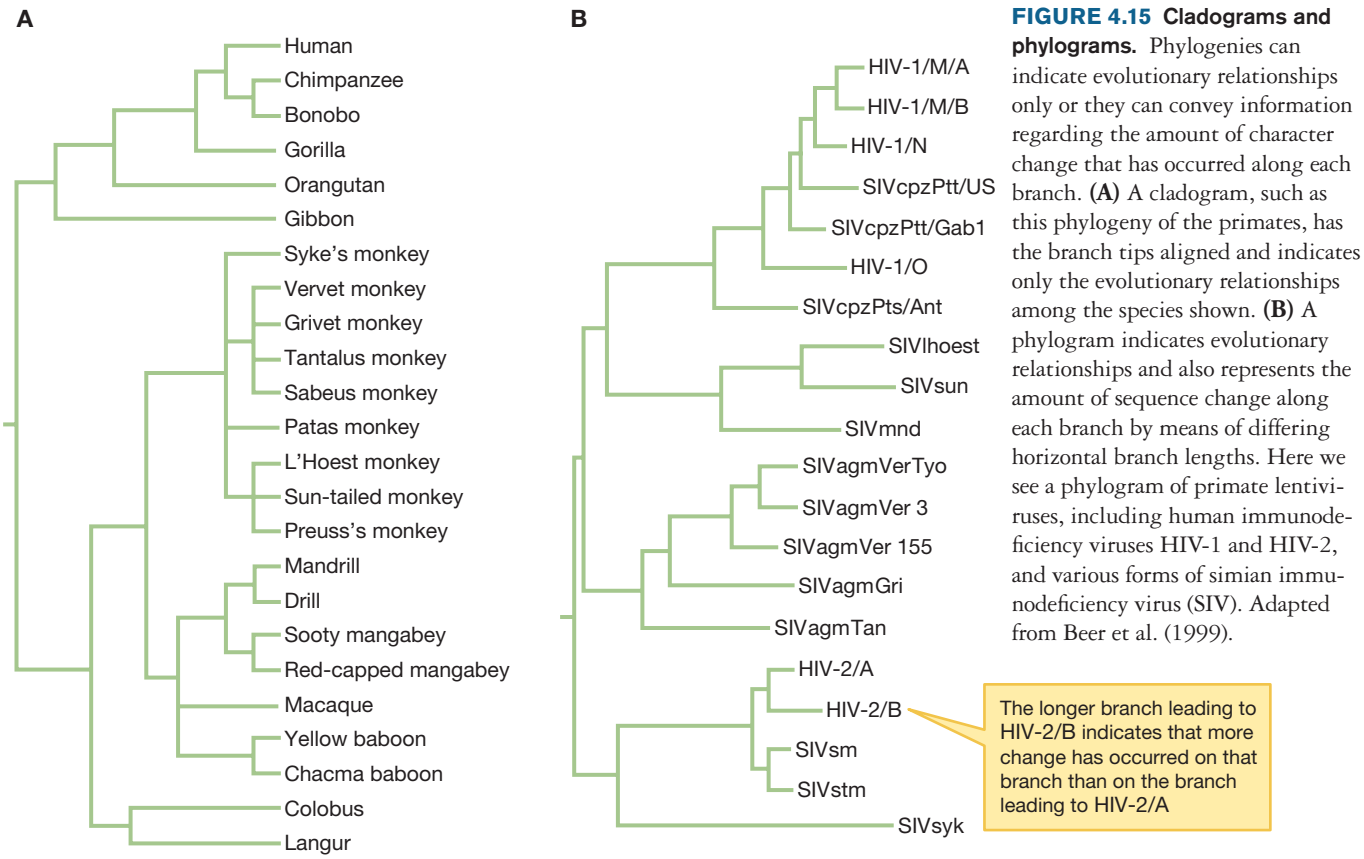


FIGURE 4.15 Cladograms and phylograms. Phylogenies can indicate evolutionary relationships only or they can convey information regarding the amount of character change that has occurred along each branch. **(A)** A cladogram, such as this phylogeny of the primates, has the branch tips aligned and indicates only the evolutionary relationships among the species shown. **(B)** A phylogram indicates evolutionary relationships and also represents the amount of sequence change along each branch by means of differing horizontal branch lengths. Here we see a phylogram of primate lentiviruses, including human immunodeficiency viruses HIV-1 and HIV-2, and various forms of simian immunodeficiency virus (SIV). Adapted from Beer et al. (1999).

Technically, trees that do not have different branch lengths are known as **cladograms**, whereas trees that represent evolutionary change with branch lengths are called **phylograms**. We occasionally see a third type of tree in which branch lengths represent actual time rather than the amount of evolutionary change. Such trees, called **chronograms**, are most common in paleontology. The chronogram in **Figure 4.16** depicts the evolutionary history of the orchids (Orchidaceae). This clade arose in the late Cretaceous period. Two of its subfamilies, the Orchidoideae and the Epidendroideae, underwent rapid bouts of speciation about 60 million years ago, shortly after the K–P (Cretaceous–Paleogene) boundary (until recently this was known as the Cretaceous–Tertiary, or K–T, boundary).

Just as we can generate and test hypotheses using the evolutionary relationships indicated by the structure of a phylogenetic tree, we can also generate and test hypotheses using the branch lengths on a phylogenetic tree. Stephen Smith and Michael Donoghue did this in order to study the question of whether a plant's generation time affects its rate of evolution (Smith and Donoghue 2008). Ever since DNA sequence data became widely available, evolutionary biologists have hypothesized that species with shorter generation times experience more rapid rates of evolution as measured by changes in DNA sequence (Wu and Li 1985; Martin and Palumbi 1993). The primary reason is thought to be that germ-line

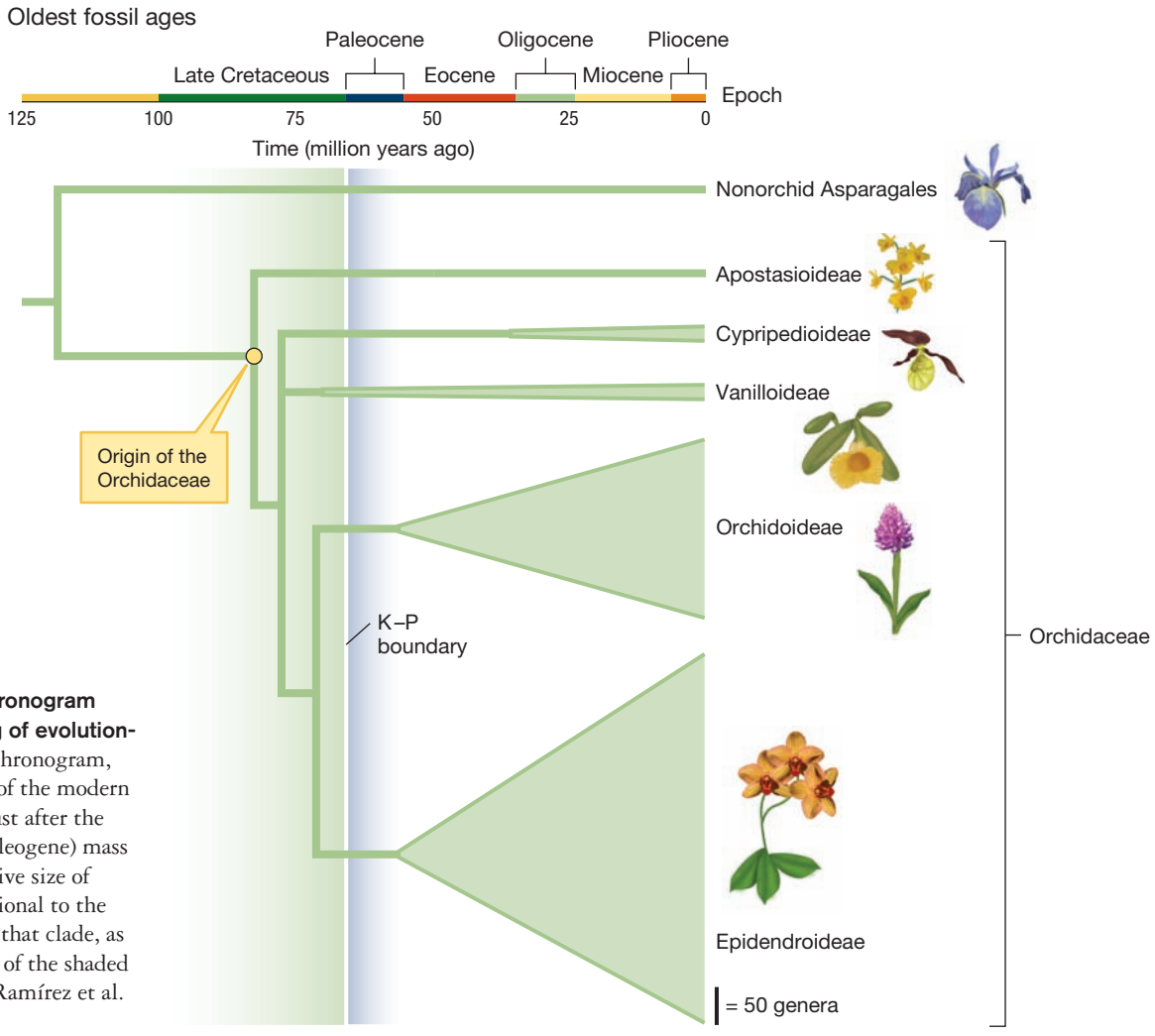


FIGURE 4.16 A chronogram indicates the timing of evolutionary events. In this chronogram, the rapid speciation of the modern orchids is dated to just after the K–P (Cretaceous–Paleogene) mass extinction. The relative size of each clade is proportional to the number of genera in that clade, as indicated by the size of the shaded area. Adapted from Ramírez et al. (2007).

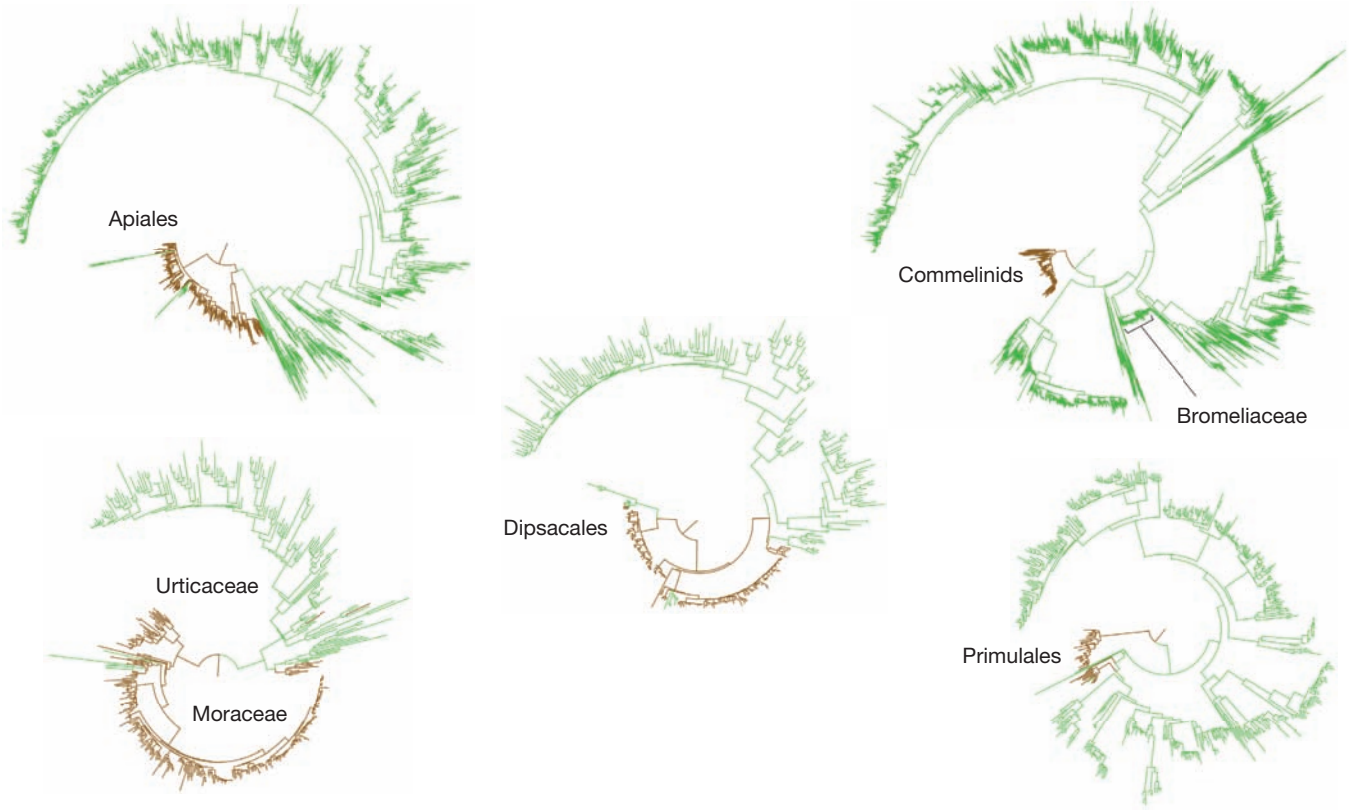


FIGURE 4.17 The rate of evolution in short- and long-lived plants. A phylogeny of five major plant clades constructed from DNA sequence data. Herbaceous species are shown in green, and shrublike or treelike species are shown in brown. For the herbaceous species, the branch lengths tend to be longer and the rates of sequence change faster. Adapted from Smith and Donoghue (2008) by permission of AAAS.

cells go through roughly the same number of rounds of replication irrespective of life span, and thus they have roughly the same opportunity for mutational change *per lifetime*. Because short-lived species have a shorter lifetime than long-lived ones, they have a higher rate of mutational change in the germ line *per year*.

To test this generation time hypothesis—that the rate of evolution is faster for shorter-lived species—Smith and Donoghue constructed phylogenetic trees for five large clades of plants, encompassing more than 7000 species. Because precise generation time data were not available for these species, Smith and Donoghue divided the species into two categories: (1) herbaceous plants and (2) shrubs/trees. Plants in the former category tend to have shorter generation times than those of plants in the latter category. Smith and Donoghue reasoned that if the generation time hypothesis is correct, there will be a slower rate of DNA sequence change along the branches of the phylogeny that represent the long-lived shrubs and trees than that along the branches that represent the short-lived herbaceous plants.

Smith and Donoghue's phylogenies are shown in **Figure 4.17**. In these phylogenies, herbaceous species are colored in green, whereas trees and shrubs are colored in brown (the interior branches are colored as well; the authors inferred the lifestyle—herbaceous or treelike—for each ancestor using a statistical model). These trees, which look somewhat different from any we have seen thus far, are rooted trees drawn using a method that lays out the phylogeny in an arc to make the best use of the space on the page.

Even at a glance, Smith and Donoghue's trees appear to support the generation time hypothesis: The brown tree-and-shrub branch lengths tend to be shorter than the green herbaceous branch lengths. Statistical analysis confirms this impression: The rates of evolution differ significantly between the herbaceous

groups and the treelike groups. Indeed, the herbaceous groups have median rates of evolution 2.7 to 10 times as high as the median rates in shrub and tree species.

KEYCONCEPT QUESTION

4.3 Figure 4.17 presents phylograms of several plant groups, colored to indicate whether they are herbaceous (green) or shrublike/treelike (brown). Within the group Commelinids, do the shrublike/treelike species form a monophyletic group? How about in the Dipsacales? The Apiales? In each of these three clades, if the shrublike/treelike species are not a monophyletic group, explain why not.

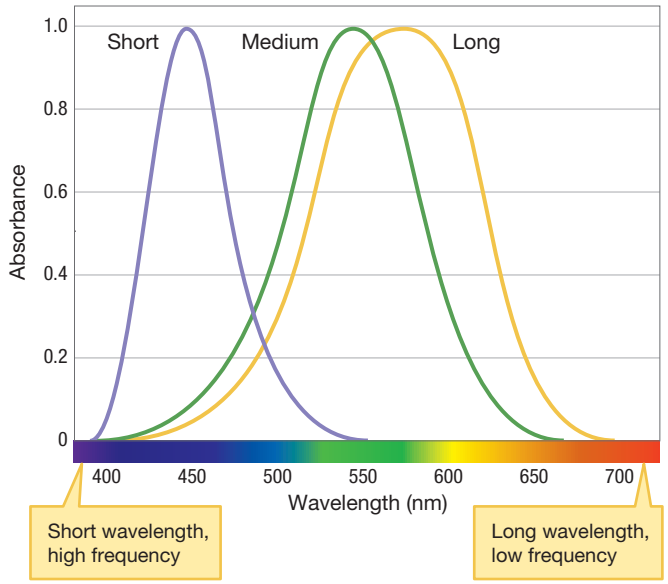
4.3 Traits on Trees

If a phylogenetic tree represents a hypothesis about the evolutionary history of a set of populations, then by looking at where a given trait appears on a tree, we can generate a hypothesis about when and how this trait has evolved. To get a feel for how we can place traits on a tree and then make inferences about the evolutionary history of these traits, we will begin with an example in which we look at the evolution of color vision in vertebrates.

Opsins are the visual pigments that facilitate color vision. It is because we have several different opsins that respond differently to various wavelengths of light that we can distinguish among a spectrum of colors. Humans, for example, have three different cone opsins: a short-, a medium-, and a long-wavelength opsin, with peak sensitivities in the indigo, green, and yellow regions of the color spectrum, respectively. The spectral sensitivity of these human cone opsins is illustrated in **Figure 4.18**.

Figure 4.19 shows a hypothesis for the evolutionary history of cone opsins in tetrapod vertebrates. At the tips of the tree are a number of representative tetrapod groups: squamate reptiles, birds, rodents, New World primates, baboons, and humans. The presence or absence of each kind of opsin is a character that we can map onto the tree. Shown along with each branch tip are the cone opsins present in that group. Thus, we see that humans and baboons are *trichromats* with three different cone opsins; rodents and New World primates, like most other mammals, are *dichromats* with only two different cone opsins; birds and squamate reptiles are *tetrachromats* with four different cone opsins. At the base of the tree, we see the hypothesized state of the common ancestor to these groups: The figure indicates that the common ancestor was most likely a tetrachromat like the birds and lizards.

FIGURE 4.18 Spectral sensitivity of the human cone opsins. Normalized spectral sensitivity of the short-, medium-, and long-wavelength opsins found in human cones.



In addition to placing traits at the tips and root of the tree, we can indicate where along the branches of the tree we think each trait has arisen or has been lost. Along the branch leading from the common ancestor to the mammalian clade, we see the loss of two medium-wavelength opsins (the dark-blue and light-blue triangles in Figure 4.19). These evolutionary losses were perhaps associated with the nocturnal lifestyle of the early mammals, which had limited use for color vision (Goldsmith 1990). After the

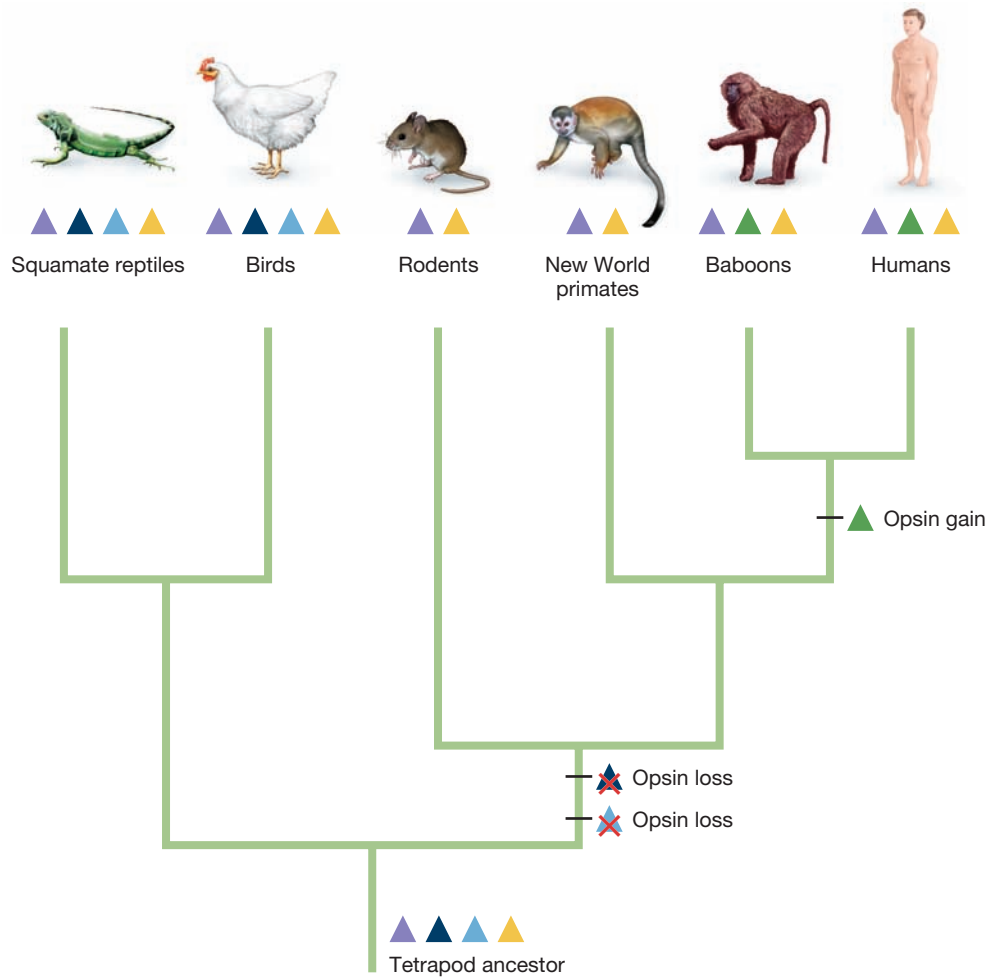


FIGURE 4.19 Evolution of tetrapod visual opsins. Evolutionary history of the tetrapod visual pigments known as opsins. Each triangle represents a particular visual pigment that facilitates color vision, with colors indicating peak spectral sensitivity. While a dichromat ancestor would be equally parsimonious based on only the data shown here, other lines of evidence reveal that the ancestral tetrapod likely had four opsins. Two were subsequently lost along the lineage leading to mammals, perhaps because early mammals were nocturnal and had limited use for color vision. In the Old World primate lineage leading to baboons and humans, a new opsin was gained because of a gene duplication of the long-wavelength opsin. Adapted from Frentiu and Briscoe (2008).

divergence of the New World primates and Old World primates (including humans and baboons), we see the gain of a new medium-wavelength opsin (the green triangle in Figure 4.19) due to the duplication and subsequent divergence of the gene coding for the long-wavelength opsin. This addition is thought to have been favored because it allowed primates better to detect and identify ripe fruit or tender young leaves, each of which may have a reddish cast (Surridge et al. 2003).

Thus, by placing traits on a tree that we have already constructed using other data, we represent a hypothesis about the evolutionary history of those traits and the species in which they occur.

4.4 Homology and Analogy

When we look at the range of living forms that populate our planet, we notice not only the vast diversity, but also many similarities that are shared across species and larger groups of organisms. Some—but not all—of these similarities are the consequence of shared ancestry. Others are the consequence of natural selection operating in similar ways on divergent groups of organisms. If we want to use similarities among organisms to deduce the historical relationships among them, we need to distinguish between these two basic sources of similarity—homology and analogy—in the traits of different species.

A **homologous trait** is a trait that is found in two or more species because those species have inherited this trait from an ancestor. All female mammals produce milk for

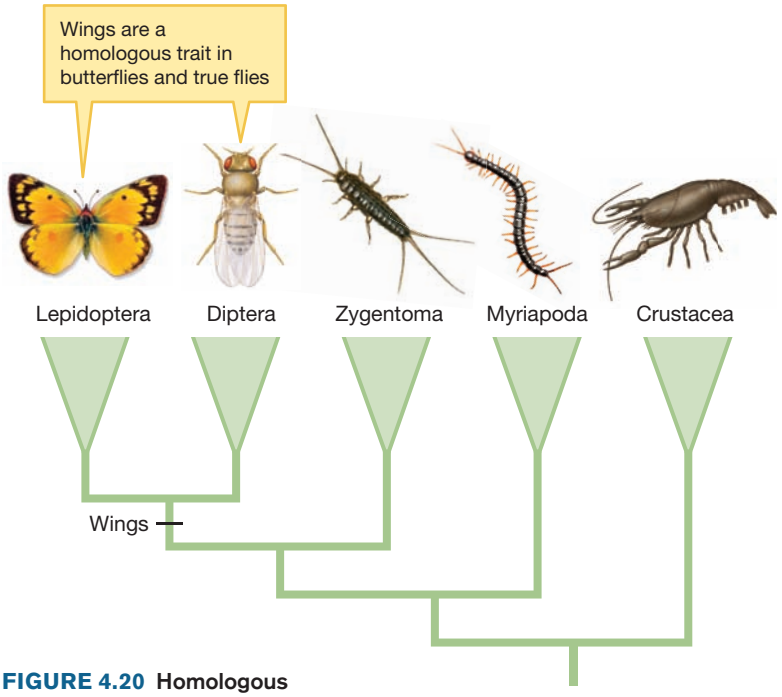


FIGURE 4.20 Homologous traits. Butterflies and true flies both exhibit wings because their common ancestor had wings. Thus wings area homogolous trait in these two groups.

their young, and they all possess this homologous trait because mammals share a common ancestor that produced milk. Similarly, all vertebrates have a vertebral column because the common ancestor to vertebrates had a vertebral column (or something like it).

In contrast to homologous traits, **analogous traits** are shared by two or more species, not because of a history of common descent, but instead because some other evolutionary process, usually natural selection, has independently fashioned similar traits in each species. **Figures 4.20** and **4.21** illustrate phylogenies that contain homologous and analogous traits.

Recognize that when considered by itself, a given trait of a single species cannot be said to be homologous or analogous. These terms refer to the comparison between a trait of one organism and a similar trait of another. As illustrated in Figures 4.20 and 4.21, wings are homologous if we are making a comparison between butterflies and true flies, but they are analogous if we are making a comparison between moths and hummingbirds.

Both homologous and analogous traits are used as evidence for Darwin's theory of evolution by natural selection—but they are typically used as evidence for different parts of the theory. The presence of homologous traits indicates that species have

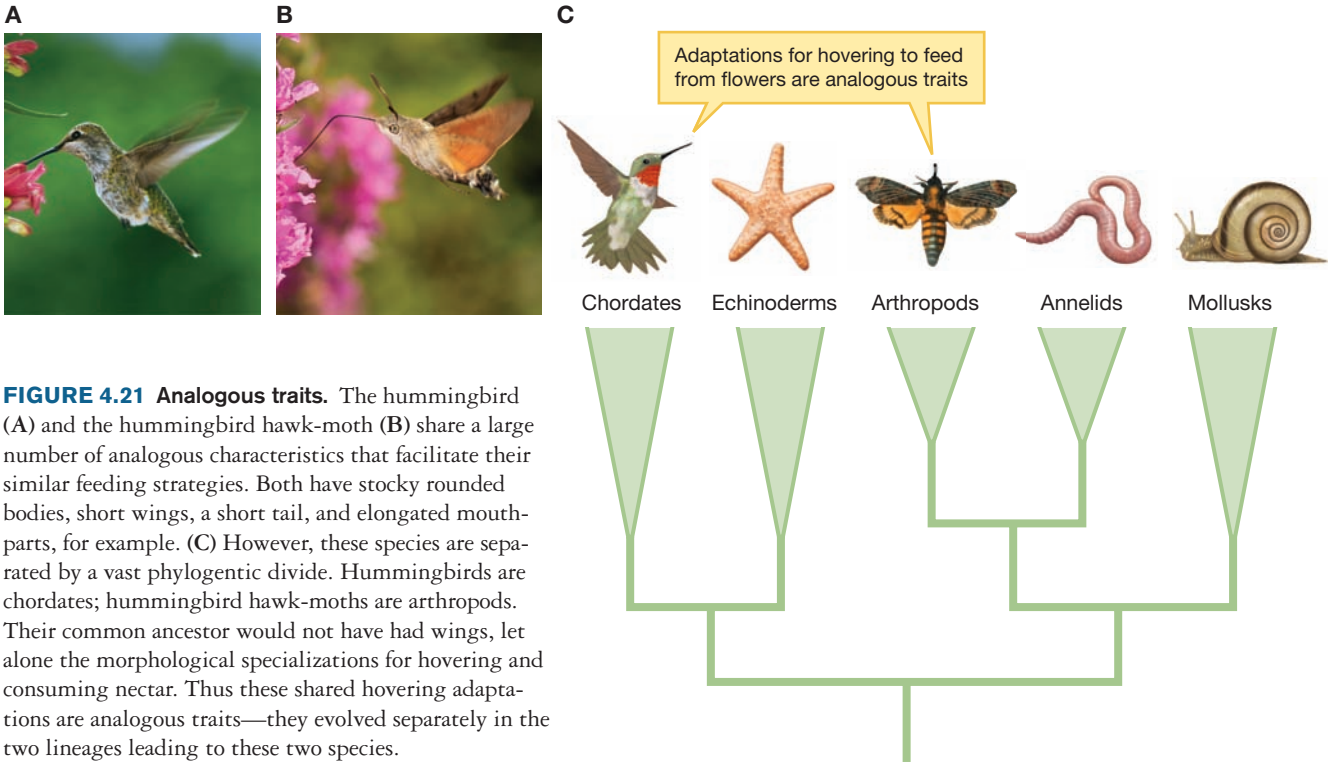


FIGURE 4.21 Analogous traits. The hummingbird (**A**) and the hummingbird hawk-moth (**B**) share a large number of analogous characteristics that facilitate their similar feeding strategies. Both have stocky rounded bodies, short wings, a short tail, and elongated mouthparts, for example. (**C**) However, these species are separated by a vast phylogentic divide. Hummingbirds are chordates; hummingbird hawk-moths are arthropods. Their common ancestor would not have had wings, let alone the morphological specializations for hovering and consuming nectar. Thus these shared hovering adaptations are analogous traits—they evolved separately in the two lineages leading to these two species.

a shared ancestry and thus supports Darwin's hypothesis that all organisms have descended from one or at most a few common ancestors. The presence of analogous traits reveals that natural selection generates structurally or functionally similar solutions to similar problems, often many times in parallel. This provides support for Darwin's hypothesis that the process of natural selection leads to organisms that are well adapted to their environments—and that natural selection can act as a creative force in generating these adaptations.

A discussion of homology and analogy leads us to the concepts of divergent and convergent evolution. **Divergent evolution** occurs when closely related populations or closely related species diverge from one another because natural selection operates differently on each of them. We have already seen a striking example of divergent evolution in the coat color variations of the oldfield mouse *Peromyscus polionotus* (Chapter 3). Inland, where the mice must hide against dark soils, dark coat coloration has evolved. In dune habitats along the coast and on the barrier islands, where mice must hide against light soils, lighter coat colors have evolved.

Convergent evolution occurs when two or more populations or species become more similar to one another because they are exposed to similar selective conditions; that is, convergent evolution leads to analogous traits in whatever populations or species we are examining. We can again look at coloration for an example of convergent evolution. This time, however, rather than comparing the coloration of mice in one habitat to that of mice in another, we will compare the coloration of pocket mice (*Chaetodipus intermedius* and *Perognathus flavescens*) in various habitats to the coloration of fence lizards (*Sceloporus undulatus*) in those same habitats (Hoekstra 2006; Rosenblum et al. 2010). Within a span of less than 20 miles in the Tularosa Basin of New Mexico, we see three distinctly different soil types: light-colored dunes, mid-toned desert grasslands, and dark lava fields. The mouse and lizard inhabitants of these areas have evolved

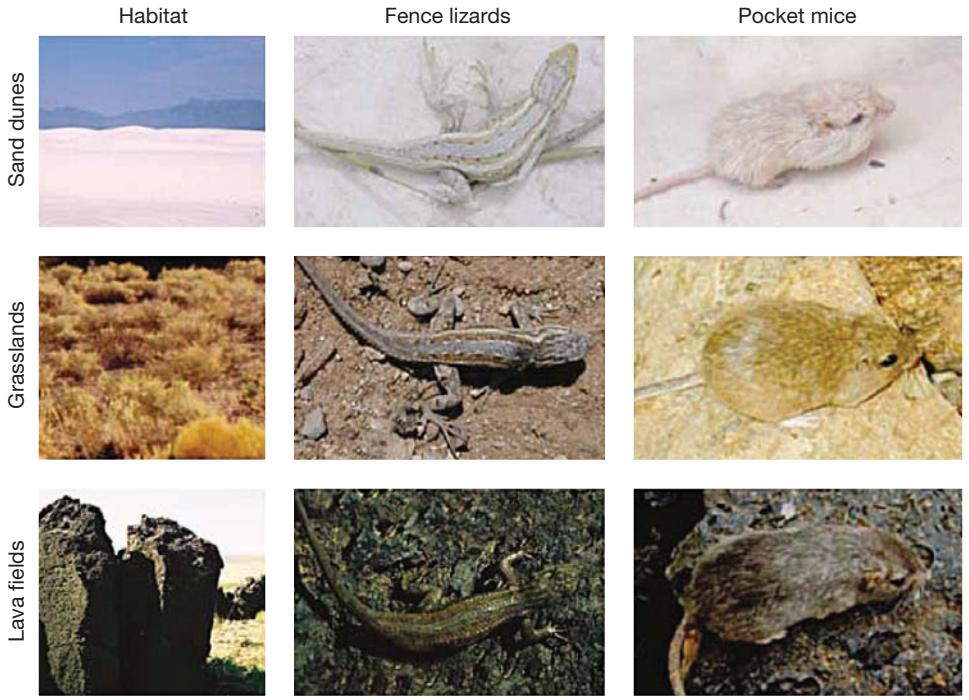


FIGURE 4.22 Convergent evolution for coloration. Fence lizards and pocket mice have evolved similar patterns of cryptic coloration in each of three different habitats.

remarkably similar coloration patterns that render these animals hard to detect against their surroundings (**Figure 4.22**).

A second example of analogous traits arising by convergent evolution comes from the poison frogs of Central and South America, a group of species famous for providing the poisons used on dart tips by indigenous hunters. Many of these frog species have *aposematic* coloration: They are highly conspicuous with bright warning colors that advertise their toxicity (**Figure 4.23**).

Historically, biologists have considered the origin of aposematism to be a difficult evolutionary step (Fisher 1930). It poses something of a chicken-and-egg problem: without predators that know to avoid the warning colors, aposematic coloration increases predation rather than decreasing it. But until warning colors are common, the predators cannot learn to avoid them. So which came first, the warning colors or the informed predators? To resolve this apparent paradox, researchers had hypothesized that the aposematic poison frogs compose a monophyletic clade, with a single origin of both toxicity and warning colors. But when Juan Santos and his colleagues constructed a detailed phylogeny of the dendrobatid frogs using DNA sequence data, they found something surprising: Aposematic coloration and toxicity were polyphyletic (Santos et al. 2003; Summers 2003). The combination of toxicity and bright coloration evolved multiple times within the family Dendrobatidae (**Figure 4.24**).

If we used these analogous traits in building a phylogenetic tree of these frogs, we might incorrectly infer too close a phylogenetic relationship between various aposematic species. Indeed, this is exactly what happened in previous phylogenies of this group: The presence or absence of toxicity and of aposematic coloration were used as a single character to construct the tree. As a result, the aposematic species were clustered together in the phylogeny, and from this, researchers incorrectly inferred that warning colors and toxic skin were monophyletic. This is one reason why it is important to use multiple characters when developing phylogenetic trees.

KEYCONCEPT QUESTION

4.4 Figure 4.12 in Section 4.2 illustrates a phylogenetic tree of the vertebrates. Based on this tree, is endothermy (warm-bloodedness) likely to be analogous or homologous in birds and mammals?

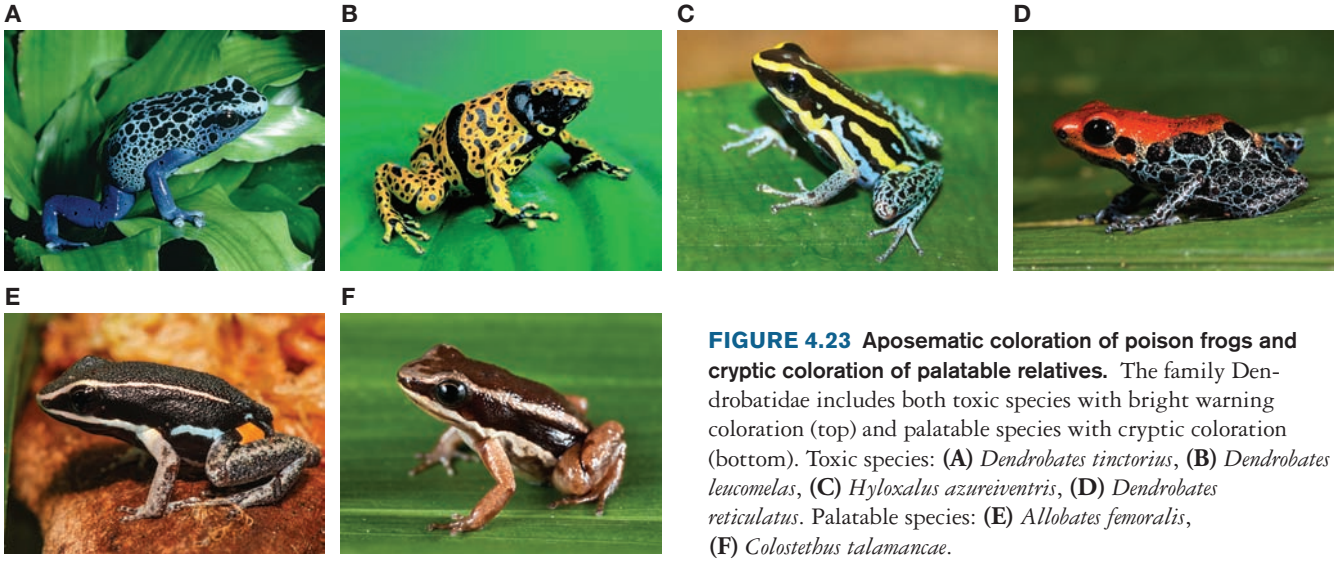


FIGURE 4.23 Aposematic coloration of poison frogs and cryptic coloration of palatable relatives. The family Dendrobatidae includes both toxic species with bright warning coloration (top) and palatable species with cryptic coloration (bottom). Toxic species: **(A)** *Dendrobates tinctorius*, **(B)** *Dendrobates leucomelas*, **(C)** *Hyloxalus azureiventris*, **(D)** *Dendrobates reticulatus*. Palatable species: **(E)** *Allobates femoralis*, **(F)** *Colostethus talamancae*.

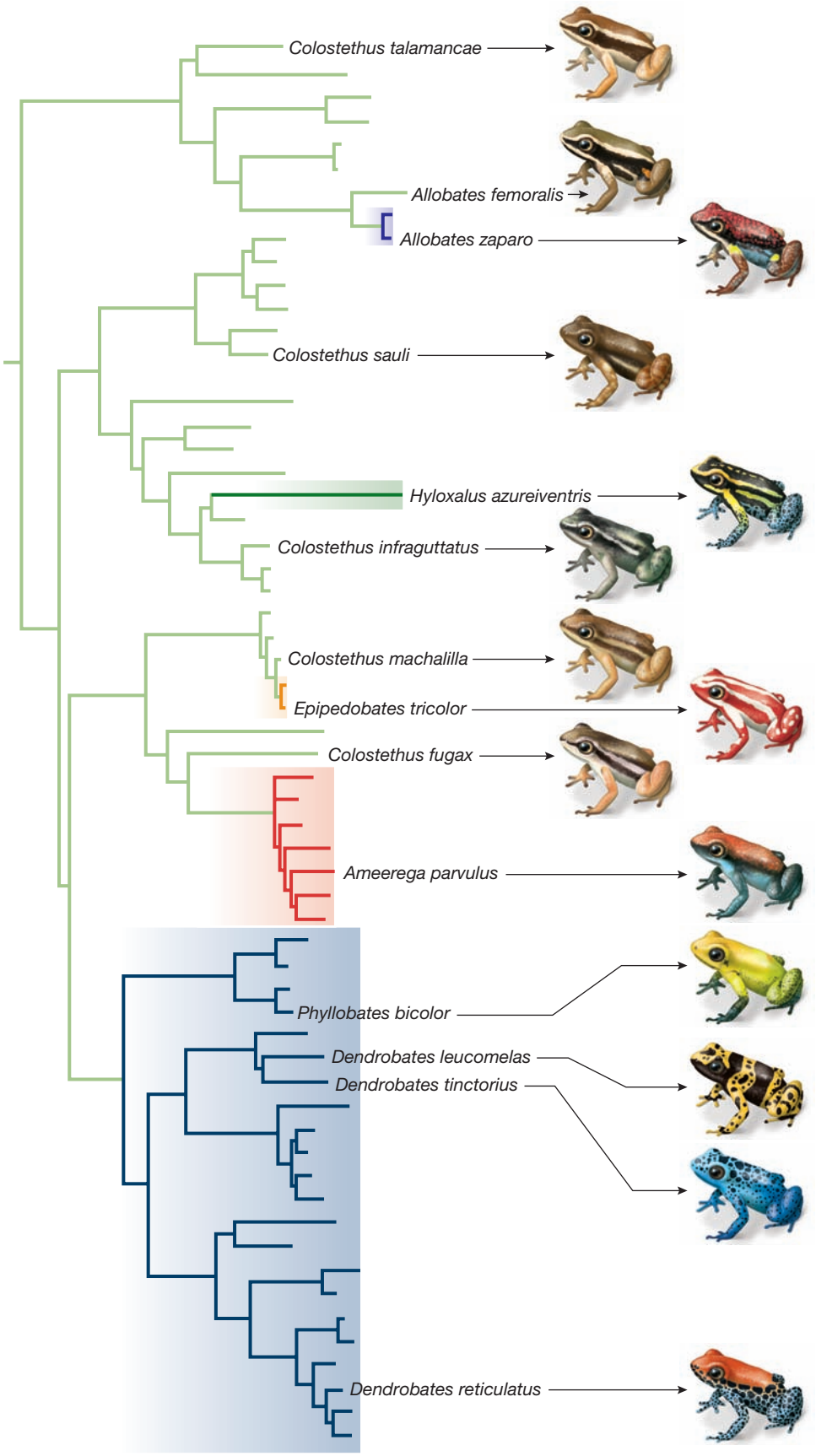


FIGURE 4.24 Convergent evolution in the Dendrobatidae. A phylogeny of the Dendrobatidae with aposematic clades shaded reveals multiple origins of aposematism. Frogs in the left column are cryptic and palatable; frogs in the right column are brightly colored and, with the exception of the mimic *A. zaparo*, toxic. Adapted from Santos et al. (2003), nomenclature following Grant et al. (2006).

Synapomorphies, Homoplasies, and Symplesiomorphies

The distinction between homologous and analogous traits is crucial when we aim to use traits to construct evolutionary trees. For example, consider a character such as coat color. We might observe a population in which, over evolutionary time, the coat color trait changes from light to dark, as in **Figure 4.25**. Here, dark coloration is a **derived trait**: It has been derived from an ancestor with a light coloration trait.

In Figure 4.25, the population splits into two descendant populations prior to the evolution of dark coloration and splits once again after the evolution of dark coloration. Here, the change in coat color tells us something about the evolutionary history of these populations. Dark coloration is not only a derived trait; it is also shared by two populations as a result of their shared ancestry. We call a shared derived trait such as this a **synapomorphy**.

When building evolutionary trees, we look for synapomorphies because they help us uncover the evolutionary relationships among groups on the tree. If we could arrange to use only synapomorphies to reconstruct evolutionary trees, the entire process of constructing phylogenies would be relatively straightforward. The more traits that two species had in common, the more closely related they would be. The problem is that not all shared traits are synapomorphies—there are other ways that two species can share a common trait. Let us see how.

One problem is that the dark coloration trait could be analogous rather than homologous. We call an analogous trait like this a **homoplasy**. (*Confusion alert*: A *homology* is a trait that is shared by two or more species because it has been inherited from a common ancestor. A *homoplasy* is a trait that is similar in two or more species even though it was not present in their common ancestor. Thus, a homoplasy is an *analogous* trait, not a homologous one.) Homoplasies can be misleading when we try to reconstruct an evolutionary tree. In **Figure 4.26**, species 1 and 2 share a common trait—dark coloration—that is not shared by species 3, but species 1 and 2 are *not* more closely related to one another than they are to species 3. If we mistakenly thought that this trait was a synapomorphy, we would conclude otherwise.

For another problem, consider the tree in **Figure 4.27**. Here we have a trait—light coloration—that is so recently derived that it is not shared. This leaves us with a shared

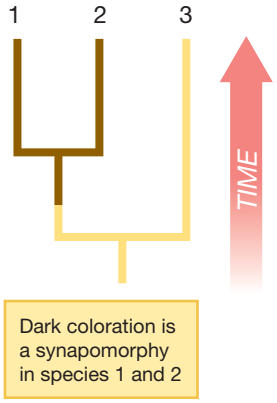


FIGURE 4.25 Synapomorphy. The trait of dark coloration is a *derived trait* because it evolved from another trait, light coloration. When the derived trait is shared because of common ancestry, we call it a shared derived trait, or *synapomorphy*.

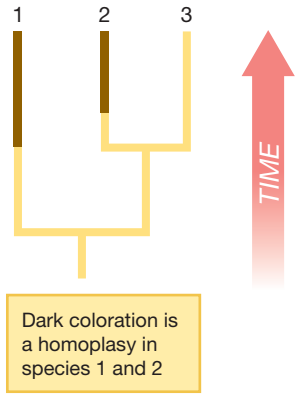


FIGURE 4.26 Homoplasy. In this tree, dark coloration is an analogous trait in species 1 and 2 because it evolved twice in parallel, once along each branch. We call such a trait a homoplasy.

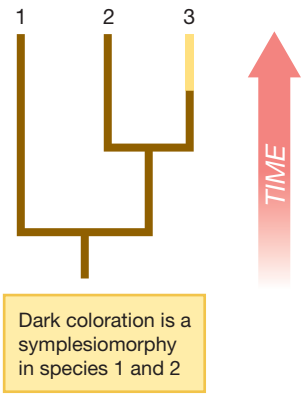


FIGURE 4.27 Symplesiomorphy. If a derived trait (here, light coloration in species 3) has arisen recently and appears in only one of the two most closely related species, the two more distantly related species (species 1 and 2) share an ancestral trait (dark coloration). We call the shared ancestral trait a *symplesiomorphy*.

trait—dark coloration—that is ancestral and in fact is not shared by the two most closely related species (species 2 and 3). A trait of this type is called a **symplesiomorphy**. Using such a trait in reconstructing a tree would incorrectly cause us to think that species 1 and species 2 were more closely related to one another than to species 3.

So, if using traits other than synapomorphies poses such a problem for phylogenetic inference, what can we do about it? Several strategies can help us avoid falling into this trap. First, we can try to pick traits that are likely to be synapomorphies rather than symplesiomorphies. Particularly when using phenotypic traits for building trees, we can use a thorough knowledge of the natural history of the organisms we are studying to select characters that are prone to change slowly rather than those that are prone to fluctuate rapidly over evolutionary time. This will help us avoid inadvertently choosing homoplasies and symplesiomorphies.

Second, we can use a large number of characters in reconstructing a phylogeny. If we use a sufficient number of characters, we might expect the synapomorphies to outweigh any homoplasies or symplesiomorphies accidentally included in the set of characters. When we build trees based on genetic sequence data, we rely heavily on this approach.

A third approach is to use an **outgroup**, a group with a known evolutionary relationship to the taxon we are studying. By including multiple outgroups, we can better estimate the **polarity**—the order of appearance in evolutionary time—of the traits we are using. This can be particularly useful in helping us avoid symplesiomorphies.

The idea of using outgroups is that when we begin the process of phylogenetic reconstruction, we do not know the relationship among the species in the taxon we are studying, but we do know the relationship of this taxon to the outgroups. Consider the incompletely resolved tree in **Figure 4.28**. The outgroups O1 and O2 have the light coloration trait. The polytomy between species 1, 2, and 3 indicates our uncertainty about the evolutionary relationships among these three groups, but the well-resolved branches for the outgroups indicate that we know they diverged from species 1, 2, and 3 before species 1, 2, and 3 diverged from one another. With this information in place, we can infer the most likely ancestral state for this tree: the state found in the outgroups. Thus, we infer that the polarity of the trait is light color → dark color.

How does this help us resolve the branching pattern among species 1, 2, and 3? **Figure 4.29** allows us to answer that question. Suppose that the common ancestor to species 1, 2, and 3 was light colored. Then if species 1 and 2 are more closely related to one another than to any other species—that is, if they are sister groups—we can explain the observed characters by a single evolutionary event (indicated by the red arrow in Figure 4.29A). But, if species 1 and 2 are not sister groups—that is,

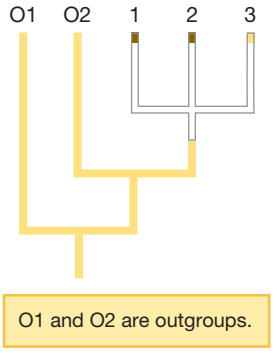


FIGURE 4.28 Using outgroups to infer the ancestral state. Outgroups O1 and O2 provide information about a trait's polarity. We assume that the ancestral trait is the trait shared by the outgroups and some members of the clade of interest.

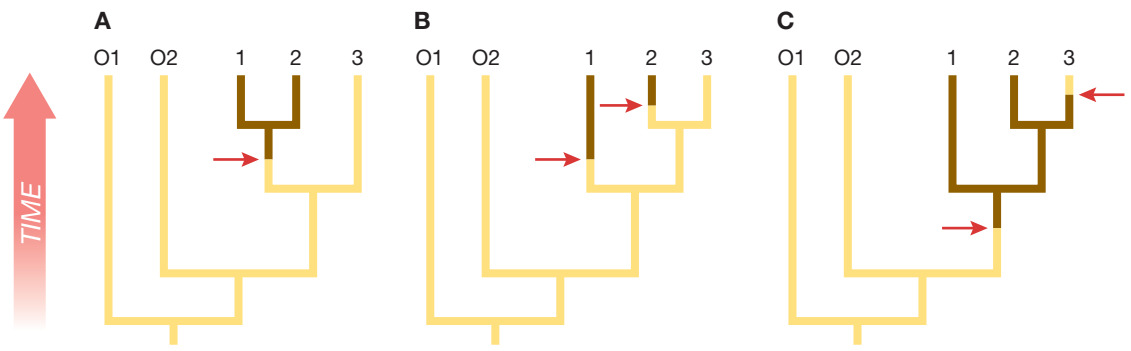


FIGURE 4.29 Outgroups help resolve the polytomy. If species 1 and 2 are sister groups, we can explain the observed traits with a single evolutionary event **(A)**. If species 2 and 3 are sister groups, we require two evolutionary events, either **(B)** two independent arisals of dark coloration or **(C)** the evolution of dark coloration early, with a subsequent reversion to light coloration in one lineage later. Red arrows indicate evolutionary changes in the trait.

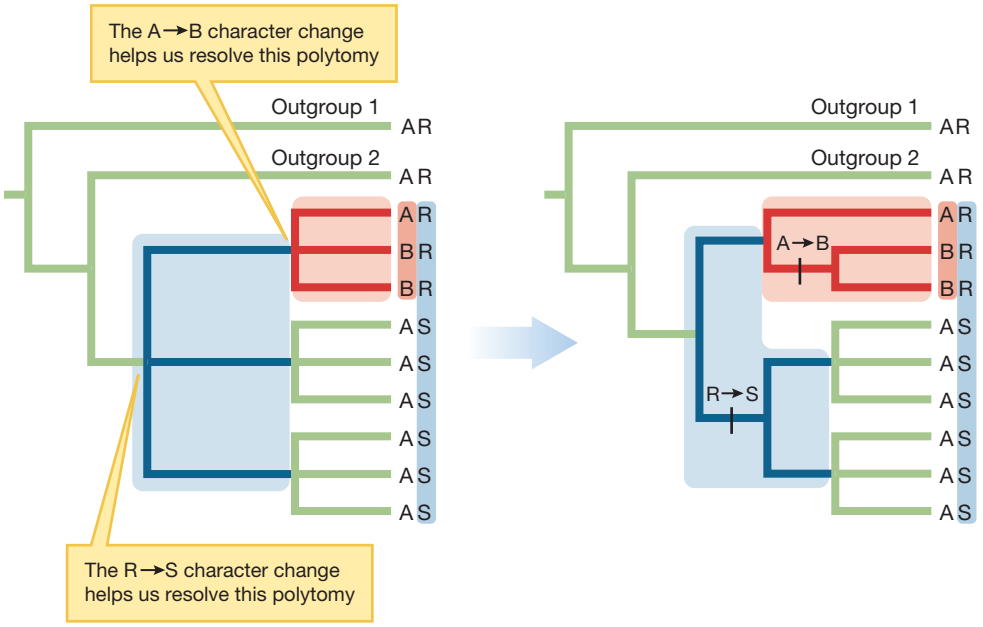


FIGURE 4.30 Synapomorphies at different levels. Synapomorphies at different levels help us resolve the polytomies at different places in the phylogeny. The first character (with character states A and B) resolves the recent polytomy in red. From the other members of the blue clade, we see the polarity of the trait: A is ancestral and B is derived. As a result, we conclude that two species with the B character state are sister groups. The outgroups reveal the polarity of the second character: R is the ancestral trait and S is derived. This resolves the deeper polytomy in blue. The two clades with the S character are sister groups.

if species 1 and 2 are not more closely related to one another than to any other species—then we require at least two evolutionary events (Figure 4.29B, C).

In Figure 4.29, knowing that light coloration is the ancestral character supports the inference that species 1 and 2 are likely to be sister groups. This approach of trying to explain the observed character states by a minimum number of evolutionary changes is known as parsimony. We will explore parsimony, along with other methods for inferring evolutionary trees, in Chapter 5.

In the preceding examples, we have shown how synapomorphies at one level of the tree can help us resolve the branching pattern among three groups on the tree. As we try to reconstruct the evolutionary history of larger numbers of groups, we need to have synapomorphies at different levels of the tree. **Figure 4.30** illustrates how. At left, we see an incompletely resolved phylogeny with a recent polytomy indicated in red and an earlier polytomy indicated in blue. To resolve the red polytomy, we look to the sister clades to determine the polarity of the first character: A is ancestral and B is derived. As a result, we conclude that the two species with the B character state are sister groups. The blue clade would have no sister clades if it were not for the outgroups. These outgroups reveal the polarity of an earlier evolutionary event: R is the ancestral state and S is derived. This earlier event allows us to resolve the polytomy shown in blue.

4.5 Using Phylogenies to Generate and Test Evolutionary Hypotheses

Evolutionary trees, or phylogenies, are hypotheses about historical relationships among organisms. Evolutionary biologists test and refine these hypotheses when new sources of information about relationships and descent—for example, new fossils, new molecular data, or new phylogeographic data—become available.

The Evolutionary History of the Shoebill

When considering the aquatic birds, the evolutionary history of a spectacular wading bird called the shoebill (*Balaeniceps rex*) poses a particular puzzle (**Figure 4.31**). Superficially, the shoebill looks quite similar to the storks, and for this reason, the species is often called the "shoebill stork." But a phylogeny developed in the 1980s and based on morphological characters suggests otherwise (**Figure 4.32A**). This phylogenetic hypothesis places the shoebill as a sister group to the herons. In other words, this phylogeny implies the hypothesis that the closest living relative of the shoebill is a heron (Cracraft 1981; Van Tuinen et al. 2001).

FIGURE 4.31 Shoebill and possibility affinities. Shoebills **(A)** are sometimes called "shoebill storks," suggesting a relationship with storks such as the painted stork (*Mycteria leucocephala*) **(B)**. In the 1980s, a phylogeny based on morphological characters placed the enigmatic shoebill as a sister group to herons such as this gray heron (*Ardea cinerea*) **(C)**. A subsequent DNA sequence tree placed the shoebill as a sister group to the pelicans including this Australian pelican (*Pelecanus conspicillatus*) **(D)**.

However, phylogenies derived from morphological characters can be misleading, particularly when multiple characters have undergone convergent evolution. To address this problem, researchers today often turn to molecular phylogenies based on DNA sequence data. **Figure 4.32B** shows a molecular phylogeny of the same group (Van Tuinen et al. 2001; Hackett et al. 2008; Jarvis et al. 2014). This tree represents a different hypothesis about the relation among aquatic bird groups. Here, the shoebill is a sister group to the pelicans. In other words, this tree poses the hypothesis that the shoebill's closest living relatives are pelicans—just as the famous ornithologist John Gould speculated when first describing the species in 1851.

Which tree correctly represents the phylogenetic history of the shoebill? The question is not settled, but the evidence is lining up in favor of the hypothesis that pelicans and shoebills are sister groups. While a recent morphological study suggests a more distant relationship between shoebills and pelicans (Mayr 2003) and a comparison of bile acids favors herons as the sister group to shoebills (Hagey et al. 2002), multiple DNA sequencing studies (Van Tuinen et al. 2001; Hackett et al. 2008) and a DNA hybridization study support the pelicans as a sister group (Van Tuinen et al. 2001). Phylogenetic reconstruction is an ongoing process. As

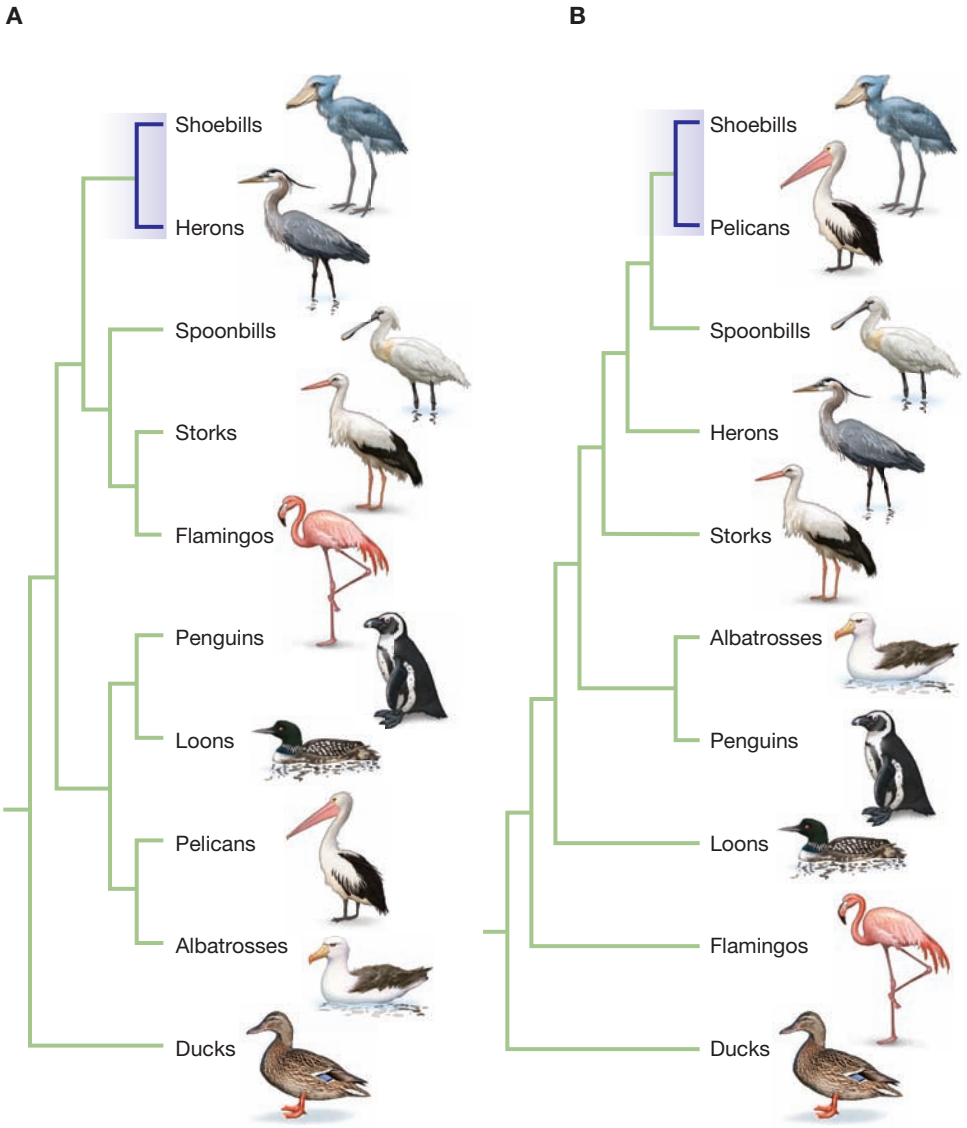


FIGURE 4.32 Two hypotheses about evolutionary relationships. These trees represent two different hypotheses about evolutionary relationships among aquatic birds. The tree on the left **(A)** is based on morphological characters; according to this tree, the herons are the closest living relatives of the shoebill. The tree on the right **(B)** is based on DNA sequence data and posits that the pelicans are the closest living relatives of the shoebill. Adapted from Van Tuinen et al. (2001), Hackett et al. (2008), and Jarvis et al. (2014).

new data become available, researchers reject previous hypotheses and pose new ones. It will take more data—perhaps in the form of whole-genome sequence information—before the shoebill's evolutionary history is definitively known.

KEYCONCEPT QUESTIONS

4.5 In Figure 4.32A, shoebills and herons are hypothesized to be sister groups. Describe two other hypotheses implied by this figure, but not by Figure 4.32B.

4.6 In Figure 4.32A, what is the smallest monophyletic group that includes shoebills and herons? How about in Figure 4.32B?

The Evolutionary Origins of Snake Venom

When evolutionary biologists place traits on a preexisting phylogenetic tree, they are generating hypotheses of a different kind—hypotheses about when traits evolved and which traits may be shared among which groups of relatives. For example, a phylogenetic picture of snake and lizard venom led to the hypothesis

that many supposedly nonvenomous snakes, and even nonvenomous lizards, actually produce and use venom in capturing their prey.

Commonly, only two families of snakes were thought to be venomous: the Viperidae (vipers) and the Elapidae (including sea snakes and cobras); a third family, Atractaspididae, may also have advanced venom-delivery systems. Snake species in both Viperidae and Elapidae commonly have hollow or grooved fangs through which the venom is delivered from a venom gland that can produce and store sizable quantities of venom, as illustrated in **Figure 4.33**.

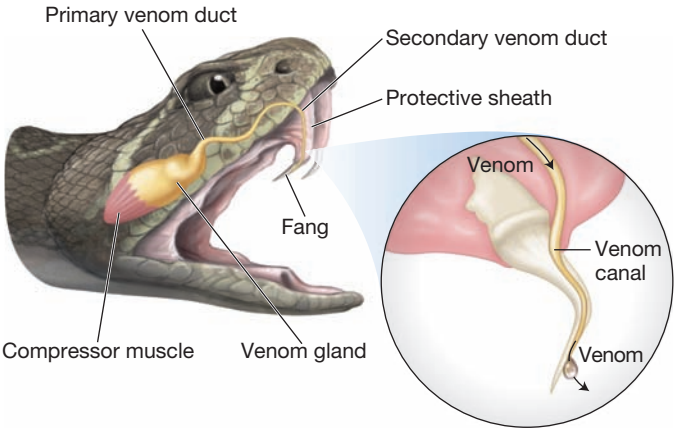
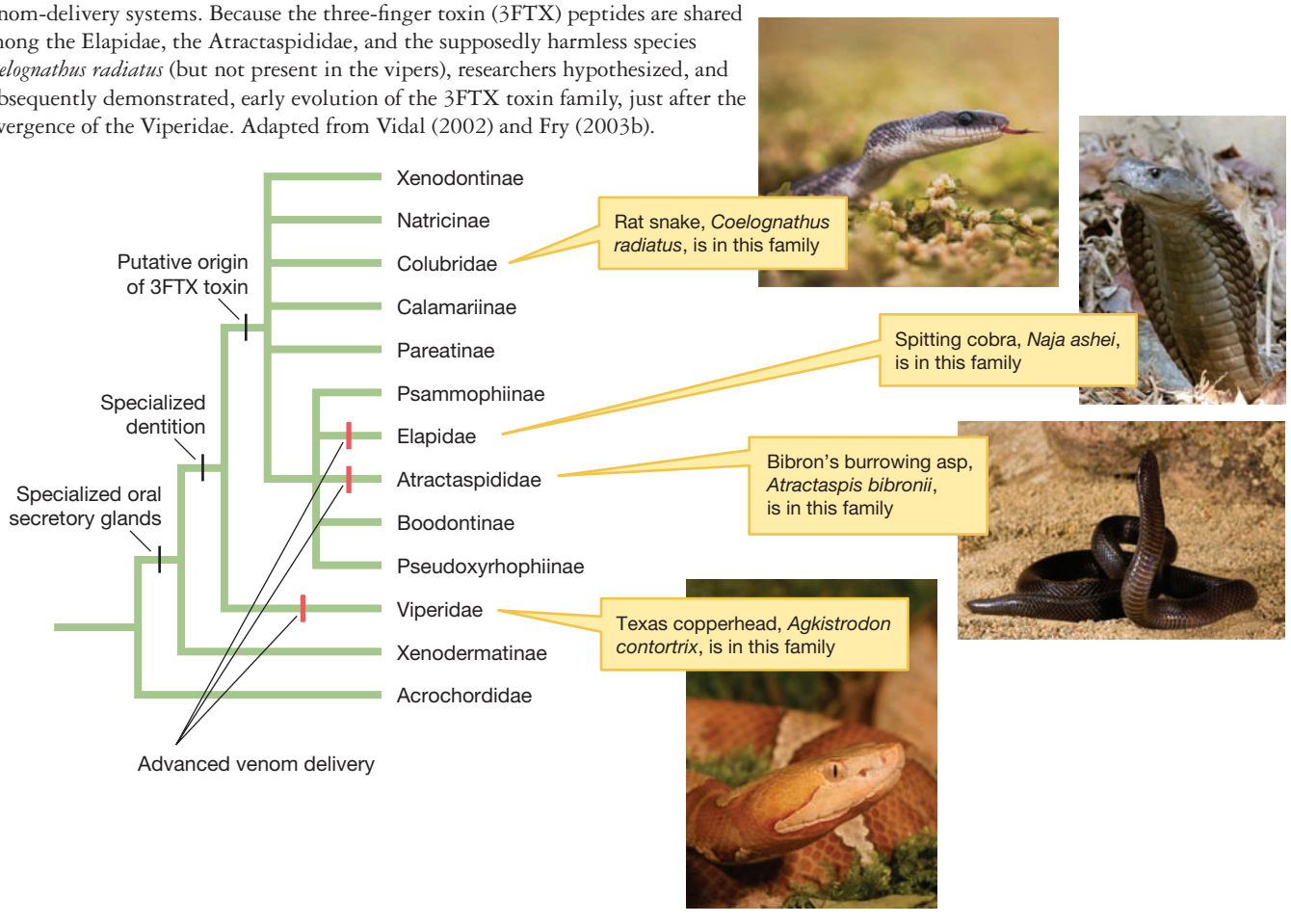


FIGURE 4.33 Snake fangs and venom. The morphology of the venom-delivery system in a venomous viperid snake.

Early phylogenetic analysis suggested that these advanced venom-delivery systems evolved independently in each family of snakes; that is, that they were analogous traits. Researchers assumed that there was no venom without a delivery system, and so they concluded that venomousness must be a highly derived trait seen in a relatively small fraction of all snake species. But more recent phylogenetic analysis, combined with careful morphological study, has forced herpetologists to reevaluate and revise this conclusion (**Figure 4.34**). This work suggests that

FIGURE 4.34 Phylogeny of advanced snakes (Caenophidia). A partial phylogeny of the Caenophidia indicates the distribution of (1) specialized oral secretory glands (for example, Duvernoy's gland), (2) specialized dentition, and (3) advanced venom-delivery systems. Because the three-finger toxin (3FTX) peptides are shared among the Elapidae, the Atractaspididae, and the supposedly harmless species *Coelognathus radiatus* (but not present in the vipers), researchers hypothesized, and subsequently demonstrated, early evolution of the 3FTX toxin family, just after the divergence of the Viperidae. Adapted from Vidal (2002) and Fry (2003b).



numerous other families of snakes are able to produce salivary toxins in an organ known as Duvernoy's gland, even though they lack grooved or hollow fangs or advanced venom-delivery pumps (Vidal 2002; Fry 2003b).

Given the broad distribution of basic toxin production capacity, herpetologists have hypothesized that toxin production is homologous among snakes, having arisen once rather than repeatedly over the evolutionary history of this group. Evolutionary biologist and venom expert Bryan Fry reasoned that if this hypothesis was correct, many so-called nonvenomous snakes should actually be capable of producing toxic venom. Based on this phylogenetic reasoning, Fry and his colleagues decided to study the salivary secretions of a purportedly nonvenomous snake common in the pet trade, the rat snake *Coelognathus radiatus*. They obtained a number of individuals of the species and milked the snakes to obtain their salivary secretions. Surprisingly—but in line with Fry's conjecture—they found that the most abundant peptide in the salivary secretions of this supposedly harmless snake is a close homologue of the three-finger toxins (3FTXs) produced by the highly poisonous elapid snakes (Fry 2003a) (Figure 4.34). The supposedly harmless rat snake turned out to be producing a potent neurotoxin closely related to that in cobra venom!

Buoyed by their successes finding toxins in the saliva of purportedly nonvenomous snakes, Fry and his colleagues decided to see if they could trace the origin of venom production even further back into evolutionary history (Fry et al. 2006). In addition to venomous snakes, the helodermatid lizards (Gila monsters and beaded lizards) are known to be venomous. But venomousness in snakes and venomousness in lizards were thought to be analogous traits; that is, snakes and helodermatid lizards were thought to have independently evolved the capacity to produce and deliver venom. The venomous snakes produce their venom in specialized glands in the upper jaw and deliver it through hollow or grooved fangs on the upper jaw, whereas the helodermatid lizards produce their venom in glands in the lower jaw and deliver it through a row of grooved teeth on the lower jaw. But after discovering homologies in snake venoms, Fry hypothesized that perhaps some snake and lizard venoms are homologous as well.

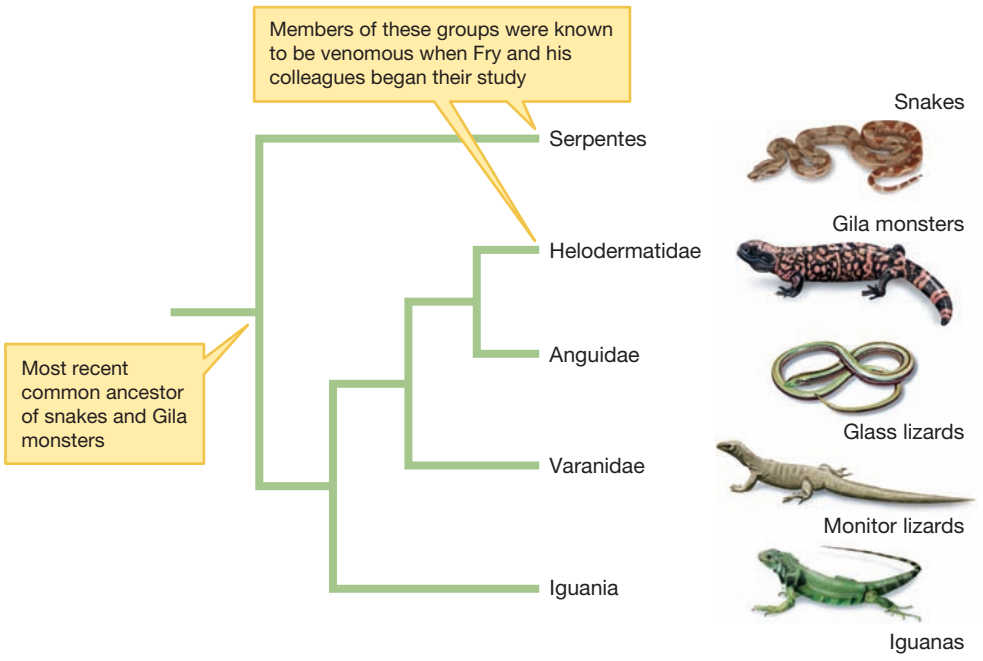


FIGURE 4.35 Venomousness as a homologous trait between snakes and Gila monsters. Phylogeny of snakes, venomous helodermatid lizards, and their relatives. The most recent common ancestor of these venomous species is indicated. If venomousness is a homologous trait in snakes and Gila monsters, we should expect to see venom production in some of the other descendants of this common ancestor, such as the monitor lizards and iguanas shown in the tree. Adapted from Fry et al. (2006).

Again, this hypothesis generated a strong testable prediction. If venom had evolved early, so that it was a homologous trait in snakes and these venomous lizards, other descendants of their common ancestor might share the ability to produce venom. So, Fry and an international team of herpetologists used genetic data to refine the phylogeny of the order Squamata (snakes and lizards) and thereby identify those common descendants who might also have venom. According to their phylogeny, shown in **Figure 4.35**, the common ancestor of snakes and

Gila monsters had descendants that include the Anguidae (glass lizards), Varanidae (monitor lizards), and Iguania (iguanas, chameleons, anoles, and relatives). Thus, these species are plausible candidates for where we might find venom production if venomousness is a homologous trait between snakes and Gila monsters.

To determine whether or not species in these other groups also produced venoms or venomlike proteins, the researchers sampled cells from the salivary glands or secretions of these species. They then looked at the genes that are expressed in those cells. They found nine genes coding for toxins that were shared between lizard species and snakes: Seven of these were previously known only from snakes. An Australian lizard, the eastern beaded dragon (*Pogona barbata*), produces a toxin previously known only from rattlesnake venom. The lace monitor produces toxins that inhibit blood clotting and induce a catastrophic drop in blood pressure. Even the gigantic Komodo dragon—the largest living lizard—may have such a devastating bite because of secreted toxins, rather than because of bacterial sepsis as previously thought (Fry et al. 2009). While these various toxins may not be lethal or even severely debilitating when smaller lizard species bite humans, they may be delivered at high enough doses to be extremely effective in disabling the smaller prey of the lizards.

All in all, these studies provide very strong evidence of an early emergence of venom production capability in the squamate reptiles, and phylogenetic thinking was the key to the discovery of the other lizards' venoms. Phylogenetic reasoning suggested to Fry and his team that lizards other than the Helodermatidae may also produce venom—and the phylogeny that these researchers constructed gave them a map of where in the lizard group to look for other venomous species. In the end, this discovery may be of more than general biological interest. Compounds derived from snake venoms are used extensively in medicine; for example, they are used as anticoagulants, in diagnosing various blood-related disorders, and to lower blood pressure (Koh et al. 2006). The diverse lizard toxins that Fry and his colleagues identified will offer a new array of potentially useful molecules for medical researchers to explore.

Vestigial Traits

One interesting class of homologous traits used in phylogenetic reconstruction are known as **vestigial traits**—Darwin often referred to these as "rudimentary" characteristics. Vestigial traits are those that have no known current function but appear to have been important in the evolutionary past. In *The Descent of Man and Selection in Relation to Sex*, Darwin wrote of the upper incisor teeth that never break through the gums of some ruminants as an example of a vestigial trait, because ruminant herbivores likely descended from carnivores, whose incisor teeth are very important in prey capture and consumption (Darwin 1871).

Why vestigial traits remain in place when they serve no current function will probably vary from trait to trait. There are at least three possible explanations: (1) the trait is not costly to the organism, and so natural selection does not act against it; (2) there is some natural selection against a vestigial trait—it is on its way out, and eventually it will be lost; or (3) the trait has some function that we have simply failed to identify. In this last instance, the trait would not really be vestigial, so let's confine ourselves to the former two cases.

Vestigial traits allow evolutionary biologists to trace common descent by comparing a now functionless trait in species 1 to the same trait in functional

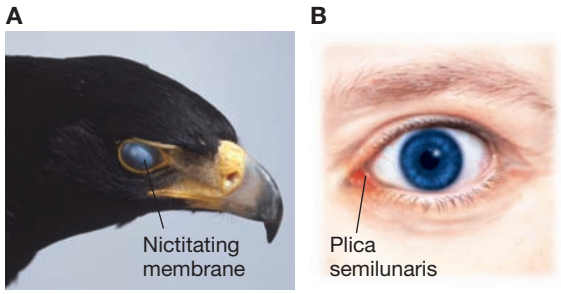


FIGURE 4.36 The nictitating membrane. The nictitating membrane in an eagle **(A)** is homologous to the plica semilunaris in a human **(B)**. The plica semilunaris has no known function in humans, while the nictitating membrane serves many functions in birds.

form in species 2—our hypothesis being that species 1 and 2 share this trait because of descent from a common ancestor who also possessed it. For example, consider the nictitating membrane—or inner eyelid—found in birds and mammals. This membrane can be drawn across the eye of birds. It can moderate incoming light, clean the eye of dust, and (in birds) prevent excessive drying of the eye during flight. Most mammalian species, including humans, also have a vestigial version of the nictitating membrane called the plica semilunaris, or semilunar fold (**Figure 4.36**). As far as we know, this membrane has no working function in humans and most other mammals. But it tells us something about common descent. The fact that birds and mammals share the complex trait of a nictitating membrane/semilunar fold, even though this trait has no known function in the latter group, is indicative of their common ancestry; that is, it suggests that an ancestor common to both these groups had some version of this trait. Indeed, we can say more, because reptiles also have a functioning nictitating membrane, which suggests that birds, reptiles, and mammals share a common ancestor that had such a membrane, and it was only when mammals diverged from these other groups that the nictitating membrane lost its function.

Evolutionary biologists have also examined vestigial traits and phylogeny in the context of limblessness in snakes. The evidence from vestigial limbs suggests that modern snakes evolved from a limbed reptilian ancestor (Carroll 1988; Lee and Caldwell 1998). Evidence from limb structure in both modern and extinct snake species, including fossil evidence, is most consistent with the following evolutionary history: The common ancestor to all snakes had fully developed hind limbs and forelimbs and a skeleton with distinct regions. The earliest snakes had already lost forelimbs, but they had functional hind limbs. Modern snakes then went through three stages: (1) a reduced pelvic area (with hind limbs present), (2) the reduction of the hind limbs to vestigial buds, and then (3) the complete loss of hind limbs. The data on the phylogeny of snakes as it relates to vestigial traits can be summarized in the evolutionary tree shown in **Figure 4.37**.

Vestigial traits serve as a strong test of Darwin's theory of evolution by common ancestry. If all organisms have arisen from one or a few common ancestors by a branching process of descent, we would expect to see vestigial traits shared with species that share a common ancestor subsequent to the evolution of that trait—but not among species whose most recent common ancestor predates the evolution of that trait. For example, think about where on the tree of life we might expect to find vestigial tetrapod limbs. Under the explanation provided here, we might expect to see vestigial limbs in some of the currently limbless descendants of ancestral tetrapod vertebrates. But we would not expect to find vestigial limbs in species that diverged prior to the origin of limbs. Thus, Darwin's theory predicts that we may find vestigial limbs in snakes but that we should not find them, for example, in earthworms (**Figure 4.38**). Indeed, such predictions have been borne out time and again in the study of comparative morphology.

In this chapter, we have emphasized the central role that common descent and phylogenetic history play in evolutionary biology. In the next chapter, we will move on to a more detailed analysis of how phylogenetic trees are constructed in the first place.

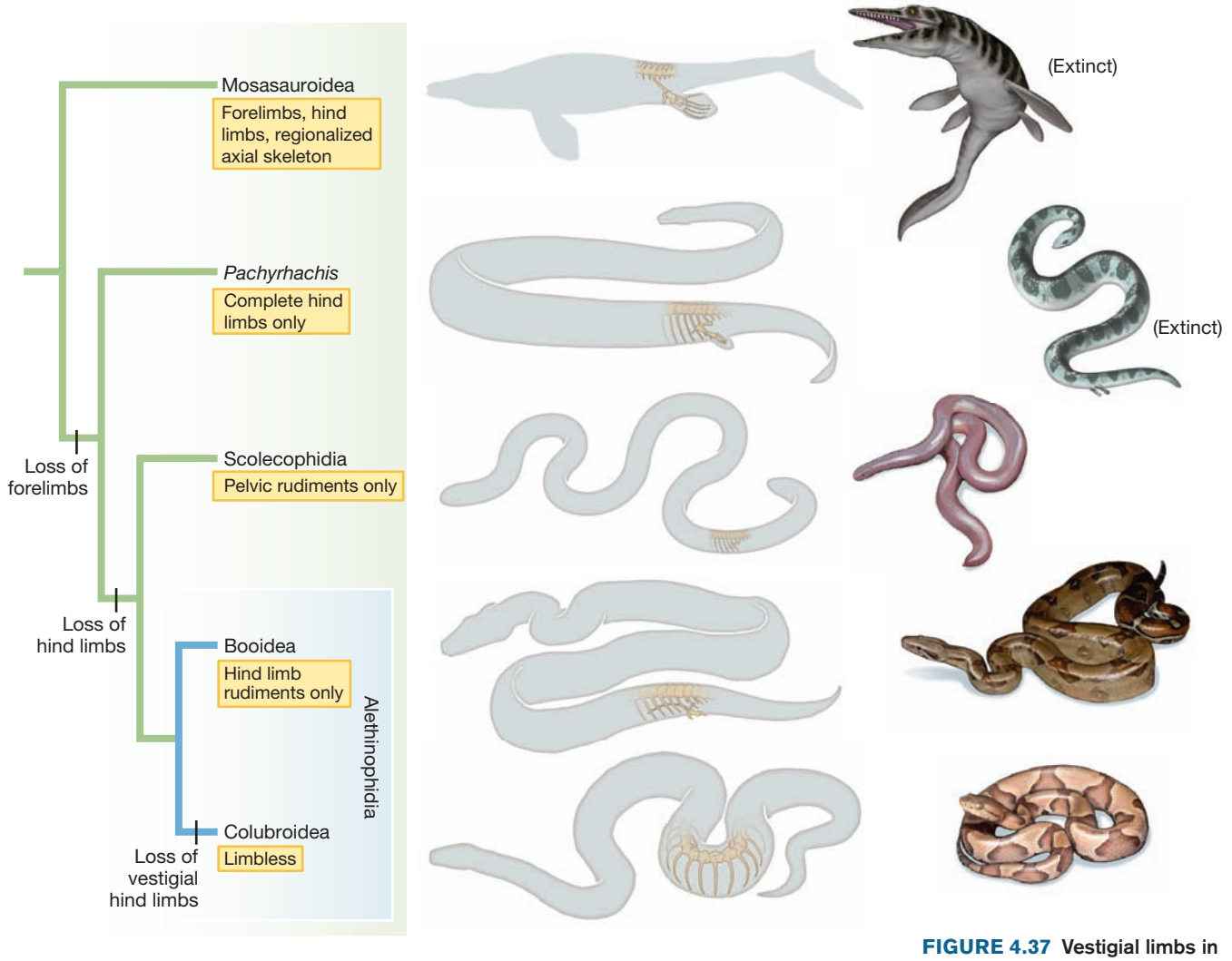


FIGURE 4.37 Vestigial limbs in snakes. A phylogenetic history of snakes shows the gradual loss of limbs from their reptilian ancestors. Species in the superfamily Booidea (boas and pythons) retain vestigial hind limbs, whereas developmental changes in the colubrid snakes have eliminated even these vestigial hind limbs. Adapted from Cohn and Tickle (1999).

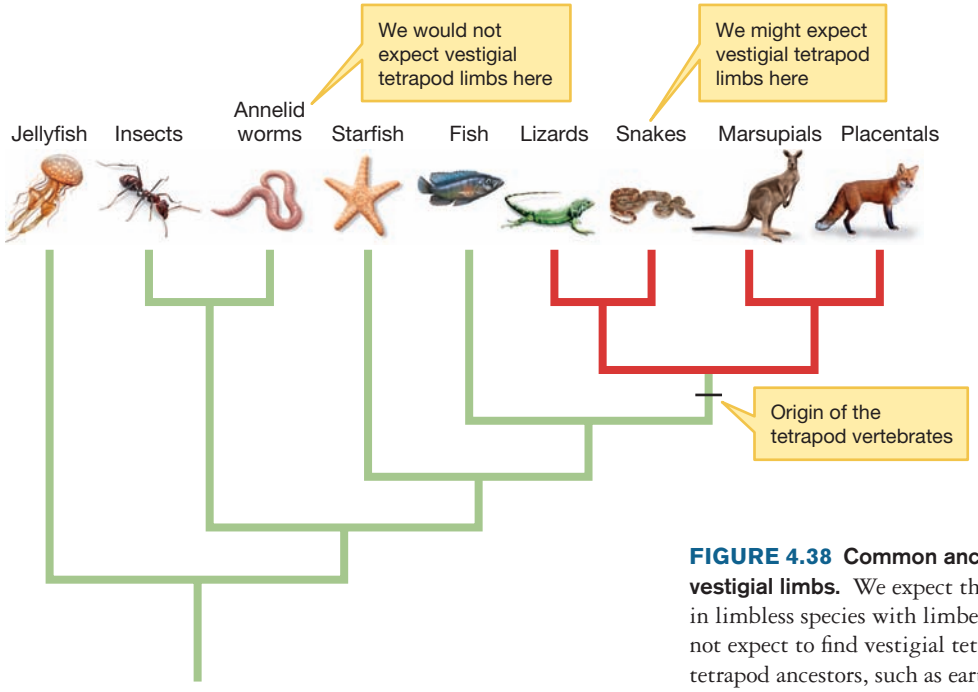


FIGURE 4.38 Common ancestry predicts where we should find vestigial limbs. We expect that we may find vestigial tetrapod limbs in limbless species with limbed ancestors, such as snakes. But we do not expect to find vestigial tetrapod limbs in limbless species without tetrapod ancestors, such as earthworms.

SUMMARY

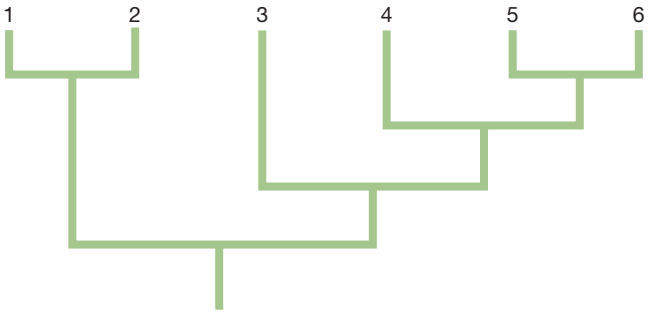
1. Darwin's idea of branching descent with modification provided a theoretical foundation for the hierarchical patterns of classification that Linnaeus suggested. The study of phylogeny is the study of these branching relationships of populations as they give rise to descendant populations over evolutionary time. Phylogenetic systematics casts that classification scheme in terms of evolutionary history.
2. The study of phylogeny rests on our observations of traits displayed by organisms. A homologous trait is a trait that is found in two or more species because those species share a common ancestor. Analogous traits are shared by two or more species because the traits have arisen independently in each species, not because of a history of common descent.
3. Both the process of reconstructing phylogenetic trees and the process of mapping evolutionary events onto trees generate hypotheses. For example, by looking at where a given trait appears on a tree, we can generate a hypothesis about when and how this trait has evolved.
4. Evolutionary biologists use synapomorphies—shared derived traits—to infer the structure of phylogenetic trees.
5. There are many equivalent ways to draw the same phylogenetic tree.
6. The points where a phylogenetic tree branches—the nodes—represent common ancestors to the species that come after the branching point. All branch tips arising from a given branching point are descendants of the common ancestor at that branching point.
7. A monophyletic group, or clade, is defined as a taxonomic group that consists of a unique common ancestor and each and every one of its descendant species, but no other species. A clade always consists of a group of species that share a single common ancestor.
8. A paraphyletic group is one that does include the common ancestor of all its members but does not contain each and every species that descended from that ancestor.
9. Rooted trees indicate the direction of time; unrooted trees do not. The base of a rooted tree is called the root: This is the common lineage from which all species indicated on the tree are derived. We can "root" an unrooted tree at different points on the tree, generating different rooted trees in each case. Each of these different rooted trees represents a different hypothesis about which nodes are most ancestral.
10. Many trees are shown with all of the branch tips aligned. Such trees, called cladograms, convey only the pattern of relationships among the various species displayed. Phylograms are drawn with branches of different lengths; in a phylogram, branch lengths represent the amount of evolutionary change—measured as the actual or estimated number of changes in DNA sequence or other characters—that has occurred along a given branch.
11. Vestigial traits are those that have no current function but appear to have been important in the evolutionary past. Such traits allow us to test evolutionary hypotheses about common origin.

KEY TERMS

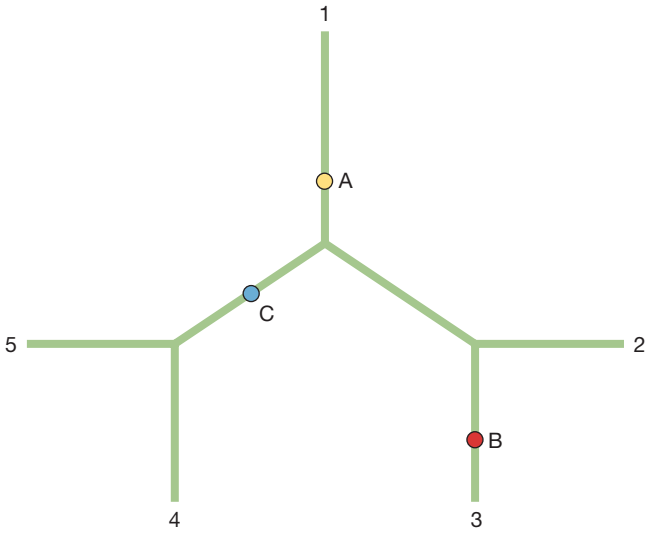
analogous traits (p. 126)
characters (p. 111)
chronograms (p. 122)
clade (p. 118)
cladograms (p. 122)
convergent evolution (p. 127)
derived trait (p. 130)
divergent evolution (p. 127)
homologous trait (p. 125)
homoplasy (p. 130)
monophyletic group (p. 118)
node (p. 116)
outgroup (p. 131)
paraphyletic group (p. 119)
phylogenetic systematics (p. 111)
phylogeny (p. 111)
phylograms (p. 122)
polarity (p. 131)
polyphyletic group (p. 119)
polytomy (p. 118)
root (p. 116)
rooted tree (p. 120)
sister taxa (p. 117)
symplesiomorphy (p. 131)
synapomorphy (p. 130)
taxon (p. 115)
traits (p. 111)
unrooted tree (p. 120)
vestigial traits (p. 137)

REVIEW QUESTIONS

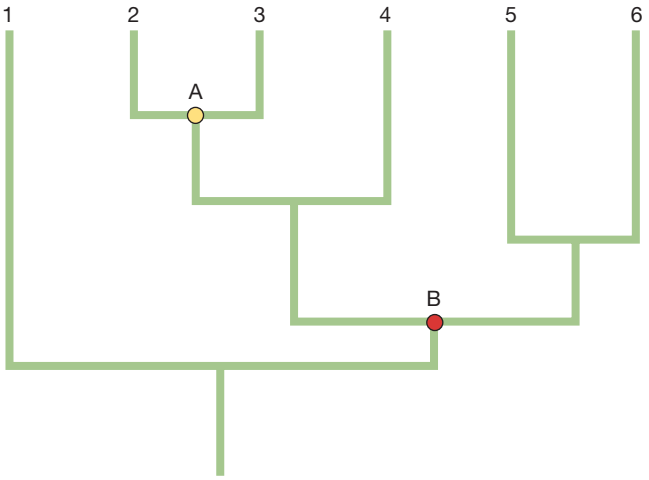
1. Find the common ancestor of species 3, 5, and 6 on the tree below. Find the common ancestor of species 1, 2, and 4.



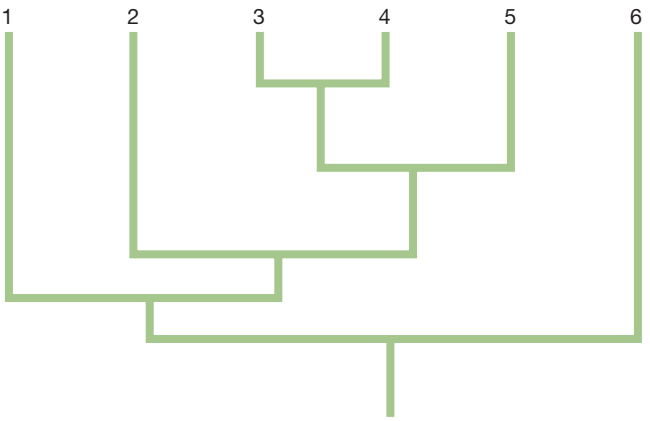
2. The tree below is an unrooted tree. Draw the three corresponding rooted trees if this tree is rooted at points A, B, and C, respectively.



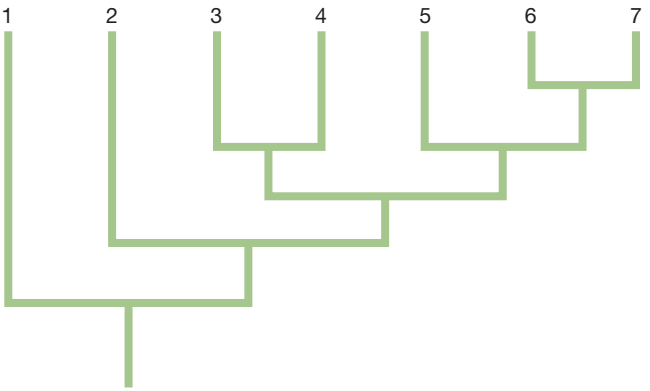
3. For the tree below:
   a. Draw how it would appear after rotating around node A.
   b. Draw it after rotating around node B.
   c. Draw it after rotating around both nodes A and B.



4. Depict the following tree in slanted (ladder) form:



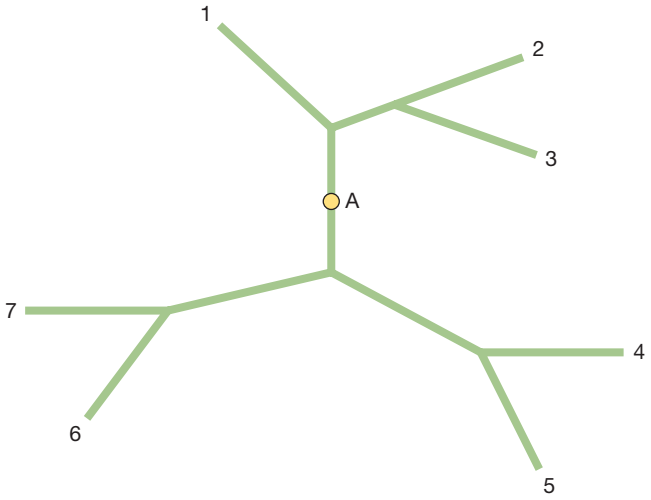
5. On the tree below, the numerals 1–7 represent seven different species.
   a. Which pair of species is more closely related: 4 and 5 or 5 and 7?
   b. Which pair is more closely related: 1 and 2 or 2 and 7?
   c. Which pair is more closely related: 3 and 5 or 2 and 4?



6. How are organisms classified in phylogenetic systematics?
7. Describe the difference between a phylogram, a cladogram, and a chronogram.
8. Contrast synapomorphies, homoplasies, and symplesiomorphies. Which are most informative for phylogenetic reconstruction?
9. Explain how outgroups can help establish the polarity of a trait.
10. How do vestigial traits serve to test Darwin's theory of common ancestry?

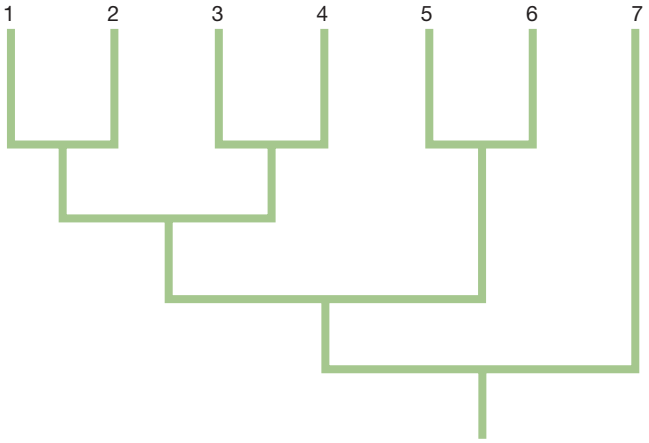
KEY CONCEPT APPLICATION QUESTIONS

11. This unrooted tree shows the evolutionary relationships between species 1–7. If species 1, 4, 5, 6, and 7 form a monophyletic clade, and species 2 and 3 form a monophyletic clade, where should the tree be rooted? Draw the rooted tree.

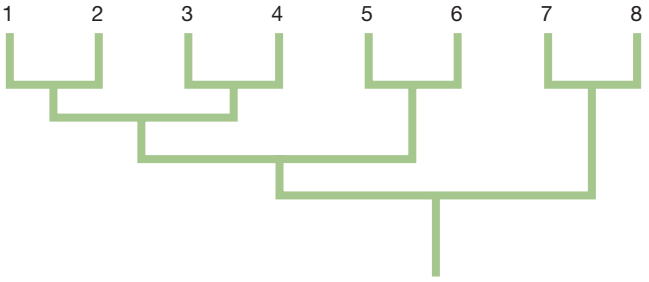


12. Suppose that the tree in question 11 is rooted around point A. What groups with three or more members are monophyletic clades in this case?

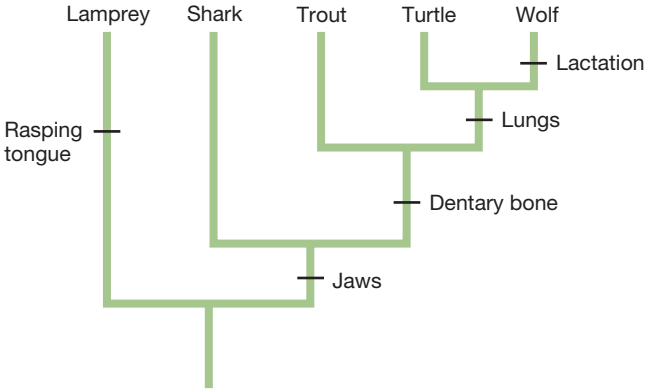
13. On the tree below, what is the smallest monophyletic clade that includes species 4, 5, and 6? What node is the most recent common ancestor of the members of this clade?



14. The tree below shows the phylogenetic relationships among eight species. How many monophyletic clades are there with exactly two members? How many with exactly three members? How many with exactly four?



15. The origins of five traits—a rasping tongue, jaws, the dentary bone, lungs, and lactation—are shown on the tree below. According to the diagram, which of these five traits do sharks have?



16. Come up with a counterexample to show that the following claim is false:

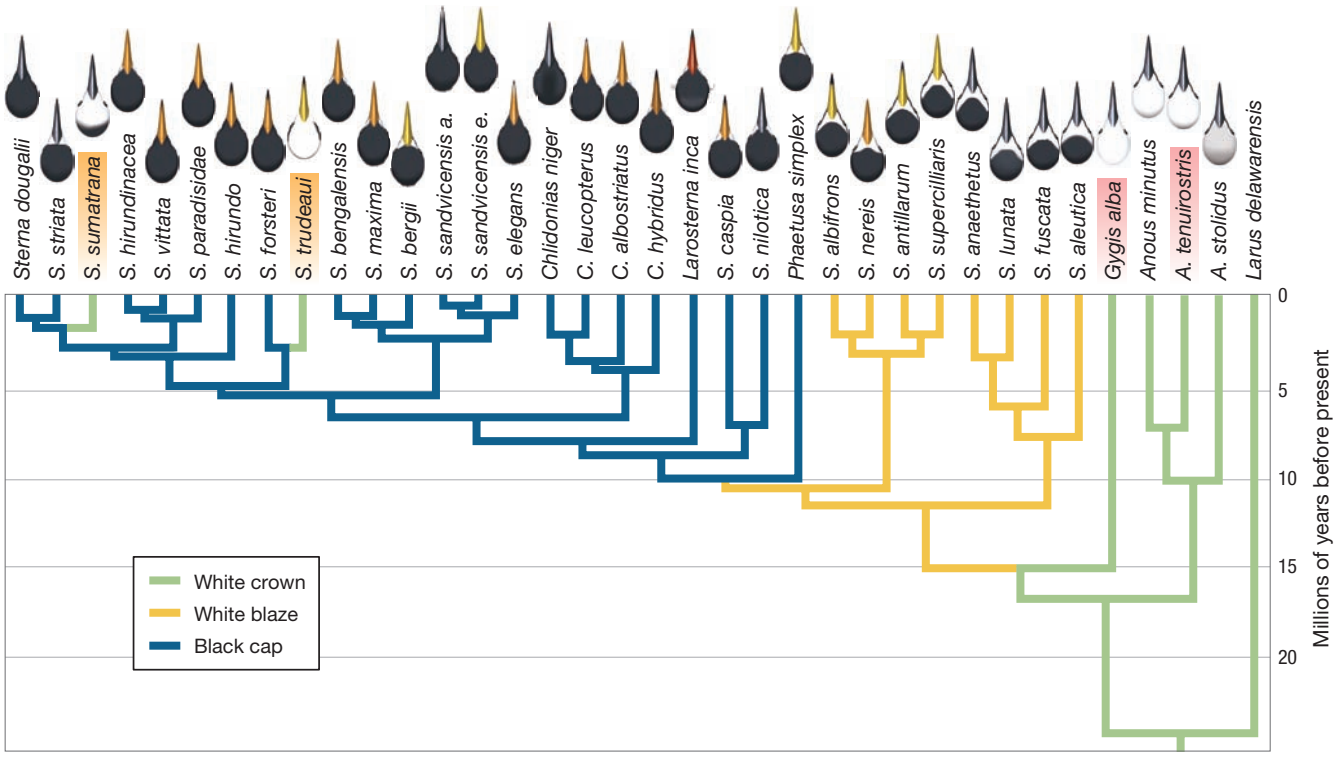
Species separated by fewer nodes are always more closely related than species separated by more.

17. Bridge et al. (2005) developed a chronogram of tern species based on a mitochondrial DNA sequence. The figure below illustrates this chronogram, with the head and beak color of each bird shown.

    a. *Sterna sumatrana* and *Sterna trudeaui* both have white head coloration. According to this phylogeny, is this a homoplasy or a homology?

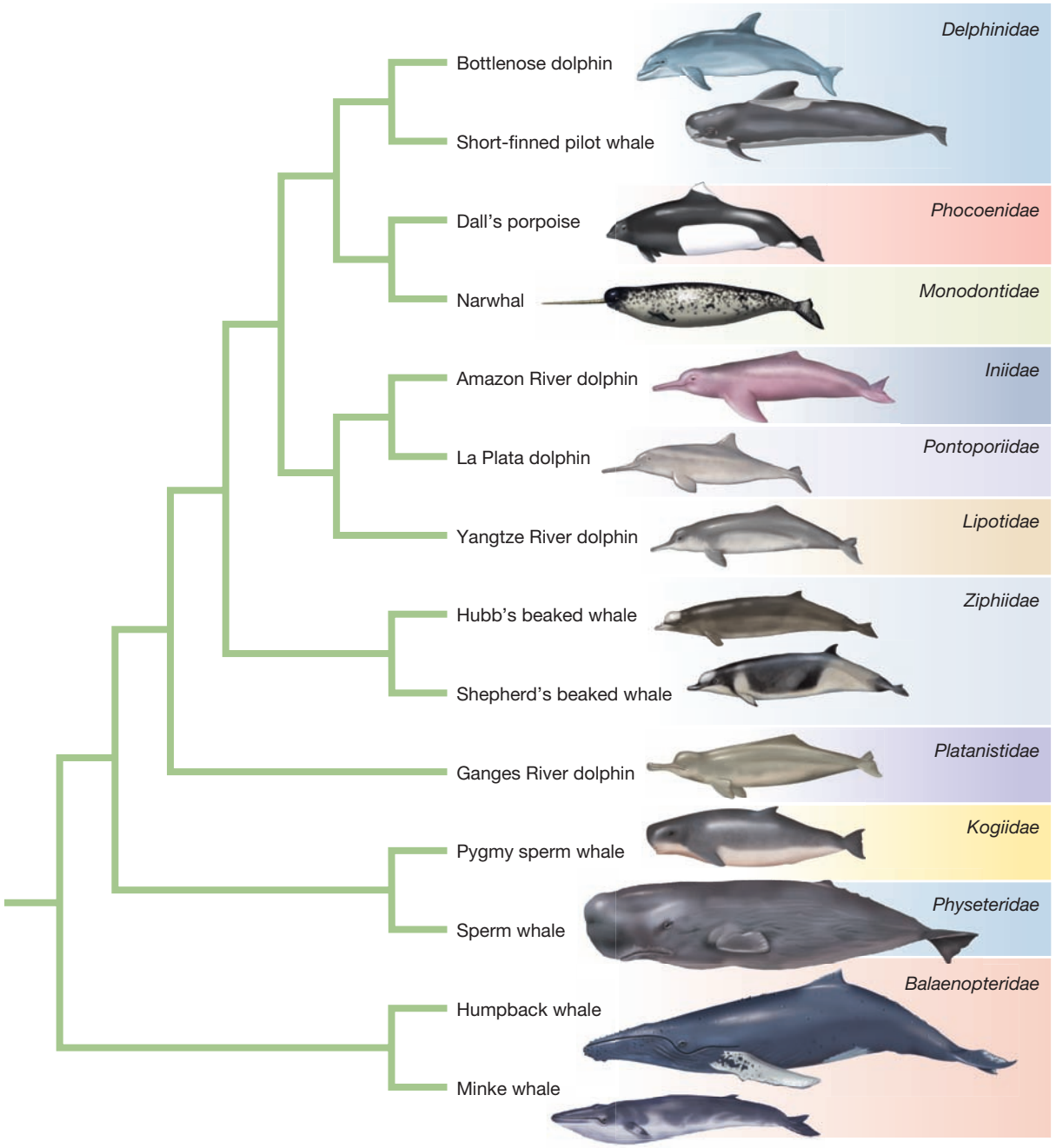
    b. *Gygis alba* and *Anous tenuirostris* also both have white head coloration. Is this a homoplasy or a homology?

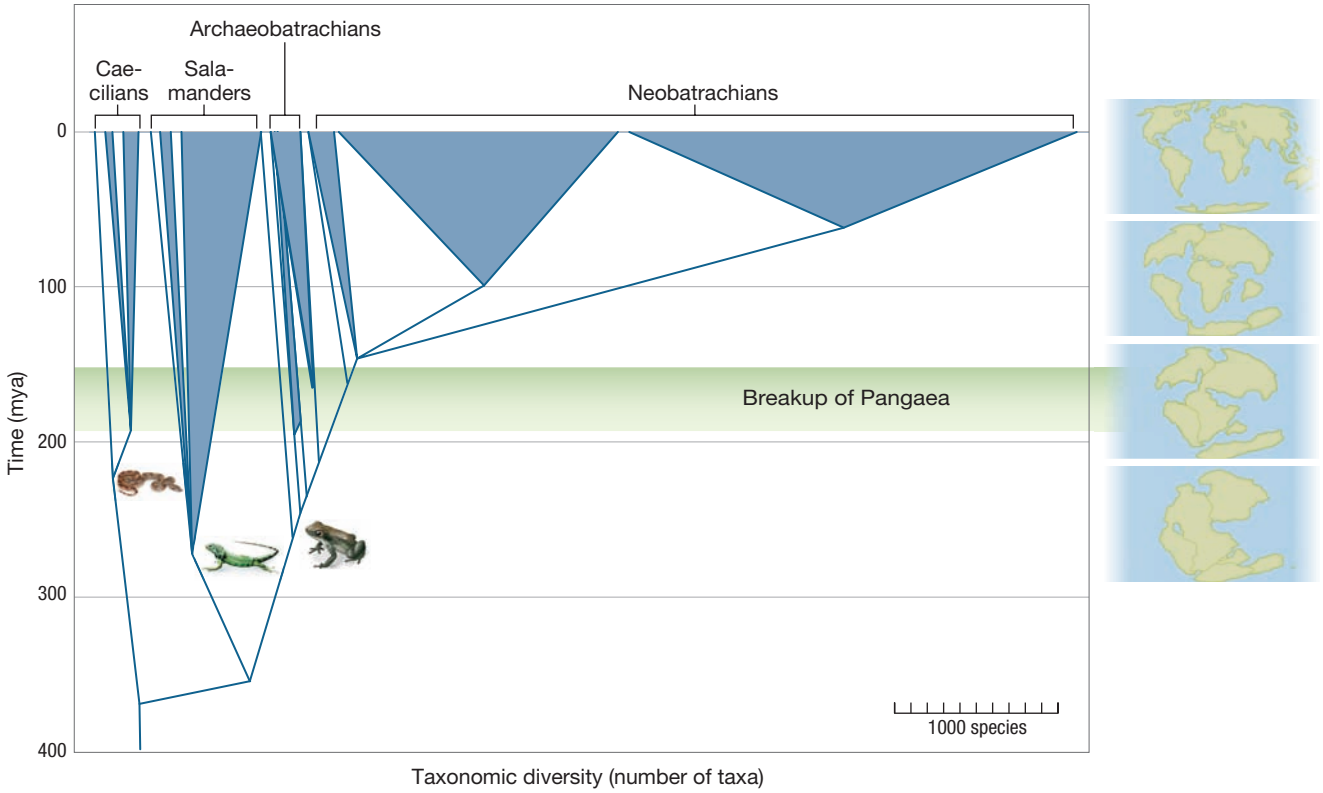
    c. Which character is more highly conserved in this clade: beak color or head color?



18. Nikaido et al. created the cladogram below for cetacean species. Based on this tree, are river dolphins a monophyletic group, a polyphyletic group, or a paraphyletic group? Adapted from Nikaido et al. (2001).

19. Researchers have proposed two different hypotheses for the origin and radiation of the living species of amphibians (Lissamphibia). The Pangaea fragmentation hypothesis posits that these species initially radiated after the breakup of the supercontinent Pangaea. The early origin hypothesis suggests a much earlier evolutionary diversification and radiation for these species. To distinguish between these hypotheses, Diego San Mauro and colleagues created a phylogenetic tree for these species based on nuclear DNA, and they used molecular clock methods to estimate the divergence times for amphibian groups. Their results are summarized in the chronogram on the next page (mya, million years ago). Which hypothesis—Pangaea fragmentation or early origin—is supported by these data? Explain.





SUGGESTED READINGS

Baum, D. A., and S. D. Smith, 2013. *Tree Thinking: An Introduction to Phylogenetic Biology*. Roberts & Company, Greenwood Village, Colorado. An in-depth but accessible text on creating and interpreting phylogenetic trees.

Meyer, A., and R. Zardoya. 2003. Recent advances in the (molecular) phylogeny of vertebrates. *Annual Review of Ecology, Evolution, and Systematics* 34: 311–338. A technical review of the role of genetics in building phylogenetic trees.

Shadwick, R. E. 2005. How tunas and lamnid sharks swim: An evolutionary convergence. *American Scientist* 93: 524–531. A short, popular article on convergent evolution.

Surridge, A. K., D. Osorio, and N. I. Mundy. 2003. Evolution and selection of trichromatic vision in primates. *Trends in Ecology & Evolution* 18: 198–205. A more in-depth review of an example covered in this chapter.

Vonk, F. J., J. F. Admiraal, K. Jackson, R. Reshef, M. A. G. de Bakker, K. Vanderschoot, I. van den Berge, M. van Atten, E. Burgerhout, A. Beck, et al. 2008. Evolutionary origin and development of snake fangs. *Nature* 454: 630–633. More on the evolution of venom-related traits in snakes, as per the example discussed in the chapter.

5

Inferring Phylogeny

◀ Avian diversity is shown in this sample of bird eggs from the Western Foundation of Vertebrate Zoology, Los Angeles, California.

In spring 1999, five Bulgarian nurses and a Palestinian medical intern working at Benghazi Hospital in Libya were accused of a horrifying crime. More than 400 children at the hospital had become infected with the HIV virus, and these six medics were alleged to have deliberately infected those children with a genetically engineered strain of HIV. Prosecutors claimed that the entire outbreak was masterminded by an unknown foreign secret service—perhaps the CIA or the Israeli Mossad—as part of a conspiracy to cause civic disruption in Libya.

Did these six medics really commit this unspeakable act? Or were they merely scapegoats for a tragedy that resulted from inexcusably poor hygienic practices in the hospital? Multiple lines of evidence suggest the latter. *If* the medics were guilty, then all of the infections should have been noted after the medics began working at the hospital, but the evidence shows that some of the infections were recorded as occurring before these medics came to Libya (more on this in a moment). Moreover, one child was even infected after the medics had already been imprisoned. Nonetheless, the "Benghazi six" were convicted in a Libyan court in May 2004 and sentenced to death by firing

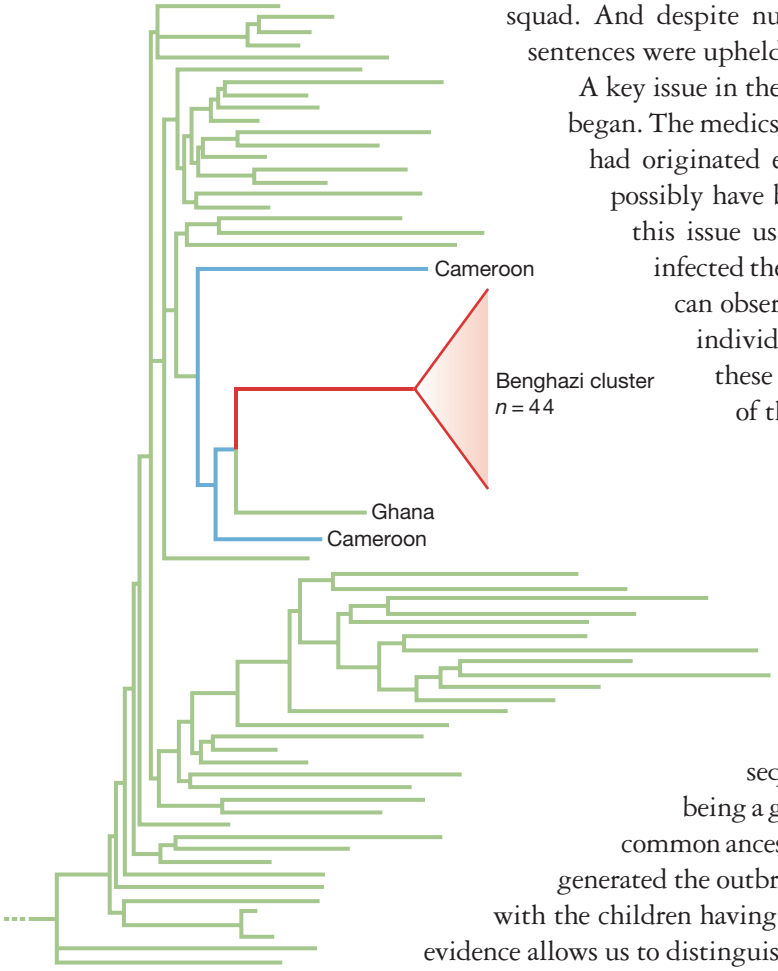


FIGURE 5.1 The Libyan HIV sequences. A phylogeny of HIV sequences that infected the Libyan children form a single clade (red), and this clade is closely related to strains from Ghana and Cameroon. (Outside the red clade, each branch tip represents a single HIV sample.) This suggests that a single introduction was responsible for the outbreak in Benghazi Hospital and that West Africa was a likely source of the strain that caused the outbreak. Adapted from de Oliveira et al. (2006).

squad. And despite numerous legal appeals, the convictions and death sentences were upheld by the Libyan Supreme Court in December 2006. A key issue in the trial was the timing of when the outbreak actually began. The medics had arrived in Libya in March 1998. If the outbreak had originated even earlier—say in 1997—the medics could not possibly have been responsible. Evolutionary biology can address this issue using phylogenetic analysis of the HIV strains that infected the children. The HIV virus changes so rapidly that we can observe differences in the genome sequence even among individual patients infected from a common source. Using these differences, biologists can reconstruct a phylogeny of the virus at a very fine level of resolution.

In 2006, Tulio de Oliveira and a team of researchers used genetic sequence data from the HIV strains infecting 44 of the Libyan children to reconstruct such a phylogeny (de Oliveira et al. 2006). Their phylogeny reveals the relationships among the individual HIV strains infecting each of the children and also the relationships between the infections in these children and other known strains of HIV (**Figure 5.1**). The Libyan sequences form a single clade (in this case, the clade being a group of strains that were all descended from the same common ancestor), as would be expected if a single infected patient generated the outbreak in Benghazi Hospital. But this is also consistent with the children having been infected by a single medic. Fortunately, other evidence allows us to distinguish between these possibilities. For example, the HIV strains in this clade are most closely related to strains observed in areas of West Africa from which numerous migrants have come to Libya seeking employment—strongly suggesting accidental introduction from the Libyan population.

It is also possible to estimate the timing of the infection from the phylogenetic information. The older a clade is, the more time it has had for phylogenetic diversification. In a very recent clade, all members would be expected to share very similar sequences, whereas in an older clade, we would see more **sequence divergence**—greater differences between DNA sequences—among the clade members. The team of researchers measured the sequence divergence among the HIV strains in the Libyan clade. Given the rate at which the HIV sequence changes over time, they concluded that the Libyan clade was too diverse to have arisen as late as March 1998. Rather, the infections must have started early, possibly in 1997, and almost certainly prior to the arrival of the medics in Libya. Comparable analysis of the hepatitis C virus strains also infecting many of the children revealed the same thing: The infections were too diverse to have begun spreading as late as March 1998.

While the Libyan courts were unwilling to heed this scientific evidence, the clear and powerful science behind the case intensified international political pressure on the Libyan government. Not the least of those campaigning on behalf of the "Benghazi six" were 114 Nobel laureates in the sciences, who, based on the scientific evidence we have detailed, published an appeal for their release in the journal *Nature* (Roberts and Nobel Laureates 2006). These pleas from the scientific community, coupled

with continued diplomatic efforts, paid off. On July 16, 2007, the Libyan Supreme Council for Judicial Authority commuted all six death sentences to sentences of life imprisonment. A week later, after having spent 8 years in a Libyan prison, the six medics were returned to Bulgaria to serve out their terms. Back in Bulgaria, they immediately received a pardon from the Bulgarian president and were released. This is a happy ending, of a sort—but of course no such eleventh-hour reprieve was possible for the more than 400 HIV-positive children who were also victims of this tragedy.

It was, in part, because of the construction of phylogenetic trees and the ability to make inferences from such trees that innocent lives were spared in this case. Of course, in most instances, no lives will be spared when phylogenetic trees are constructed and interpreted, but they are still an extraordinarily powerful tool for understanding evolutionary history.

KEYCONCEPT QUESTION

5.1 How would the sort of analysis described above be helpful when epidemiologists are responding to an ongoing epidemic?

As we will explore in much more depth, evolutionary biologists use many different methods for constructing phylogenetic trees and use various types of data when they do so. Phylogenetic trees are used both to construct hypotheses about common ancestors and how various species are related to each other and to test hypotheses about such relationships.

In this chapter, we will examine the following questions:

- What are some of the methods used to construct phylogenetic trees, and what are their limitations?
- How do different sources of information—including information derived from molecular genetic sequences, the fossil record, and geographic patterns—enable evolutionary biologists to build phylogenetic trees?
- How do biologists use phylogenetic thinking to handle the problem that data from closely related species often cannot be considered independent observations when testing questions related to adaptation?

5.1 Building Trees

The task of constructing a phylogenetic tree is fundamentally a problem in statistical inference; that is, we wish to make inferences about the world from a data set. In the case of phylogenetic inference, we typically have information about characters of the species we are studying, such as morphological measurements, behavioral patterns, or genetic sequences. From these data we aim to infer the historical evolutionary relationships among these species. Before we look at how this is done, take a moment and think about how powerful such techniques can be in principle. What we are aiming to do is use data we can measure *right now* to make inferences about events in the evolutionary past, often millions of years in the past.

The basic conceptual approach to phylogenetic tree building is straightforward. We select a number of species (or other taxa) for which we wish to build a tree. We collect information about the characters of individuals of these species, and we look at which species have which traits in common. The logic of tree building is that species with many traits in common are more likely to be closely related to one another than are species with fewer traits in common. For example, we presume that mammalian species—species in which females produce milk and feed their young, and in which all individuals have hair, have a middle ear with three bones, and share numerous other traits—are more closely related to one another than they are to species that lack these traits, such as lizard species.

This logic assumes that shared traits are homologies; that is, traits that are shared because of shared common ancestry. Otherwise, we would not expect species with more traits in common to be more closely related phylogenetically. Although this logic seems straightforward, the devil is in the details. How do we test the possibility that common traits are analogous rather than homologous? How do we resolve conflicts in the data regarding the evolutionary relationships among the species we are studying? How—by what algorithm or procedure—do we go about actually finding the best tree corresponding to a given set of character data? Evolutionary biologists have developed a number of different *phylogenetic methods*, each of which handles these challenges in a different way. In this chapter, we will look at a number of these methods, with an aim to understand both the logic of each approach and its particular strengths and weaknesses.

We begin by looking at what are called *parsimony methods*, in which we search for trees that minimize the number of evolutionary changes. We touched briefly on parsimony analysis in Chapter 4 when we examined phylogenies in which the character of interest was coat coloration; here, we explore the topic in more depth. Advantages of the parsimony approach include its conceptual simplicity and the existence of straightforward algorithms for constructing parsimonious trees.

Next, we turn to *distance methods*. As we mentioned, the basic logic of phylogenetic reconstruction is that species with a large number of traits in common tend to be more closely related to one another than are species with smaller numbers of common traits. One of the simplest approaches to reconstructing trees is simply to count up the number of commonalities and to use this information directly to cluster closely related species together. This is what distance methods do.

While both parsimony methods and distance methods can be quite effective in inferring evolutionary history, neither incorporates an explicit statistical model of how evolutionary change takes place. Parsimony methods assume that the fewer changes required, the more plausible the tree; distance methods assume that more similar species are more closely related. By contrast, **maximum likelihood** methods use explicit models of how traits change through the evolutionary process by applying conventional techniques of statistical inference to find the phylogenetic tree that best explains the data. **Bayesian inference** methods do something similar. The difference between the maximum likelihood and Bayesian inference methods lies in the interpretation of what "best explains" should mean. Maximum likelihood methods and Bayesian inference methods require a modest background in probability theory, so we will defer our treatment of these topics to the appendix entitled "Likelihood Methods and Bayesian Methods for Phylogenetic Inference," located at the end of this book.

5.2 Parsimony

The fundamental idea behind **parsimony** is that the best phylogeny is the one that explains the observed character data by positing the fewest evolutionary changes. To find the best phylogenetic tree, one first must be able to evaluate a given tree and calculate how many character changes are necessary to explain the observed character pattern on that particular tree. An example helps. Suppose we are trying to decide between the two phylogenetic trees in **Figure 5.2**. Which of these two hypotheses about the evolutionary relationships among species 1–4 is better supported by our observations of trait values?

If we have a character that differs in only one species on our tree—say, tail length—it can always be explained by a single character change, regardless of what tree we examine. In both trees in **Figure 5.3**, long tails arose by a single evolutionary change occurring after our long-tailed species diverged from the other species on the tree. Therefore, this character does not help us distinguish between different phylogenetic hypotheses.

Now imagine our character of interest is coat coloration and that it can be either dark or light. If two species have dark coats and two have light coats, matters get interesting. Suppose that species 2 and 3 share the common trait of dark coats, and species 1 and 4 share the other trait, light coats. Notably, there are multiple ways to explain this pattern with two character changes. One possibility is that dark coats arose twice from a light-colored ancestor; another is that light coats arose twice from a dark-colored ancestor. Either way, under hypothesis I in **Figure 5.4**, we require two distinct evolutionary events to obtain the observed character states. But, under hypothesis II, we can explain the pattern with a single evolutionary event. Thus, we say that hypothesis II provides a more *parsimonious* explanation of our character-state observations.

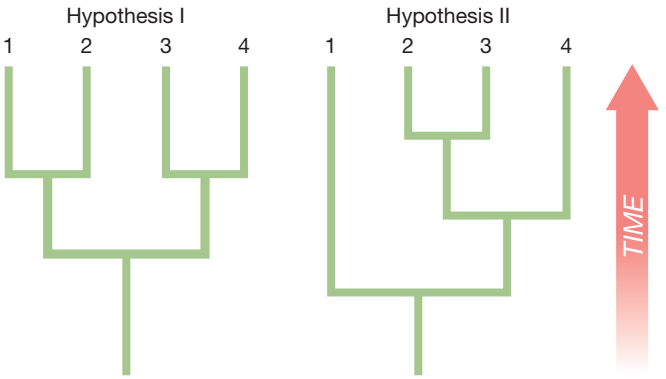


FIGURE 5.2 Phylogenetic trees as hypotheses. These two phylogenies represent two different hypotheses about the evolutionary relationships among species 1–4.

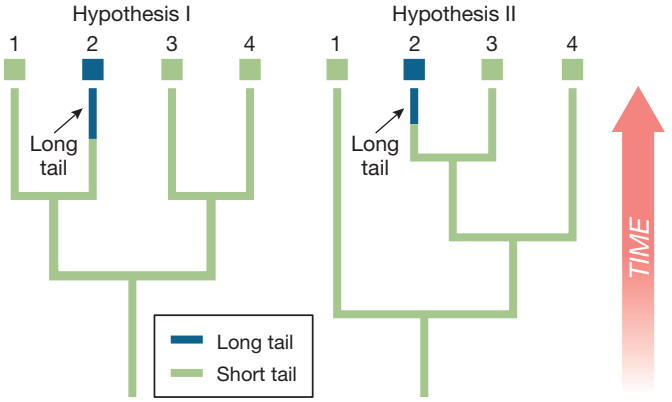


FIGURE 5.3 A single species differs from the others. Here, the tail-length character has the long character state in one species and the short character state in the others. If only one species has a different character state from the rest, only one evolutionary change will be required irrespective of the phylogenetic tree. Thus, tail length does not help us distinguish between the two hypothesized trees.

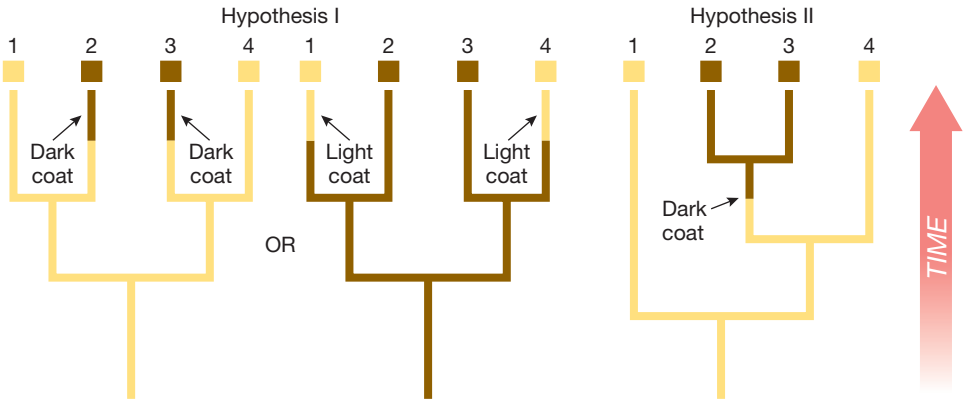


FIGURE 5.4 Two species differ from the others. Here, two species have light coat coloration and two have dark coat coloration. In this case, a tree shaped like those in hypothesis I requires two changes in character state to explain the observed coat colors. However, a tree shaped like the one in hypothesis II requires only a single character change, from a light-colored ancestor to a dark-colored one along the branch leading to species 2 and 3.

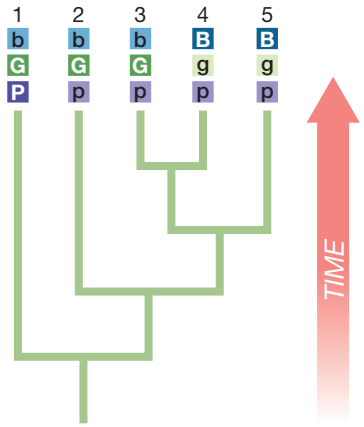


FIGURE 5.5 A phylogeny with three observed characters in five taxa. Three character states—dark/light blue (B/b), dark/light green (G/g), and dark/light purple (P/p)—and a hypothetical phylogenetic tree relating the species. We want to evaluate this tree using a parsimony approach.

The process is the same for larger trees with more species. Given a tree and a set of character states for a particular character, we can figure out how many evolutionary changes are necessary to explain our observations. In Figure 5.4, we only looked at a single character, but in practice there are usually multiple characters to consider. In the parsimony framework, working with multiple characters is straightforward. We look at each character in turn, determine how many changes are necessary for that character, and sum up the total number of changes necessary for all characters in order to find the total number of changes required.

For example, suppose we have information about three different characters, as shown in **Figure 5.5**. To use the parsimony approach, we need to know the minimum number of changes in each character that are needed to explain our data. To do this, we tally the number of changes required, given our tree. In this case, our tree requires one, two, and two character changes, respectively, to explain the purple (P/p), green (G/g), and blue (B/b) characters. In **Figure 5.6**, we show one way in which each of the character states could be explained by the minimum number of changes.

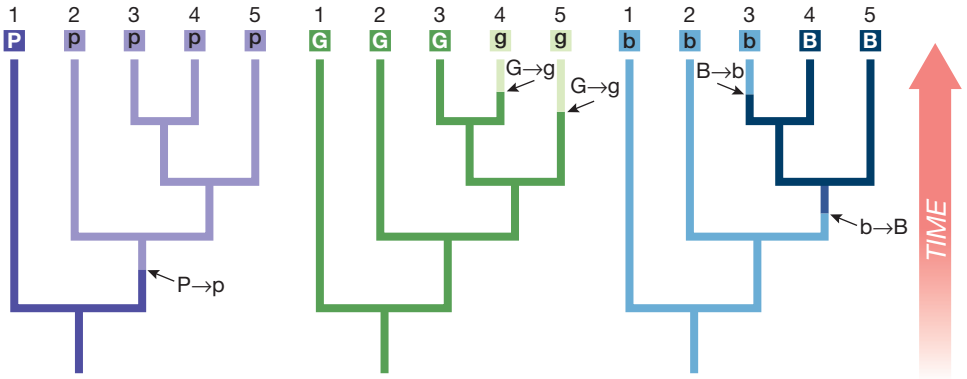


FIGURE 5.6 Explaining character states with a minimal number of changes. Possible locations of character changes for three character states along the hypothetical tree shown in Figure 5.5. For this particular tree, the purple character requires only a single change, whereas the green and blue characters each require two changes in character state.

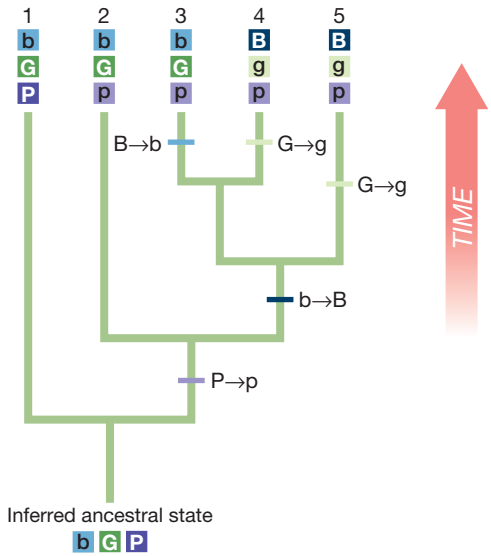


FIGURE 5.7 Showing multiple characters on a single phylogeny. We can show all of the changes on a single diagram by indicating the inferred ancestral state and then marking each change in character state.

Notice that while each tree in Figure 5.6 shows how a minimal number of changes can be placed on our trees to explain character changes, these patterns of change are not unique. For example, the purple tree could alternatively be explained with a single change if light purple (p) were the ancestral state and the dark purple character (P) arose via a change along the branch leading to species 1 alone.

Because it is inconvenient to have to draw out a separate tree for each character, we often summarize the changes in all characters with a diagram like that shown in **Figure 5.7**. We saw this sort of representation when we looked at the process of placing traits on trees in Chapter 4.

Once we have found a way to represent the minimum number of character-state changes on a tree, we can define this number as a parsimony score *for that particular tree.* To use maximum parsimony to infer phylogenetic history, we look at various possible trees and select the one with the lowest parsimony score.

In Figure 5.7, for example, it took five character changes to explain the character data on that particular tree. But we can explain the same character data with fewer changes by means of a different phylogenetic tree. **Figure 5.8** illustrates this. For this tree, only three character changes are necessary to explain the character data. Under the logic of maximum parsimony—that is, minimizing the number of evolutionary changes required to explain our tree—we prefer this tree to the previous one because it can explain our data with fewer changes. Sometimes several different trees may be tied for the lowest parsimony score. In this case, each is said to be equally parsimonious: The parsimony approach does not give us cause to prefer any one of these most parsimonious trees over any other.

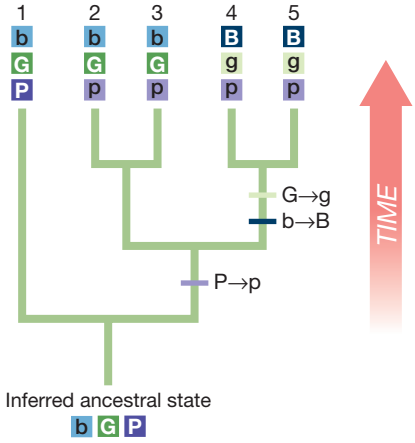


FIGURE 5.8 A more parsimonious tree for our character data. Only three character changes are necessary to explain the character data using this phylogenetic tree.

Any time we have multiple candidate phylogenies, whether a set of equally parsimonious phylogenies from a single analysis or a set of alternative phylogenies from separate analyses, a *consensus tree* can be constructed to represent the multiple possible phylogenies in a single tree (Adams 1972; Swofford 1991). A *strict consensus tree* reflects the monophyletic groups that appear in all of the phylogenies and depicts the uncertain relationships—those that differ from one tree to another—as polytomies. A *majority rule consensus tree* resolves these polytomies according to majority vote, by featuring the monophyletic groups that appear in a majority of the phylogenies. Researchers have also developed a number of additional methods for generating consensus trees (Bryant 2003).

How do we know when we have found the most parsimonious tree? In Figure 5.8, it is straightforward to tell: We have only one change per variable character, so we know we cannot possibly do better. But we still need a general way to figure out how many changes a tree will require given a certain set of characters. Fortunately, there are a number of algorithms that allow us to determine the number of changes necessary to explain a given character pattern on a given tree. **Box 5.1** describes one of the simplest of these, the Fitch algorithm.

Parsimony has the advantage of conceptual simplicity, but parsimony approaches are not without problems. The worst of these problems is that parsimony is not a *consistent estimator*; that is, an estimation procedure that, given enough data, will ensure that we get the right answer. Thus, if we use parsimony to reconstruct a phylogeny, it is possible for us to get the wrong tree, no matter how much data we have available. Sequencing additional loci or tabulating additional morphological characters may not help us in the least. Parsimony is most likely to run into trouble when evolutionary changes occur at different rates on different branches of the phylogeny, as illustrated in **Figure 5.9**. In that case, parsimony methods may incorrectly infer too close a relationship between the rapidly evolving branches. This tendency is known as **long-branch attraction**, because species on long branches of the phylogenetic tree are "pulled together" by the inference procedure used in parsimony analysis (Felsenstein 1978; Bergsten 2005).

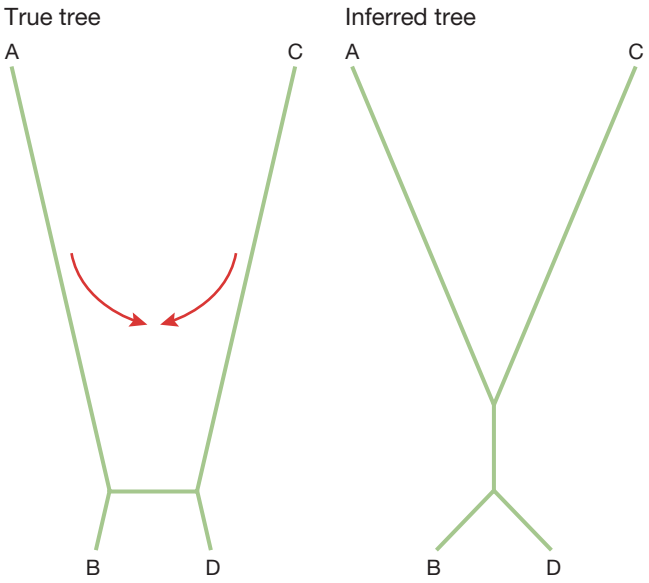


FIGURE 5.9 Long-branch attraction. The true tree is shown in the left panel. Because evolutionary change is occurring more quickly in taxa A and C, the corresponding branches are much longer. As a consequence, the branches A and C "attract" each other (red arrows), and parsimony methods may incorrectly infer a tree of the form shown in the right panel.

BOX 5.1 The Fitch Algorithm

Parsimony algorithms search trees to explain the observed character data with a minimum number of changes. But given a hypothetical tree and the character states for a given character, how many evolutionary changes are required? Evolutionary biologist Walter Fitch developed a method to answer this question (Fitch 1971). The *Fitch algorithm* applies to a given tree and a single character trait at a time: The number of changes required to explain multiple characters on that tree is simply the sum of the number of changes required to explain each individual character.

The Fitch algorithm does not find the best tree; it simply tells us how many character changes are required for a given tree. We then would need to repeat the process for other plausible trees to find the most parsimonious. In this box, we illustrate the application of the Fitch algorithm to a single character on one sample tree.

Figure 5.10 illustrates a tree in which we wish to evaluate the character values red, blue, or yellow for each of seven species on that tree. The Fitch algorithm proceeds in a series of steps (Felsenstein 2004). We begin at the branch tips, taking sister groups and working downward to the base of the tree. Beginning with zero, we keep a running count of how many character changes are necessary. As we work our way down the tree, each internal node is assigned one or more character states, and we update the tally of character changes where appropriate. The rules for assigning these character states and tallying character changes are as follows:

1. If each of the two *daughters* (immediate descendants) of a node share one or more possible states for our trait, assign those shared states to the node in question. In other words, the possible traits at the node are the *intersection* of the set of possible traits of daughter 1 and the set of possible traits of daughter 2; that is, any possible trait shared by both daughter 1 and daughter 2. In this case, we do not increase our tally of necessary character changes.
2. If the two daughters share no possible states in common, assign to the node in question all of the possible states for both daughters. In other words, the set of possible traits at the node is given by the *union* of the set of possible traits of daughter 1 and the set of possible traits of daughter 2; that is, any possible trait from either daughter 1 or in daughter 2. In this case, we augment the tally of necessary character changes by one.

We then repeat until we have worked all the way to the root of the tree.

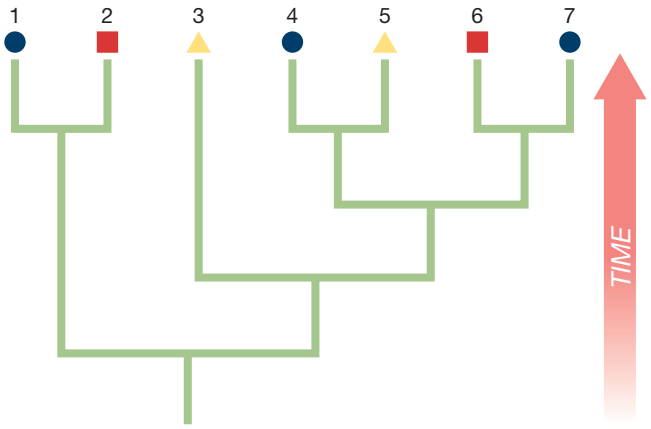


FIGURE 5.10 How many character changes are necessary for this tree? We will use the Fitch algorithm to determine the minimum number of evolutionary changes required to explain the character states of the seven species on this tree.

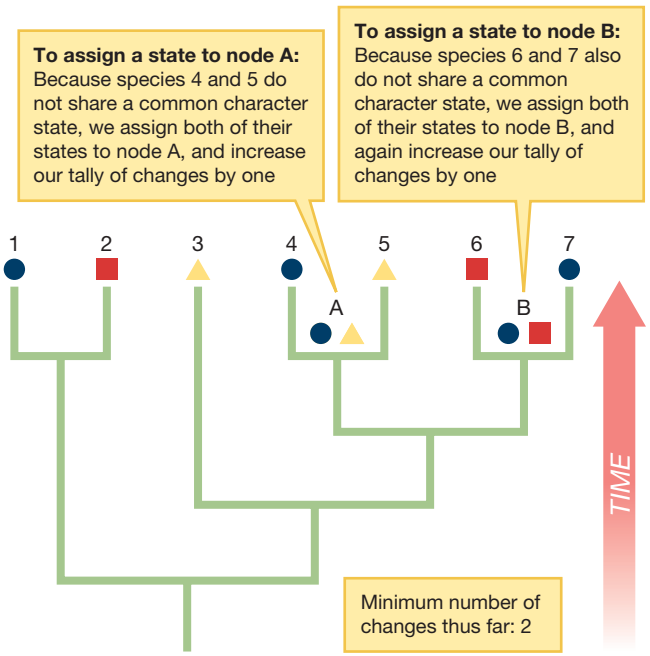


FIGURE 5.11 Assigning possible character states to nodes A and B. Here we see how to use the Fitch algorithm to assign possible character states to nodes A and B.

In the figures that follow, we carry out this process for our example tree. In **Figure 5.11**, we assign character states to nodes A and B. In each case, the daughter nodes share no possible character states in common. We thus take the union of the daughters' character states and increase our tally of character changes by one each time. Node A has two daughters: species 4, which is blue, and species 5, which is yellow. Thus, node A is assigned both blue and yellow as possible character states. Node B has two daughters: species 6, which is red, and species 7, which is blue. Thus, node B is assigned both red and blue as possible character states. In each case, the daughters share no possible traits in common, and so we have to augment our tally of character changes each time. This gives us a total of two necessary character changes thus far.

Figure 5.12 illustrates how we continue downward along the tree. Node C has two daughters: node A with states blue and yellow, and node B with states blue and red. These share a common possible state, blue, and so we assign that state to node C. Because its daughters share a common state, we do not have to augment our tally of character changes to account for node C. We then move on to node D. Node D has two daughters: species 3 with state yellow, and node C with state blue. Because these daughters share no common character states, we assign to node D the union of their character states, blue and yellow, and we increase our tally of character changes by one more, to a total of three.

In **Figure 5.13**, we assign character states to the two remaining nodes, node E and node F. Node E has two daughters: species 1, which is blue, and species 2, which is red. We thus assign the possible character states of blue and red to species E, and we augment our tally of changes again, giving us a total of four. Node F has two daughters: node E and node D. These daughters share blue as a possible character state, so we assign blue to node F, and we do not need to increase our tally of changes further.

At this point we have assigned character states to each node of the tree, and the algorithm is complete. Our tally of character changes is four. By the algorithm, this is guaranteed to be the minimum number of changes necessary to explain the character data on this particular tree.

It is important to realize that the Fitch algorithm does not tell us the most likely character states for each ancestral node. In the algorithm, the process of assigning states to interior nodes is simply a way to count the number of changes, not a reconstruction of ancestral types.

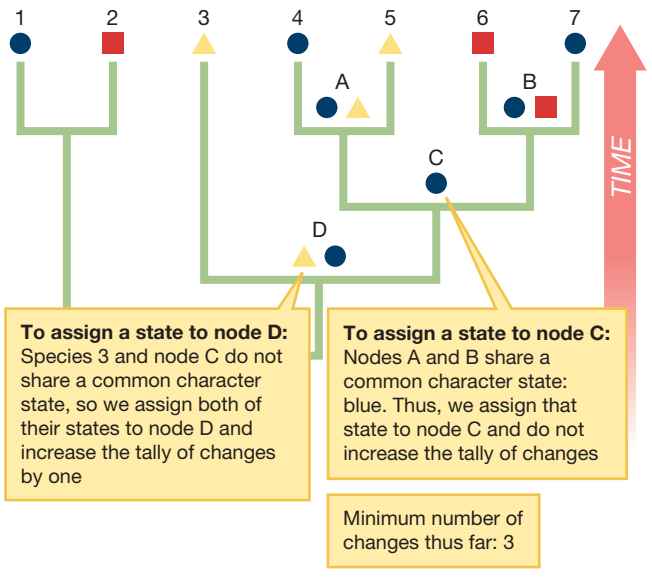


FIGURE 5.12 Assigning character states to nodes C and D. Here, we see how to use the Fitch algorithm to assign character states to nodes C and D.

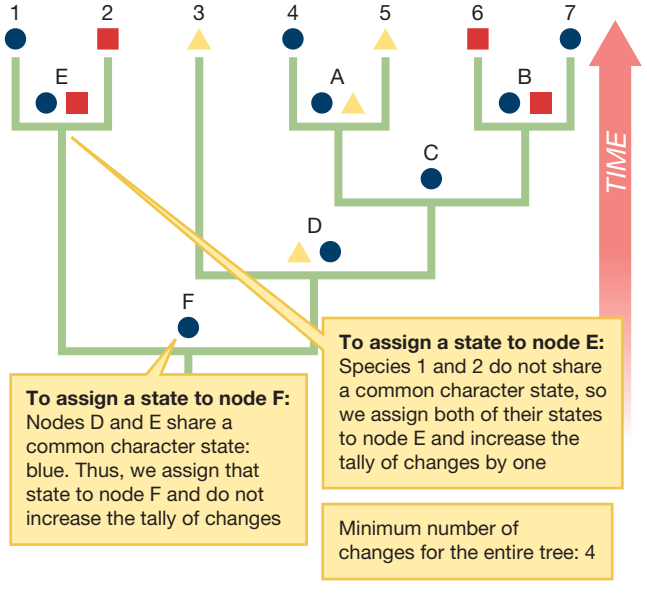


FIGURE 5.13 Assigning character states to the two remaining nodes in the tree, nodes E and F. Here we use the Fitch algorithm to assign character states to nodes E and F.

5.3 Distance Methods

Phylogenetic distance methods provide a second approach that evolutionary biologists use to infer phylogenetic trees. The basic idea behind distance methods is that if we can measure the pairwise "distances" between species, then we can use these distances to reconstruct a tree. A warning here: "Distance" is not being used in the literal geographic sense of feet, miles, and so on. Instead, it is a measurement of morphological or genetic differences between species. Our aim is to find a phylogenetic tree with branches arrayed such that the distance along the branches between any two species is approximately equal to the distance that we measured between those two species.

To do this, we need to address two questions: (1) how do we measure distance between species, and (2) once we have these distance measurements, how do we find the best tree given these distance data? We will address these in turn.

Measuring Distances between Species or Population

There are a number of different ways we can measure the distance between any two species or, more generally, between any two populations. Prior to molecular systematics, distances were often computed from morphological measurements or by tallying the number of character differences between species. Such methods remain important when using fossil data to build phylogenies for extinct organisms. But when we study living species, it is now far more common to use DNA sequences from the two species, suitably aligned (**Box 5.2**). One of many ways to do this is simply to count up the number of base pair differences and to use this tally as the molecular distance between the two species. **Figure 5.14** illustrates one of the earliest DNA sequence comparisons for influenza A. Influenza virus proteins that are recognized by the immune system, such as the hemagglutinin (HA) protein, can evolve rapidly from year to year to escape immune memory, and therefore even sequences from the same strain can show sizable year-to-year variation. Shown here are the initial coding regions of the hemagglutinin (HA) genes from the H3N2 strains circulating in 1968 and 1975. The regions shown differ by only a single highlighted base pair and thus are separated by a genetic distance of 1 (Verhoeyen et al. 1980). If we have amino acid sequence data instead of DNA sequence data, we can look at the number of amino acid substitutions between the two species and use this tally as the molecular distance between them. **Figure 5.15** illustrates the amino acid distance between two more recent strains of the Influenza A virus. Here we compare a short segment of the HA protein in the dominant 2008 H1N1 strain of the virus with the equivalent segment in the 2009 H1N1 strain responsible for the 2009 swine flu pandemic. In the region illustrated, the two proteins differ by four amino acids, for a genetic distance of 4. Due to genetic reassortments among bird, swine,

```
H3N2 1968   A U G A A G A C C A U C A U U G C U U U G
H3N2 1975   A U G A A G A C U A U C A U U G C U U U G
```

FIGURE 5.14 Measuring distance based on nucleotide sequence. The DNA sequences here represent a small region at the start of the hemagglutinin protein of the influenza A virus. These two segments differ by only a single highlighted base pair, for a genetic distance of 1. Data from Verhoeyan et al. (1980).

```
H1N1 2009   Val - Lys - Ser - Thr - Lys - Leu - Arg - Leu - Ala - Thr - Gly - Leu
H1N1 2008   Val - Arg - Ser - Ala - Lys - Leu - Arg - Met - Val - Thr - Gly - Leu
```

FIGURE 5.15 Measuring distance based on amino acid sequence. The two amino acid sequences here represent a small region toward the end of the hemagglutinin protein of the influenza A virus. These two segments differ by the four highlighted amino acids, for a molecular distance of 4. Data from Gallaher (2009).

BOX 5.2 Sequence Alignment

If we want to use any phylogenetic method that relies on DNA or amino acid sequence data, we face the problem of *sequence alignment.* Because of insertions, deletions, and other changes to the structure of the DNA, the sequences from species from the various groups being studied may not line up—or align—cleanly, making comparison very difficult. To see this more concretely, let's first look at a case where sequence alignment is *not* a problem, as in **Figure 5.16**.

Now suppose there has been a deletion in the DNA sequence of species A. **Figure 5.17** illustrates the consequences. Because of this deletion, the species A sequence doesn't align with the others directly; it would have to be adjusted, leaving a gap at this position, to align correctly. In general, there can be multiple deletions at different places in different species, as well as multiple insertions. Alignment becomes more difficult as the number of such instances increases. As such, evolutionary biologists have created various computer program methods for handling this alignment problem, although many sequences are still frequently aligned by hand for verification (Feng and Doolittle 1987; Higgins and Sharp 1988; Baldauf 2003).

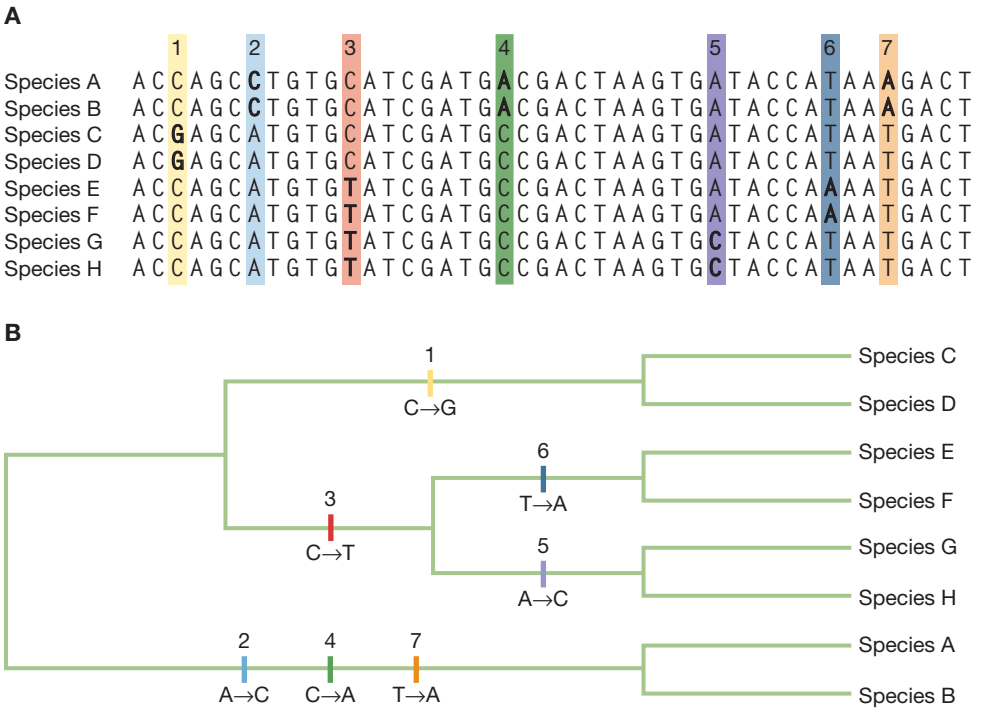


FIGURE 5.16 Sequence alignment and construction of a phylogeny. **(A)** A case where sequence alignment is not a problem. Here we have nucleotide sequence data for eight species, and the data align. We see differences across species at the seven shaded positions. **(B)** From the data in panel A, we can construct a phylogenetic tree. Adapted from University of Illinois (2011).

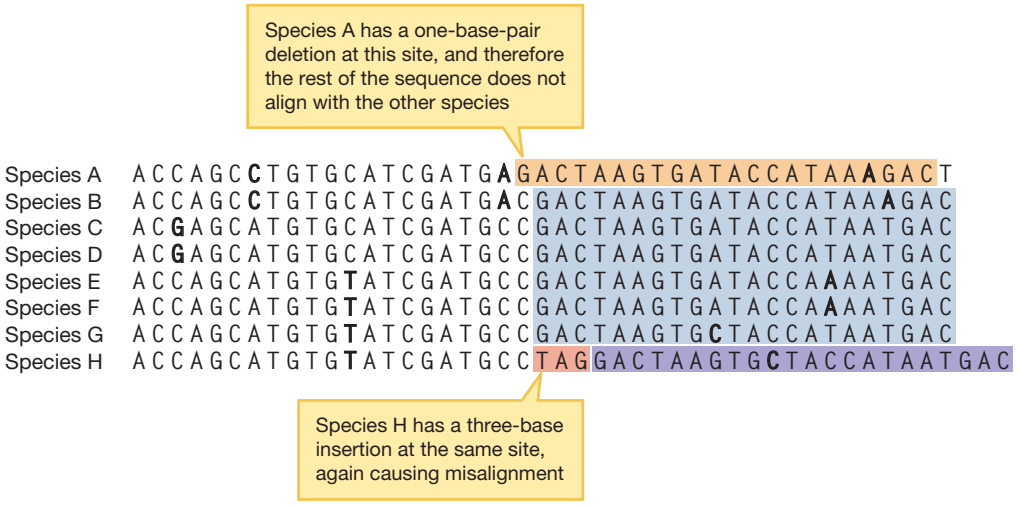
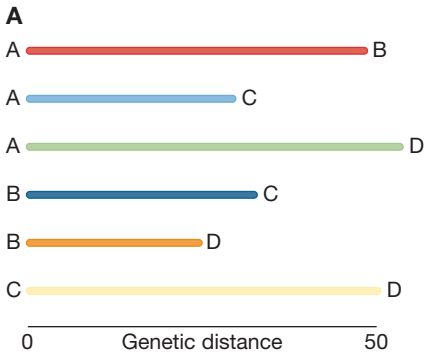


FIGURE 5.17 Deletions or insertions affect sequence alignment. Here we see the same sequences as in Figure 5.16, but with a single base pair deletion at the indicated position in species A and a three-base insertion (red base pairs) in species H. Notice that the subsequent base pairs in species A are now shifted relative to those in the other species. To see this, shift the orange shaded area one position to the right and observe how sequences in the blue and orange shaded areas will once again align. Similarly, in species H the purple shaded area is shifted three bases to the right. Adapted from University of Illinois (2011).



B

	A	B	C	D
A		48	29	53
B			32	24
C				50
D				

FIGURE 5.18 Genetic distances between species A and D. **(A)** There are six pairwise distances among four species. Here, each distance is indicated by a colored band. **(B)** The distance matrix for these genetic distances.

and human influenza viruses, the hemagglutinin protein 2009 strain differed at a remarkable 89 of 327 amino acid positions from its 2008 predecessor (Gallaher 2009). As a consequence, many humans were susceptible to this new strain.

In the examples of this subsection, we assumed that the individuals in each population are similar with respect to the character we are measuring, or at least that we have a characteristic sequence from that population. If instead we have information about allele frequencies in each population, we can look at the differences in allele frequencies and use these differences to compute a **genetic distance** between the two populations. The idea is that populations with similar allele frequencies may be more closely related than are those with more divergent allele frequencies. This approach is more commonly used when attempting to construct phylogenetic trees showing the relationships among different populations of a single species. This is a topic of great interest to evolutionary biologists—for example, those studying the process of speciation or trying to infer patterns of recent migration—and so population geneticists have developed a number of different ways to compute distances based on allele frequencies.

Constructing a Tree from Distance Measurements

Regardless of which type of distance measure we are using, the process of constructing a phylogenetic tree from distance information proceeds as follows: After measuring our distances between species, we have a list of the distances between each species pair in our sample. For example, if we are trying to infer the relationships among four species, A, B, C, and D, we use six pairwise measurements, as shown in **Figure 5.18A**.

Researchers often represent these in the form of what is called a *distance matrix*; that is, a table that lists the distance between each species pair. The distance between each species and itself is zero, so the diagonal entries of this matrix are all zero (shaded on Figure 5.18A). Because the distances are symmetric—it is as far from A to B as it is from B to A—we only need to fill in the upper half of the matrix to fully specify all distances. **Figure 5.18B** is the distance matrix corresponding to the genetic distances shown in Figure 5.18A.

Once we have these measurements, our aim is to find a way of arranging all six segments along a single tree. One way to envision the problem is to imagine that each of the six colored line segments in Figure 5.18A is a cable made of rubber. We want to lay these out along a four-species phylogenetic tree such that the cables undergo a minimum of compression or stretching. To try to make this work, we get to choose the shape of the tree, which species go on which nodes, and how long to make each branch of the tree.

For a phylogenetic tree relating four species, there is only one basic tree shape, the one shown in **Figure 5.19A**. Given this tree shape, there are three distinct ways to arrange the four species on the four branch tips. These are shown in **Figure 5.19A–C**. All other arrangements can be reached by rotating the tree around one of the interior nodes, and so they do not represent distinct trees. They are just different visual perspectives on the three ways that are shown in Figure 5.20.

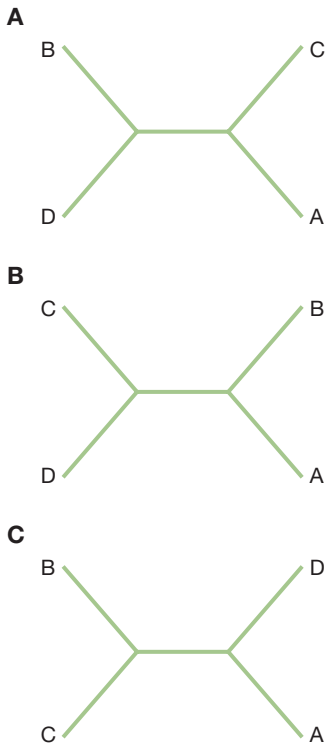


FIGURE 5.19 Three different arrangements of four species. Four species can be assigned to an unrooted phylogenetic tree in three different ways, as shown.

KEYCONCEPT QUESTION

5.2 Show how we can obtain five different rooted trees corresponding to the unrooted tree in Figure 5.19A.

Our job is now to choose which of these three arrangements is best and how long each branch should be to minimize the stretching necessary as we lay out our imaginary cables. There are a number of different algorithmic procedures for doing this, including what are called *weighted least squares*, *UPGMA* (unweighted pair group method with arithmetic mean), and *neighbor-joining* methods. Each has its strengths and weaknesses; we illustrate the weighted least squares solution in this example.

Because we are looking at only four species, we can already guess which tree shape is most appropriate without even using the weighted least squares algorithm. Looking at our distances in Figure 5.18, we see that species A is more closely related to species C than to any other species, while species B is more closely related to species D than to any other species. This means that the assignment of species to nodes on our tree will be that shown in Figure 5.19A. Now we want to lay down the six distances with a minimum of stretching. In doing so, we can adjust the lengths of the five line segments that make up the tree. **Figure 5.20** illustrates the best way to do this.

Evolutionary biologists have readily available phylogenetic inference software (one of the most common is a program named PHYLIP), which can be used to construct such trees, given both the tree topology—the shape and assignment of species to branch tips—and the branch lengths. **Figure 5.21** shows the weighted least squares tree for our example.

To explore an example of distance methods in action, we turn to a recent study of infectious disease. In 2014, the largest-ever outbreak of Ebola virus disease raced through the West African countries of Guinea, Liberia, and Sierra Leone, infecting more than 16,000 people and killing about 38% of them as of June 2015. In addition to the cost in human lives, the scale of the outbreak gave researchers and public health workers cause to worry that the virus might evolve in dangerous ways. It might evolve to transmit more readily from human to human, perhaps even acquiring the capacity for airborne transmission (Osterholm 2014). Or the virus might mutate sufficiently that the diagnostics in place and the vaccines under development would be ineffective (Hoenen et al. 2015).

A study of 99 viral genomes sampled through June 2014 revealed that the current virus had several hundred genetic differences from previous Ebola viruses. Moreover, it appeared to be rapidly accumulating novel mutations at about twice the rate observed in previous Ebola outbreaks (Gire et al. 2014). Finally, many of

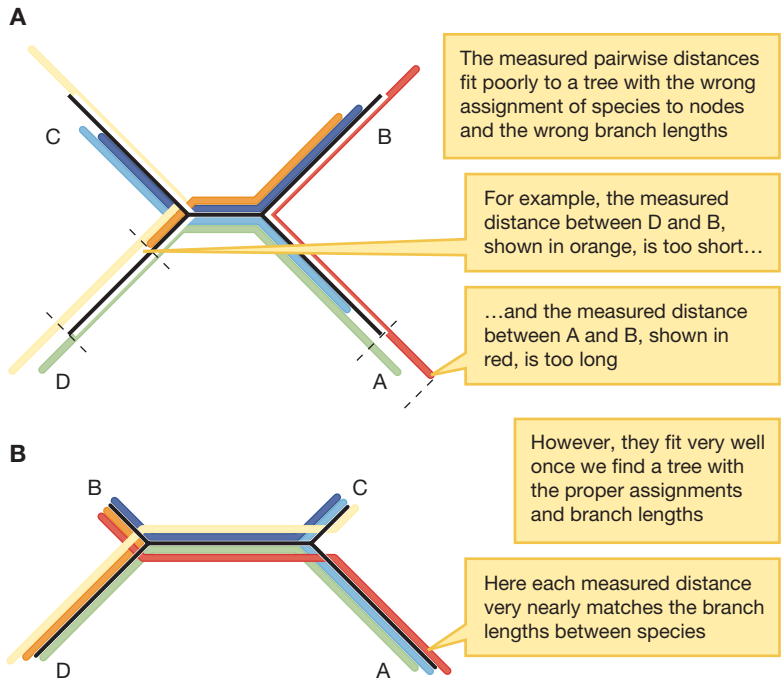


FIGURE 5.20 Assigning distances to the tree. When we use a distance-based method to infer tree topology and branch lengths, our aim is to find a tree topology in which each pairwise distance is as close as possible to that inferred from the data. For this example, with four species and six pairwise distances, our aim is to arrange the six measured distances, or "cables," to best fit together in a phylogenetic tree. **(A)** If we pick the wrong tree, the fit will be very poor: Some of the cables representing each pairwise distance will be much too long, and others will be much too short. **(B)** For the best tree, the cables are too long or too short by only a small margin.

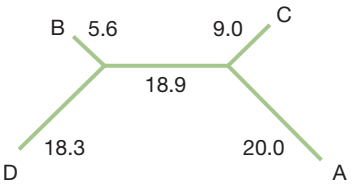


FIGURE 5.21 Weighted least squares tree for our example. Branch lengths are indicated by the values listed alongside each branch.

these new mutations changed the amino acid sequence of viral proteins and thus were likely to have phenotypic effects. (As we will explore in greater detail in the next chapter, each animo acid is encoded by several nucleotide triplets, and thus not all nucleotide sequence changes will alter the amino acid sequence as well.) But was the virus actually evolving to spread more easily among humans? Or were most of the changes that the researchers observed merely transient deleterious mutations that were likely to disappear?

As the epidemic continued, Thomas Hoenen and his colleagues set out to answer these questions and determine how rapidly the virus was changing over a longer timescale. In October and November of 2014, Ebola virus disease was introduced into the nation of Mali twice, both times from Guinea. Hoenen's team sequenced four samples of the virus from these two introductions into Mali. They then compared these to Guinean samples taken near the start of the epidemic in March 2014 and to samples from Sierra Leone taken in June. They created distance matrices indicating the number of nucleotide differences between viral genomes and the number of amino acid differences between viral genomes (**Figure 5.22**).

To understand the phylogenetic relationships among the isolates taken at different times, the team also used this distance matrix to construct a phylogeny of these strains, using a distance method known as *neighbor joining*. This approach produces an unrooted tree. The resulting phylogeny, shown in **Figure 5.23**, reveals that three sets of samples represent three different clades, ordered in relation to one another according to the time of sampling. This is somewhat surprising given that the Mali isolates represent two independent introductions into that country from widely spread geographic sources. While this could be coincidence, it hints that there may be a dominant genome in the Guinean population from which these introductions occurred.

The next step was to use this distance information to understand how the virus was evolving. Based upon the relatively small number of nucleotide sequence changes separating the Mali strains from the earlier Sierra Leone strains, Hoenen and his colleagues were able to make their own estimates of the

FIGURE 5.22 Matricies of nucleotide and amino acid sequence differences between 2015 Ebola isolates. Researchers created distance matrices showing the distances between strains collected in Guinea in March, Sierra Leone in June, and Mali in October and November. **(A)** The relatively small number of nucleotide sequence differences between the Sierra Leone sequences and the Mali sequences indicates that the viral genome was was not rapidly evolving. **(B)** The minimal number of amino acid sequence differences between these isolates indicates that the virus was not quickly acquiring beneficial mutations.

A Nucleotide differences

	Guinea March 2015			Sierra Leone 2015					Mali October/November 2015			
	G1	G2	G3	SL1	SL2	SL3	SL4	SL5	M1	M2	M3	M4
G1		4	5	14	9	11	12	13	15	18	19	20
G2			3	12	7	9	10	11	13	16	17	18
G3				9	4	7	7	8	10	13	14	15
SL1					7	3	4	5	11	14	15	16
SL2						6	7	8	10	13	14	15
SL3							1	2	8	11	12	13
SL4								3	9	12	13	14
SL5									10	13	14	15
M1										7	8	9
M2											1	2
M3												3
M4												

B Amino acid differences

	Guinea March 2015			Sierra Leone 2015					Mali October/November 2015			
	G1	G2	G3	SL1	SL2	SL3	SL4	SL5	M1	M2	M3	M4
G1		2	3	6	4	5	5	5	6	6	6	7
G2			1	4	2	3	3	3	4	4	4	5
G3				2	0	1	1	1	3	3	3	4
SL1					2	1	1	1	2	2	2	3
SL2						1	1	1	2	2	2	3
SL3							0	0	1	1	1	2
SL4								0	1	1	1	2
SL5									1	1	1	2
M1										0	0	1
M2											0	1
M3												1
M4												

rate at which new mutations were occurring in the population of Ebola viruses. They found a much lower rate than was estimated by the previous study and concluded that the virus was not in fact evolving significantly faster than in previous outbreaks. Moreover, the nucleotide sequence changes that they did see tended not to result in amino acid sequence changes. Finally, a paper by another research group noted that most of the animo acid changes that did occur were in parts of the viral protein where we would not expect substantial phenotype effects (Olabode et al. 2015). All three of these observations suggest that much of the non-silent (amino acid-changing) variation observed in the previous study either had no effect or consisted of transient deleterious mutations that would eventually be lost to selection. This was good news. It appeared that the virus was not rapidly acquiring mutations that could enhance human-to-human transmission and hamper efforts to control and eradicate the outbreak.

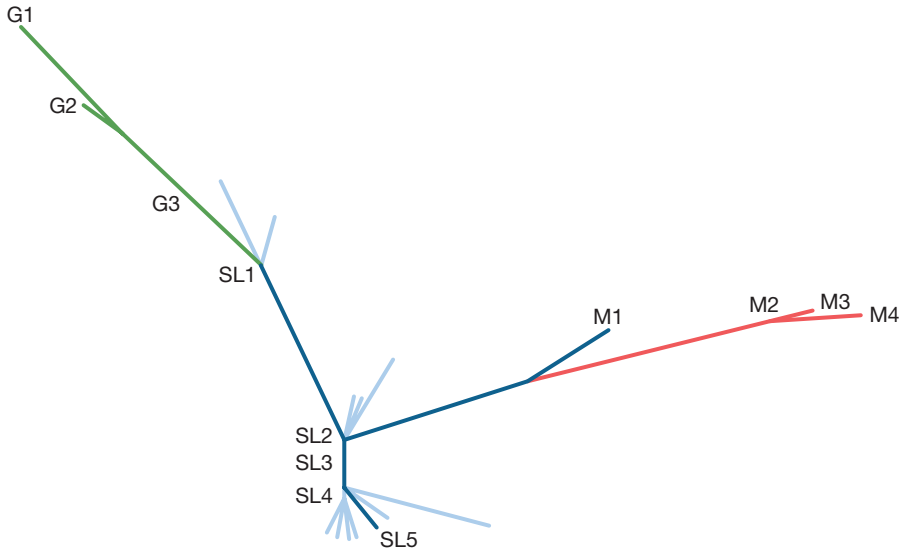


FIGURE 5.23 A phylogenetic tree of Ebola virus isolates. Using the nucleotide distance matrix in Figure 5.22A, the researchers created this phylogeny of the Ebola virus strains. Unlabeled branches correspond to other isolates measured but omitted from the distrance matrices for simplicity. The phylogeny reveals that three distinct clades were responsible for the three outbreaks.

We conclude with a caveat. As encouraging as it was that the virus was not rapidly evolving, public health authorities cannot afford to let their guard down when something like the 2014 Ebola outbreak occurs. Any time a virus emerges from an animal population and undergoes sustained transmission in humans, there is a real risk that it will evolve to transmit more efficiently among humans (Antia et al. 2003). This is the major fear surrounding H5N1 avian influenza (bird flu), a strain that is rarely transmitted between humans but kills a high fraction of the people that it does infect (Guan et al. 2004). On top of the immediate human cost of disease, the risk of viral evolution provides yet another reason that we need to move aggressively to control such outbreaks wherever they arise.

One of the major advantages of distance methods is that they are computationally very fast, allowing researchers to construct very large phylogenies that include many species. Another advantage of distance methods is their conceptual simplicity. But distance methods are not without problems. One of the biggest concerns to many researchers is a philosophical one: Distance methods lack any sort of underlying evolutionary model. Rather, they are fundamentally *phenetic* in their approach, meaning that they group species together according to similarity without attempting to reflect the underlying historical evolutionary relationships among those species. The assumption being made here is that the similarity we are measuring is a reflection of homology rather than analogy. Sometimes this is correct, and sometimes it is not. When we work with these methods, we accept the risk that some traits we use are analogous, in order to obtain the benefit of having many easily measurable characters available when building our tree. Many contemporary evolutionary biologists prefer *cladistic* methods, which aim to reconstruct evolutionary relationships explicitly.

There is another problem with distance methods as well. When we use genetic distances in the process of building phylogenies, we are assuming that the more DNA sequences differ from each other, the more distantly related our species are. But what if some species in our taxa of interest are evolving faster than others (as in Figure 5.9)? In that case, it is possible that quickly evolving species cluster together because of the speed at which they evolve, rather than because of true phylogenetic history. Although this is beyond the scope of what we will cover in this chapter, we note that evolutionary biologists have developed a number of statistical techniques is an attempt to deal with these difficulties.

5.4 Rooting Trees

In our treatment of parsimony in Section 5.2, we illustrated our trees as if they were rooted. Strictly speaking, however, a maximum parsimony approach does not distinguish among the multiple alternative rooted trees that correspond to the same unrooted tree. Any two rooted trees corresponding to the same unrooted tree will require the same number of changes, so there is no way to distinguish among them using parsimony criteria alone. If we want to work with rooted trees, then, it is important to have ways of *rooting*—assigning a root to—the unrooted tree that we get from a maximum parsimony analysis.

The most common approach to rooting a tree is to use an outgroup. Suppose we have an unrooted phylogenetic tree of several magpie species, as shown in **Figure 5.24**, and from this we wish to derive a rooted phylogenetic tree for these populations (Lee et al. 2003).

To root this tree using the outgroup method, we pick another species that we know in advance to be an outgroup; that is, a related species that branched off earlier in evolutionary history from the entire clade that we are considering. In this case, the azure-winged magpie (*Cyanopica cyana*) works well. The azure-winged magpie is a fairly close relative of the group we are considering, but this species is less closely related to the members of the *Pica* genus than the *Pica* species are to one another. We can therefore construct another phylogenetic tree that includes our outgroup, as shown in **Figure 5.25A**.

We can form a rooted tree from an unrooted tree simply by picking a branch around which to root the tree. Using the outgroup method, we select the branch leading to the outgroup; namely, the branch connecting the *Pica* magpies to the azure-winged magpie. We then draw a tree rooted around a point (the

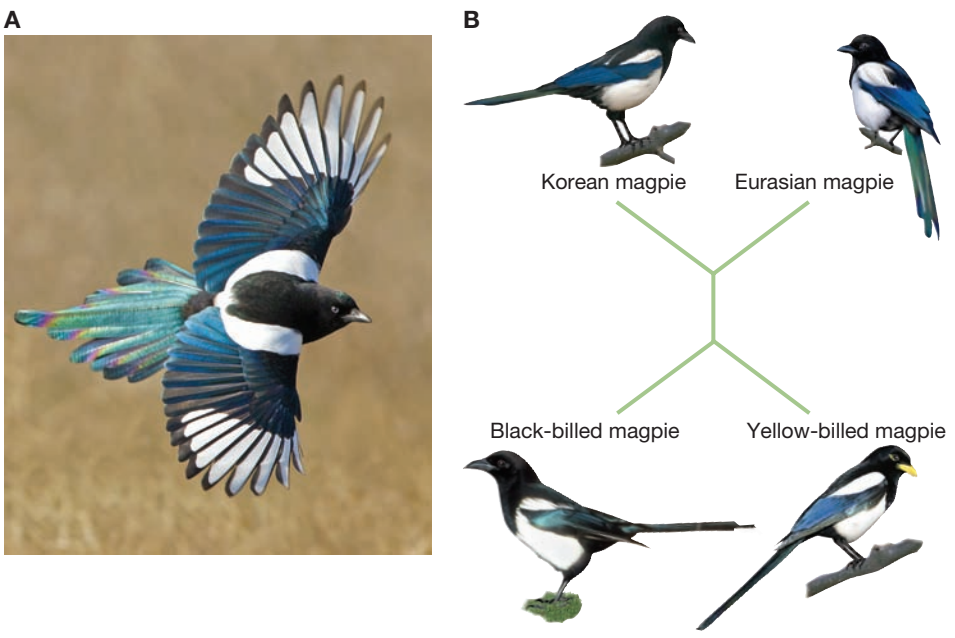


FIGURE 5.24 Phylogeny of magpie populations. **(A)** The black-billed magpie (*Pica hudsonia*). **(B)** An unrooted phylogenetic tree showing relationships among four magpie populations: the Korean magpie (*Pica pica sericea*), the Eurasian magpie (*Pica pica pica*), the black-billed magpie (*Pica hudsonia*), and the yellow-billed magpie (*Pica nuttalli*). This phylogeny is based on a maximum parsimony phylogeny derived using mitochondrial DNA sequences. Panel B adapted from Lee et al. (2003).

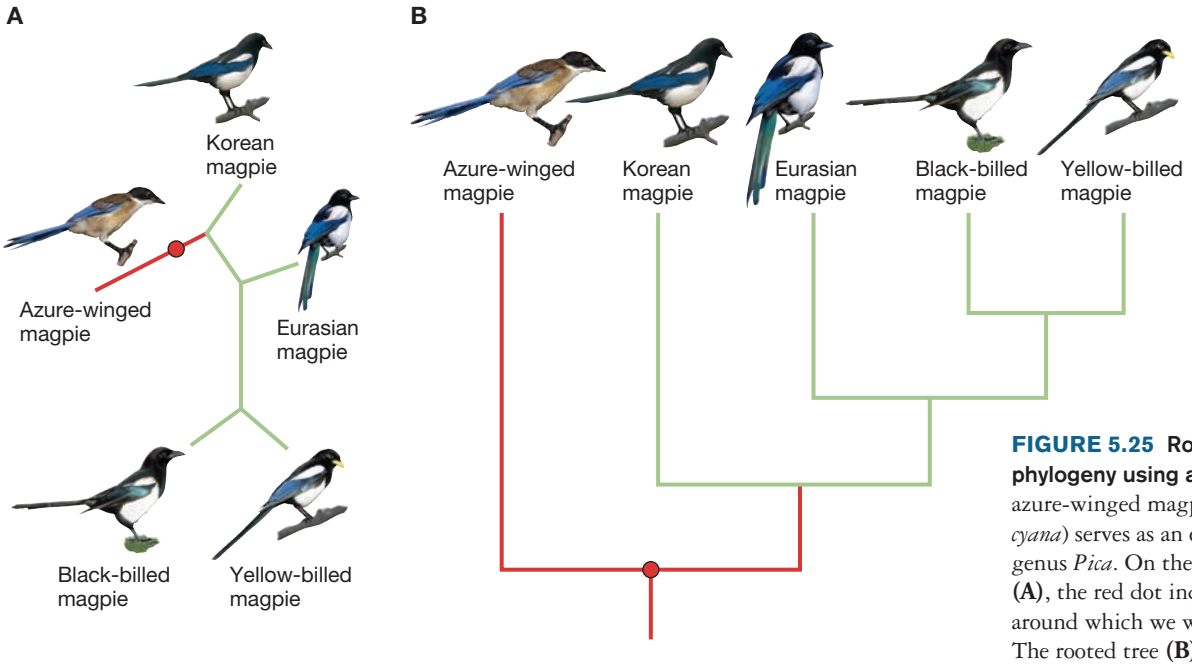


FIGURE 5.25 Rooting the magpie phylogeny using an outgroup. The azure-winged magpie (*Cyanopica cyana*) serves as an outgroup for the genus *Pica*. On the unrooted tree **(A)**, the red dot indicates the point around which we will root the tree. The rooted tree **(B)** has the azure-winged magpie as an outgroup. Adapted from Lee et al. (2003).

red dot in Figure 5.25A) on this branch. **Figure 5.25B** shows the rooted tree that we get by this process.

As we discussed in Chapter 4, rooting the tree can be useful because a rooted tree informs us about something that evolutionary biologists are keen to know—the polarity of character changes. For example, consider the light-colored beak that is unique to the yellow-billed magpie. From the unrooted tree in Figure 5.24B, we cannot tell whether having a light bill is ancestral or derived, because we do not know along which branch the root lies. If the tree were rooted along the branch between the yellow-billed magpie and the rest of the tree, having a yellow beak could have been the ancestral state, which was then lost in the branch leading to the other magpie populations. But once we find the root, we see that a yellow beak is very likely to be a derived character. Even ignoring the fact that the outgroup also has a dark beak, we see that we would require multiple character changes to explain the beak color character if yellow beaks were ancestral, whereas we can explain this character with a single character change given the likelihood that yellow beaks are derived (**Figure 5.26**).

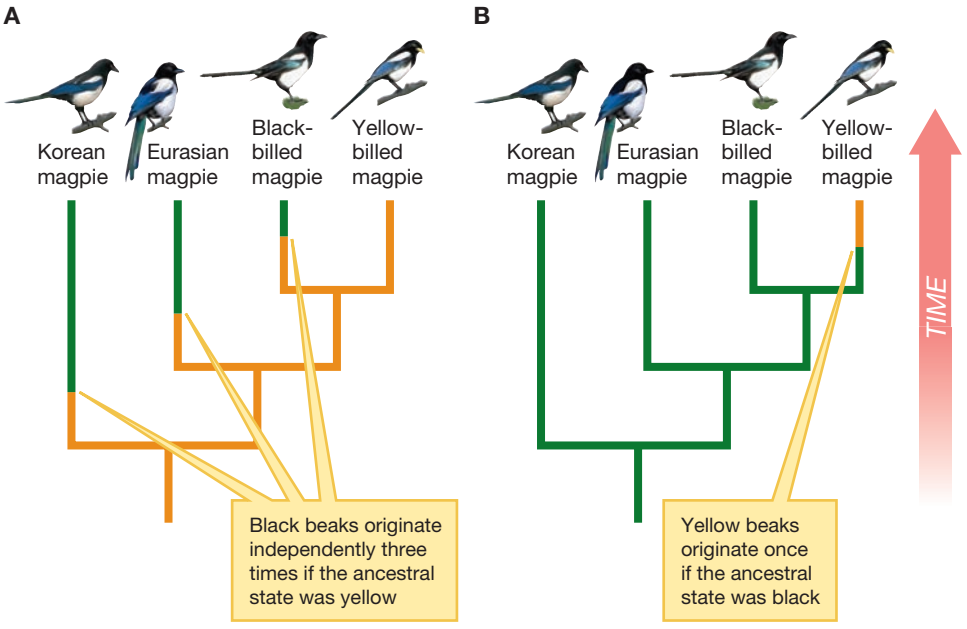


FIGURE 5.26 Parsimony suggests that yellow beaks are a derived character. (A) If yellow beaks are ancestral, multiple character changes are required to explain the distribution of beak color on the phylogeny. **(B)** If yellow beaks are derived, we can explain the distribution of beak color with a single change.

Knowing the root of the tree can also tell us about **phylogeography**: the story of how a group of populations or species moved across the globe over the course of their

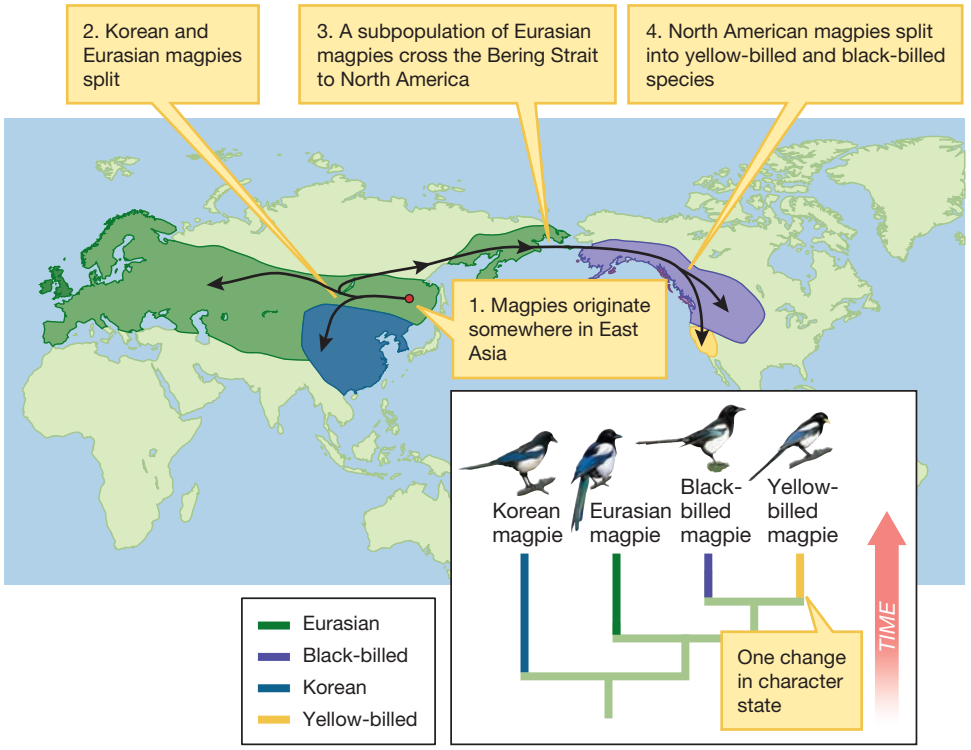


FIGURE 5.27 Magpie phylogeography as inferred from the rooted phylogeny. Magpies appear to have originated in East Asia, where they diverged into the Korean magpie lineage and the Eurasian magpie lineage. A subpopulation from the Eurasian lineage then crossed the Bering Strait to the New World and subsequently underwent speciation, producing the black-billed and yellow-billed species now found in North America.

evolutionary history. The conventional explanation of magpie evolution had been that magpies arose in Asia and subsequently colonized North America in two separate waves, once early leading to the yellow-billed magpie, and again later as the black-billed magpie. But the form of the rooted tree suggests an alternative hypothesis (Lee et al. 2003). It suggests that a subpopulation of Eurasian magpies invaded North America a single time, and that their descendant lineages branched into the black-billed and yellow-billed magpie species found there (**Figure 5.27**).

Of course, we could only follow this outgroup rooting procedure because we knew that the azure-winged magpie is a suitable outgroup to the genus *Pica*. In other words, we already knew quite a bit about the patterns of evolution in the larger corvid clade that includes the genus *Pica*, and this knowledge helped us get a more detailed picture of evolution within the magpies. There are other methods of rooting trees. For example, molecular clock rooting methods assume a constant rate of molecular evolution along each branch of the tree and then locate the root at a point that is evolutionarily equidistant from each of the branch tips (Huelsenbeck et al. 2002). We will not treat these here.

5.5 How Many Different Trees Are There?

We have discussed several ways of inferring phylogenies. In each of these cases, biologists can use computational algorithms to determine how strongly any particular phylogeny is supported by the data. Why, then, is phylogenetic inference a difficult and computationally intensive problem? The answer lies in the fact that there are simply too many possible phylogenetic trees to search, even with the fastest of computers. Instead, researchers must devise clever ways to search within the "space" of possible trees.

In this section, we will develop a basic intuition for the problems evolutionary biologists face regarding the number of possible trees: Just how big is the space of possible trees, and how rapidly does the space grow as we add species or other taxa (Felsenstein 2004)? We will begin by considering unrooted trees. There is only one unrooted tree relating three species A, B, and C, as shown in the center of **Figure 5.28**.

Now think about the different ways we could add a fourth branch to this tree to create an unrooted tree for four species. Our three-species tree has three branches, each leading from the internal node to one of the three tips. To create a four-species tree, we could add a new branch leading to a new species D to any of these three branches. Each point of attachment creates a *different* four-species tree, as illustrated in Figure 5.28. Thus, there are three different unrooted four-species trees.

Each of the four-species trees has five branches. We can create a five-species tree by adding a new branch, with a new species E, to any of those five branches. Each choice of attachment location again produces a different tree. Thus, from *each* of our three four-species trees, we can produce five different five-species trees. This gives us a total of $3 \times 5 = 15$ different five-species trees.

We can continue adding branches in this way and counting the resulting trees. Each time we add a new branch, we get a tree with two additional branches: One of these is the one we just added, and the other comes from splitting the branch to which our new branch is attached. This means that our five-species trees will have seven branches and seven potential attachment points, our six-species trees will have nine branches, and so forth. There will be $3 \times 5 \times 7 = 105$ six-species trees and $3 \times 5 \times 7 \times 9 = 945$ seven-species trees. As shown in **Table 5.1**, even a relatively small number of species can be arrayed on unrooted trees in an exceptionally large number of ways.

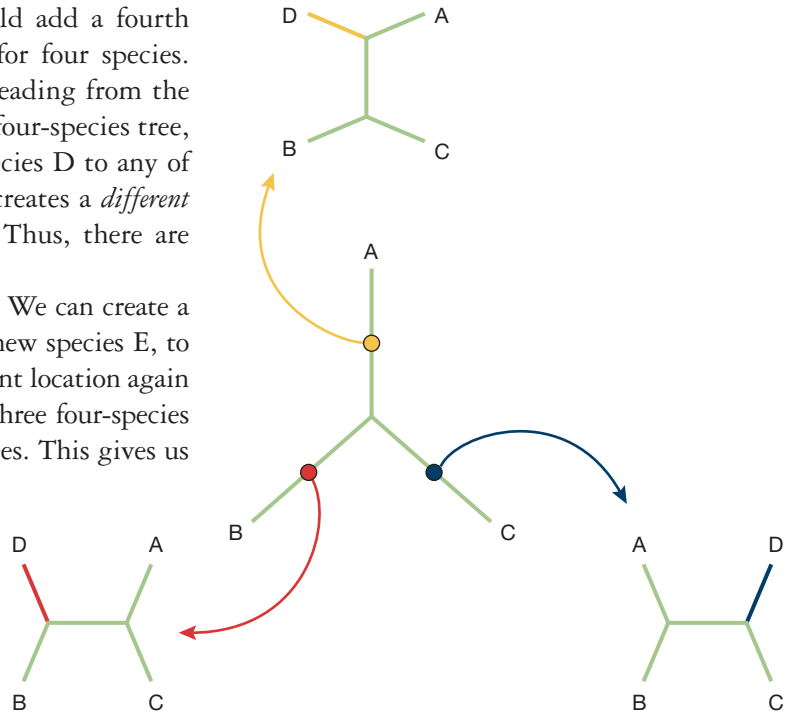


FIGURE 5.28 A fourth species can be added to a three-species tree in three different locations. An unrooted tree with three species is shown at the center of the figure. From this tree, we can make three different unrooted trees relating four species. Each is constructed by adding a branch (for species D) to a different branch of the three-species tree.

To give you a sense of just how rapidly these numbers increase, there are more 13-species trees than there are people on the planet (somewhat over 7 billion at present). There are more 22-species trees than there are stars in the universe (approximately 10^{23}). There are more 36-species trees than there are water molecules in all of Earth's oceans (approximately 10^{47}). There are more 53-species trees than there are atoms in the universe (approximately 10^{80}).

This is just the number of possible *unrooted* trees. As we have seen, each unrooted tree corresponds to numerous rooted trees. From an initial unrooted tree, we can form a distinct rooted tree by rooting on each of its branches. An unrooted tree with k species has $2k - 3$ branches, which means that there will be $(2k - 3)$ times as many rooted trees as there are unrooted trees. So, for our 53-species taxon, there are about 10^{80} (the unrooted case) $\times$ 103 (that is, $2k - 3$) possible rooted trees.

Clearly, with so many possible trees for even a few dozen species, it is not feasible to check each and every tree to see how well it explains a given set of character data. As a result, computer programs for reconstructing phylogenies have to be very clever in the way that they search the set of possible trees, only checking a very small fraction of those trees. Researchers continue to develop increasingly good algorithms for selecting which trees to check and which can be safely ignored; this *search problem* makes up much of the challenge of phylogenetic inference.

TABLE 5.1

The Number of Different Unrooted Trees for 3 to 30 Taxa

Number of Taxa	Unrooted Trees
3	1
4	3
5	15
6	105
7	945
8	10,395
9	135,135
10	2,027,025
11	34,459,425
12	654,729,075
13	13,749,310,575
14	316,234,143,225
15	7,905,853,580,625
16	213,458,046,676,875
17	6,190,283,353,629,375
18	191,898,783,962,510,625
19	6,332,659,870,762,850,625
20	221,643,095,476,699,771,875
21	8,200,794,532,637,891,559,375
22	319,830,986,772,877,770,815,625
23	13,113,070,457,687,988,603,440,625
24	563,862,029,680,583,509,947,946,875
25	25,373,791,335,626,257,947,657,609,375
26	1,192,568,192,774,434,123,539,907,640,625
27	58,435,841,445,947,272,053,455,474,390,625
28	2,980,227,913,743,310,874,726,229,193,921,875
29	157,952,079,428,395,476,360,490,147,277,859,375
30	8,687,364,368,561,751,199,826,958,100,282,265,625

5.6 Phylogenies and Statistical Confidence

Throughout this chapter, we have stressed that constructing a phylogenetic tree involves sampling characters and making assumptions about homology, and that any phylogeny is a hypothesis about the true evolutionary history of a group of organisms. As a result, it is essential that we develop statistical measures of support for our phylogenetic hypotheses. Yet, thus far, we have only looked at how we find a "best estimate" of the real phylogeny, and not at another component of statistical inference: how we measure our confidence in that best guess.

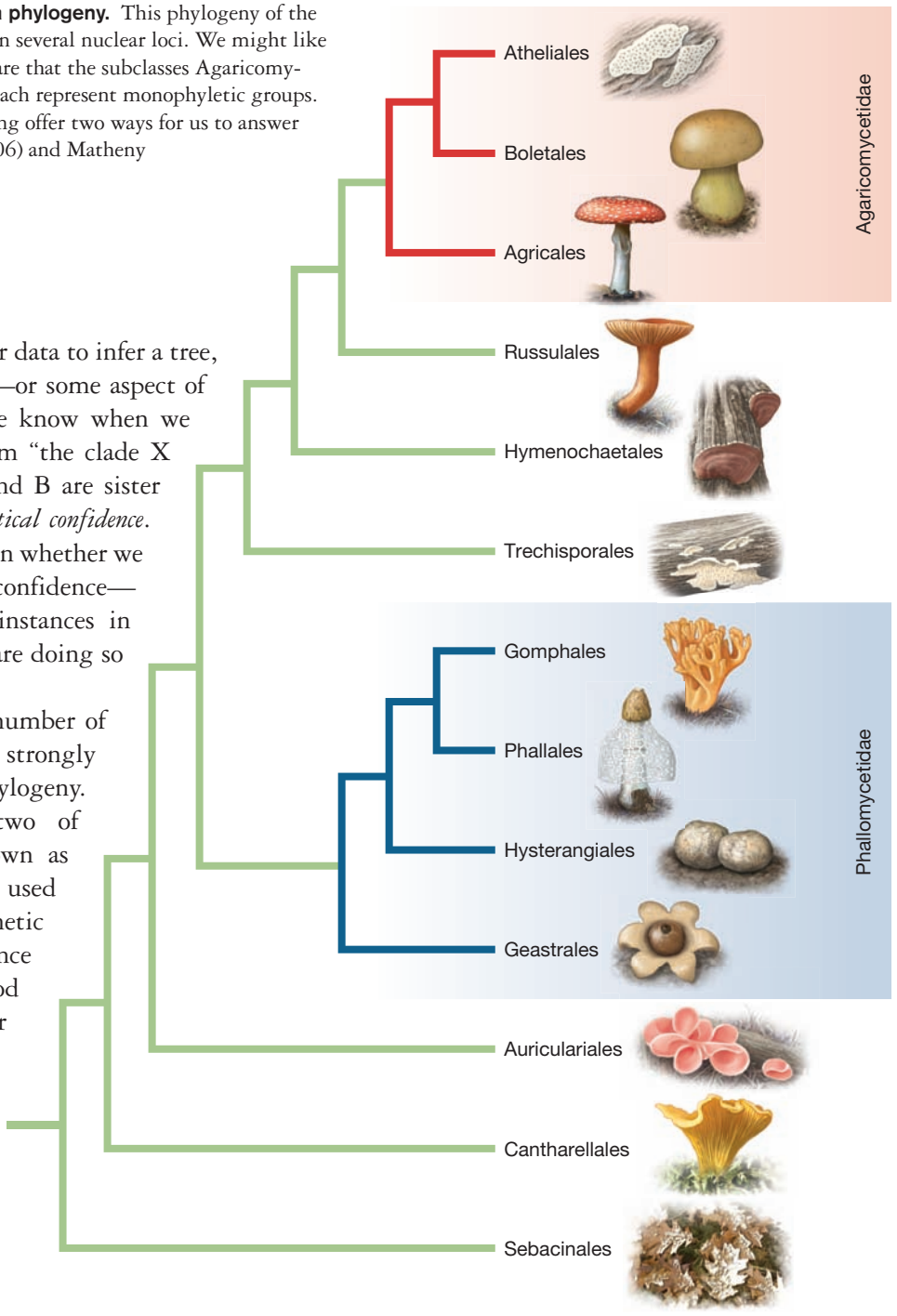


FIGURE 5.29 Statistical certainty for a phylogeny. This phylogeny of the Agricomycetes, a class of fungi, is based on several nuclear loci. We might like to know, given this data, how certain we are that the subclasses Agaricomycetidae (red) and Phallomycetidae (blue) each represent monophyletic groups. Bootstrap resampling and odds ratio testing offer two ways for us to answer this question. Adapted from Hibbett (2006) and Matheny et al. (2007).

Once we have used our character data to infer a tree, how certain are we that this tree—or some aspect of this tree—is correct? How do we know when we can reject a hypothesis of the form "the clade X is monophyletic" or "species A and B are sister groups"? These are issues of *statistical confidence*. Typically, we might aim to ascertain whether we can reject a hypothesis with 95% confidence—that on average, for every 100 instances in which we reject a hypothesis, we are doing so correctly in 95 instances.

Researchers have developed a number of techniques for quantifying how strongly our data support a given phylogeny. In this section, we explore two of these approaches. The first, known as **bootstrap resampling**, can be used with any technique for phylogenetic inference, be it parsimony, a distance method, or a model-based method such as maximum likelihood or Bayesian inference. The second, **odds ratio testing**, can only be used with the model-based frameworks of maximum likelihood or Bayesian inference that we describe in the appendix to this book.

Bootstrap Resampling

Suppose we infer a phylogenetic tree such as that in **Figure 5.29** from a set of character data. How certain are we that this is the "correct" tree; that is, the actual phylogeny of the groups we are studying? If we are looking at even modest numbers of species, we will rarely be sure—our statistical confidence is low—that we have *exactly* the right tree. Because there are so many possible trees, and because many of them may be very similar, it is rare that we will have a single tree that is 95% likely given our data.

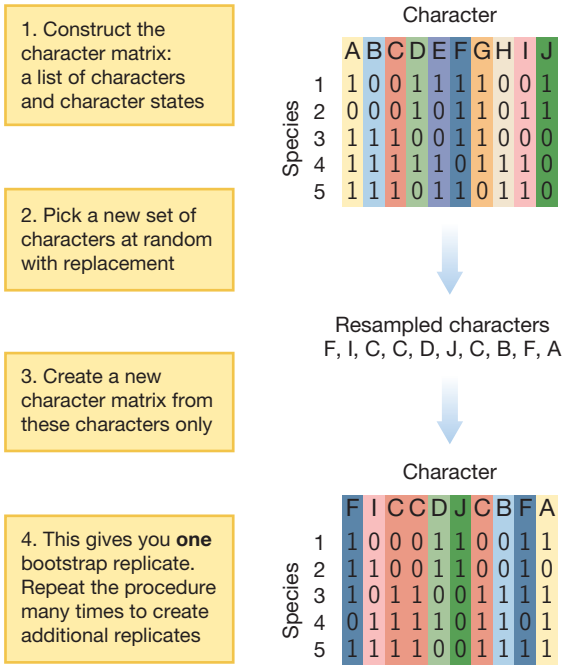


FIGURE 5.30 Resampling character data. Here we have a character-state matrix made up of binary character data for 10 characters in 5 species. A single bootstrap replicate is created by resampling—by picking characters one at a time from the original data set to include in the replicate data set. Because sampling occurs with replacement, it is possible to draw the same character more than once and to draw other characters not at all. In the illustration here, character C appears three times in the replicate data set, and character F appears twice. Characters E, G, and H do not appear at all. Note that for each species, the character states do not change when resampling occurs. This procedure resamples at the level of which characters are included in the analysis, but it does not cause changes in character-state assignments.

What this means is that, typically, we will not want to make confidence statements about the entire tree. Instead, we will make statements about *features* of the tree. In essence, we can break down our problem into more manageable bits. Because we are interested in inferring patterns of shared ancestry, one of the most important features of a tree is the set of monophyletic clades that it implies. Thus, a common aim of confidence assessment in phylogenetics is to say how strongly the data support a given monophyletic clade. In Figure 5.29, for example, how certain are we that the subclass Agaricomycetidae is indeed monophyletic? How certain are we that the subclass Phallomycetidae is monophyletic?

Bootstrap resampling offers a powerful way to answer questions of this sort, by creating many new data sets from the observed data to get a representative distribution of results. To illustrate, suppose we have observed 10 different characters for 5 species. For this example, we will assume that these are *binary characters*; namely, characters that have two possible states, which we will call 0 and 1. For example, binary characters include whether individuals in a species engage in parental care, whether they have cryptic coloration, and whether their sex determination depends on chromosomes or on environmental factors. We can represent our observations as a *character-state matrix*, a table that lays out the states for each character in each species. Such a matrix is shown at the top of **Figure 5.30**.

To carry out a bootstrap analysis, we *resample* from our original character-state matrix to create a collection of *bootstrap replicate* data sets; that is, a set of alternative character-state matrices. Essentially, this procedure involves picking a set of characters, *with replacement*, from the original set of characters and using these picks to form a new data set. Figure 5.30 illustrates the basic type of procedure that we might follow to generate a single replicate character-state matrix. In a bootstrap analysis, we create several hundred such replicate matrices.

We then apply the same tree-building methods that we used on our original data set to each replicate character-state matrix. This gives us a collection of *bootstrap replicate phylogenies*. Finally, we look to see how often the feature we are interested in—say, one particular set of species forming a monophyletic clade—occurs among our replicate phylogenies (**Figure 5.31**). If, for example, these species form

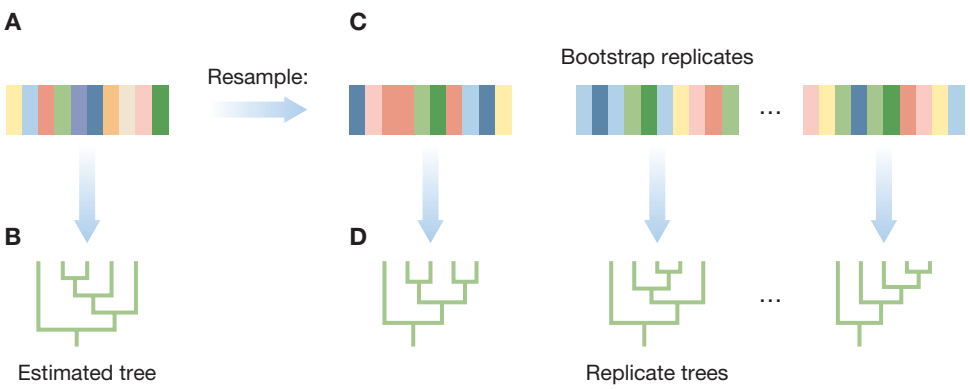


FIGURE 5.31 An overview of a bootstrap analysis. Given our character data **(A)**, we construct our estimated phylogeny **(B)**. We also resample from the original character data to create multiple bootstrap replicate data sets **(C)**. For each replicate data set, we construct a phylogenetic tree using the same procedure that we used on the original character data. This gives us a replicate tree for each replicate data set **(D)**. To assess the support for any feature of our original tree, we count up the percentage of replicate trees that also display this feature.

a monophyletic clade in 90% of the replicate phylogenies, we say that this clade has 90% *bootstrap support*. In the phylogeny illustrated in Figure 5.29, the subclass Agaricomycetidae has 96% bootstrap support and the subclass Phallomycetidae has 98% bootstrap support. Thus the data strongly support the hypothesis that each is a monophyletic group.

Often, when presenting a phylogenetic tree, researchers will indicate the level of bootstrap support for each clade. This is done by placing a percentile number along the branch leading to that clade, as in **Figure 5.32**. Here, the number 90 indicates that the highlighted clade, just above the number, appears as a monophyletic clade in 90% of the bootstrap replicates.

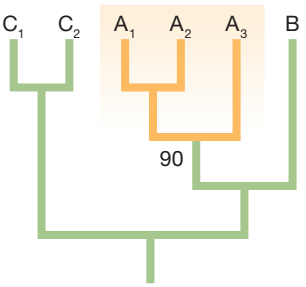


FIGURE 5.32 Numbers at a branch point indicate bootstrap support. The number 90 indicates that the highlighted clade (species A_1, A_2, and A_3) appears as a monophyletic clade in 90% of the bootstrap replicates.

Although bootstrap support levels and statistical significance levels (statements such as "We can reject the hypothesis that A is not a monophyletic clade with 98% confidence") are both percentages used to indicate the support that our data provide for our conclusions, they are not the same thing and should not be confused with one another. Note that we sometimes see clades with bootstrap support values of 100%. This means that the clade in question appears in all bootstrap replicates—but it does not mean that we can reject the hypothesis that this is not a monophyletic clade with 100% certainty.

Odds Ratio Testing

Bootstrap support levels are not statistical significance levels, but there are other procedures by which we can construct statistical confidence tests for whether we have correctly depicted various features of our phylogenetic tree. When using likelihood or Bayesian methods for phylogenetic inference, we can do this using an approach known as odds ratio testing.

Suppose that once we reconstruct a phylogenetic tree, we want to determine how strongly our character data support a given feature of this phylogenetic tree. For example, suppose that again we want to know how strongly the data support whether clade A is monophyletic, as shown in Figure 5.32. To answer this question, we can compare the best possible tree overall against the best possible tree in which clade A is not monophyletic. We have already found the former. This is simply the tree that we constructed in the basic process of phylogenetic inference. We can find the latter by *constraining* our search of phylogenetic trees to consider only those in which clade A is not monophyletic.

We can then see *how much better* is the best tree with clade A monophyletic, relative to the best tree without clade A monophyletic. Various statistical procedures have been developed for making this comparison and determining when the difference is statistically significant.

Testing Hypotheses about Phylogenetic Structure

In Chapter 4, we looked briefly at two different hypotheses for the phylogenetic relationships among mammalian groups. According to the *Theria hypothesis*, placental mammals (Eutheria) and marsupials (Metatheria) are sister groups, with monotremes (Prototheria) more distantly related (**Figure 5.33A**). By contrast, the *Marsupionta hypothesis* places the marsupials and monotremes as sister groups, with

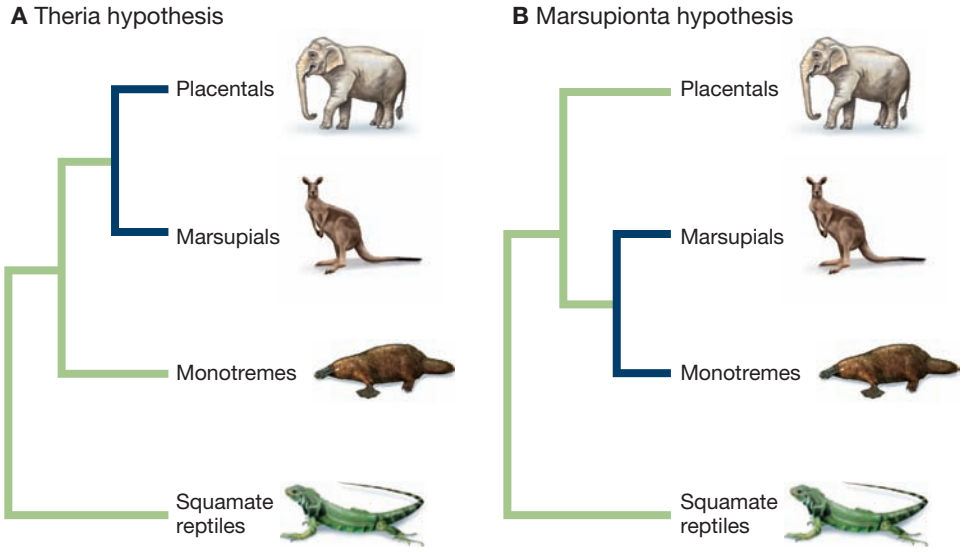


FIGURE 5.33 Two competing hypotheses for the evolutionary relationships among mammalian groups. **(A)** Under the Theria hypothesis, the placentals and marsupials are sister groups and thus form a single monophyletic clade, whereas marsupials and monotremes are not a monophyletic clade. **(B)** Under the Marsupionta hypothesis, the marsupials and monotremes are sister groups and form a monophyletic clade, but placentals and marsupials together are not monophyletic. Adapted from Meyer and Zardoya (2003).

the eutherian mammals more distantly related (**Figure 5.33B**). Prior to widespread genomic analysis, there was considerable controversy as to which of these two hypotheses was correct. Morphological evidence tended to support the Theria hypothesis, whereas molecular evidence from mitochondrial DNA (mtDNA) sequences tended to support the Marsupionta hypothesis.

In an effort to bring a new source of data to bear on the problem of distinguishing between these alternative hypotheses, Keith Killian and his colleagues obtained DNA sequences of a large nuclear gene known as *M6P/IGF2R* from 11 placental, 2 marsupial, and 2 monotreme species (Killian et al. 2001). They used this DNA sequence data to construct a phylogeny of the mammals. They reasoned that if the Theria hypothesis was correct, the placentals and marsupials would form a single monophyletic clade, whereas marsupials and monotremes would not form a monophyletic clade. If the Marsupionta hypothesis was correct, the reverse pattern would hold: Marsupials and monotremes would be a monophyletic clade, but placentals and marsupials together would not be monophyletic.

When Killian and his colleagues constructed a maximum likelihood tree, they found a pattern of relationships consistent with the Theria hypothesis. Their tree, shown in **Figure 5.34**, places Eutheria and Metatheria as sister groups.

But how much should we make of this result? Does the Theria hypothesis do a much better job of explaining the data from the *M6P/IGF2R* gene or is the Marsupionta hypothesis a close second? In other words, can we quantify how strongly the data support the Theria hypothesis relative to the Marsupionta hypothesis? This is where the method of bootstrap resampling comes in. Killian and his colleagues created 100 bootstrap replicate data sets by performing the resampling procedure we have described. When they constructed phylogenetic trees for each replicate, they found that the placental mammals and marsupials formed a monophyletic clade in every one of the 100 replicate trees (shown by the magenta number 100 on the tree). This indicates that *these particular data* very strongly support the Theria hypothesis. As shown in Figure 5.34, other clades are much less well supported. For example, the bat and hedgehog formed a monophyletic clade in only half of the bootstrap replicates (shown by the magenta number 50 on the tree).

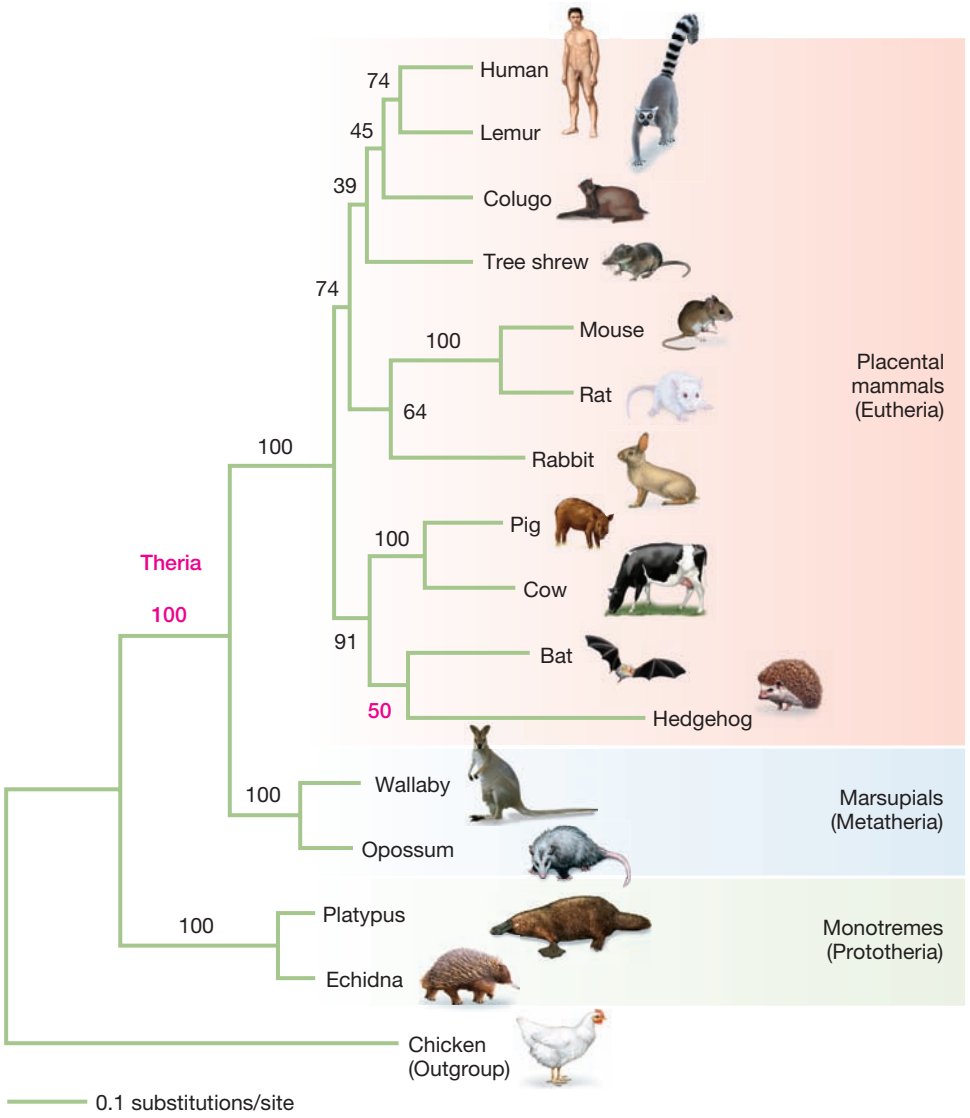


FIGURE 5.34 A maximum likelihood tree for the mammals. Killian and his colleagues inferred this maximum likelihood tree based on sequence data from the *M6P/IGF2R* gene. Numbers represent bootstrap support values for each clade. Theria—the group comprising placentals and marsupials but not monotremes—has 100% bootstrap support as a monophyletic clade. Other clades, such as that comprising bats and hedgehogs, have much lower bootstrap support. Adapted from Killian et al. (2001).

Because Killian and his colleagues were using maximum likelihood to construct their phylogeny, they could also use an odds ratio test to evaluate the strength of support for the Theria hypothesis. To do so, they compared the maximum likelihood tree shown in Figure 5.34 with the maximum likelihood tree *given the constraints of the Marsupionta hypothesis*. That is, they compared their maximum likelihood tree with the highest-likelihood tree in which the marsupials and monotremes formed a monophyletic clade. A likelihood ratio test allowed them to reject (at the $p < 0.001$ level) the hypothesis that there is no difference in likelihood between the maximum likelihood tree (which happens to support the Theria hypothesis) and the best tree that is consistent with the Marsupionta hypothesis. Like the bootstrap resampling approach, the odds ratio test approach showed that Killian's data strongly supported the Theria hypothesis over the Marsupionta hypothesis.

Since the publication of Killian's paper, numerous additional mammalian phylogenies have been constructed using nuclear DNA. These have overwhelmingly supported the Theria hypothesis, and today the majority of researchers would agree that placental mammals and marsupials are sister groups, and that monotremes are more distantly related.

KEYCONCEPT QUESTION

5.3 In the phylogeny shown in Figure 5.34, which monophyletic clade or clades have the strongest statistical support? Which have the weakest support?

5.7 Fossil Evidence of Evolutionary History

Evolutionary biologists use many different kinds of traits to reconstruct evolutionary trees, from fossil evidence to anatomical features of modern organisms, from embryological processes to genetic sequence data, from behavioral patterns to chromosome structure. DNA sequences are the most frequently used character for phylogenetic construction today, but DNA may not always be available, as in the case of the fossil record (although recent advances in extracting DNA from some types of fossilized remains are making molecular phylogenetics possible even for extinct groups). Even when DNA sequences are available, alternative characters—morphological, behavioral, or otherwise—can provide additional lines of evidence with which to test the evolutionary hypotheses that our molecular trees represent. In general, we see a high degree of concordance (agreement) among phylogenies constructed using various types of traits, although often some of the smaller details can vary, depending on the choice of characters.

In this section, we will explore how evolutionary biologists can use fossil evidence to understand evolutionary history.

The Fossil Record

Especially for extinct taxa, the fossil record is a primary source of data for constructing phylogenetic trees. Scientists can use these data to formulate hypotheses about phylogenetic relationships. For example, Wallace, Darwin, and others recognized that extant (that is, not extinct) species from a given location tend to resemble fossils uncovered at that same spot more so than fossils found at other locations. From this and other sources of evidence, 4 years before Darwin published *On the Origin of Species*, Wallace concluded that "Every species has come into existence coincident both in space and time with a pre-existing closely allied species" (Wallace 1855, p. 186).

Indeed, this pattern of local resemblance among fossils has been observed so often, and at so many locations, that it is sometimes called the law of succession. Moreover, it generates a hypothesis: Common ancestry explains the similarity between extant and fossil species at location 1 and the similarity between extant and fossil species at location 2, and so on. What's more, if common ancestry explains the similarity of fossil and living forms at a given location, then by knowing enough about the geological and ecological conditions at this location at various points through evolutionary time, we can generate and test hypotheses about how natural selection and other evolutionary processes may have been responsible for many of the differences between fossil and extant species. If, for example, the type of prey consumed in the group we are studying has changed over time, that might help us explain why the modern and fossil species are generally very similar but have differences in morphological traits associated with foraging (tooth shape, beak size, and so on).

To understand better the many ways that evolutionary biologists have used the fossil record to reconstruct phylogenies, we will examine two examples. The first

focuses on the use of fossil data to reconstruct the evolutionary history of horses, and the second examines how fossil evidence explains an important development in the history of animals—the transition from life in the sea to life on the land.

Phylogenetic Relationships in the Equidae

The reconstruction of the phylogenetic relationships in the Equidae, the family that includes the modern horse, is largely but not exclusively based on fossil evidence. Although there is some debate on the details of this phylogeny (Weinstock et al. 2005), the overall picture is clear (MacFadden 1992; Martin 2004) (**Figure 5.35**). The earliest Equidae fossils are between 50 million and 60 million years old, dating from the Eocene. Evidence from fossilized bones and teeth indicate that these "dawn horses," or *Eohippus*, were small compared to modern-day horses. They weighed only about 5 kilograms (modern horses weigh about 500 kilograms), and they were primarily browsers (feeding on leaves) rather than grazers (feeding

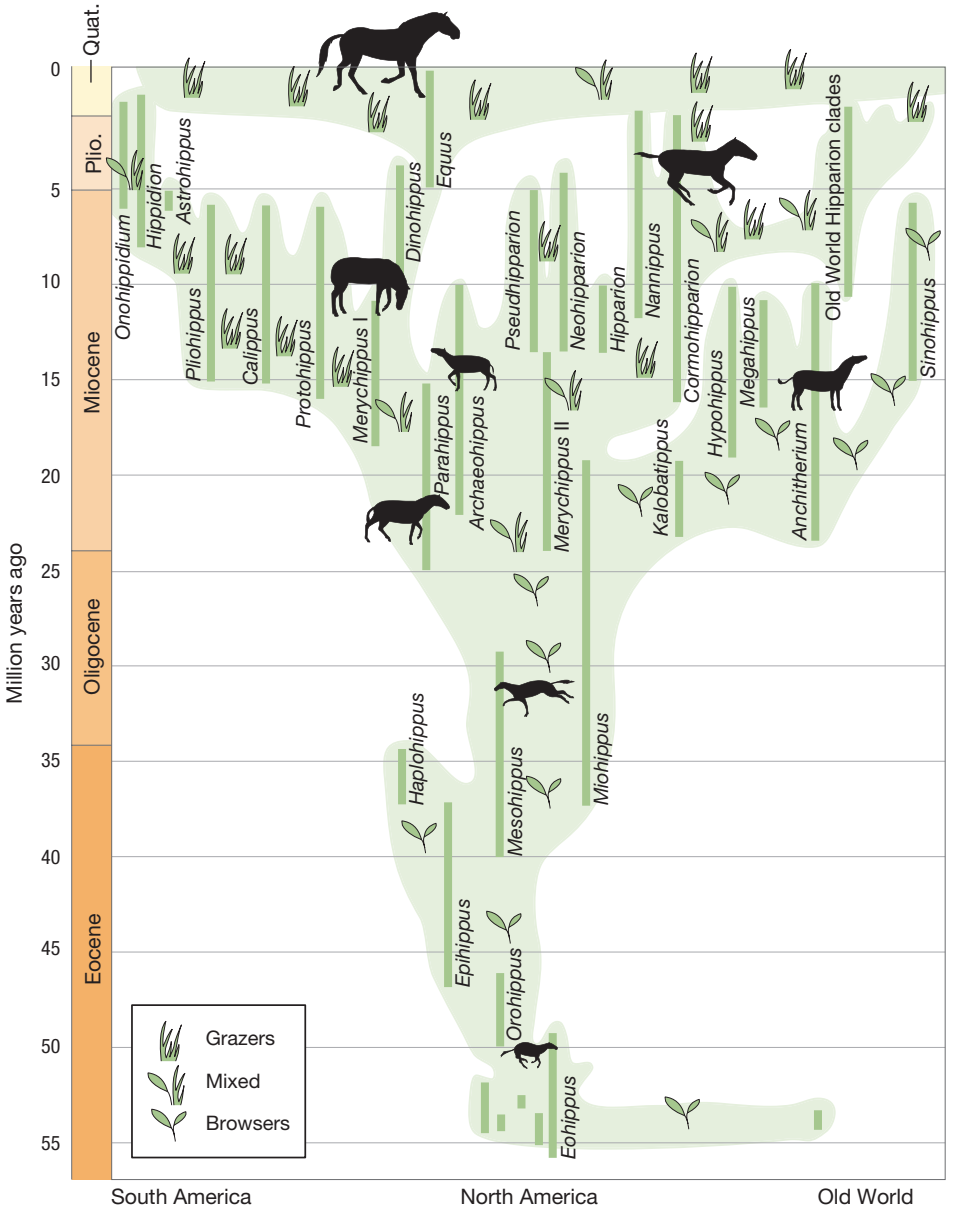


FIGURE 5.35 The evolutionary history of horses from 58 million years ago to the present. While not an explicit phylogeny, this diagram helps us understand the evolutionary origin of modern horses. Horse lineages increased in size, speed, and limb morphology, and their snout shape changed as they adapted to life in emerging grasslands. Adapted from MacFadden (2005).

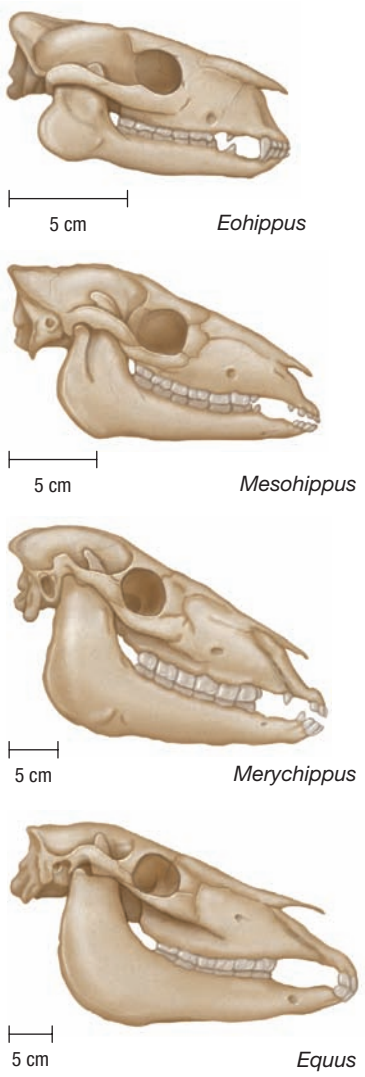


FIGURE 5.36 Changes in cranial shape in horse lineages. *Eohippus* existed about 50 million years ago, *Mesohippus* about 30 million years ago, *Merychippus* about 15 million to 20 million years ago, and *Equus* from about 4.5 million years ago to the present. From Martin (2004).

on grasslands), with teeth adapted to that mode of foraging (**Figure 5.36**). Most strikingly, *Eohippus* had hind limbs with three toes and forelimbs with four toes, rather than the hooves of modern horses.

KEYCONCEPT QUESTION

5.4 Why do we say that the diagram in Figure 5.35 is "not an explicit phylogeny"?

As we move forward in evolutionary time (toward the present) to the Oligocene, the fossil record shows a general trend in which equid lineages such as *Miohippus* and *Mesohippus* became somewhat larger in body size (approximately 10–50 kilograms), with a more elongated snout and larger molars than those of *Eohippus*. The general anatomy of these lineages also changed in a way that suggests that natural selection favored the ability to run more swiftly. But the story of horse evolution is not one in which a single series of species changes gradually and unidirectionally from the tiny *Eohippus* to the large modern horse. During the Miocene, horses underwent a large-scale radiation, with different lineages evolving a diversity of body sizes, some larger and some smaller than those of their Oligocene ancestors. The family Equidae then comprised a number of different species, evolving simultaneously and often changing in morphology in opposite directions.

The feeding ecology of the Equidae changed during the Miocene as well. With grassland ecosystems becoming more common, we can see from the structure of their molars that many, although not all, horse lineages became better adapted to either a combination of browsing and grazing or grazing alone. The fossil evidence also reveals that, along the lineage leading to modern horses, a number of forelimb bones fused together, and the early stages of hooves became evident.

The genus of modern horses, *Equus*, includes domestic horses, zebras, donkeys, and asses; *Equus* appeared in the fossil record about 4.5 million years ago, emerging from just one of the lineages of late Miocene horses. Around this time, natural selection appears to have favored larger animals with teeth better designed for grazing in their new environments. These animals also had fused forelimbs and fused hind limbs, with a muscle and tendon system that gave them the "springing" motion we see in trots and gallops.

We end with a somewhat cautionary note. When working with fossils, it is sometimes tempting to use post hoc—after the fact—explanations of how natural selection produced the changes in the lineage being studied. This becomes much less of a problem, however, when we have a good understanding of how the biotic and abiotic environments changed over the period associated with the fossils under investigation. When we have that sort of information—and we do for the case of the Equidae fossils—we can test whether the changes we see in the traits of the fossils we are studying are consistent with the sorts of changes that we expect would have been favored by natural selection, given environmental changes during that period.

Tetrapod Evolution

The fossil record has also been used to reconstruct phylogenies with the specific purpose of finding species that represent transitions between major life-forms, such as the transition from aquatic to terrestrial animal species. We will examine

such a case in this subsection, but as we do, keep in mind that "transitional" species are past forms, not current ones. After all, no living species is a direct ancestor of another living species. Also keep in mind that being transitional is a relative distinction. All species, even those we now view as transitional, were once extant—and at that time they were on the tips of their respective phylogenetic trees. Likewise, species that today are depicted as the tips of modern phylogenetic trees will some day in the future be viewed as "transitional."

FIGURE 5.37 ***Tiktaalik roseae.*** This species, draws here based on fossil remains, ranged in length from about 1.2 to 3 meters.

The origin of the tetrapods has been a long-standing topic of interest in evolutionary biology (Ruta et al. 2003; Coates et al. 2008). Evolutionary biologists wondered what species filled the phylogenetic gap between fish and tetrapods and what these transitional species actually looked like. Did these transitional species possess both fish- and tetrapod-like features, and if so, which features, and why? In 2005, researchers took a big step toward answering these questions when paleontologist Ted Daeschler and his colleagues uncovered a set of striking fossils on Ellesmere Island, 800 miles from the North Pole in northern Canada (Daeschler et al. 2006; Shubin et al. 2006).

Daeschler was examining the evolution of tetrapods from lobe-finned fish (sarcopterygians) in the Late Devonian period (385 million to 359 million years ago). This evolutionary transition represents not only the emergence of the group that would one day contain our own species, but also the evolution of new forms of locomotion, respiration, and hearing. Daeschler lists the remarkable changes that occurred during this transition:

> The proportions of the skull were remodeled, the series of bones connecting the shoulder and head was lost, and the region that was to become the middle ear was modified . . . , robust limbs with digits evolved, the shoulder girdle and pelvis were altered, the ribs expanded, and bony connections between vertebrae developed. (Daeschler et al. 2006, p. 757)

Evolutionary processes were dramatically reshaping this lineage. So, what did organisms look like when these modifications were under way? The fossil remains of three individuals from a recently discovered species called *Tiktaalik roseae* provide some answers to this question (**Figure 5.37**).

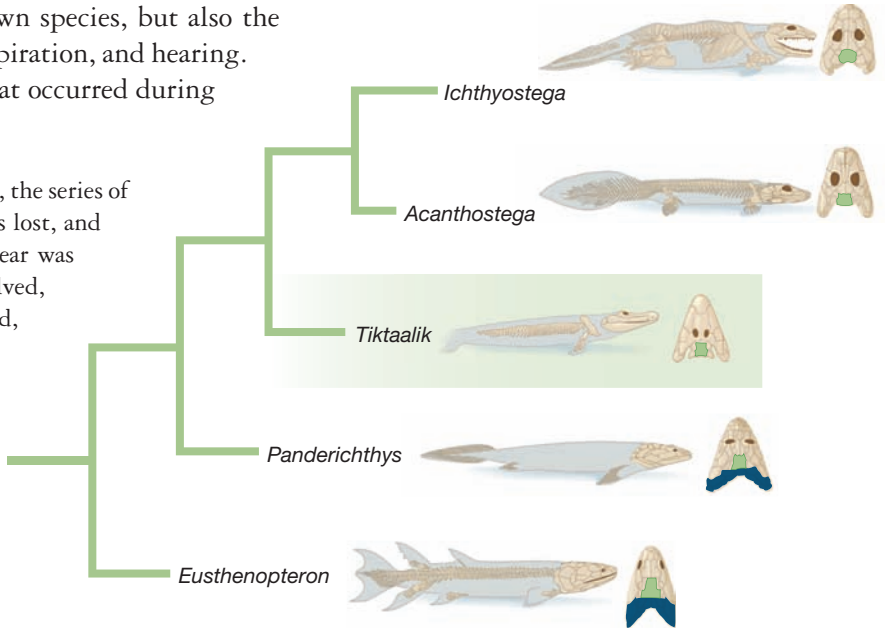


FIGURE 5.38 **A bridge between fish and tetrapods.** The lineage that led to modern tetrapods includes several animals—for example, *Tiktaalik*—that are morphologically intermediate between fish and tetrapods. Skull roofs show the loss of the gill cover (blue) and a size reduction in postparietal bones (green), as well as a reshaping of the skull. Adapted from Ahlberg and Clack (2006) and Clack (2009).

By comparing anatomical traits such as scales, gills, fins, ribs, neck, and limbs in *T. roseae* to those species in the fossil record that came before and after, evolutionary biologists have been able to produce a more comprehensive tree depicting the transition from fish to tetrapods (**Figure 5.38**).

At the time when *Tiktaalik roseae* lived, the land that now lies near the North Pole was located near the equator, and *T. roseae* lived in shallow water on a floodplain in a subtropical or tropical climate. Unlike its lobe-finned fish ancestors, *T. roseae* had a flattened body that was capable of complex movements. Its ribs were modified in

a way that allowed it to support itself on the solid substrate at the bottom of the shallow waters it inhabited, as well as on land (at least for short periods of time). The anatomy of *T. roseae* had been modified so it could move its head in a much more independent fashion than can lobe-finned fish, perhaps allowing it to feed in novel ways at the water–land interface. This species was also intermediate between lobe-finned fish and tetrapods in terms of its respiration, and anatomical analysis of the fossil evidence suggests that it was capable of breathing both in the water and in the air.

5.8 Phylogeny, Natural Selection, and the Comparative Method

One of the principal ways to understand the large-scale effects of natural selection and other evolutionary processes is by taking a *comparative* approach. By comparing traits across groups of species, we can look for trends and patterns in evolutionary events. Do ecological generalists speciate at lower rates than ecological specialists? Do species with parental care have delayed sexual maturation? Do long-lived species evolve larger brains and increased cognitive capacity? Do chromosome duplications lead to more rapid morphological differentiation? These are the types of questions that we can approach using the comparative method in evolutionary biology.

To apply the comparative method properly, it is critical to recognize that the species we study share a common evolutionary history and that historical relationships among them are represented by a phylogeny. A simple example illustrates this point (Felsenstein 2004). Suppose we are interested in understanding whether two traits, say, nocturnal activity and an arboreal (tree-based) lifestyle, tend to evolve together. We might think simply to collect information about the lifestyle of a number of species and enumerate these in a table (**Table 5.2**). Suppose we find the pattern of characters in **Figure 5.39**.

At first glance, Figure 5.39 appears to offer strong support for the hypothesis that nocturnal and arboreal lifestyles go hand-in-hand. A statistical test known as a *chi-square test* reveals that this correspondence is significant at the $p < 0.0016$ level.

TABLE 5.2
An Association between Activity and Habitat[a]

	Nocturnal	Diurnal
Arboreal	4	0
Terrestrial	0	6

[a]A chi-square test reveals an association between time of activity and habitat, significant at the $p < 0.0016$ level.

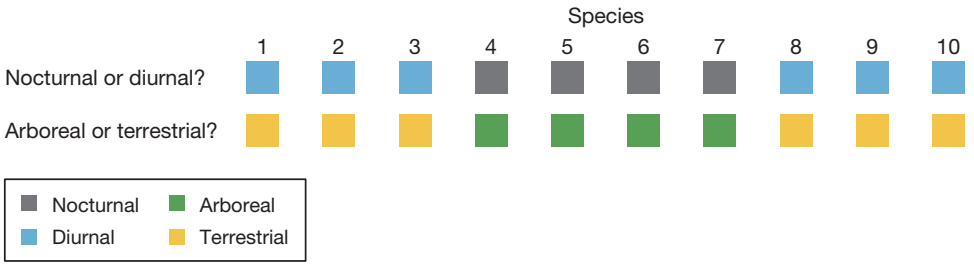


FIGURE 5.39 Character states for 10 species. Characters are shown as nocturnal in dark gray, diurnal in blue, arboreal in green, and terrestrial in gold. Adapted from Felsenstein (2004).

But there is a problem. The chi-square test assumes that each species evolved independently from every other—in other words, the test does not account for any shared evolutionary history among these species. Suppose that we discover that the phylogenetic history of these species is as depicted in **Figure 5.40**. Now we can infer the evolutionary changes that gave rise to the characters that we observe. The most parsimonious assignment of characters is shown in the figure.

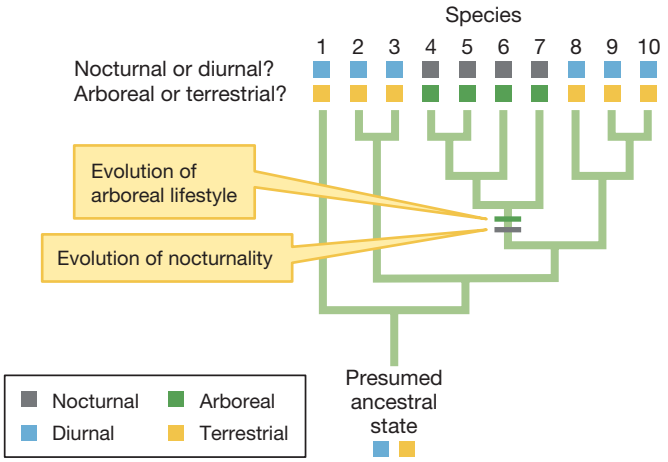


FIGURE 5.40 Traits on a phylogeny are not independent. The relationship among our 10 species is indicated by this phylogenetic tree. The most parsimonious assignment of character changes has nocturnal activity and arboreal living each evolving a single time. Adapted from Felsenstein (2004).

Knowing what we know from Figure 5.40, we might take a different view of the character pattern that we've observed. Rather than representing 10 independent samples, we note that the entire pattern has arisen from a *single pair* of evolutionary changes, one for each character. We still have some evidence that nocturnal behavior and arboreal life go hand-in-hand, because the two changes both occurred on the same branch. But is this a statistically unlikely event or could it have happened by chance? To answer that question, we need to find the probability that both changes happened on exactly the same branch. There are 18 branches on this tree, so, ignoring branch lengths, this probability is 1/18, or 5.5%, a value that is no longer significant at the 5% level (that is, with $p < 0.05$). If we fail to consider the phylogenetic relationships among the species we are studying, the comparative method can give misleading estimates of the significance of the patterns that we observe.

A similar problem arises if we try to look at comparative relationships among continuous quantitative characters without regard for the underlying phylogeny. **Figure 5.41** shows a hypothetical set of measurements of testes size and age at first reproduction for 20 species of mammals. Interpreted independently from the phylogeny, it appears that there is a positive relationship between these quantities: Species with an earlier age at first reproduction also have a larger testes size. One might therefore conclude that these two traits are selected to change together: As one increases, the other increases as well.

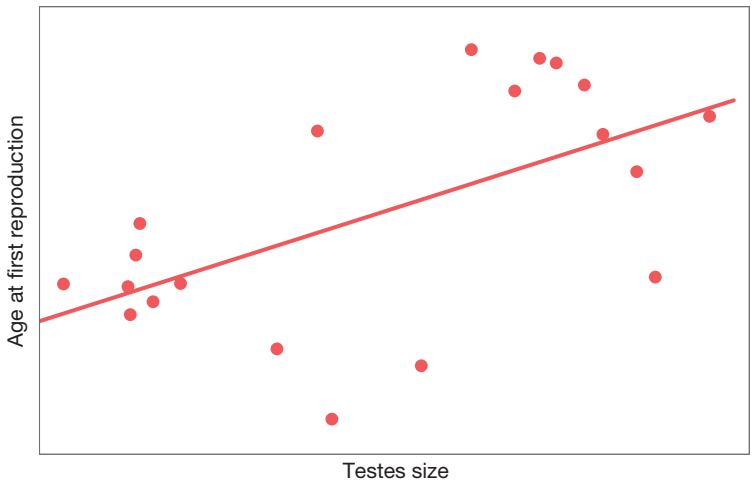


FIGURE 5.41 Testes size versus age at first reproduction. The solid line is the best-fit linear regression for the 20 hypothetical species.

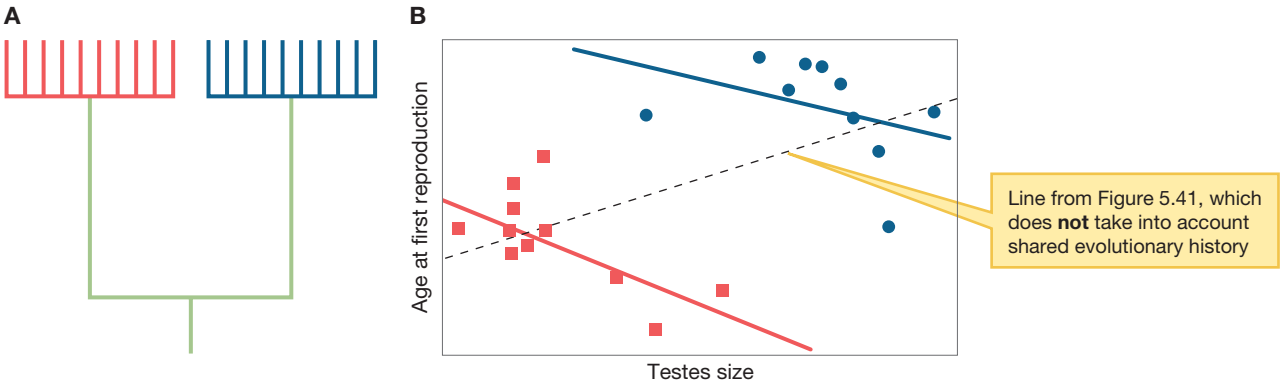


FIGURE 5.42 The phylogenetic relationship among the 20 species and evolutionary trends within each clade. **(A)** The partially resolved phylogeny (that is, there are polytomies) reveals that an early divergence event created two separate clades, which recently radiated to form 10 species per clade. Adapted from Felsenstein (1985). **(B)** Testes size versus age at first reproduction, with clade membership indicated by color and symbol shape. Lines indicate the best-fit linear regressions for each 10-species clade considered independently. Once each clade is considered separately, we observe a negative relationship between testes size and age at first reproduction, rather than the positive relationship (dashed line) we found in Figure 5.41, when the clades were grouped together.

But again, suppose that these points are not statistically independent observations, but rather are linked by a shared evolutionary history. Suppose that the phylogenetic tree shows a single early divergence event, as in **Figure 5.42A**. This information radically changes our interpretation of the pattern in Figure 5.41. We now see that a single evolutionary event led to the separation between the two major clades. Moreover, within each clade, the trend is now exactly the reverse of what we had originally thought: Testes size tends to decrease with increasing age of first reproduction. **Figure 5.42B** illustrates our reinterpretation of the data, coloring each species according to its clade membership and looking at the trend within each clade separately.

Thus far, we have seen the ways that we could potentially be misled by applying the comparative method without properly accounting for phylogeny. How do we cope with this problem? The method of **independent contrasts** provides a solution (Felsenstein 1985). The solution is not to look at each species as an independent data point, but rather to look at estimated changes that occur along various branches of the tree, and to pick these branches in such a way that evolution along each segment can be considered independently of every other segment.

Figure 5.43 illustrates how we can find four independent comparisons to make in a five-species tree. The key here is that we are not looking at the absolute character states, but rather at the differences in character states between each pair that we are considering in a given contrast. That is, if we are studying testes size and age at first reproduction as our characters of interest, we look at the *difference* between testes size for species A and B, and at the *difference* between age of first reproduction for species A and B. This pair of *differences* becomes our first "data

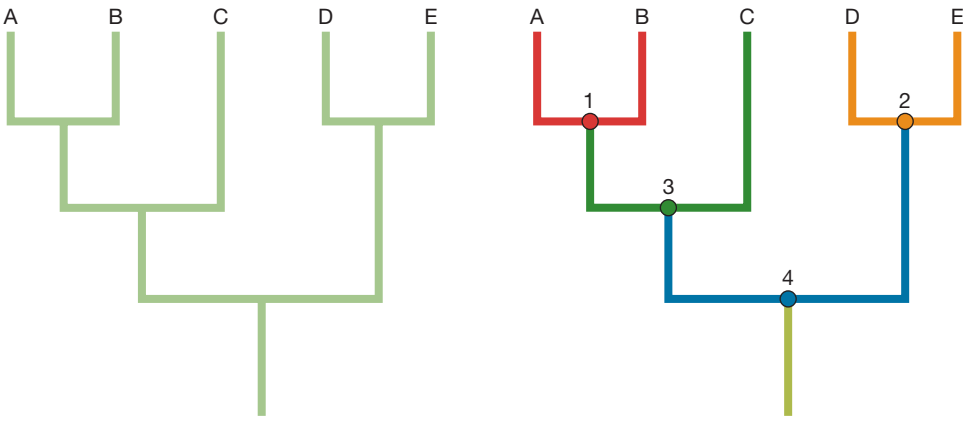


FIGURE 5.43 Independent contrasts. This five-species tree features four independent contrasts: A versus B, D versus E, 1 versus C, and 2 versus 3. Here the labels 1–4 represent the inferred character states of the internal nodes.

point"; this data point is a difference, or *contrast*. For our second data point, we can look at the differences in these characters between species D and species E. As we see from the figure, the evolutionary path along which D and E diverged from one another is entirely disjointed from the evolutionary path along which A and B diverged from one another; the two contrasts, A versus B and D versus E, are thus said to be *independent contrasts*.

At this point, we cannot form any additional independent contrasts that involve only the branch tips A–E; any other path between two species A–E will include a segment of the A-to-B or D-to-E path, and thus it will not be independent from the two contrasts that we have already accumulated. We are not finished, however. We can form additional independent contrasts by considering internal nodes. The comparison between internal node 1 and branch tip C follows an evolutionary path that is disjoint from those traced by the A-to-B and D-to-E paths, and it provides us with a third independent contrast. Although we do not know the character state of internal node 1 directly, we can and do infer it from the character states of nodes A and B using a model of evolutionary change. Finally, by using similar logic, we can find a fourth and final independent contrast in the comparison between internal node 2 and internal node 3.

Having accumulated a set of independent contrasts in this way, we can now proceed with well-established statistical analyses, such as linear regression, on the contrasts to test our hypothesis of interest.

Independent Contrasts: A Test of the Flammability Hypothesis

Organisms are not merely the passive victims of external environmental conditions; rather, they actively affect the environment around them. The role of organisms in this process of **niche construction**—shaping their own environmental conditions—can feed back into evolutionary processes in interesting and complex ways. Fire ecology provides an excellent example. Trees, shrubs, and other plants not only suffer the effects of fire but also provide the necessary fuel for fire, and thus it is reasonable to say that an ecosystem's flora create the conditions for their own immolation. Certain physiological traits—thin twig structure, low needle density, and high oil content—tend to enhance the rate and intensity of fire. Trees that retain their dead branches on the trunk make a particularly large contribution to the potential for frequent and severe fire. Dead branches are drier and burn much hotter than living branches; thus, by retaining dead branches instead of dropping them to the forest floor to decompose, branch-retaining trees greatly add to the volume of highly combustible fuel in the forest.

Dylan Schwilk and David Ackerly hypothesized that when plant species construct the fire conditions around them, this has evolutionary consequences (Schwilk and Ackerly 2001). Specifically, they conjectured that those plants that create the conditions for frequent and severe fire also induce natural selection *on themselves* for traits that allow rapid regeneration after fires have passed through (**Figure 5.44**).

To test this hypothesis, Schwilk and Ackerly used a comparative approach, looking to see if pine species that create conditions for frequent and severe fire also tend to have traits that allow rapid regrowth after fire, such as the ability to resprout from surviving underground tissue or *serotiny*, the fire-induced release

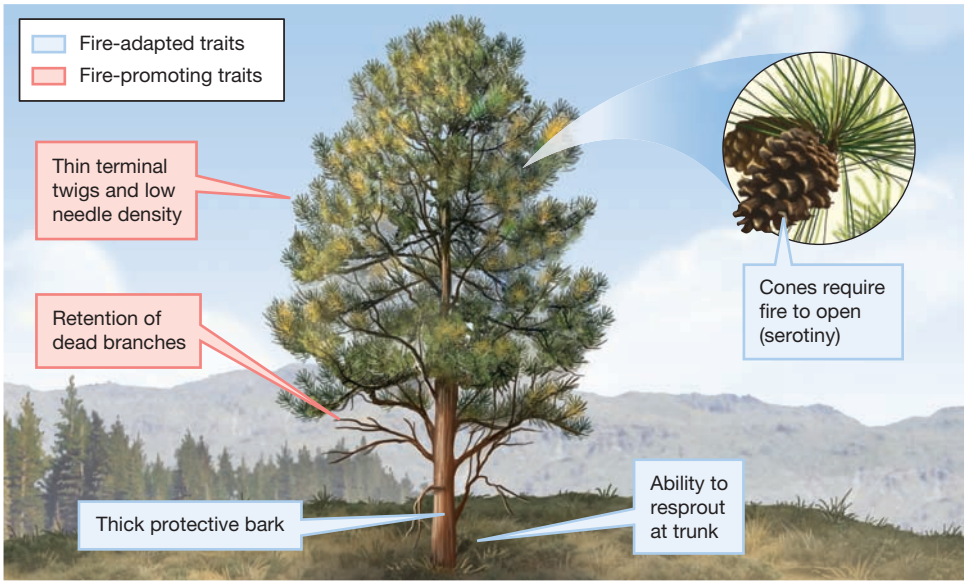


FIGURE 5.44 Fire-adapted traits and fire-promoting traits. Many pines have traits that promote fire in the environment; these species also tend to have traits that help them deal with the frequent occurrence of fire.

of seeds from seed cones. They reasoned that if their hypothesis was correct, they would observe an association between traits that promote fire and traits that promote regeneration after fire.

For 38 pine species in the subgenus *Pinus*, the researchers collected data on a number of traits that affect the fire ecology of the landscape and on a number of traits that indicate regenerative ability after fire. Here, we will focus on one particular pair: the retention of dead limbs on the tree as a fire-affecting trait and serotiny as a regenerative trait.

Because pines are linked by evolutionary history, Schwilk and Ackerly faced a classic case of the phylogenetic nonindependence we have discussed throughout this section. To correct for this, the method of independent contrasts was necessary. They constructed a phylogenetic tree of their study species, and from this phylogeny they identified a set of independent contrasts between the species therein (**Figure 5.45**). Then, for the characters of branch retention and serotiny, they calculated each of the contrasts for the 38 species.

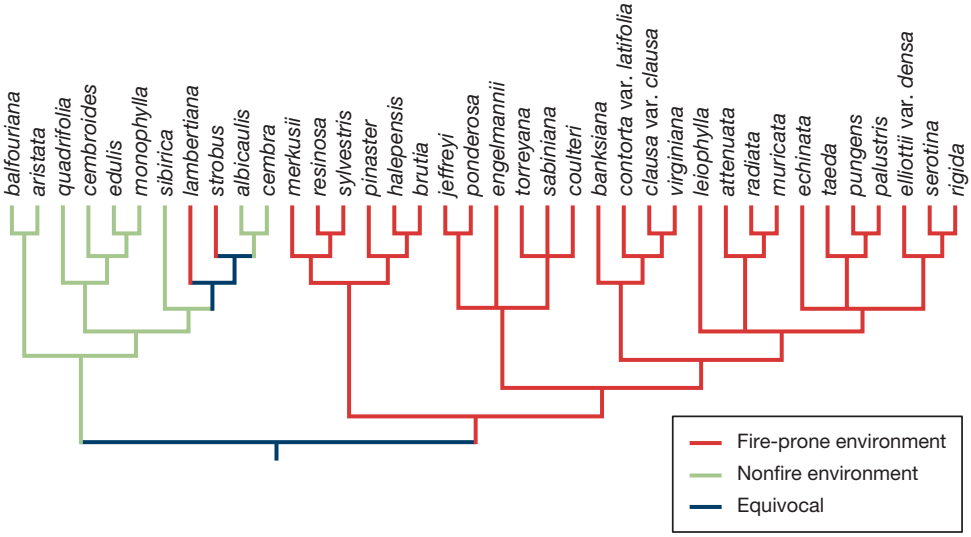


FIGURE 5.45 Phylogeny and the independent contrasts method. A consensus phylogeny of the 38 species of *Pinus*. This phylogeny allowed researchers to apply the method of independent contrasts to their hypothesis regarding traits that promote fire and traits that promote regeneration after fire. Adapted from Schwilk and Ackerly (2001).

By applying the method of independent contrasts, Schwilk and Ackerly were able to demonstrate a statistically significant association between branch retention and serotiny, accounting for the shared phylogenetic histories of their study species. They found similar associations between numerous other flammability-enhancing traits and regenerative traits. These associations support their hypothesis that flammability-enhancing tree species are selected for the ability to regenerate rapidly after fire.

In this chapter and the previous chapter, we have learned how to read and interpret phylogenetic trees. We have seen how phylogenetic trees can be used to generate and test hypotheses, and we have explored the methods that evolutionary biologists use to infer or reconstruct phylogenies from character data. We will not be leaving phylogeny behind with the close of this chapter, however. Phylogenetic reasoning is a fundamental ingredient in almost every area of evolutionary biology, as we will see throughout the remainder of this book.

SUMMARY

1. The task of reconstructing a phylogenetic tree is a problem in statistical inference. That is, we wish to make inferences about the historical evolutionary relationships among populations based on some data set.
2. At the most basic level, to build a phylogenetic tree, we collect information about the characters of some species, and we look at which species have which traits in common. We begin by assuming that species with many traits in common are more likely to be closely related to one another than are species with fewer traits in common. This logic assumes that common traits are homologies—traits that are due to shared common ancestry.
3. Evolutionary biologists have developed a number of different phylogenetic methods to test whether characters that are shared across species are analogous rather than homologous.
4. Parsimony methods search for trees that have the minimum number of evolutionary changes. The best phylogeny is assumed to be the one that both explains the observed character data and posits the fewest evolutionary changes.
5. Phylogenetic distance methods are a second approach to inferring trees. The idea behind distance methods is that if we can measure the pairwise "distances" between species, then we can use these distances to reconstruct a tree. First, researchers have to measure these distances, and then they have to use statistical methods to find the best tree given these distance data. The goal is to find a tree with branches arrayed so that the distance along the branches between any two species is as close as possible to the distance that we measured between those two species.
6. Maximum likelihood methods and Bayesian inference methods use explicit models of how characters change through the evolutionary process. By applying techniques of statistical inference, they attempt to find the phylogenetic tree that best explains the data.
7. For any comparison involving more than a few species, there are too many possible phylogenetic trees to search exhaustively, even with the fastest computers, and so researchers have devised clever ways to search within the "space" of possible trees.
8. Evolutionary biologists have developed numerous statistical measures of support to test their phylogenetic hypotheses. Once they have used character data to infer a tree, they can test how certain they are that a tree—or some component of a tree—is correct. Bootstrap resampling is one technique for doing this; the odds ratio test is a second technique used to address such questions.
9. When using the comparative method for studying how natural selection operates, we must account for any shared evolutionary history among the species we are studying. The method of independent contrasts allows evolutionary biologists to do this.

KEY TERMS

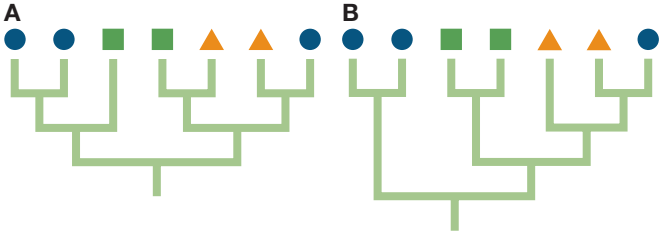
Bayesian inference (p. 150)
bootstrap resampling (p. 167)
genetic distance (p. 158)
independent contrasts (p. 178)
long-branch attraction (p. 153)
maximum likelihood (p. 150)
niche construction (p. 179)
odds ratio testing (p. 167)
parsimony (p. 151)
phylogenetic distance methods (p. 156)
phylogeography (p. 163)
sequence divergence (p. 148)

REVIEW QUESTIONS

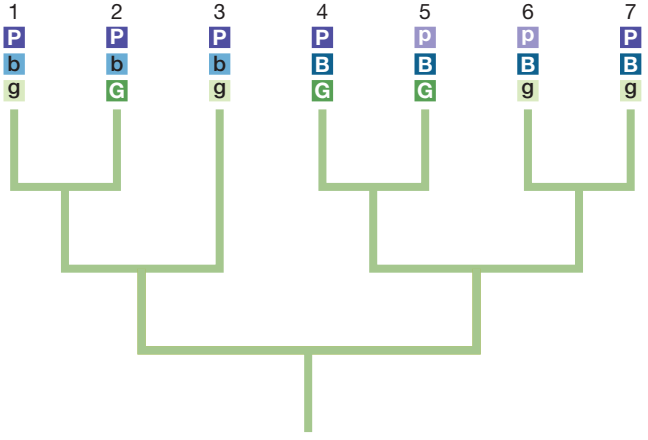
1. The introductory story of the chapter involves an HIV outbreak in a children's hospital in Benghazi. List two pieces of evidence provided by phylogenetic analysis that this infection was accidental rather than a deliberate action by the accused medics.
2. What kind of information do researchers use to create a phylogenetic tree?
3. Concisely describe the core idea underlying parsimony methods of phylogenetic reconstruction.
4. What type of phylogenetic reconstruction uses a distance matrix?
5. What is the purpose of sequence alignment?
6. Why is it computationally difficult to infer a phylogeny for even a few dozen species?
7. When using the outgroup method to root a phylogenetic tree, on what branch do you place the root?
8. What does it mean when a node in a phylogeny has a bootstrap value of 95?
9. Why do we say that *Tiktaalik* is a transitional species?
10. Why do comparative biologists need to use independent contrasts when looking at evolutionary trends?

KEY CONCEPT APPLICATION QUESTIONS

11. Which of the two trees illustrated below offers a more parsimonious explanation for the observed character states?

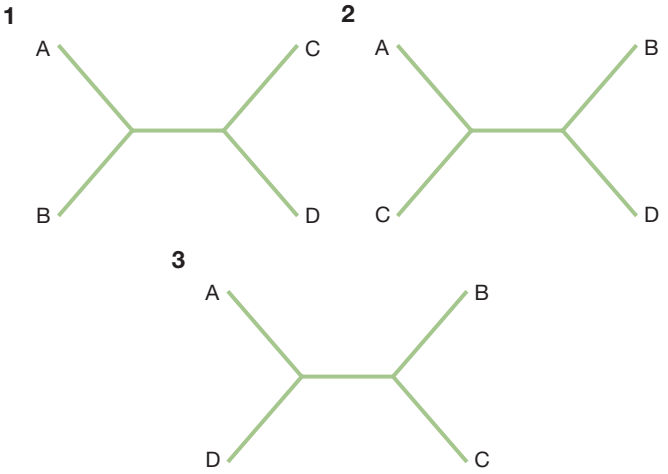


12. For the same character data in question 11, can you draw an even more parsimonious tree than either of the two shown? If so, draw it. If not, explain why it is not possible to do so.
13. Given the tree that follows and the character states for the three characters illustrated, assign possible locations of character changes on the tree. Be sure to indicate the presumed ancestral state.



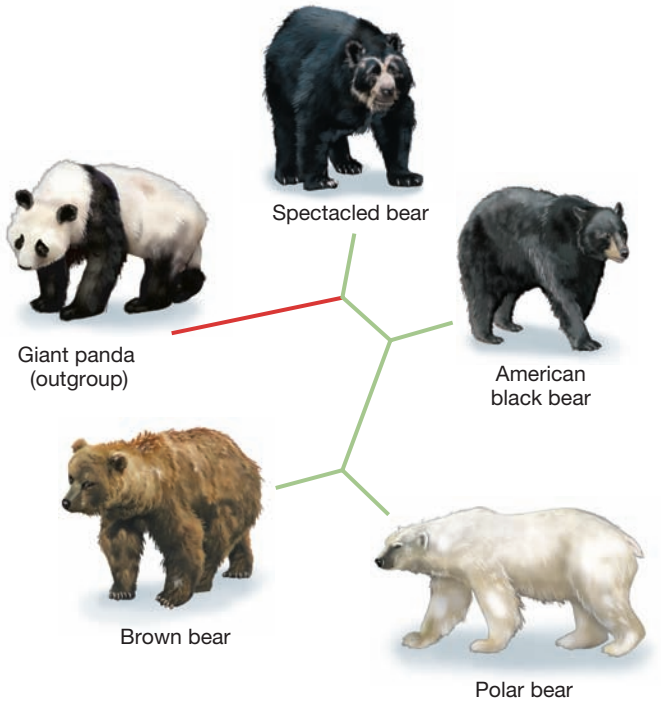
14. Are your assignments of state changes from question 13 parsimonious? How do you know?
15. Is there only one maximally parsimonious way to assign state changes to the tree in question 13? If so, why? If not, show two different ways.

16. Which of these three assignments of taxa to branch tips—1, 2, or 3—is most likely given the distance matrix that follows?

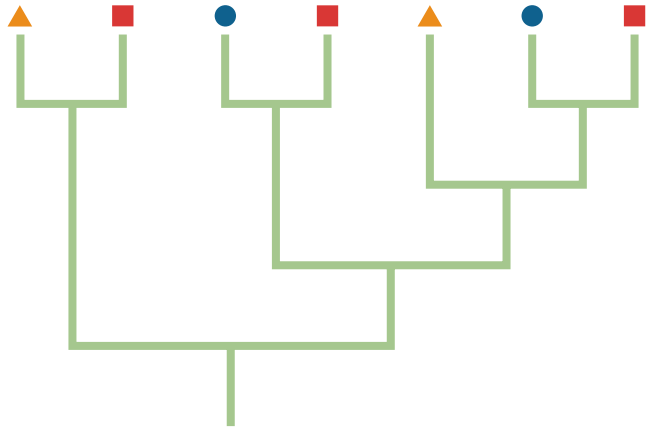


	A	B	C	D
A		18	8	17
B			16	6
C				17
D				

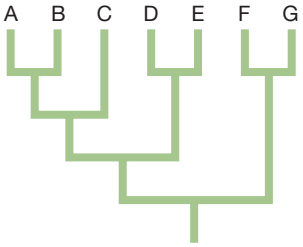
17. The figure below illustrates an unrooted phylogeny (after Zhang and Ryder 1994) of several bear species: the polar bear (*Ursus maritimus*), the brown bear (*Ursus arctos*), the American black bear (*Ursus americanus*), and the spectacled bear (*Tremarctos ornatus*), with the giant panda (*Ailuropoda melanoleuca*) as an outgroup. Using the outgroup method, redraw this unrooted phylogeny as a rooted phylogeny.



18. Use the Fitch algorithm to find the minimum number of character changes necessary to explain the distribution of the character states indicated on the tree below.



19. Indicate how six independent contrasts can be obtained from the tree below.



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PART II

Evolutionary Genetics

Gold *Plusiotis optima* beetles are displayed at the National Institute of Biodiversity, San José, Costa Rica.

6

Transmission Genetics and the Sources of Genetic Variation

◀ A collection of strikingly patterned eggs from the Common Murre (*Uria aalge*). Their unusual shape causes them to roll in tight circles and may help prevent eggs from falling off the bare rock ledges where they are laid.

About 12,000 years ago, people began selectively planting certain varieties of seeds to improve their crops. Those involved in these early attempts at artificial selection must have possessed a basic understanding that traits present in the parental stock of one generation somehow affected the traits in offspring generations. Millennia later, the Greek philosopher and physician Hippocrates suggested that offspring contained the blended "seeds" from their two parents, and that these seeds made the offspring what they were. Over the subsequent centuries after Hippocrates, theories of heredity took some interesting twists and turns, including a hypothesis that all individuals contain within them "preformed" tiny versions of all the individuals that will ever come from their lineage. But until the turn of the twentieth century, most scholars envisioned heredity as operating by some form of blending inheritance.

At almost the same time that Charles Darwin published his book *On the Origin of Species* in Great Britain, Gregor Mendel, an Augustinian monk and amateur plant breeder in the Austro-Hungarian Empire, was examining tens of thousands of pea plants that he had bred. In doing so, Mendel was quietly

undertaking some of the most important studies ever performed in biology (Henig 2001). Mendel was the only child of peasant farmers, and at age 21 he entered the St. Thomas Augustinian monastery. After a short stint with pastoral duties, he became a student at the University of Vienna, where he studied mathematics and biology, hoping to teach these subjects as part of his duties as a monk. It was at the University of Vienna that Mendel became practiced in scientific research.

In his now famous experiments of the 1850s and 1860s, Mendel bred pea plants and examined the way that traits were passed down across generations. His discoveries set the foundation for the field of genetics, as we will see in Section 6.1.

In this chapter, we will review what DNA is and how it directs the synthesis of proteins. We will also include an overview of **transmission genetics**—the mechanisms by which genes are passed from parents to offspring—and a discussion of genetic variation and mutation. In the course of this brief review, we will address the following questions:

- How does an understanding of DNA, amino acids, and proteins help us understand the evolution of life?
- What is transmission genetics, and how does our understanding of this topic affect the way that we study the process of evolution?
- How does mutation generate genetic variation, and how do mutations affect the evolutionary process?

When discussing these topics, our goal is not simply to provide a refresher on basic genetics, but rather to emphasize how knowledge of fundamental genetic mechanisms is critical for a comprehensive understanding of evolution. This chapter also sets the stage for the next four chapters, which focus on population genetics.

6.1 Mendel's Laws

We begin by briefly summarizing Mendel's famous experiments on pea plants. Mendel examined seven different characters of pea plants, including flower color; specifically, he looked at whether the flowers were purple or white. He began 2 years of breeding experiments to determine if his pea plants always bred "true," that is, always produced a specific type of offspring: purple-flowered offspring when a purple-flowered parent was self-fertilized, and white-flowered offspring when a white-flowered parent was self-fertilized. By using pea varieties that bred true, Mendel ensured that his plants were what today we would call homozygotes; that is, each plant contained alleles (gene variants) for only one trait for any given character, in this case, a specific flower color.

Mendel's protocol was simple but powerful. In the parental generation, he crossed a true-breeding parent plant homozygous for purple flowers with a true-breeding parent plant homozygous for white flowers. All of the offspring from these matings—known as the F_1 generation (the first generation of offspring)—produced purple flowers. Mendel then self-fertilized the F_1 plants to produce an F_2 generation (the second generation of offspring). The F_2 generation exhibited a distinctive ratio of flower colors: three-quarters had purple flowers, while one-quarter had white flowers (**Figure 6.1**).

Mendel was able to derive a number of important conclusions about the genetics of diploid organisms—organisms with two copies of each chromosome—from these experiments. These conclusions have come to be known as *Mendel's laws*.

The Law of Segregation

From his experiments, Mendel could infer the genetic contributions of both parents to their offspring. He deduced that even though all F_1 plants produced purple flowers, they must have received and retained genetic information from *both* parents; otherwise, he would not have seen white flowers return in the F_2 generation. Mendel's results demonstrated that each parent plant had two copies of what we now call genes, and that the two gene copies separate with equal probability into the **gametes** (sex cells) of the pea plants. Much work has confirmed this finding, and we now speak of Mendel's first law, or the **law of segregation**, which states that each individual has two gene copies at each **locus** (the physical location of gene copies on the chromosome) and that these gene copies segregate during gamete production, so that only one gene copy goes into each gamete.

Moreover, Mendel concluded that because all F_1 plants were purple-flowered but contained a copy of genetic information from both parents, purple color in flowers was **dominant** to white color; that is, purple flower color appeared when both gene copies coded for purple flowers or when one coded for purple and the other for white flowers. White flower color was **recessive**; that is, it appeared only when both gene copies coded for white flowers. Hence, each gene copy retained its particulate individuality, whether or not it was expressed in the external appearance of the flowers.

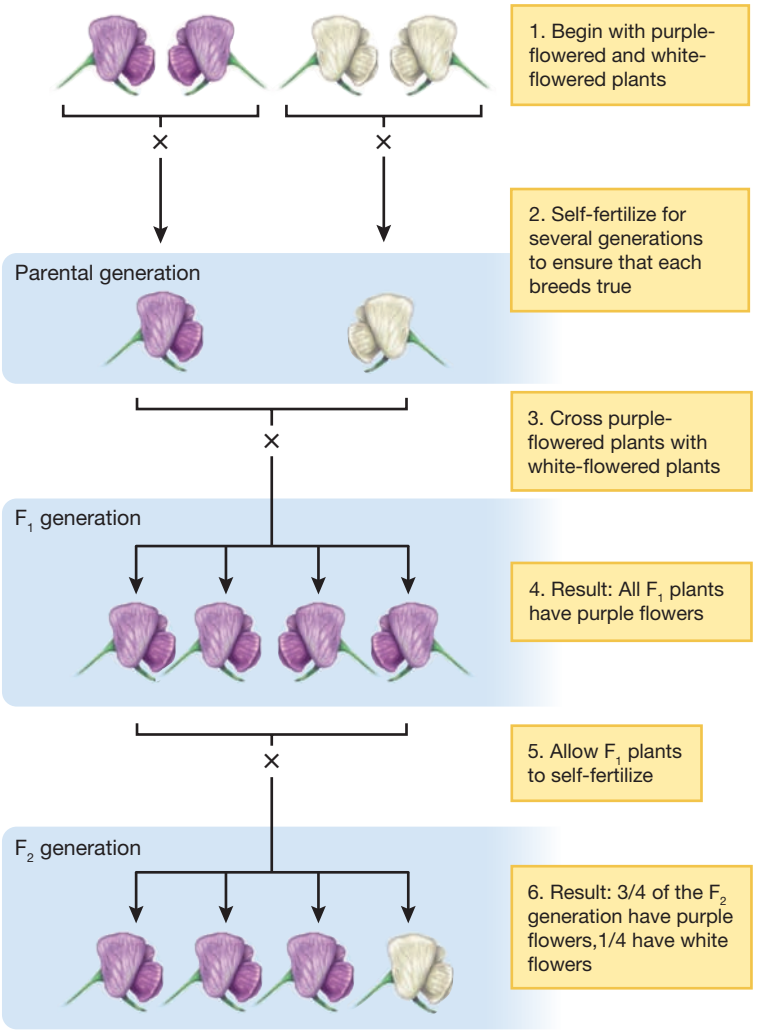


FIGURE 6.1 Mendel's experiments. Mendel's experiments on the genetics of flower color and other traits in peas helped reveal the laws of genetic inheritance. Mendel found that when he crossed true-breeding purple-flowered plants with true-breeding white-flowered plants in the parental generation, all of the F_1 offspring had purple flowers. But if he allowed the F_1 offspring to self-fertilize to produce an F_2 generation, approximately 3/4 of the F_2 plants had purple flowers, while approximately 1/4 had white flowers.

The Law of Independent Assortment

Mendel also conducted breeding experiments in which he tracked other characters, such as seed shape (whether seeds were round or wrinkled). From these studies, he discovered what has since become known as Mendel's second law, or the **law of independent assortment**. This law states that which allele is passed down to the next generation at one locus (for example, the locus associated with seed shape) is independent of which allele is passed down to the next generation at another locus (for example, the locus associated with flower color). Today, we know that this holds true only for genes on different chromosomes, known as *unlinked loci*, and not for genes close together on the same chromosome, known as *linked loci*.

To illustrate the distinction between linked and unlinked loci, let's consider two cases, both of which involve seed shape (round or wrinkled) and flower color (purple or white) in pea plants. In both cases, we assume that natural selection favors purple flowers over white flowers. For case 1, suppose that the loci for seed shape and flower color are unlinked. In this case, selection can operate independently on each character. Purple flowers should increase in frequency regardless of which seed shape is favored by natural selection. For case 2, instead suppose that the loci for seed shape and flower color are linked. Changes in the frequency of the alleles at one locus will then affect the frequency of the alleles at the other locus. Now, to determine if purple flowers will increase in frequency, we need to know whether purple color is more often associated with round or wrinkled seeds, and which seed shape is favored by selection. This is not always a straightforward problem, as we will discuss when we explore the population genetics of linked and unlinked loci in Chapter 9. For now, our point is that whether the loci are linked or unlinked has important implications for predicting how natural selection will operate.

KEYCONCEPT QUESTION

6.1 Why is it fortunate that Mendel picked characters in the pea plant that were, for the most part, unlinked? Why would it have been much more difficult for Mendel to come up with his law of independent assortment if he had chosen some linked characters?

As we learned in Chapter 2, Mendel's work remained unnoticed until about 1900. And even when his results were rediscovered, there was an intense debate about what Mendel's findings meant for our understanding of evolution by natural selection. But today we recognize that Mendel's results provide us with a basic understanding of one of the three prerequisites for a trait to evolve by natural selection; namely, that the trait must be passed down across generations. Mendel's work also provided empirical evidence disproving once and for all the early idea that traits from the two parents were permanently blended in the offspring. Rather, he clearly demonstrated that genes are particulate; that is, they are passed down across generations as separable entities and they can persist across generations even when they are not visibly expressed in the phenotype.

Blending versus Particulate Inheritance

The demonstration that biological heredity was fundamentally particulate resolved one of the major challenges to Darwin's theory. As we noted in Chapter 2, one substantial problem for Darwin was to explain how sufficient variation could be maintained in populations to allow natural selection to continue to operate. Not only does natural selection itself reduce variation by favoring some forms over others, but according to Darwin's view of heredity, the very mechanism of genetic transmission would also reduce variation.

Darwin, like most of his contemporaries, envisioned heredity as a blending process in which the characteristics of the parents were averaged in some way to determine the characteristics of each offspring. It is true that mechanisms of blending inheritance would result in the sort of resemblance between parent and offspring that is needed for heredity, and thus for evolution. The problem is that blending of this sort also eliminates variation (**Figure 6.2**).

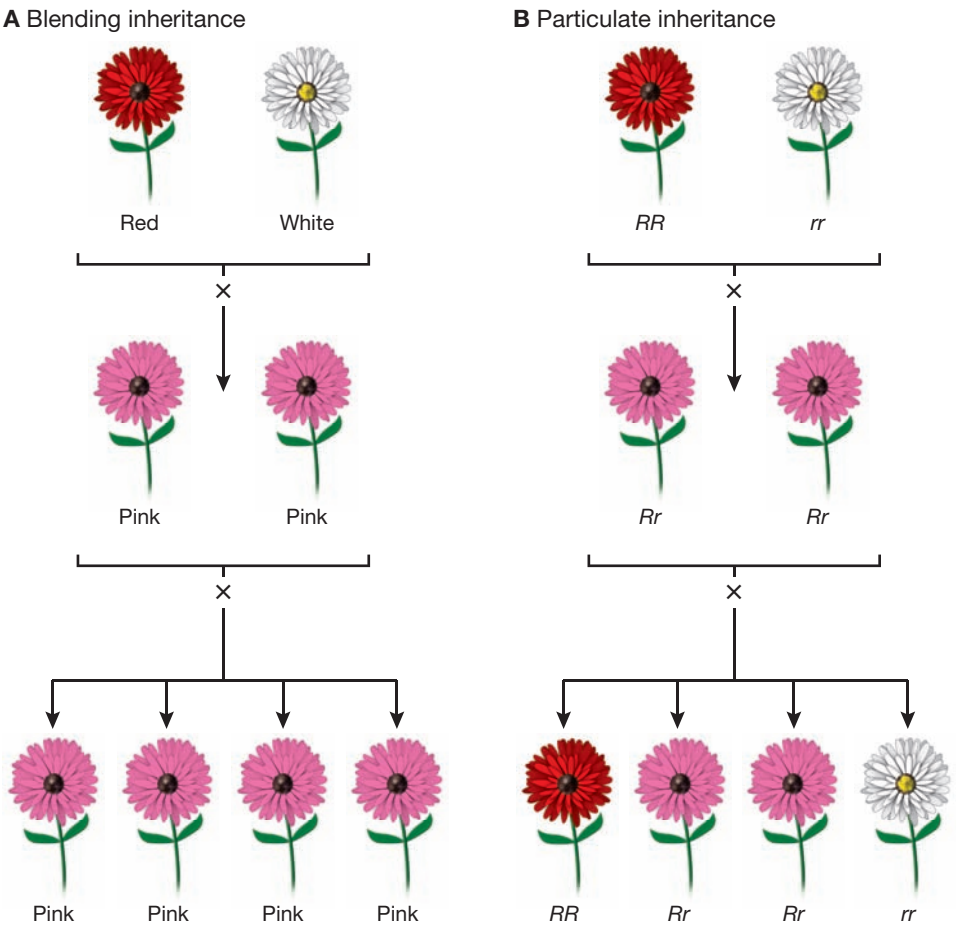


FIGURE 6.2 Blending inheritance versus particulate inheritance. Darwin, like most of his contemporaries, viewed heredity as a blending process. In this view, offspring tend to resemble their parents—and where parents differ in phenotype, offspring exhibit an intermediate value. Under this blending model, the process of inheritance irreversibly reduces variation in the population. **(A)** Under blending inheritance, the offspring of a red-flowered and a white-flowered parent would be pink; the offspring of these pink-flowered individuals would also be pink. **(B)** Under particulate inheritance, the offspring of red-flowered (*RR*) and white-flowered (*rr*) parents might also be pink (*Rr*). But these pink offspring, when crossed, could re-create the red and white phenotypes among their offspring.

Mendel's theory of inheritance suggested that the hereditary determinants of phenotype were particulate. While the phenotypic effects of the particles carrying heritable information may blend, the particles themselves remain distinct, and they can be separated again in future reproductive events. Instead of thinking about the hereditary determinants blending irreversibly like colored dyes, a better metaphor is colored filters, which blend in appearance but are readily separated into new future combinations (**Figure 6.3**).

The theory of particulate inheritance thus resolved a major concern with Darwin's theory, which was first raised in 1867 by the engineer Fleeming Jenkin (Morris 1994). Jenkin's objection was this: Given the supposed blending nature of inheritance, how can new mutations ever have significant effects on the characteristics of a population? Under theories of blending inheritance, a favorable new mutation in a large population would, over the course of many generations, be swamped as it blended with the more prevalent character. As a result, natural selection could never take a new allele to fixation, because the new allele would blend away before selection could increase its frequency enough to make a lasting difference. With Mendelian inheritance, this problem disappears. A new mutation retains its particulate nature and is not blended into obscurity. As we saw in Figure 6.1, phenotypic variation can be masked in one generation and yet reappear in the next. Then, if the mutation has positive effects on fitness, its frequency can increase via natural selection.

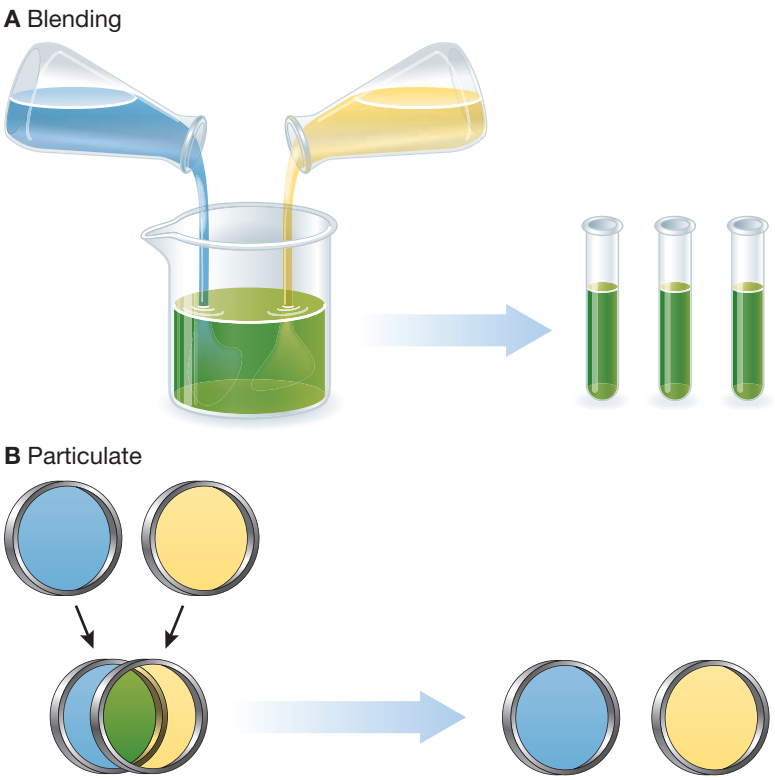


FIGURE 6.3 A color-mixing metaphor for blending and particulate inheritance. Mendel showed that inheritance was particulate. The hereditary particles responsible for inherited physical characteristics behaved not like **(A)** colored dyes, but rather like **(B)** colored filters for a camera lens. Just as blue and yellow dyes can come together to make green, so can blue and yellow filters be combined to make a green one. But unlike colored dyes, filters are not irretrievably blended when they are combined. They can be separated again with ease, so that the variation in filter colors is not lost.

6.2 Transmission Genetics

For most of the past 4 billion years, deoxyribonucleic acid—DNA—has been the chemical underpinning of life on Earth. At a very basic level, it is changes in DNA sequences and DNA expression that underlie the process of biological evolution by driving changes in phenotype and causing differences in fitness. We primarily will be considering DNA as it relates to transmission genetics in this chapter. But, for now, keep in mind two things that we have already seen numerous times in this book. First, a small change to DNA that is passed down across generations can have a large effect on fitness. We saw this in Chapter 3 in our example of dark and light coat coloration in oldfield mice. The avian influenza virus offers another good example: A change to just one component of a single protein in the H5N1 virus makes this virus much more dangerous to mammalian hosts (Li et al. 2009). Second, as we saw in Chapters 4 and 5, changes in DNA sequences across populations and species are used by evolutionary biologists to reconstruct phylogenetic relationships.

DNA and Chromosomes

DNA is a polymer; that is, it is a macromolecule composed of repeating units linked together in a chain. The building blocks of this macromolecule are four nucleotides. Each nucleotide is composed of a pentose (a five-carbon sugar) known as deoxyribose, a phosphate group (a phosphorus atom and four oxygen atoms), and a nitrogenous base. The four nitrogenous bases are adenine (A), guanine (G), cytosine (C), and thymine (T). Adenine and guanine are purines: nitrogenous bases that contain a six-sided ring and a five-sided ring. Cytosine and thymine are pyrimidines: nitrogenous bases that consist of only a six-sided ring. It is a triumph of modern biology that we are capable of describing the stuff of life in such succinct terms. There remains much more to learn about DNA, but we have a basic understanding of the biochemical basis of the genetic material underlying the phenotypes on which natural selection acts.

DNA is a double-stranded molecule: Two strands of connected nucleotides are wound around one another, held in place with hydrogen bonds. Chemically, each strand has what is called a 5′ (five prime) end with a terminal phosphate group, and a 3′ (three prime) end with a terminal hydroxyl group. The two strands are oriented in opposite directions with respect to each other in what is called an antiparallel fashion. The nitrogenous bases A, T, C, and G are positioned on the interior part of each strand. The two strands of DNA are complementary in sequence: Adenine on one strand always pairs with thymine on the other strand,

and cytosine on one strand always pairs with guanine on the other strand (Figure 6.4).

Within cells, DNA is arranged into tightly coiled structures known as chromosomes. Most prokaryotes have a single circular chromosome; most eukaryotes have multiple linear chromosomes. Haploid organisms have a single copy of each chromosome. Diploid organisms have two copies of each chromosome; humans, for example, have 23 pairs of chromosomes. Eukaryotic cells contain a nucleus and organelles, which are smaller units within the cell. Bound by a phospholipid membrane, organelles perform specific functions, such as generating energy for the cell. Some organelles, including mitochondria and chloroplasts, have their own haploid genomes, which are typically made up of a single chromosome with a circular structure.

From DNA to Proteins

For natural selection to operate, the genetic information encoded in DNA must produce an effect on an organism's phenotype—its observable physical, developmental, and behavioral characteristics. This is a complicated process, and we are still uncovering many of the finer details. The basic process of going from DNA to the phenotype is as follows: The double strands of DNA are "unzipped" when the hydrogen bonds that keep the strands wound around one another are broken. When the sections of DNA are unwound, portions are copied into RNA by the process of **transcription**.

Transcription occurs when a complementary and antiparallel strand of RNA is synthesized from a strand of DNA (Figure 6.5). RNA is a nucleotide polymer similar to DNA, but it is single-stranded, and it uses a nucleotide called uracil (U) in the place of thymine. To determine which portions of the DNA are to be transcribed and when, an enzyme called RNA polymerase binds to a **promoter**—a short DNA sequence before the transcribed part of the gene—and this serves as a signal to begin transcription.

Once RNA polymerase is bound to the promoter, the enzyme unwinds the double helix, separating the two strands of DNA. One of the separated DNA strands—called the template strand—is then used to synthesize a complementary RNA strand, with DNA nucleotides binding to RNA nucleotides (T in DNA binds with A in RNA, G in DNA binds with C in RNA, C in DNA binds with G in RNA, and A in DNA binds with U in RNA). The nucleotides compose a sequence of bases that encodes genetic information. Changes to this nucleotide sequence may have effects on the synthesis of proteins, which ultimately may affect the organism's phenotype. We discuss this in more depth in a moment.

The RNA that is synthesized during transcription has numerous functions. Some types of transcribed RNA act directly without being translated into proteins. These include *ribosomal RNA* (rRNA), which is a key component of the ribosomes that guide the process of protein production, making the covalent bonds that link amino acids together to form proteins; *transfer RNA* (tRNA), which is used to transport amino acids to ribosomes and to recognize and associate

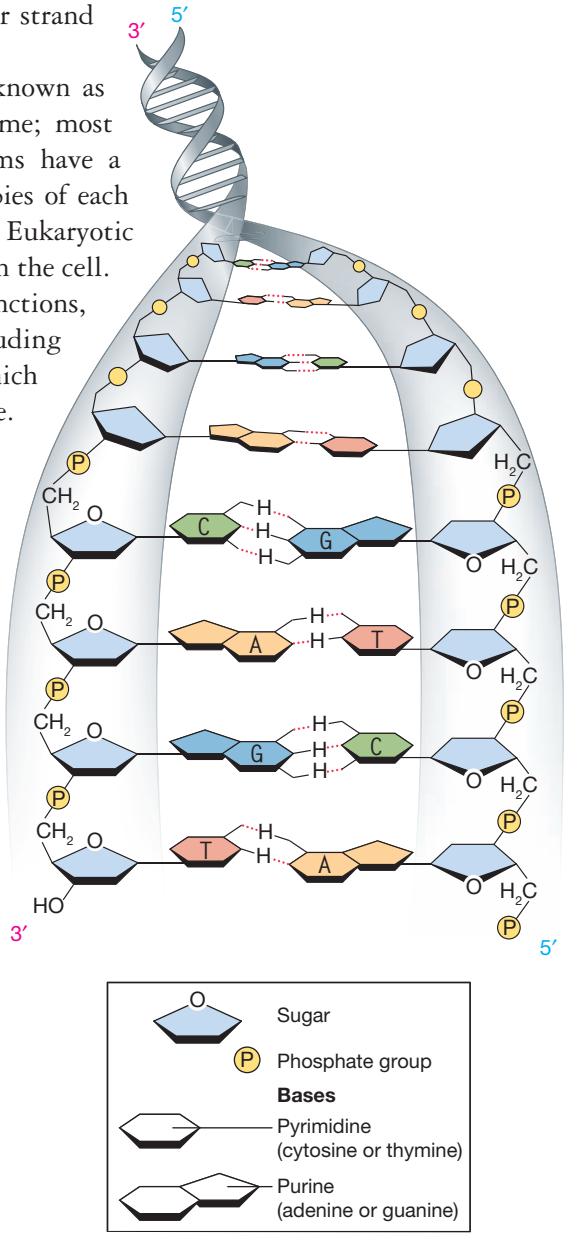


FIGURE 6.4 The chemical structure of DNA. DNA is a double-stranded molecule held in place with hydrogen bonds, denoted here by red dotted lines. The two strands are wound together so that they are oriented in opposite directions. The nitrogenous bases (A, T, C, and G) are positioned on the interior part of each strand. This figure is increasingly magnified as you move downward. Adapted from Slonczewski and Foster (2011).

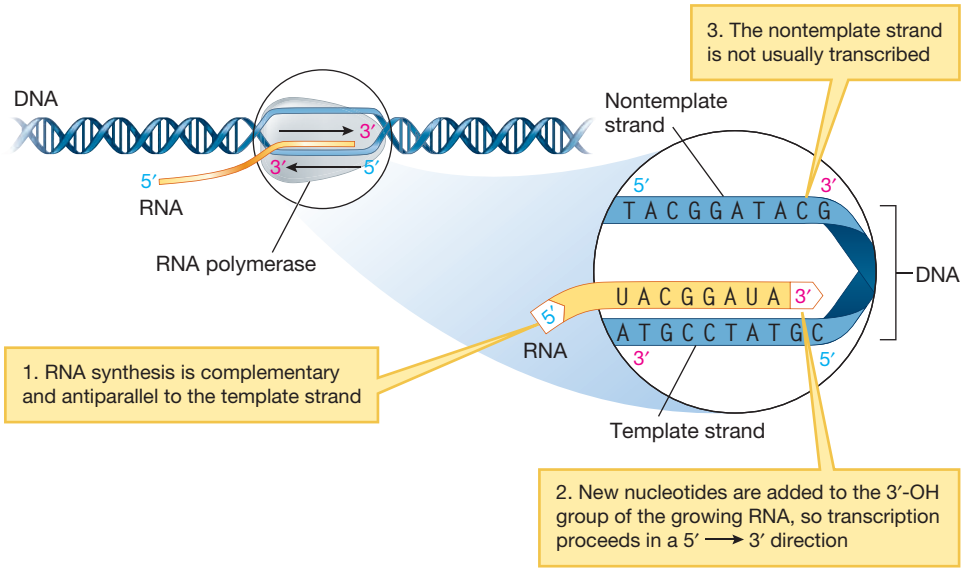


FIGURE 6.5 The process of transcription. When RNA polymerase binds to a promoter, double-stranded DNA is unwound, allowing the polymerase to access that segment of DNA and to synthesize a complementary RNA molecule. The polymerase shown here is moving to the right. Adapted from Pierce (2010).

genetic information with the appropriate amino acids; and *microRNA,* short RNA molecules that play a number of roles in gene regulation (that is, when genes are "switched" on or off).

But perhaps the most important role of RNA is as the template in the **translation** process, where a nucleotide sequence of *messenger RNA* (mRNA) specifies the sequence in which **amino acids** are linked together to form proteins (**Figure 6.6**). There are 20 different amino acids specified by nucleotide triplets in mRNA; these three-base sequences are called **codons**. Collectively, this specification is known as the **genetic code** (**Figure 6.7**). The process of translation begins when a ribosome attaches to the mRNA strand and, moving in the 3′ direction, reaches a start codon (AUG, which codes for the amino acid methionine). At this point, the ribosome facilitates the pairing of an appropriate tRNA molecule with each successive codon. The amino acids associated with each codon are linked

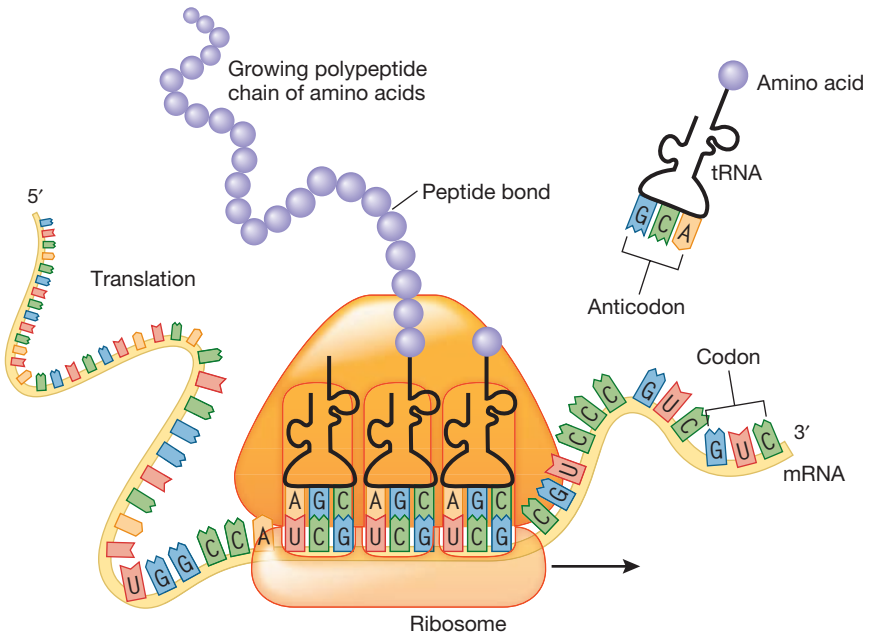


FIGURE 6.6 The process of translation. During the process of translation, the ribosome moves in the 3′ direction along an mRNA strand. Successive tRNA molecules, matching successive codons on the mRNA strand, dock with the ribosome. The amino acids carried by these tRNA molecules link together to form a growing amino acid chain, which will subsequently fold into an active protein. Adapted from National Human Genome Research Institute.

in a growing chain by an enzyme known as peptidyl transferase. When the ribosome reaches a stop codon (UAA, UAG, or UGA), it dissociates from the mRNA. The amino acid chain is released and folds into an active protein, possibly with some additional changes known as *post-translational modification*.

As illustrated in Figure 6.7, most amino acids can be encoded by more than one nucleotide triplet; for this reason, we say that the genetic code is redundant, or *degenerate*. Given the redundancy of coding for amino acids, many nucleotide changes at the third position of a codon do not change the amino acid that is specified by the codon.

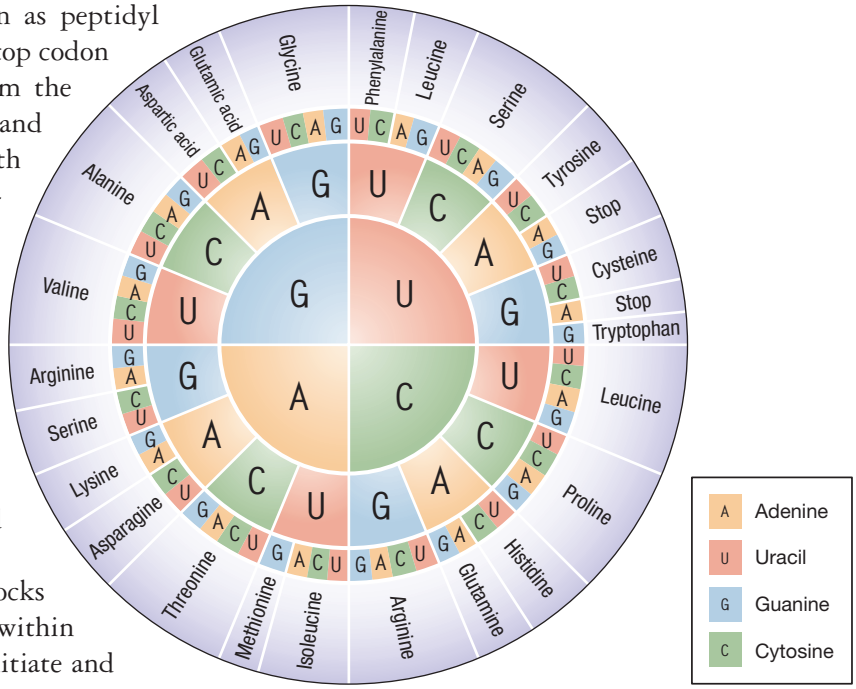


FIGURE 6.7 The genetic code. The genetic code specifies the relation between codon triplets and the amino acids for which they code. To read this figure, begin at the inside of the circle and move out, reading off three nucleotides followed by the amino acid or stop codon that they specify. For example, CCU specifies the amino acid proline, whereas UAG specifies a stop codon.

Proteins are the essential building blocks of life and serve many different functions within cells. Some proteins act as enzymes that initiate and regulate chemical reactions, while other proteins serve as chemical signals that are used in communication within and between cells. Some proteins bind to DNA and help to regulate when and how DNA is expressed; others serve structural functions, forming the cytoskeleton or elements of the extracellular matrix. Still other proteins transport materials within and between cells. All of these processes are critical for virtually every stage of development for most life-forms. Producing the wrong protein may affect when a signal occurs for DNA to be expressed or turned off or it may affect the kind of structure that is made, and hence have significant effects on fitness.

While there are many definitions of a **gene**, most reflect the notion that a gene is a sequence of DNA that specifies a functional product. This product is most often a protein, but it can also be rRNA or tRNA. In eukaryotes, protein-coding genes are typically composed of **exons** (stretches of DNA that code for protein products) interspersed with **introns** (stretches of DNA that do not normally encode proteins) (**Figure 6.8**). After transcription of a primary RNA strand, the introns are spliced—that is, they are cut out, typically by an RNA–protein complex called the spliceosome—and the remaining exons are linked together. The product of this splicing is an mRNA strand, which is then translated into a chain of amino acids. A single gene can be and often will be spliced in different ways: Many human genes encode multiple different proteins that are produced by this process of alternative splicing.

Alleles and Genotypes

As noted earlier in the chapter, different variants of the same gene are known as **alleles**, and the physical location of a gene on a chromosome is known as a locus. The combination of alleles that an individual has at a given locus is known as its **genotype** at that locus (sometimes the term *genotype* may instead refer to the combination of alleles that an individual has at *all* loci).

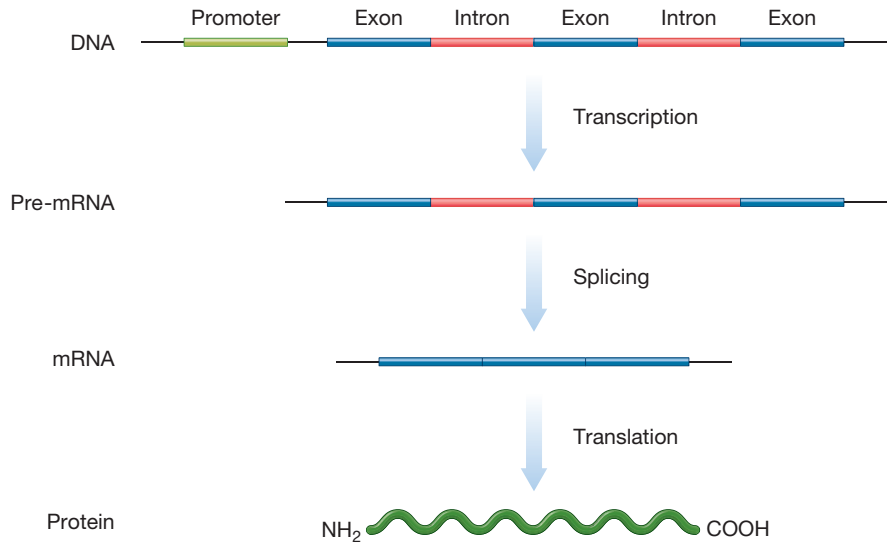


FIGURE 6.8 The processes of transcription, RNA splicing, and translation in eukaryotes. A gene is first transcribed in its entirety, including both the coding exons and the noncoding introns. The introns are subsequently excised during RNA splicing, and the remaining exons are linked together to form a mature mRNA. This mRNA is in turn translated to produce a protein.

In diploid species, individuals with two copies of the same allele at a locus are called **homozygotes** (for that locus), and those with two different alleles at a locus are referred to as **heterozygotes**. If an allele generates the same phenotype when present in a heterozygote as it does when present in homozygous form, we say it is dominant (as in the dominant alleles for purple flowers in Mendel's peas). If an allele does not generate its corresponding phenotype unless it is homozygous, we say that allele is recessive (as in the recessive alleles for white flowers in Mendel's peas). If two alleles generate a heterozygote phenotype that is intermediate between the homozygotes for each allele, the alleles are said to exhibit **incomplete dominance** (Figure 6.9).

KEYCONCEPT QUESTION

6.2 In the case of incomplete dominance, the heterozygote phenotype is intermediate between the homozygote phenotypes. How is this different from the idea of blending inheritance?

At the turn of the twentieth century, British geneticist Reginald Punnett (1875–1967) devised the Punnett square, an elegant but simple diagram that can be used to predict the results of genetic crosses between two individuals (Figure 6.10). Along the top row and down the left column, a Punnett square shows the alleles that are present in the gametes produced by each parent. In the main part of the Punnett square, each possible offspring genotype is represented.

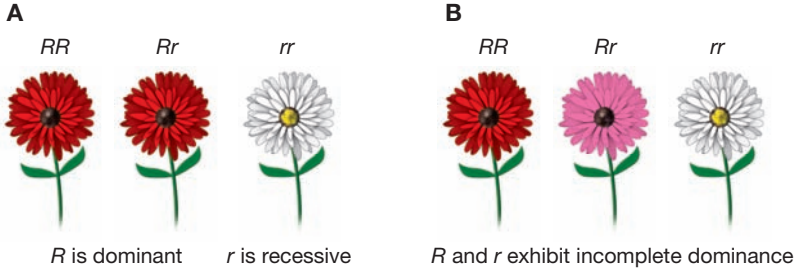


FIGURE 6.9 Dominance relationships. (A) If the *R* allele is dominant and the *r* allele is recessive, then the *RR* homozygote and the *Rr* heterozygote reveal the same phenotype. **(B)** If the *R* and *r* alleles exhibit incomplete dominance, then the *Rr* heterozygote manifests a phenotype that is intermediate between that of the *RR* homozygote and the *rr* homozygote.

Regulatory Elements

Stretches of DNA called **regulatory elements** influence the rate at which RNA molecules are transcribed from DNA, thereby affecting levels of gene expression and, ultimately, the organism's phenotype. For example, regulatory elements affect the color of the body and wings of fruit flies, and these color patterns are critical in the context of the evolution of morphology and sexual behavior. This process is known as transcriptional regulation. Regulatory elements that increase the rate of transcription are called *enhancers*, and those that decrease the rate of transcription are known as *silencers*.

When regulatory elements affect genes at nearby sites on the same chromosome, they are called ***cis* regulatory elements**. By contrast, ***trans* regulatory elements** modify the expression or activity of genes on a different chromosome. *Trans* regulatory elements often do so by encoding soluble proteins that can act at remote locations on DNA.

Epigenetic Inheritance

One of the most important developments in the study of genetics over the past several decades is the growing appreciation of heritable mechanisms that alter gene expression without changes in DNA sequence. These are collectively known as mechanisms of **epigenetic inheritance**. Epigenetic mechanisms may be heritable across mitosis from one cell generation to the next or even across meiosis from one organismal generation to the next.

Gene expression in eukaryotes is strongly influenced by the local structure of the chromosome. The chromosome is not made up of free DNA but rather is structured as **chromatin**: DNA wound around proteins called **histones**. Where the chromatin is condensed—packed tightly—a gene's promoter is inaccessible to RNA polymerase, and thus the gene is unexpressed. Where the chromatin is decondensed, RNA polymerase can bind to the promoter, and the gene can be transcribed (**Figure 6.11**).

Tt × *tt* cross

	t	*t*
T	*Tt* Tall	*Tt* Tall
t	*tt* Short	*tt* Short

FIGURE 6.10 A Punnett square. When Mendel examined the genetics for the height of his pea plants, he found that the allele for tall plants (*T*) was dominant and the allele for short plants (*t*) was recessive. A Punnett square allows us to predict the proportion of tall and short plants, given a set of parental genotypes. Here, we cross a heterozygous tall individual with a recessive homozygous short individual. To predict genotype proportion in their offspring, the law of segregation allows us simply to fill in the four boxes with the corresponding alleles expected in possible gametes of the parents. Our prediction in this example is a 1 : 1 ratio of short to tall plants. Adapted from Pierce (2010).

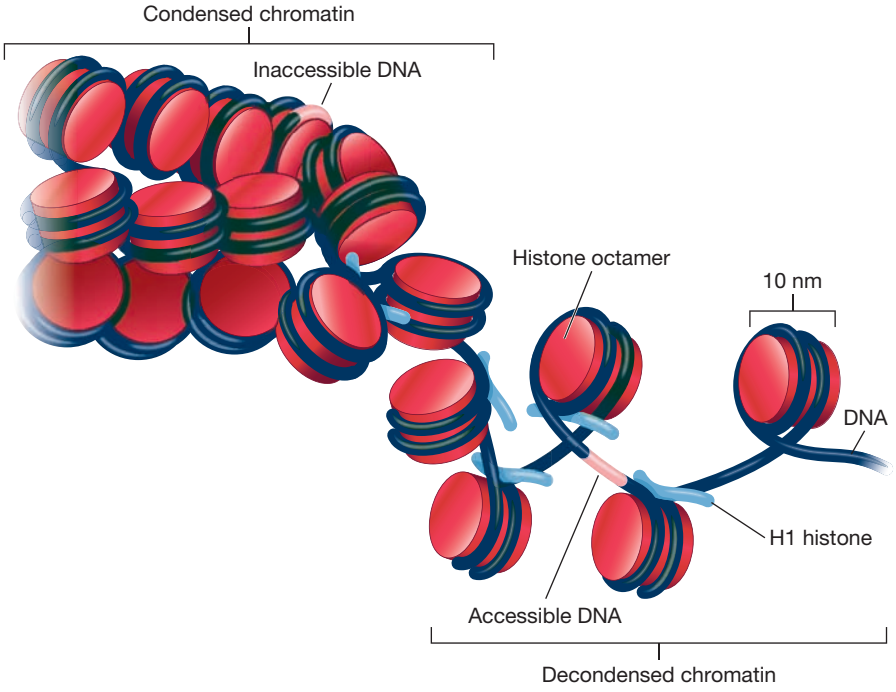


FIGURE 6.11 Chromatin structure influences gene expression. RNA polymerase cannot readily access promoters in the condensed chromatin. Therefore, the chromatin must be decondensed before DNA in a given region can be expressed.

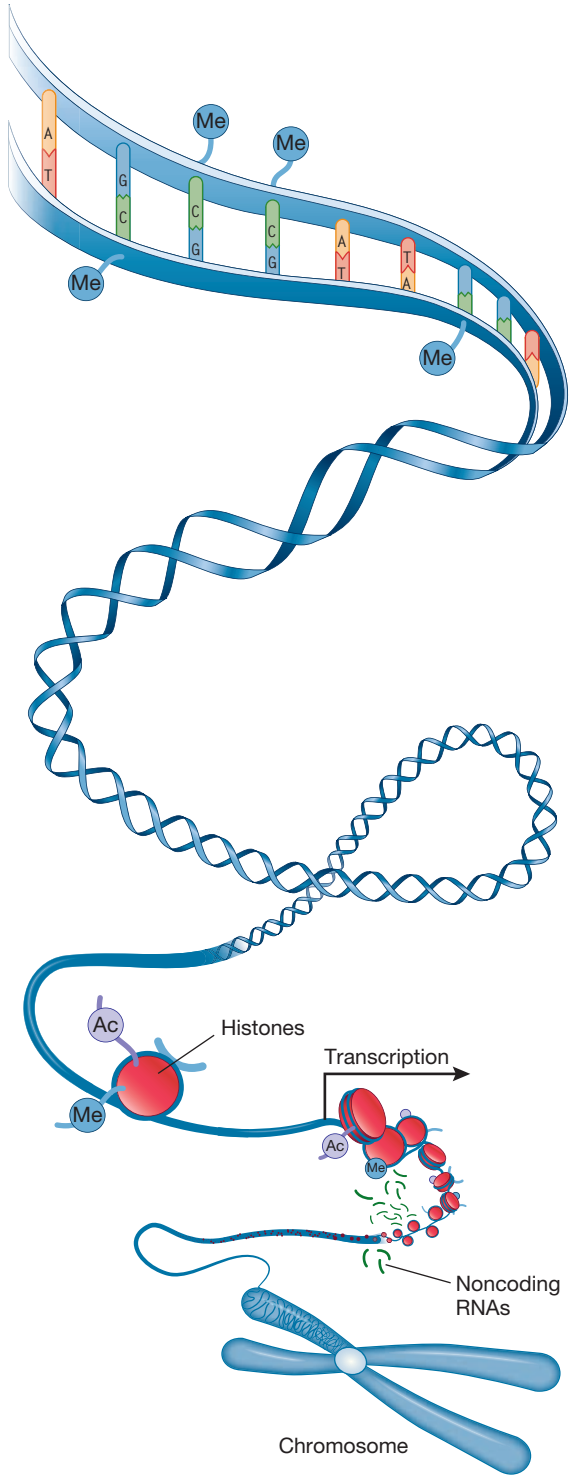


FIGURE 6.12 Mechanisms of DNA methylation. Gene expression is influenced by heritable secondary modifications to the DNA and associated proteins. These include methylation of C–G dinucleotide pairs, modifications to the histones including the additions of acyl groups (Ac) and methyl groups (Me), and the presence of noncoding RNAs. Adapted from Jones et al. (2008).

Many epigenetic mechanisms involve secondary modifications to the DNA molecule or to the histones that affect chromatin structure (**Figure 6.12**). One of the most important of these is DNA methylation, the addition of a methyl group to a C–G base pair in DNA. Methylation affects the ability of transcription factors to bind to DNA, and methylated regions interact with proteins that determine chromatin structure. Highly methylated regions tend to be inaccessible to RNA polymerase and thus are unexpressed. Another form of epigenetic modification involves changes to the histones themselves; for example, when histones are modified by adding an acyl group, the chromatin in that region is often decondensed.

Epigenetic changes play important roles in organismal development. One role involves *cell differentiation*: The DNA in all the cells of a complex multicellular organism's body is the same, but its cell types differ because of differences in gene expression. Many of these differences are driven by epigenetic differences—methylation patterns and histone modifications—inherited across cell generations in the developing organism. Epigenetic mechanisms also play a role in *X chromosome inactivation*: In XX mammalian females, one of the X chromosomes is inactivated by methylating the histone proteins. This ensures that genes on the X chromosome are expressed at the same rate in females with two X chromosomes as they are in males with one X chromosome. Like the epigenetic markers involved in cell type differentiation, X chromosome inactivation is heritable along cell lines. Daughter cells inactivate the same X chromosome that was inactivated in the parent cell. In addition, epigenetic mechanisms play a role in genomic imprinting, which we will discuss in detail in Chapter 17: In mammals, some genes are differentially methylated according to whether they are inherited from the mother or from the father. In this way, one parental copy may be highly expressed while the other is mostly or entirely suppressed.

Epigenetic modifications during prenatal development or early in life are responsible for aspects of *developmental plasticity*—adjustment of the phenotype to suit the environment. In humans for example, a mother's diet during gestation influences her offspring's risk of metabolic diseases, such as type 2 diabetes, later in life. Growing evidence links this pattern to early-life epigenetic changes that affect gene expression at loci associated with these diseases (Kuzawa et al. 2008; Gluckman et al. 2009). Most epigenetic changes to the genome are reset each generation, but some epigenetic information can be passed across generations. The mechanisms are not well understood, but they probably involve transmission of small RNA molecules rather than DNA methylation and chromosome modification (Daxinger and Whitelaw 2012).

6.3 Variation and Mutation

As we discussed in Chapter 3, natural selection requires genetic variation to operate. New genetic variation—in the form of new alleles or new allelic combinations—enters a population from one of four sources: mutation, recombination, migration, or lateral gene transfer (we discuss how sexual reproduction creates new genetic variation at the level of the *genotype* in Chapter 16). In the cases of mutation and recombination, which we will discuss here, new variation arises within a population. In the cases of migration and lateral gene transfer, new variation enters the population from outside. In Chapter 7, we will discuss migration. In Chapters 10 and 11, we will explore the process and evolutionary importance of lateral gene transfer, in which new gene clusters are transferred among members of the same species or even across species boundaries.

Genetic Variability and Mutation

Mutation, defined as a change to the DNA sequence of the organism, is the primary source of all genetic variation. In species such as humans that have a well-defined separation between germ-line cells (sex cells) and somatic cells (body cells), it matters a great deal where mutations occur. When a mutation occurs in a somatic cell, it can have fitness consequences for the individual—for example, most cancers result from somatic mutations—but the mutation itself will not be transmitted to the next generation. Thus, somatic mutations do not generate the type of heritable variation required for evolution by natural selection at the level of the organism. When a mutation occurs in the germ line, however, it can be transmitted to the next generation: It is these germ-line mutations that provide the underlying variation on which natural selection operates.

Mutations include many different kinds of changes to DNA. The most basic form of mutation is a single base change, in which one base is altered; for example, from a cytosine to a thymine or from a guanine to an adenine. When a purine (adenine or guanine) is replaced by a purine or when a pyrimidine (cytosine or thymine) is replaced by a pyrimidine, we call it a **transition**. When a purine replaces a pyrimidine or vice versa, we call it a **transversion** (Figure 6.13).

Figure 6.13 shows that there are twice as many ways to change a base by a transversion as by a transition. Thus, if all mutations were equally likely, we would see twice as many transversions as transitions. However, changes from one base pair to another do not all occur at the same rate; in fact, transitions generally occur more frequently than transversions. Most species, including humans, exhibit roughly twice as many transitions as transversions (Gojobori et al. 1982; Zhang and Gerstein 2003).

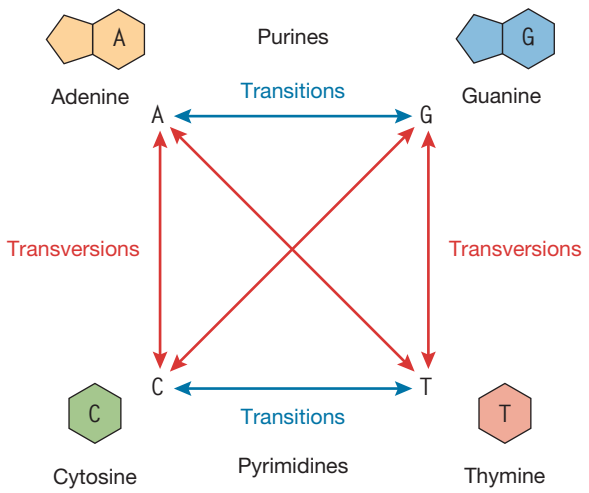


FIGURE 6.13 Transitions and transversions. A transition occurs when a purine is replaced by another purine or a pyrimidine is replaced by another pyrimidine. A transversion occurs when a purine replaces a pyrimidine or vice versa. In most organisms, transitions occur at about twice the rate of transversions.

We can also categorize base changes by their effects on the resulting amino acid sequence. If a base change does not alter the amino acid that a codon specifies, it is known as a **synonymous mutation**, also called a *silent mutation*. (We will discuss synonymous mutations in much more detail in Chapter 8.)

If the base change specifies the production of a different amino acid, it is known as a **nonsynonymous mutation**. For example, a nonsynonymous mutation in

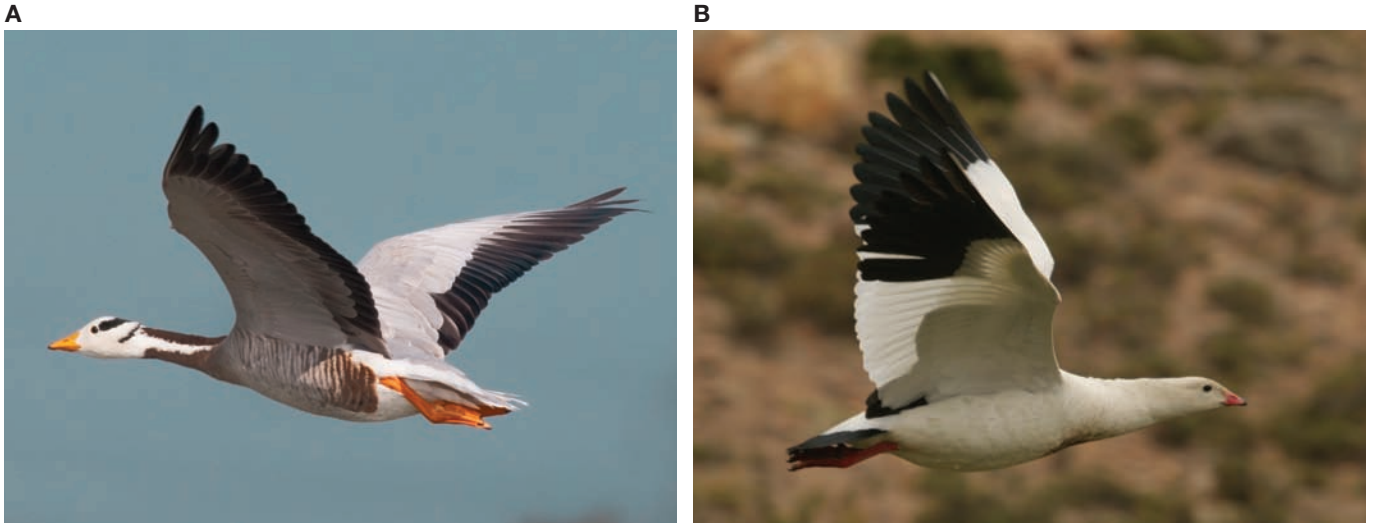


FIGURE 6.14 Convergent evolution in high-flying geese.
Nonsynonymous substitutions in two different species of goose improve oxygen binding and thus allow better physiological performance at high altitude. **(A)** The bar-headed goose (*Anser indicus*) migrates across the Himalayas. **(B)** The Andean goose (*Chloephaga melanoptera*) inhabits the Andes.

mice has been shown to lead to a degeneration of the neural pathways associated with locomotion (Martin et al. 2002). Sometimes, by chance, a nonsynonymous mutation can prove beneficial. For example, twice a year the bar-headed goose (*Anser indicus*) migrates across the Himalayas, flying at altitudes where the oxygen pressure is very low. In these geese, a nonsynonymous mutation leading to the substitution of the amino acid proline by the amino acid leucine allows these birds to better bind oxygen during their migrations, and so this mutation has been favored over evolutionary time by natural selection. A similar scenario has been documented in the Andean goose (*Chloephaga melanoptera*), which spends long periods of time in the low-oxygen environment of the Andes (**Figure 6.14**). In the case of the Andean goose, the mutation allowing the birds to bind oxygen better involved a change from the amino acid leucine to serine, a change that was subsequently favored by natural selection (Jessen et al. 1991; Weber et al. 1993; McCracken et al. 2010).

KEYCONCEPT QUESTION

6.3 Even though bar-headed geese and Andean geese are both species of goose, why should we nevertheless view their increased ability to bind oxygen as a case of convergent evolution?

If a base substitution creates a stop codon where there was not one previously, it is known as a **nonsense mutation**. For example, a nonsense mutation in a protein kinase involved in signal transduction interferes with growth and development of a number of mammalian species including cattle, leading to dwarfism (Koltes et al. 2009). Synonymous, nonsynonymous, and nonsense mutations are summarized in **Figure 6.15**.

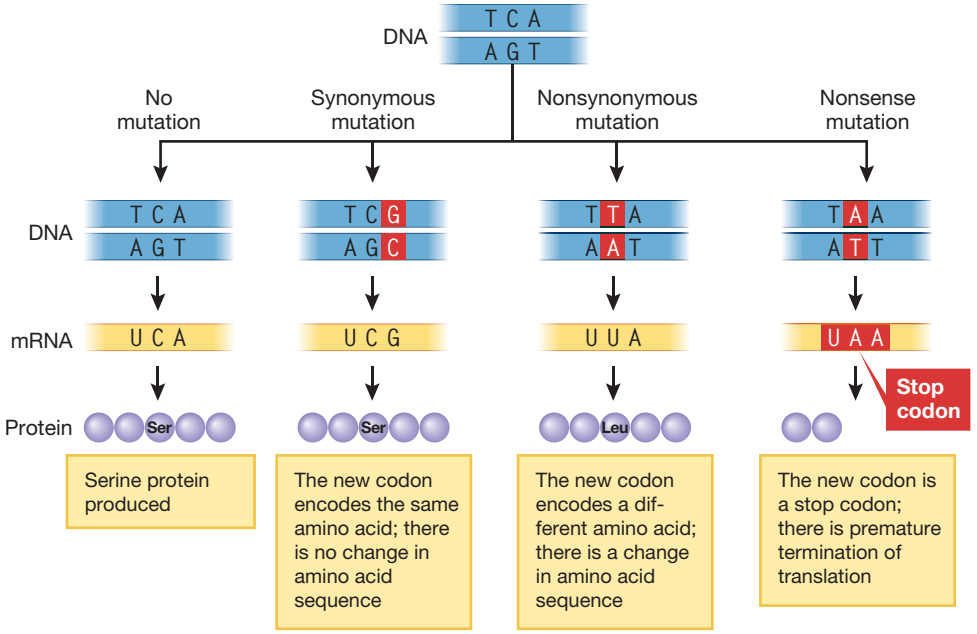


FIGURE 6.15 Synonymous, nonsynonymous, and nonsense mutations. The original DNA sequence is TCA, coding for the amino acid serine. If the A is converted to a G, we have a synonymous mutation: The new sequence TCG still codes for serine. If the C is converted to a T, this generates a nonsynonymous mutation: The new sequence TTA produces the codon UUA in mRNA, which codes for the amino acid leucine. If the C is converted to an A, we have a nonsense mutation: A stop codon UAA is created, terminating the protein. Adapted from Pierce (2010).

Not all mutations involve the substitution of one nucleotide for another. An *insertion mutation* involves the addition of one or more nucleotides to a sequence, while a *deletion mutation* occurs when one or more nucleotides are deleted from a sequence. Because codons are made up of three nucleotides, when an insertion or deletion mutation involves a multiple of three nucleotides, it does not disrupt the *reading frame*—the way in which adjacent base pairs are grouped into triplets and translated into amino acids. On either side of the mutation, the base pair triplets remain grouped as before. Such insertions and deletions are known as in-frame mutations. If an insertion or deletion does not occur in a multiple of three nucleotides, however, it produces a **frameshift mutation**, which affects the translation of other codons and, therefore, the production of amino acids and proteins (**Figure 6.16**). For example, at least eight frameshift mutations are associated with Tay–Sachs disease in descendants of European Jewish populations (Myerowitz 1997).

Mutations can also occur at the whole-gene or chromosome level. Gene duplications involve the duplication of regions of DNA that contain entire genes. For example, a gene duplication event has been linked to the ability to digest new food types in a primate species called the douc langur (Zhang et al. 2002). We will discuss the evolutionary implications of gene duplication in Chapters 10 and 13.

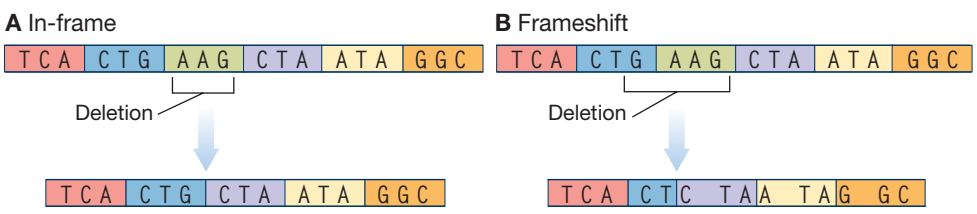


FIGURE 6.16 In-frame and frameshift mutations. **(A)** Insertions or deletions of three nucleotides, or multiples of three nucleotides, do not shift the reading frame. **(B)** An insertion or deletion of any other length generates a frameshift mutation.

Chromosomal rearrangements are large-scale mutations at the level of the chromosome. A **chromosomal duplication** occurs when a section of a chromosome is duplicated. A **chromosomal deletion** entails the loss of a large section of a chromosome. Another form of chromosomal rearrangement is an **inversion**, which involves a 180° flip in a section of a chromosome. A **translocation** is a mutation in which a section of one chromosome moves to another chromosome. Chromosomes can also break apart into stable new configurations (chromosomal fission) or fuse together to create new chromosomes (chromosomal fusion). Chromosomal duplications, deletions, inversions, and translocations are depicted in **Figure 6.17**.

On a still larger scale, errors in the process of meiosis can result in a change in ploidy—the addition or loss of an entire *set* of chromosomes. Changes in ploidy in animals are typically fatal, in that they disrupt the normal developmental process. But this is not always the case. For example, related species of some frogs differ primarily in the fact that some species are diploid, with two copies of each chromosome, while others are tetraploid, with four copies of each chromosome (Holloway et al. 2006). And for reasons that we do not completely understand, changes in ploidy are *often* maintained in plant populations. For instance, many crops that humans rely on as food sources are species that have changed ploidy in the past (**Figure 6.18**).

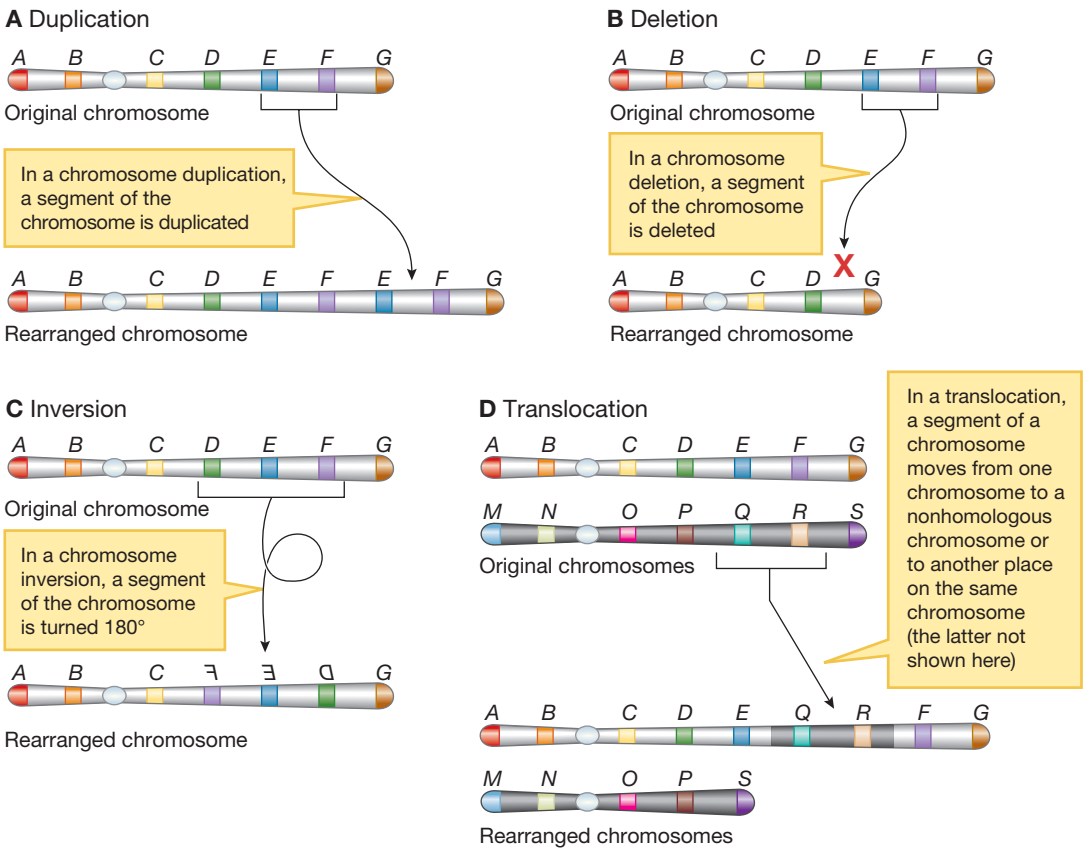


FIGURE 6.17 Chromosomal duplications, deletions, inversions, and translocations. In a duplication **(A)**, a second copy of a gene region, here the *E* and *F* loci, is inserted into the chromosome. In a deletion **(B)**, a region is excised from the chromosome. In an inversion **(C)**, the direction of a chromosomal region is inverted. In a translocation **(D)**, a section of one chromosome is moved to a different chromosome. Adapted from Pierce (2010).

KEYCONCEPT QUESTION

6.4 Sally Otto and Jeannette Whitton (2000) looked at the haploid number of chromosomes in a large sample of fern species. They found that 1092 species had an even haploid number of chromosomes, while only 637 species had an odd haploid number. Propose a hypothesis for why fern species have even haploid numbers more often than odd ones.

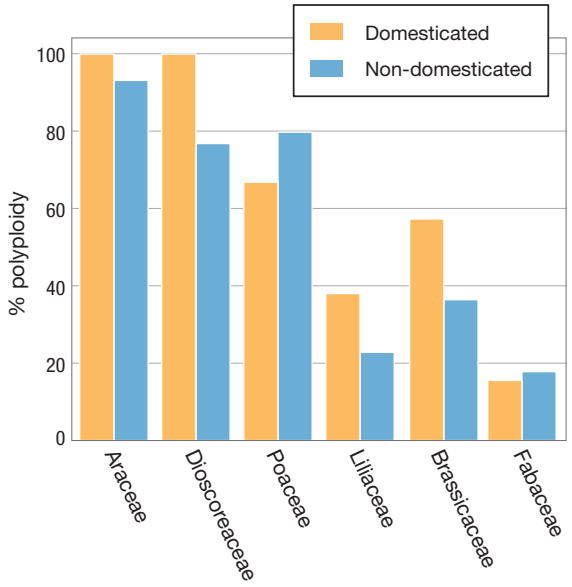


FIGURE 6.18 Polyploidy in crop plants. For five of six plant families with numerous domesticated (crop) members, the incidence of polyploidy is higher among domesticated species than among non-domesticated species. Adapted from Hilu (1993).

Genetic Variability and Recombination

In sexually reproducing organisms, another source of genetic variation is available. Recombination remixes existing variation, present in parents, into new genetic combinations that appear in their offspring.

In most diploid eukaryotic organisms, each cell has a fixed number of chromosomes. With the exception of sex chromosomes, these chromosomes typically come in homologous pairs, so called because two homologous chromosomes each consist of the same loci (although they often carry different alleles at some of those loci). One copy of a homologous pair of chromosomes in an individual comes from each parent via the gametes, haploid sex cells that have one set of chromosomes. In animals, these gametes are the egg from the mother and the sperm from the father.

The gametes are produced through the process of **meiosis**. This process begins with a single diploid cell. One round of DNA replication, followed by two rounds of cell division, produces the four haploid gametes. Later, when fertilization occurs—that is, when two individuals mate and their gametes fuse in a process called *syngamy*—diploidy is restored. The offspring produced have a full complement of pairs of homologous chromosomes, with one chromosome in each pair coming from each parent.

Sexually reproducing organisms generate huge amounts of genetic variability among their offspring through a type of recombination called **crossing-over**—the physical exchange of segments of DNA on homologous chromosomes. Crossing-over occurs during meiosis, after the chromosomes have duplicated, when sections of one homologous chromosome may swap positions with corresponding sections on the other homologous chromosome (**Figure 6.19**). Because of crossing-over, the chromosomes in each gamete may differ from the chromosomes in the original parental cell.

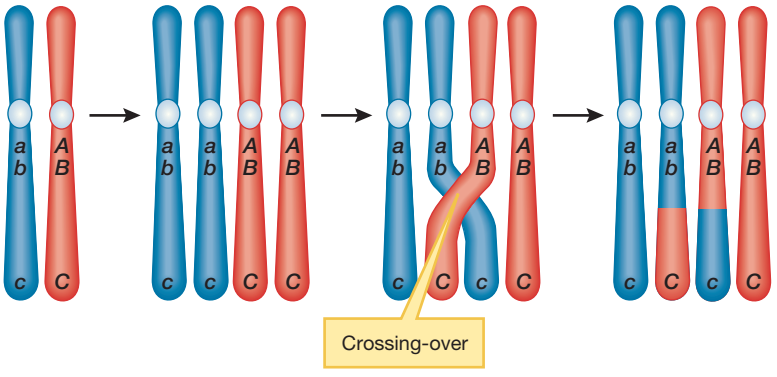


FIGURE 6.19 Crossing-over and recombination during meiosis. Here we have three genes (*A*, *B*, *C*), each with two alleles (*A*, *a*; *B*, *b*; *C*, *c*). Crossing-over occurs between one of the red *ABC chromatids* (that is, chromosome copies) and one of the blue *abc* chromatids at a location between the *B* locus and the *C* locus. As a result, four different daughter chromatids are produced: *abc*, *abC*, *ABc*, and *ABC*. Thus, recombination generates new allele combinations not present on the original chromosomes. Adapted from MacAndrew (2003).

6.4 Mutation Rates and Fitness Consequences

From an evolutionary perspective, perhaps the most important way to categorize mutations is in terms of their effect on fitness. With respect to changes in relative fitness, mutations can be beneficial, deleterious, or neutral. One common sort of neutral mutation would be the synonymous mutation we discussed earlier in the chapter; we will discuss neutral mutations in greater depth in Chapter 8.

Before we discuss the frequency and distribution of different types of mutations, it is important to understand one of the most basic principles in evolutionary genetics, which is that mutations are *undirected*. In other words, mutations are generated *at random* with respect to their effects on fitness. There are no known mechanisms that preferentially generate mutations with a positive effect on fitness or that avoid generating mutations with a negative effect on fitness. For example, imagine a population of dark mice introduced into a beach environment, as in the mouse example we discussed in Chapter 3. Lighter coat color would make it more likely for a mouse to survive and reproduce in its new environment. When it comes to mutations that affect coat color, however, there is no way for mice preferentially to produce mutations that result in a lighter coat color or to avoid mutations that result in a yet darker coat color. Thus, natural selection operates as a two-stage process: the *random* generation of variation, followed by the *differential* replication of certain variants.

The random nature of mutation was established through one of the most elegant experiments in the history of biology. In 1943, before geneticists knew for certain that DNA was the hereditary material, Salvador Luria and Max Delbrück wanted to understand the nature of the mutation process (Luria and Delbrück 1943). Evolutionary biologists had proposed that mutations occurred at random, independent of whether or not they would be favored by natural selection. But was this really correct? Or did the conditions in the environment somehow induce those specific mutations that would be beneficial in that particular environment?

Luria and Delbrück had good reason to wonder. They knew that when a culture containing the bacterium *Escherichia coli* was exposed to a high density of a bacteriophage—a virus that infects *E. coli*—almost all of the *E. coli* cells would be infected and killed. But after some period of time, colonies of *E. coli* that were resistant to the phage would appear.

To explain this observation, Luria and Delbrück formulated two alternative hypotheses:

1. *Hypothesis 1: Random mutation*. Prior to exposure to the phage, a few resistant *E. coli* cells would arise by random mutation. Once exposed to the bacteriophage, most cells would be killed, but the resistant cells would not. These would reproduce and form new resistant colonies.
2. *Hypothesis 2: Acquired inherited resistance*. At the time of exposure to the phage, all *E. coli* cells would be phage-sensitive; that is, all the cells would be sensitive to the damaging effects of the phage. The process of exposure to the phage would induce mutations responsible for phage resistance in a small fraction of the bacterial cells. This resistance would then be heritable, and the cells with induced resistance would go on to produce colonies of resistant cells.

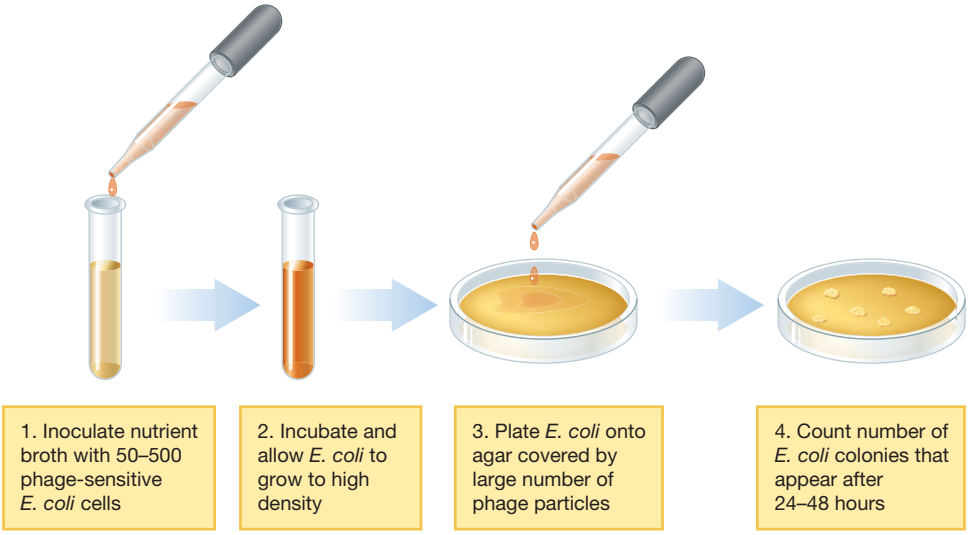


FIGURE 6.20 Luria–Delbrück experiment. To determine the distribution of phage-resistant mutants that arise from a phage-sensitive ancestor, Luria and Delbrück grew *E. coli* bacteria to high density before spreading them onto an agar plate covered with phage particles. Only the phage-resistant *E. coli* strains were able to grow on the plate, so Luria and Delbrück could count the number of resistant mutants by simply counting the colonies.

To distinguish between these two alternatives, Luria and Delbrück devised an ingenious experiment (**Figure 6.20**).

Luria and Delbrück began by inoculating multiple cultures of nutrient broth with 50–500 phage-sensitive bacterial cells each. Next, they incubated the cultures until the bacteria reached high density—approximately 10^8 to 5×10^9 cells/ml. They then took samples of each culture with its high density of bacterial cells, and they spread those samples on agar plates that had already been covered with a high density of phage particles. (Phage-sensitive bacteria grow readily on agar plates but will die if phage particles are present. Phage-resistant bacteria, however, grow readily on agar even in the presence of phage particles.) Luria and Delbrück incubated the agar plates for 24–48 hours, at which point a number of *E. coli* colonies—populated by resistant bacteria—had appeared on each plate. Each colony was composed of the descendants of a single resistant cell. The experimenters then counted the number of colonies present on each plate. From this information alone, they were able to distinguish between the two hypotheses listed above. How?

The key to understanding this experiment is to use phylogenetic reasoning. In any single culture, the large number of cells present at the time that the bacteria are transferred to the agar plate have arisen through a process of successive cell division and are therefore related by a phylogenetic pattern, as illustrated in **Figure 6.21**. Once we start thinking about this phylogeny, we can see that the random mutation hypothesis and the acquired inherited resistance hypothesis make different predictions.

Under the random mutation hypothesis, resistant cells that are present after the phage particles are added must have had their origin in mutations that occurred earlier, during the growth of the bacterial population. If one of these mutations happens to arise early in this growth process, it will become common in the population, giving rise to a large cluster of colonies full of resistant individuals, as illustrated in Figure 6.21A, top left. If, instead, the first resistant mutation arises late in the growth process, it will generate a much smaller cluster of colonies full of resistant individuals (Figure 6.21A, top right). As a result, some cultures will have a large number of resistant cells, and others will have a small number. Thus, the random mutation hypothesis predicts that the experimenters should observe a wide variation in the number of resistant colonies on each plate.

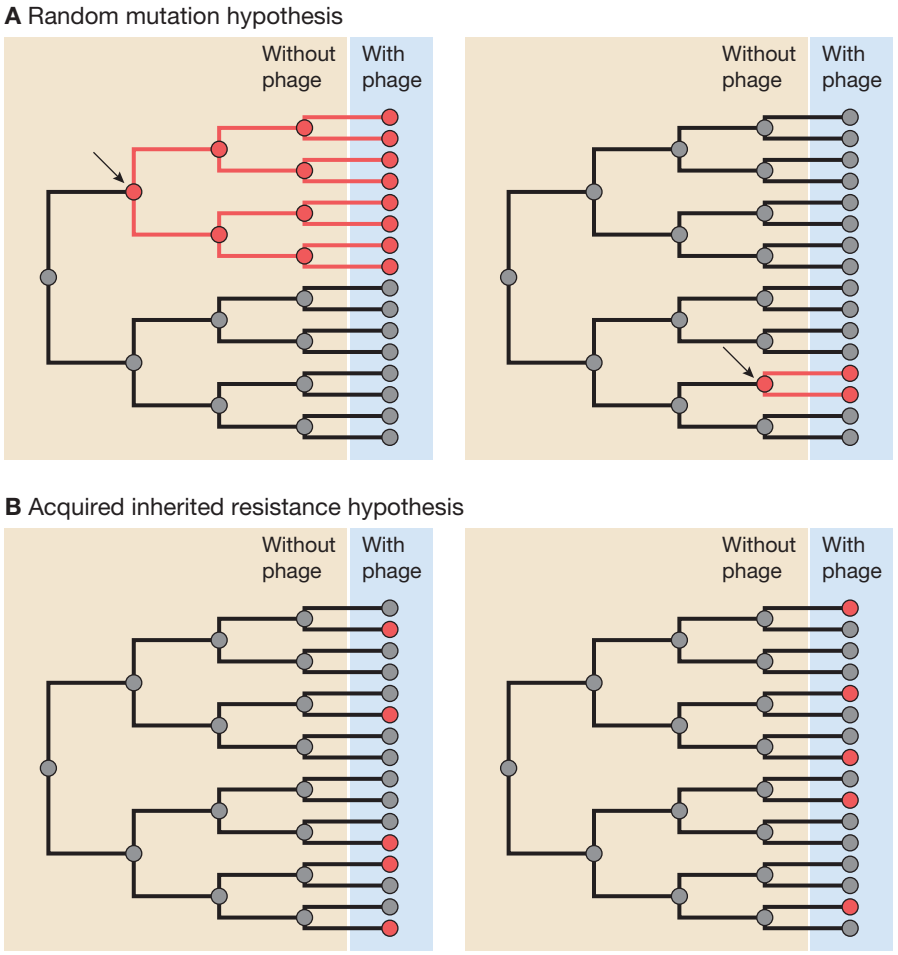


FIGURE 6.21 Random mutation or acquired inherited resistance? The random mutation hypothesis and the acquired inherited resistance hypothesis make different predictions about the distribution of resistant mutants that will be observed on exposure to the phage. **(A)** The random mutation hypothesis predicts that resistant cells arise by random mutation even before the phage is present. In some cultures, a mutation may arise early (arrow), resulting in many resistant cells, as shown in red in the top left panel. In other cultures, a mutation may occur late (arrow), resulting in few resistant cells, as shown in the top right panel. Thus, under the mutation hypothesis, the number of resistant cells fluctuates widely from culture to culture. **(B)** The acquired inherited resistance hypothesis predicts that resistance is only induced by the presence of the phage. Resistance arises independently with some probability in each cell once the phage is present, and its distribution clusters around the average, as shown in the bottom panels.

Under the acquired inherited resistance hypothesis, resistance never arises until the phage particles are added. At that point, each cell acquires resistance, or does not, independently from every other cell. Because there are a relatively large number of cells in each culture and a nontrivial fraction of these acquire resistance, then by the law of large numbers (discussed further in Chapter 8), each culture will have a similar number of resistant cells. Thus, the acquired inherited resistance hypothesis predicts that the experimenters should observe a similar number of resistant colonies on each plate (Figure 6.21B).

More precisely, the acquired inherited resistance hypothesis predicts that the number of colonies on each plate should follow a *Poisson distribution*, with its variance equal to its mean. The random mutation hypothesis predicts that the number of colonies on each plate should follow a different distribution—now known as the *Luria–Delbrück distribution* in honor of this experiment—with its variance much larger than its mean. Luria and Delbrück demonstrated this with a detailed mathematical model.

To distinguish between the two hypotheses, Luria and Delbrück carried out their protocol repeatedly, and they counted the number of resistant colonies that arose from each of a large number of cultures. As predicted by the random mutation hypothesis, they observed a dramatic variation from culture to culture in the number of resistant colonies. From this, they concluded that phage resistance was likely to be a product of random mutations that occurred at different times

prior to the presence of the phage. At least for this trait, mutation worked as evolutionary biologists had predicted: randomly and independently of selection.

For this and other contributions, Luria and Delbrück won the 1969 Nobel Prize in Medicine or Physiology. Since their original experiment, more than half a century of subsequent developments in molecular genetics have revealed that, indeed, randomly generated mutation is the rule throughout biology. But without thinking of the phylogenetic structure of a growing population, Luria and Delbrück could never have designed their beautiful experiment and made such an important leap forward in our understanding of mutation and—consequently—the evolutionary process.

Rates of Mutation

Mutation generates the genetic variation on which natural selection acts. Therefore if we want to understand how selection proceeds at the molecular level, we need to know something about the rate at which mutations occur. Human mutation rates can be estimated in a variety of ways (Kondrashov and Kondrashov 2010), and these estimates generally suggest genome-wide mutation rates in the range of 1×10^{-8} to 3×10^{-8} mutations per nucleotide per generation. Because the human genome is approximately 3.1×10^{9} nucleotides in length and the genome is diploid, this implies that each individual carries somewhere in the range of 60–180 novel mutations per diploid genome. These estimates are in close accord with direct counts of novel mutations obtained by sequencing the whole genomes of parents and their offspring (Roach et al. 2010; Kong et al. 2012).

Mutation rates vary considerably across species. RNA viruses have extremely high mutation rates per nucleotide base in the genome, while DNA viruses have somewhat lower mutation rates. Among cellular organisms, mutation rate per site increases with genome size (**Figure 6.22**) (Baer et al. 2007; Lynch 2010a).

Mutation rates also vary widely in different regions of a single genome (Wolfe et al. 1989; Ellegren et al. 2003), between nuclear and organellar genomes (Lynch et al. 2006), between sexes (Haldane 1947; Bohossian 2000; Taylor et al. 2006),

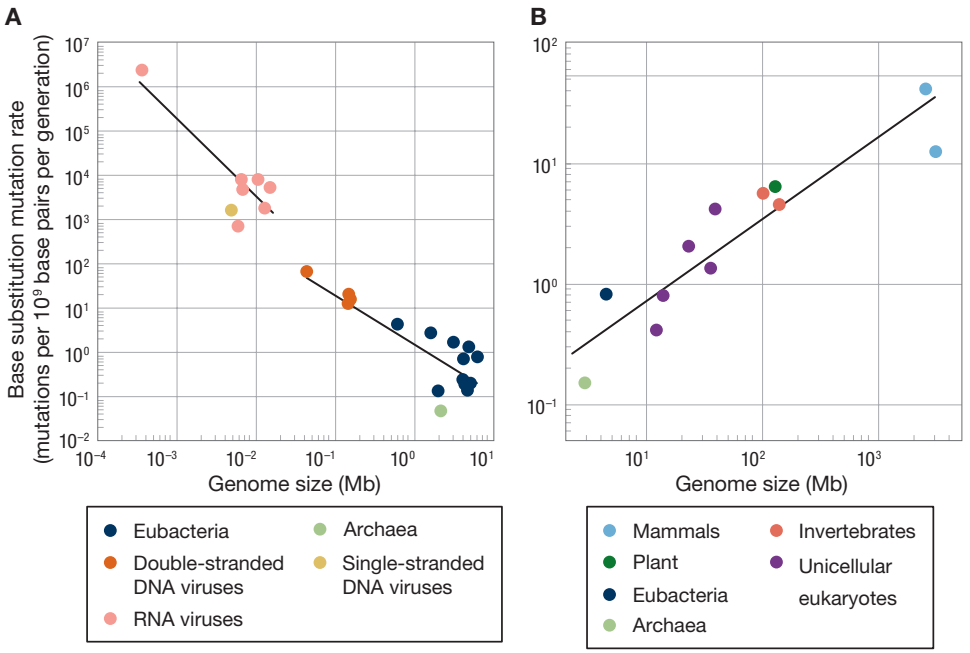


FIGURE 6.22 Mutation rates vary across species. Here, we see the base substitution mutation rate per nucleotide site per generation as a function of genome size in **(A)** non-eukaryotes and **(B)** cellular organisms including cellular microbes. Among microbes, the per-site mutation rate decreases with genome size, whereas among cellular organisms, the per-site mutation rate increases with genome size. bp, base pairs; Mb, megabase. Adapted from Lynch (2010a).

and between families (Conrad et al. 2011). Finally, they vary across tissue types within the same species (Lynch 2010a) (**Table 6.1**).

Distribution of Fitness Effects of Mutation

While beneficial mutations provide the fuel that drives adaptive change, evolutionary biologists have historically focused their studies of mutation primarily on deleterious mutations and on neutral mutations (Loewe and Hill 2010; Orr 2010). The focus on deleterious and neutral mutations is due to the fact that these are more common. Because mutations are generated at random with respect to fitness, and because most traits have been under selection for long periods of time, any single arbitrary genetic change is likely to have a negative effect on fitness or, at best, no effect on fitness.

TABLE 6.1

Mutation Rates per Nucleotide Site ($\times 10^{-9}$) in Different Tissues

Species	Tissue	Approximate Cell Divisions per Generation	ESTIMATED MUTATION RATES	
			per Generation	per Cell Division
Homo sapiens	Germ line	216	12	0.06
	Retina	55	54	0.99
	Intestinal epithelium	600	160	0.27
	Fibroblast (culture)			1.34
	Lymphocytes (culture)			1.47
Mus musculus	Male germ line	39	38	0.97
	Brain		77	
	Colon		83	
	Epidermis		90	
	Intestine		120	
	Liver		240	
	Lung		170	
	Spleen		130	
Rattus norvegicus	Colon		180	
	Kidney		170	
	Liver		180	
	Lung		220	
	Mammary gland		58	
	Prostate		450	
	Spleen		100	
Drosophila melanogaster	Germ line	36	4.6	0.13
	Whole body		380	
Caenorhabditis elegans	Germ line	9	5.6	0.62
Arabidopsis thaliana	Germ line	40	6.5	0.16
Saccharomyces cerevisiae		1	0.33	0.33
Escherichia coli		1	0.26	0.26

Adapted from Lynch (2010a).

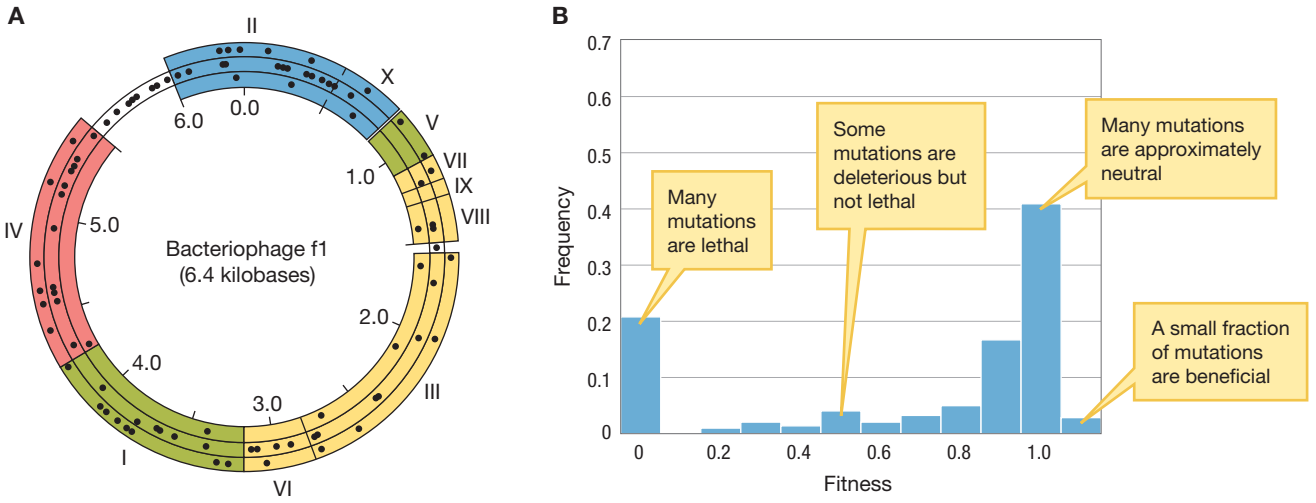


FIGURE 6.23 Distribution of fitness effects in Bacteriophage f1. **(A)** The circular genome of the Bacteriophage f1 virus. Each small black circle indicates where an experimental mutation occurred. Roman numerals indicate genes and colors indicate gene functions: Blue is associated with replication, green with maturation, yellow with capsid production, and red with extrusion. Rings indicate the type of mutation: Synonymous mutations are shown on the outer ring, nonsynonymous mutations on the middle ring, and nonsense mutations on the inner ring. **(B)** The distribution of fitnesses of Bacteriophage f1 mutants, relative to the wild type. Values greater than 1.0 indicate beneficial mutations. Values less than 1.0 indicate deleterious mutations. Mutants with fitness 0 have suffered lethal mutations. Adapted from Peris et al. (2010).

Recently, however, evolutionary biologists have begun a concerted effort to study the full spectrum of mutational effects. In 1996, population geneticist Brian Charlesworth said that if he had a single question that he could ask a fairy godmother, it would be what the relative frequencies of deleterious, beneficial, and neutral mutations were in nature (Charlesworth 1996). Today, Charlesworth's fairy godmother is beginning to deliver. Data on the **distribution of fitness effects**—the relative frequencies of mutations with various fitness consequences—are becoming available for a number of species at scales from a single protein to the entire genome.

Of course, we need to remember that the fitness effects of any individual mutation depend on genetic background and environment, but the hope is that the overall *distribution* of fitness effects for new mutations may be similar regardless of context. One recent study on *Drosophila* found similar distributions of fitness effects on different genetic backgrounds but different distributions under different environmental conditions (Wang et al. 2014).

Evolutionary biologists can estimate the distribution of fitness effects in a number of different ways. Perhaps the most straightforward of these is to create an array of mutants and then directly assess the fitness of each. Joan Peris and her colleagues took this approach using the virus Bacteriophage f1 (Peris et al. 2010). They experimentally produced base substitutions at 100 nucleotide sites (**Figure 6.23A**) and measured the frequency of deleterious, beneficial, and neutral mutations in the virus. As expected given the degeneracy of the genetic code, approximately two-thirds of the mutations caused a change in the amino acid that was specified.

The distribution of fitness effects that Peris and her colleagues observed is illustrated in **Figure 6.23B**. This distribution is much like that seen in a number of other viruses: Most mutations are either neutral or deleterious, though a few are beneficial. Moreover, the distribution is bimodal—it has two peaks. One peak corresponds to neutral or nearly neutral mutations; the other corresponds to lethal mutations that prevent successful replication entirely (Wylie and Shakhnovich 2011). The nearly neutral mutations are those that slightly change protein structure or stability without dramatically affecting folding or function. The lethal ones prevent proper folding—and in small viruses such as these, each protein encoded in the genome is essential for successful replication.

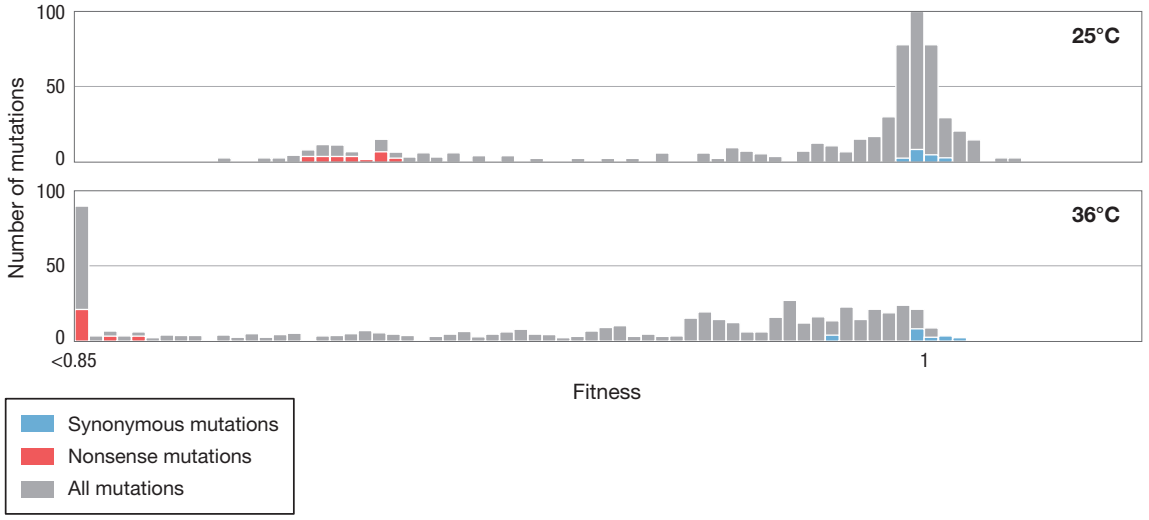


FIGURE 6.24 Distribution of fitness effects for HSP90 in *Saccharomyces cerevisiae*. The distributions of fitnesses, measured as growth rate relative to the wild type, for strains with single mutations in the heat shock protein HSP90 in low-temperature (top) and high-temperature (bottom) environments reveal that most mutations are neutral or deleterious. Both distributions are bimodal, with a lower peak corresponding to protein inactivation and a higher peak corresponding to no change in protein function. Mutations that generate premature stop codons are shown in red; these tend to have strongly deleterious effects. Synonymous mutations are shown in blue; these tend to be neutral or nearly neutral. Because the HSP90 protein facilitates thermal tolerance, mutations to this protein have a more deleterious effect in the higher-temperature environment. Adapted from Bank et al. (2014).

In eukaryotes, a similar pattern has been observed in the distribution of fitness effects for changes in a single gene. In a recent study, Claudia Bank and her colleagues estimated the distribution of fitness effects for mutations in the HSP90 heat shock protein of the yeast *Saccharomyces cerevisiae* (Bank et al. 2014). They first generated an array of cells with different single-base mutations in the coding region for this protein, and then they allowed these cells to compete with one another for a number of generations. They then measured the relative frequencies of each mutant type and from these frequencies inferred the fitnesses of the mutants. As with the virus studies, most mutations were neutral or deleterious, though a small number were beneficial (**Figure 6.24**). And as with the virus studies, the distribution of fitness effects was bimodal. At 25°C the thermal tolerance conferred by the HSP90 protein is inessential for survival and reproduction, so the lower peak comprises mutations with moderately deleterious effects. At 36°C, HSP90 is needed for thermal tolerance and thus the lower peak is composed of strongly deleterious or even lethal mutations.

Although studies such as these provide new and useful insights into the distribution of mutational effects, we are still only beginning to learn about the relative frequency of deleterious versus beneficial versus neutral mutations across species (Keightley and Eyre-Walker 2010; Loewe and Hill 2010). Considerable work awaits.

Darwin was able to develop the theory of natural selection without knowing the details of genetic transmission. He simply needed to understand that traits were passed down from parents to offspring. But much of the work on evolution that has been done since Darwin's time has relied on a solid and ever-expanding understanding of the mechanisms underlying genetic inheritance. In this chapter, we have discussed some key subjects in this area, with an emphasis on their connection to key concepts in evolutionary biology. We are now ready to proceed to a series of chapters on population genetics (Chapters 7–10), moving in turn through single-locus models in large populations, to single-locus models in small populations, to multilocus models, and, ultimately, to genome evolution.

SUMMARY

1. At almost the same time that Charles Darwin was publishing *On the Origin of Species*, Augustinian monk Gregor Mendel was breeding tens of thousands of pea plants. This work gave birth to the field of genetics, including transmission genetics. Mendel's work provided empirical evidence that traits from the two parents were not irreversibly blended in the offspring. He demonstrated that instead, the heritable factors were particulate.
2. Mendel's laws are (a) the law of segregation, which states that each individual has two gene copies at each locus and these gene copies segregate during gamete production, so that only one gene copy goes into each gamete, and (b) the law of independent assortment, which states that which of the two gene copies is passed down to the next generation at one locus is independent of which gene copy is passed down to the next generation at the other loci. The second law holds true only for unlinked loci.
3. DNA is arranged into structures known as chromosomes. Diploid organisms have two copies of each chromosome. Organisms with a single copy of each chromosome are known as haploids.
4. The sequences of nucleotides in DNA molecules specify the sequences of amino acids that make up proteins. DNA is transcribed into messenger RNA (mRNA) by an enzyme called RNA polymerase. The mRNA is then translated into protein with the help of a ribosome. Changes in DNA sequences correspond to changes in protein structure or expression and create the variation on which evolutionary processes act.
5. Proteins are constructed using 20 different amino acids. Most amino acids can be encoded by more than one nucleotide triplet (codon). The correspondence between codons and amino acids is known as the genetic code.
6. Proteins are long strings of amino acids that are essential building blocks of life and serve many different functions within cells. Some proteins act as enzymes that initiate and regulate chemical reactions. Other proteins are chemical signals that are used in communication within and between cells. Some proteins bind to DNA and help to regulate when and how DNA is expressed; others serve structural functions, forming the cytoskeleton or elements of the extracellular matrix.
7. A gene is a sequence of DNA that specifies a functional product. In eukaryotes, protein-coding genes are composed of exons and introns. An allele is a specific form of a gene. In diploid species, individuals with two copies of the same allele for a given gene are called homozygotes, and those with copies of different alleles are called heterozygotes.
8. Regulatory elements influence the rate at which RNA molecules are transcribed from the DNA. This process is known as transcriptional regulation. Regulatory elements that increase the rate of transcription are called enhancers, while those that decrease the rate of transcription are known as silencers.
9. In eukaryotic cells, the structure of chromatin—DNA wrapped around proteins called histones—also influences the transcription rate, by determining the accessibility of genes to RNA polymerase. Epigenetic modifications to the DNA and the histones further affect gene expression and are heritable across cell divisions.
10. Mutation, a change to the DNA sequence of the organism, generates genetic variation. Mutations are undirected, in that they are random with respect to their fitness consequences.
11. Changes of single nucleotides are classified as synonymous if they change the amino acid specified and nonsynonymous otherwise. Nonsense mutations introduce a stop codon where an amino acid was previously specified. Insertion or deletion of one or more nucleotides may produce a frameshift mutation. Large-scale mutations at the whole-gene or chromosome level are also possible.
12. In sexually reproducing organisms, recombination through crossing-over—the physical exchange of segments of DNA during meiosis—remixes existing variation into new genetic combinations.
13. In humans, mutations occur at rates around 1×10^{-8} to 3×10^{-8} mutations per nucleotide per generation. Mutation rates differ across species, between families, between sexes, between nuclear and organellar genomes, across regions of a single genome, and across tissue types.
14. In the systems studied to date, most mutations are deleterious or nearly neutral, while only a few are beneficial.

KEY TERMS

alleles (p. 195)
amino acids (p. 194)
chromatin (p. 197)
chromosomal deletion (p. 202)
chromosomal duplication (p. 202)
cis regulatory elements (p. 197)
codons (p. 194)
crossing-over (p. 203)
distribution of fitness effects (p. 209)
dominant (p. 189)
epigenetic inheritance (p. 197)
exons (p. 195)
frameshift mutation (p. 201)
gametes (p. 189)
gene (p. 195)
genetic code (p. 194)
genotype (p. 195)
heterozygotes (p. 196)
histones (p. 197)
homozygotes (p. 196)
incomplete dominance (p. 196)
introns (p. 195)
inversion (p. 202)
law of independent assortment (p. 189)
law of segregation (p. 189)
locus (p. 189)
meiosis (p. 203)
nonsense mutation (p. 200)
nonsynonymous mutation (p. 199)
promoter (p. 193)
recessive (p. 189)
regulatory elements (p. 197)
synonymous mutation (p. 199)
trans regulatory elements (p. 197)
transcription (p. 193)
transition (p. 199)
translation (p. 194)
translocation (p. 202)
transmission genetics (p. 188)
transversion (p. 199)

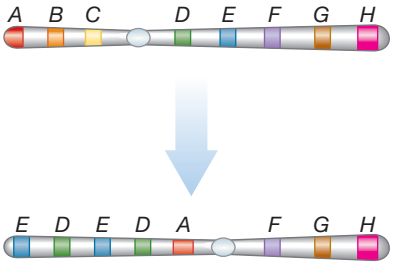
REVIEW QUESTIONS

1. Which law required Mendel to observe at least two traits in order to derive it: the law of segregation or the law of independent assortment?
2. How did the discovery of particulate inheritance resolve a serious concern about Darwin's theory?
3. Describe two chemical differences between DNA and RNA.
4. Briefly outline the processes by which DNA sequence information is used to produce proteins in eukaryotes.
5. Why is the genetic code said to be degenerate?
6. Explain how the following terms relate to one another: *gene, allele, locus, genotype*.
7. What is epigenetic inheritance?
8. Distinguish between synonymous mutations, nonsynonymous mutations, and nonsense mutations.
9. Suppose a resistant mutation arises early in the growth process during the Luria–Delbrück experiment. How does this affect the number of resistant cells once the bacteria are exposed to phage particles?
10. Is there one "human mutation rate"? Why or why not?

KEY CONCEPT APPLICATION QUESTIONS

11. We emphasized that mutation is undirected. Why is this such a critical concept? How can a misunderstanding about this lead to a complete failure to grasp how the evolutionary process operates?
12. Imagine that it had turned out that mutation was not undirected, but that instead some mechanism of directed mutation allowed organisms to generate only beneficial mutations. Sketch what Figure 6.23B might look like in this case.
13. Figure 6.9B shows a flower species that exhibits incomplete dominance for flower color: *RR* individuals produce red flowers, *Rr* individuals produce pink flowers, and *rr* individuals produce white flowers. Use a Punnett square to determine what types of offspring would be produced, in what frequencies, from a cross between two *Rr* parents.

14. Propose a plausible sequence of chromosomal rearrangements that would transform the first chromosome into the second as shown in the illustration below.

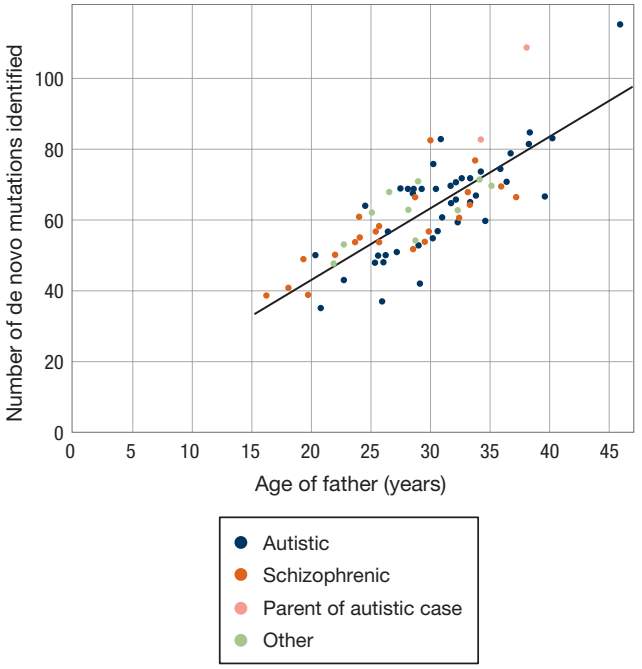


15. What sequence of amino acids is specified by the following mRNA?

AUGGCAUCACCGUGGAAGUGAGUGCGU

Assume that the reading frame begins at the start: AUG CAU and so forth.

16. In a study of children with autism and schizophrenia, Kong et al. (2012) sequenced the genomes of family members to explore the relationship between father's age and the number of de novo (newly arisen) mutations in the offspring. Their results are shown below (figure adapted from Kong et al. 2012). Describe in a sentence or two what this plot tells us.



Propose a hypothesis for this observation.

SUGGESTED READINGS

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Henig, R. 2001. *The Monk in the Garden: The Lost and Found Genius of Gregor Mendel, the Father of Genetics*. Mariner Books, Boston. A beautiful book on Mendel's work and its place in the history of science.

Luria, S. E., and M. Delbrück. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics* 28: 491–511. A classic paper using phylogenetic thinking to demonstrate that conditions in the environment did not induce specific mutations that would be beneficial in that particular environment.

McCracken, K. G., C. P. Barger, and M. D. Sorenson. 2010. Phylogenetic and structural analysis of the HbA (α^A/β^A) and HbD (α^D/β^A) hemoglobin genes in two high-altitude waterfowl from the Himalayas and the Andes: Bar-headed goose (*Anser indicus*) and Andean goose (*Chloephaga melanoptera*). *Molecular Phylogenetics and Evolution* 56: 649–658. A technical article that ties together transmission genetics, natural selection, and phylogenetics to help understand adaptation to low oxygen at high altitudes.

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7

The Genetics of Populations

◀ Sooty terns (*Onychoprion fuscata*) return to roost against a twilight blue sky at the Hawaiian Islands National Wildlife Refuge, Hawaii.

In the previous chapter, we provided an overview of Gregor Mendel's work on the nature of genetic inheritance. In Chapter 2, we mentioned its fame as one of the great "lost discoveries" in the history of science. What we did not explore in those chapters was the intense controversy that arose upon the rediscovery of this work. This controversy itself makes a fascinating story.

When, after 34 years of obscurity, Mendel's work was finally rediscovered in 1900, his ideas were met with great excitement but not with broad and immediate acceptance. Instead, the renewed attention around Mendel's paper triggered a vigorous debate about the nature of heredity. Were the peculiar rules of inheritance that Mendel described simply a strange quirk of a few characters in one particular species, the garden pea? Or were they more fundamental to biology, telling us about the process of inheritance throughout the living world?

Critics attacked Mendel's conclusions on multiple grounds. First, Mendel's examples did not seem to accord with most biological observations: The traits Mendel studied were discrete characters that take on one of a fixed set of possible values, whereas most biological variation appeared to be

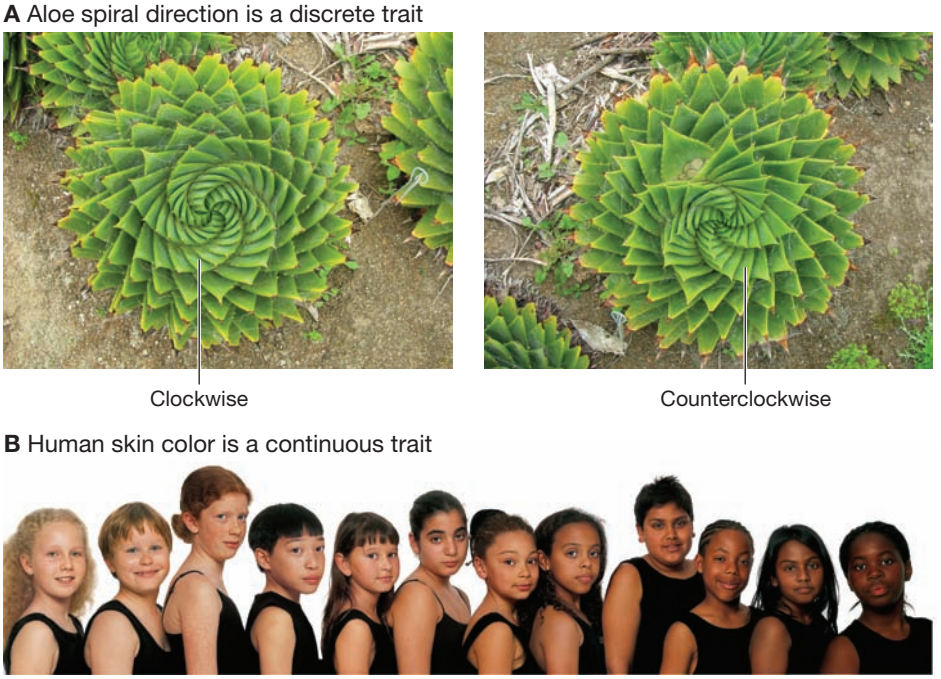


FIGURE 7.1 Discrete versus continuous traits. **(A)** The succulent plant *Aloe polyphylla* spirals either clockwise (left) or counterclockwise (right). The direction of the spiral is a discrete trait. **(B)** Human skin color is a continuous trait.

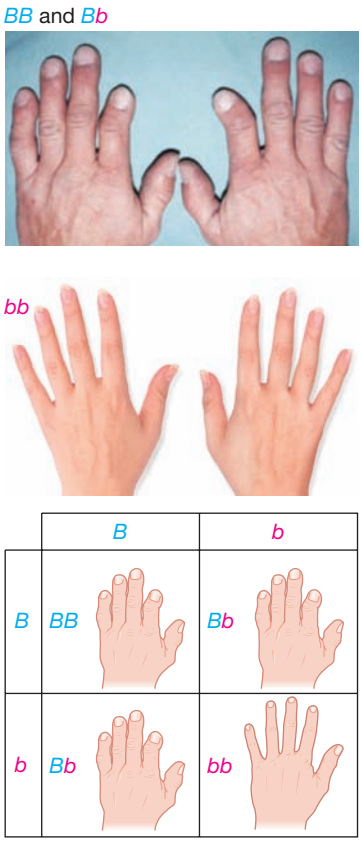


FIGURE 7.2 The genetics of brachydactyly. Brachydactyly is a malformation or shortening of the digits and is inherited as a dominant trait. *BB* and *Bb* individuals show malformed or shortened fingers, whereas *bb* individuals have normal fingers.

continuous (**Figure 7.1**). Second, at the time it was unclear whether a Mendelian system of inheritance could be consistent with Darwin's theory of evolution by natural selection. We will defer discussion of these two issues—and their ultimate resolution—until the beginning of Chapter 9.

For now, we will focus on a third critique, levied by leading biologists of the time against the best examples of so-called *Mendelian traits*—discrete traits passed on to offspring in the expected Mendelian ratios. These critics thought that trait frequencies as observed in nature were not consistent with the frequencies expected under Mendelian inheritance. A satisfactory resolution to this problem required a mathematical way of linking the rules of individual inheritance to their population consequences: It drew in one of the leading mathematicians of the twentieth century, and it led to the initial foundation of the field known as *population genetics*.

A concise version of the story centers on a 1908 paper presented by Reginald Punnett to the Royal Society of Medicine. Punnett, who is also known for introducing the Punnett square (Chapter 6), was a leading advocate of Mendel's ideas. In his paper, Punnett laid out a series of examples of human traits that were transmitted according to Mendel's laws of inheritance. Among these was *brachydactyly*, a genetically inherited condition leading to shortened or malformed fingers and toes. Based on an analysis of human pedigrees, Punnett noted that heredity of this trait was consistent with Mendel's model of inheritance. We now know that Punnett was correct: Brachydactyly is controlled by a single locus on an *autosome*—one of the chromosomes that is not a sex chromosome. The allele conferring the brachydactylous state is dominant to the typical state, or *wild type* (**Figure 7.2**).

Punnett's paper was followed by spirited discussion. G. Udny Yule (1871–1951), a British statistician who also wrote important papers on Mendelian genetics, is reported to have attacked the brachydactyly case, as he believed that it was an invalid example of a Mendelian trait. Supposedly, Yule expected that any dominant Mendelian trait should occur in a 3:1 ratio, reflecting the 3:1 ratio Mendel had found with his peas.

Across the fog of a century, it is hard to reconstruct exactly who believed precisely what, but Yule appears to have reasoned along the following lines: Mendel's rules predict that heterozygote crosses yield a 3:1 ratio of dominant to recessive phenotypes among offspring. Therefore, if Mendel's rules are correct, a heterozygous trait should be observed in a 3:1 ratio *in a population* (this inference turns out to be false, as we will see). But brachydactyly—one of the favorite examples used to support Mendelian arguments—does not occur in a 3:1 ratio in human populations. Rather, as simple observation reveals, brachydactyly remains rare in human populations. From this observation, Yule erroneously concluded that the brachydactylous trait must not be strictly Mendelian in nature. Yule reportedly took this empirical observation as evidence against the Mendelian hypothesis.

Unable to counter Yule's critique on his own, Punnett turned for help to his friend G. H. Hardy (1877–1947), a renowned British mathematician. Hardy developed a straightforward mathematical model to predict the *population-level* consequences of Mendelian inheritance. This model allowed Hardy to test mathematically—and refute—Yule's presumption that Mendel's rules necessarily produce a 3:1 ratio of dominant to recessive phenotypes at the population level. The model undercut Yule's criticism, and it showed that Punnett's examples of rare Mendelian traits, including brachydactyly, could be valid even though the ratios observed in the population as a whole were nowhere near 3:1. We return to this model in detail a bit later in the chapter.

Hardy's model also cleared up a second misperception surrounding the population-level implications of Mendel's laws. Many biologists believed that under Mendelian inheritance, dominant alleles would replace recessive alleles over time, simply by the nature of heredity. Hardy showed otherwise. According to Hardy's model, the frequency of an allele neither increases nor decreases simply because its effects are dominant or recessive. In other words, an allele's dominant or recessive mode of *expression* has nothing to do with the mechanics of its *transmission*. Other factors, such as selection or mutation, may lead to changes in allele frequencies. But in the absence of such factors, dominant alleles do not increase in frequency simply because they are dominant, nor do recessive alleles decrease in frequency simply because they are recessive.

Over the next three chapters, we will learn how to construct some simple population genetic models. In this chapter, we will limit ourselves to considering how allele frequencies change *at a single locus* in a large population. Our goal will be to understand how genotype frequencies in the offspring population relate to genotype frequencies in the parental population. We will begin our quantitative treatment of the subject by exploring the model that Hardy developed at Punnett's request. In doing so, we will see how this model serves as a null model against which we can compare observations of genotype frequencies and the way that they change over time. We will then examine natural selection, mutation, and migration to see how each can produce changes in gene frequencies and thus affect the evolution of traits. We will address the following questions:

- How do allele frequencies change over time in the absence of natural selection and other evolutionary processes?
- How do we build a mathematical model of natural selection?
- How do mutation, nonrandom mating, and migration affect genotype and allele frequencies in a population?

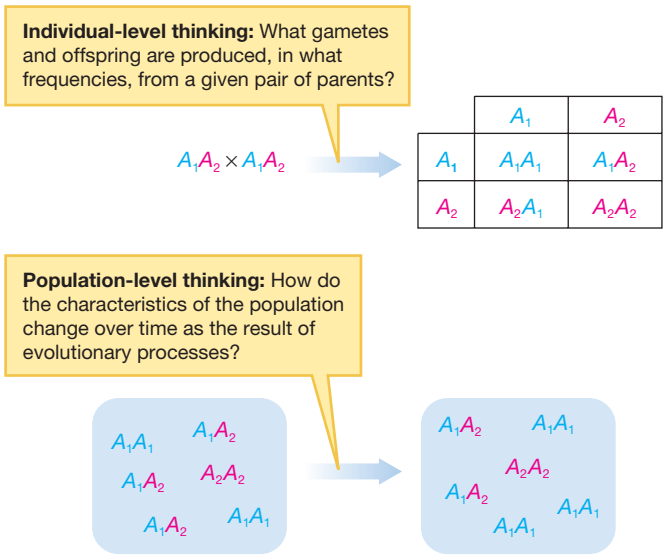


FIGURE 7.3 Individual-level thinking versus population-level thinking. Individual-level and population-level approaches ask different questions.

7.1 Individual-Level versus Population-Level Thinking

The field of transmission genetics, which we reviewed in Chapter 6, characterizes the way in which the genotype of an *individual* offspring is related to the genotypes of its parents. The field of **population genetics** then investigates how the genotype frequencies in an offspring *population* are related to the genotype frequencies in a parental population. To understand the process of evolutionary change, we have to make a shift from individual-level thinking, so prevalent in the study of genetics, to the sort of population-level thinking that we tend to associate with ecology and evolution. Individuals live but one lifetime, whereas evolution results in changes in the composition of populations across generations. We illustrate the difference between these individual-level and population-level approaches in **Figure 7.3**.

Quantitative versus Qualitative Predictions

In the previous chapters, we examined the evolutionary process and its consequences in *qualitative* terms. For example, we learned that in order for natural selection to operate on a character such as the coat color of oldfield mice, there must be variation in coat color, fitness differences associated with the different coat colors, and heritability of coat color. From this, we can then predict whether coat color in a given population is likely to change over evolutionary time. If lighter-colored mice are less likely to be eaten by predators, we expect to see the allele variants that contribute to lighter coloration become more common over evolutionary time. In essence, if any measurable trait has a genetic basis, we can make predictions about whether the alleles for that trait will increase or decrease in frequency.

Evolutionary biologists can also make *quantitative*, or numerical, predictions about evolutionary dynamics. Evolutionary change occurs because certain alleles or genotypes become more common and others become less common. At its most basic level, *biological evolution occurs when genotype frequencies change over time*. The field of population genetics provides a formal structure with which to look at this process. Using population genetics, we can develop a mathematical description of how these frequencies change over time—and thus a mathematical description of the evolutionary process itself. This greatly facilitates the testing of evolutionary hypotheses.

It is not only change that we are interested in. We also want to understand stasis; we want to understand when genotype frequencies or allele frequencies will stay the same. Are there "steady-state" frequencies for which no further change will occur? Such frequencies are known as the *equilibria* of our models. In general, we say that a physical or mathematical system is at equilibrium if the system has reached a state where it does not change in the absence of outside forces or processes acting on it. In population genetics, we typically track the genotype frequencies in a *population*. An equilibrium is then a state of the population such that genotype frequencies do not change from generation to generation. **Box 7.1** illustrates several types of equilibria.

BOX 7.1 Types of Equilibria

Typically, when we think about an equilibrium, we think about a *stable equilibrium*, for which two conditions hold:

1. When at this point, the system does not change.
2. If perturbed or displaced by some small amount, the system will return to its original position at rest.

The first condition ensures that we have an equilibrium; the second ensures that our equilibrium is stable.

Perhaps the simplest way to envision a stable equilibrium is by thinking about a marble in a rounded cup (**Figure 7.4**). The bottom of the cup is a stable equilibrium for the marble, because a marble at rest at this point does not move further, and if perturbed with a small push, the marble will return to the equilibrium point at the bottom of the cup.

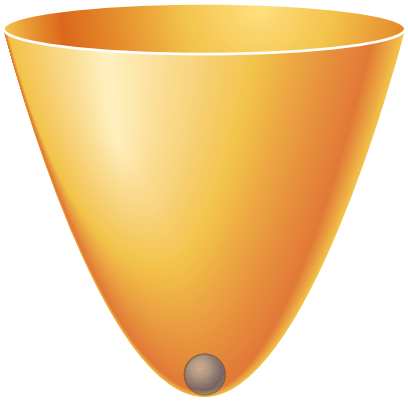


FIGURE 7.4 Stable equilibrium. A marble at the bottom of a rounded cup represents a stable equilibrium.

But stable equilibria are not the only kind of equilibria. There are also *unstable equilibria*. At an unstable equilibrium, two conditions hold:

1. When at this point, the system does not change.
2. If perturbed or displaced by some small amount, the system will move away even further from its initial position at rest.

Corresponding to our marble in a cup, we can think of an unstable equilibrium as a marble perfectly balanced on the top of a hill (**Figure 7.5**). In the absence of external forces, it is not going anywhere.

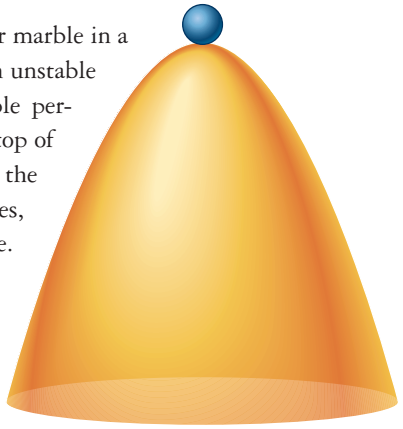


FIGURE 7.5 Unstable equilibrium. A marble balanced on top of a hill represents an unstable equilibrium.

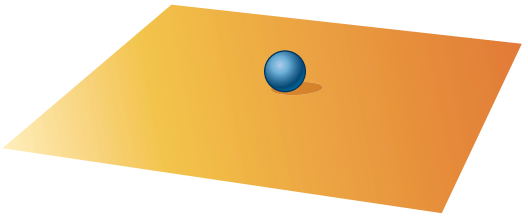


FIGURE 7.6 Neutral equilibrium. A marble at rest on a tabletop represents a neutral equilibrium.

But give it the slightest push in any direction, and it will tumble off the hill rather than return to its starting position.

In addition to stable equilibria and unstable equilibria, there are also *neutral equilibria*. A neutral equilibrium is a state of the system such that these conditions hold:

1. When at this point, the system does not change.
2. If perturbed or displaced by some small amount, the system will stay in its displaced position, rather than returning to the original position as it would in a stable equilibrium or moving further away as it would in an unstable equilibrium.

Here we can think about a marble on a flat tabletop (**Figure 7.6**). If we move it slightly to the left or right, front or back, it neither returns to its original position nor falls off the table. It will simply sit at rest in its new position.

An equilibrium can also be stable with respect to perturbations in one direction, but neutral with respect to perturbations in another. We call this a *mixed equilibrium*. One example of such an equilibrium is the position of a marble in a half-pipe (**Figure 7.7**).

When displaced leftward or rightward, up the sides of the half-pipe, the ball will return to its position in the center, as with a stable equilibrium. But when displaced forward or backward along the bottom of the half-pipe, the ball will remain in its newly displaced position.

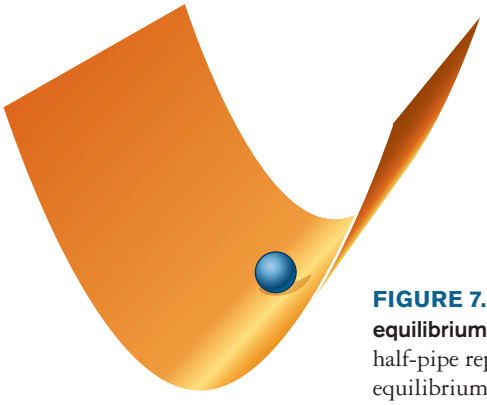


FIGURE 7.7 Mixed equilibrium. A marble in a half-pipe represents a mixed equilibrium.

7.2 The Hardy–Weinberg Model: A Null Model for Population Genetics

Population-level thinking sets the stage for the construction of a mathematical model of evolutionary change. But, in order to understand the effects of any natural process, we need a baseline model for comparison.

The Role of Null Models in Science

The role of a null model in science is to provide such a baseline. In physics, Newton's first law provides a baseline to help us understand the effects of forces acting on objects. The first law states that if no net external force is acting on an object, then an object in motion continues that motion, and an object at rest stays at rest. With this baseline in place, we can see that objects in motion speed up or slow down only when they are acted on by forces.

If we want to understand the effects of biological processes such as natural selection or mutation on the frequencies of genotypes in a population, we also need a null model. The **Hardy–Weinberg model** provides such a null model. It tells us what happens to genotype frequencies when natural selection and other important drivers of evolutionary change are not operating. Then, when we observe change in genotype frequencies relative to Hardy–Weinberg predictions, we will be able to make inferences about the sorts of evolutionary processes necessary to explain our observations.

It only became possible to construct such a null model once biologists had a rudimentary understanding of the mechanistic basis of heredity. With this understanding in place, evolutionary biologists could scale up their thinking about how genes are transmitted, using the rules of heredity at the individual level in order to model the rules of heredity at the level of populations. In other words, they could now model how the frequency of traits might change in populations.

The Hardy–Weinberg Model

Taking the most basic case, suppose that a single character at a single genetic locus is encoded by a single pair of alternative alleles. What will happen over time to the frequencies of these alleles, as well as to the frequencies of the genotypes in which they are found, in the absence of any significant evolutionary processes? While the answer may seem obvious to us today, it was by no means obvious a century ago. Population geneticists needed a formal model to answer this question definitively.

This is the question that G. H. Hardy's model addressed. The German physician Wilhelm Weinberg (1862–1937) independently developed and published a comparable model at the same time: In recognition of this parallel discovery, we commonly refer to it as the Hardy–Weinberg model. The Hardy–Weinberg model examines a character encoded by a single locus, with two alleles A_1 and A_2. In this case, there are three possible genotypes—A_1A_1, A_1A_2, and A_2A_2. Hardy and Weinberg wanted to examine what would happen to the frequencies of these three different genotypes in a simple genetic model in which natural selection—and other important evolutionary processes—were *not* operating. Their solution, now called the **Hardy–Weinberg equilibrium**, serves as a null model for studies of

allele frequencies and genotype frequencies in populations. The model provides three important conclusions:

1. The frequencies of the A_1 and A_2 alleles do not change over time in the absence of evolutionary processes acting on them. Note that these allele frequencies need not be 50%. The A_1 allele could be much more common than the A_2 allele or vice versa.
2. Given allele frequencies (the frequencies of A_1 and A_2) and random mating, we can predict the equilibrium genotype frequencies (the frequencies of A_1A_1, A_1A_2, and A_2A_2) in a population in which evolutionary processes are not acting. Today, these are referred to as *Hardy–Weinberg equilibrium frequencies*.
3. If no evolutionary processes are operating, a locus that is initially not at Hardy–Weinberg equilibrium will reach Hardy–Weinberg equilibrium in a single generation.

The first conclusion tells us how allele frequencies change in the absence of evolutionary processes. The second conclusion tells us how genotype frequencies relate to allele frequencies in the absence of evolutionary processes. The third conclusion tells us how long it takes to reach these genotype frequencies.

To biologists in the early twentieth century, the Hardy–Weinberg model revealed something very important: that the mechanics of inheritance itself do not diminish the variation present in a population. Recall Darwin's concern that blending inheritance would use up the variation present in a population and leave selection with no variation to sort upon. The Hardy–Weinberg model shows that inheritance itself does not change the allele frequencies in a population, nor does the fraction of homozygotes decrease over time once Hardy–Weinberg proportions are reached.

The Hardy–Weinberg Assumptions

Every mathematical model begins with a list of assumptions. When modelers list their assumptions, they are, in essence, laying out for the reader what will and will not be included in a model. This process of enumerating the assumptions is one of the most important aspects of any model, because it allows the reader to understand the scope, as well as the limitations, of the mathematics to follow.

The Hardy–Weinberg model begins by making a number of basic assumptions about the individuals and population under study, as well as the evolutionary processes in operation. The model envisions a population of sexually reproducing diploid organisms with the same allele frequencies in males and females. These organisms reproduce in discrete non-overlapping generations: all parents reproduce synchronously and then die. Critically, the Hardy–Weinberg assumptions state that none of five important evolutionary processes are operating:

1. Natural selection is *not* operating on the trait or traits in question.
2. There is no assortative mating: mating in the population is random with respect to the locus in question.
3. No mutation is occurring.
4. There is no migration into or out of the population.

5. The population is effectively infinite in size, so genetic drift—chance fluctuations in allele frequencies—is negligible.

We begin by developing the model using these assumptions. Later in this chapter, we will explore what happens when assumptions 1–4 are relaxed. In the subsequent chapter we will relax assumption 5 and examine genetic drift in detail. By comparing what happens when we remove one or more of these assumptions with what happens in the basic Hardy–Weinberg model when all of the assumptions are operating, we can get a sense of how processes such as natural selection, nonrandom mating, mutation, migration, and genetic drift influence genotype frequencies.

Deriving the Hardy–Weinberg Model

Every organism in our population must have one of the three possible genotypes A_1A_1, A_1A_2, or A_2A_2. Let us call the frequencies of these three genotypes $f[A_1A_1]$, $f[A_1A_2]$, and $f[A_2A_2]$. Because each individual has one of these three genotypes, the sum of genotype frequencies must be unity: $f[A_1A_1] + f[A_1A_2] + f[A_2A_2] = 1$. (**Box 7.2** summarizes the rules of probability used in this chapter.)

From these genotype frequencies, we can compute the allele frequencies directly. Allele A_1 is found only in individuals with the A_1A_1 or A_1A_2 genotypes. Because each A_1A_1 individual possesses two A_1 alleles, and each A_1A_2 individual possesses a single A_1 allele, we can devise a simple mathematical relationship between genotype frequencies and allele frequencies. This allows us to calculate the frequency of the A_1 allele, which we denote as p, from the genotype frequencies:

$$p = f[A_1A_1] + \frac{f[A_1A_2]}{2}$$

BOX 7.2 Basic Probability Calculations

In probability, we study the chance that certain outcomes—which we will call *events*—are observed. Suppose P_1 is the probability that a given outcome—call it event E_1—occurs, and suppose that P_2 is the probability that another event E_2 occurs.

Probability of a Sure Event and Probability of an Impossible Event

1. If the event E_1 is certain to occur, we say that its probability is 1.
2. If the event E_2 is certain not to occur, we say that its probability is 0.

Probability That an Event Does Not Occur

If E_1 occurs with probability P_1, the probability that E_1 does not occur is given by $1 - P_1$.

Events Can Be Assembled from Other Events

We can create new events using other events as building blocks. For example, we could define E_3 as the event that both E_1 and E_2 occur; we could define E_4 as the event that neither E_1 nor E_2 occurs.

Probability of Event 1 *and* Event 2

If event E_1 and event E_2 are *independent* events—that is, if the chance of E_2 happening does not depend on whether E_1 happened and vice versa—then the probability that both E_1 and E_2 occur is given by the product of their probabilities:

$$\Pr(E_1 \text{ and } E_2) = P_1 \times P_2$$

For example, let E_1 be the event that you roll a 1 on a fair die, and let E_2 be the event that you get heads on the flip of a fair coin. The probabilities of these events are $P_1 = 1/6$ and $P_2 = 1/2$, respectively. These are independent events: The result of the coin flip does not depend on the result of the die roll and vice versa. Therefore, the probability that you both roll a 1 on the die and get heads on the coin flip is $\Pr(E_1 \text{ and } E_2) = P_1 \times P_2 = 1/12$.

We are counting the A_1A_2 genotypes only half as much as the A_1A_1 genotypes because, in the former, only half of the alleles at the A locus are A_1 alleles, whereas in the latter, both of the alleles at the A locus are A_1 alleles. Similarly, because half of the A alleles in an A_1A_2 heterozygote are A_2 alleles, whereas all of the A alleles in an A_2A_2 individual are A_2 alleles, the frequency of the A_2 allele, which we denote as q, is given by

$$q = f[A_2A_2] + \frac{f[A_1A_2]}{2}$$

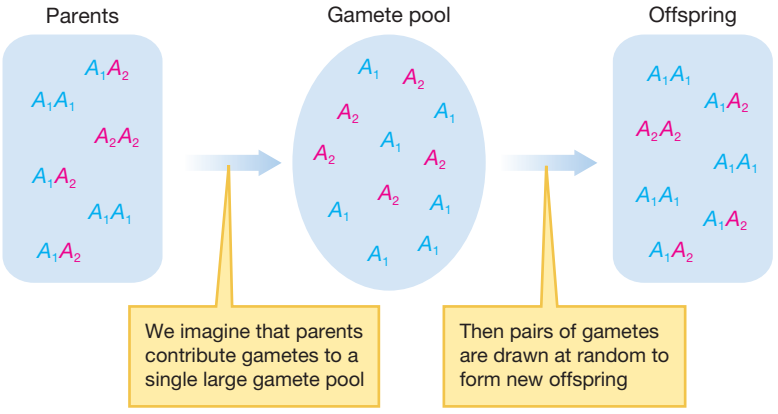


FIGURE 7.8 A gamete pool approach. When individuals mate at random with respect to the genotype we are studying, we can take a gamete pool approach. Using this approach, the frequencies of the offspring produced are equal to those expected if the parental generation were simply to contribute their gametes to a single large gamete pool, from which pairs of gametes are drawn at random to form new offspring.

Finally, because we have only two alleles in our system, it must be true that $p + q = 1$ because every A allele is either an A_1 or an A_2.

We want to see how genotype frequencies change over time, so we need to calculate the new genotype frequencies after individuals in our population mate with one another and produce offspring. One way to do this is to go through all possible mating pairs that can occur in our population, compute how common such mating pairs are, and write out Punnett squares to determine what type of offspring are produced from such matings. But doing the calculations in that way would involve a large amount of tedious algebra even in this simple one-locus, two-allele case. Fortunately, if the Hardy–Weinberg assumptions are met, we can bypass all of that algebra. We can take advantage of the very convenient fact that in this model, gametes assort at random—that is, they pair up at random to produce offspring—just as if they were all mixed together in one great gamete pool and then drawn out randomly in pairs (**Figure 7.8**). The composition of this hypothetical gamete pool is simply proportional to the frequency of the alleles in the parental generation.

Probability of Event 1 *or* Event 2

If the events E_1 and E_2 are *mutually exclusive* events—that is, if it is impossible for E_1 and E_2 both to occur—then the probability that either E_1 or E_2 occurs is given by the sum of their probabilities: $\Pr(E_1 \text{ or } E_2) = P_1 + P_2$. The probability that they both occur is, of course, 0.

For example, let E_1 be the event that you get a 1 when you roll a die, and let E_2 be the event that you get an even number on the same roll. These are mutually exclusive events. If they have probabilities $P_1 = 1/6$ and $P_2 = 1/2$, respectively, then the probability that E_1 and E_2 both occur is $\Pr(E_1 \text{ and } E_2) = 0$, and the probability that P_1 or P_2 occurs is $\Pr(E_1 \text{ or } E_2) = P_1 + P_2 = 2/3$.

More generally, for *any* two events E_1 and E_2, independent or not, mutually exclusive or not, the probability that at least one of them occurs is given by

$$\Pr(E_1 \text{ or } E_2) = P_1 + P_2 - \Pr(E_1 \text{ and } E_2)$$

We can rewrite this as a general expression for the probability that E_1 and E_2 both occur as

$$\Pr(E_1 \text{ and } E_2) = P_1 + P_2 - \Pr(E_1 \text{ or } E_2)$$

Frequencies and Probabilities

In population genetics, we often speak of the frequencies or expected frequencies of different genotypes or alleles—that is, of the fraction of the population that we expect to be composed of each genotype or allele. If we assume that each offspring is produced independently by the same random process that leads to the production of every other offspring, the *frequencies* in a very large population will be equal to the *probabilities* of producing each type of offspring in a single reproduction event. In the Hardy–Weinberg model and many (but not all) other population genetic models, we indeed make this assumption. Therefore, we can and will use the laws of probability laid out above in order to compute the frequencies of genotypes and alleles.

The offspring, produced by random draws from this gamete pool, occur with frequencies that we can calculate using the rules of probability detailed in Box 7.2:

Genotype	Hardy–Weinberg Equilibrium Frequency
A_1A_1	p^2
A_1A_2	$2pq$
A_2A_2	q^2

The frequency of the A_1A_1 genotype among the offspring is just the frequency of the A_1 allele, squared. The Hardy–Weinberg model, then, predicts that in the absence of evolutionary processes, the expected frequency of the A_1A_1 genotype is equal to the fraction of the time that we would expect a random draw from a gamete pool with A_1 at frequency p to yield two A_1 alleles: p^2. Similarly, the frequency of the A_1A_2 genotype is equal to the fraction of the time that a random draw would select one A_1 allele and one A_2 allele. This is $2pq$ rather than pq because there are two ways to draw an A_1A_2 individual: by drawing an A_1 first and an A_2 second or by drawing an A_2 first and an A_1 second. The frequency of the A_2A_2 genotype is equal to the fraction of the time that two A_2 alleles would be drawn: q^2. This is a general result: The frequencies at Hardy–Weinberg equilibrium are always those that we would find if the gametes were paired randomly.

BOX 7.3 Hardy–Weinberg Equilibrium Is a Mixed Equilibrium

The key to a deep understanding of the Hardy–Weinberg equilibrium is to recognize that it is a mixed equilibrium. How is this so?

Recall that for a single locus A with alleles A_1 and A_2 at frequencies p and q, the Hardy–Weinberg model predicts that:

1. A population not at Hardy–Weinberg equilibrium will achieve Hardy–Weinberg genotype frequencies after a single generation of random mating.
2. In the absence of evolutionary processes acting on the population, allele frequencies remain constant.

This first condition indicates that Hardy–Weinberg genotype frequencies $f[A_1A_1] = p^2, f[A_1A_2] = 2pq$, and $f[A_2A_2] = q^2$ represent a stable equilibrium, given the allele frequencies p and q.

The second condition indicates that the allele frequencies p and q are themselves a neutral equilibrium. In the absence of external processes (for example, natural selection, drift, migration, and mutation), they don't change. But once displaced from their initial values to new values p' and q', the allele frequencies do not return to the initial values, but rather they remain at the new values until further influenced by external processes.

We can represent this graphically by plotting the frequency p of the A_1 allele on the x axis and the frequency $f[A_1A_2]$ of the heterozygote on the y axis. (These two quantities are sufficient to determine all three genotype frequencies and thus the entire state of the system.) The curve in **Figure 7.9** indicates the Hardy–Weinberg heterozygote genotype frequency as a function of the frequency of the A_1 allele.

In terms of the metaphor of marbles on surfaces that we developed in Box 7.1, the Hardy–Weinberg mixed equilibrium is like a marble on a curved half-pipe, as shown in **Figure 7.10A**. The marble can be shifted left to right along the bottom of the half-pipe, and it simply stays in its new position: any value of the allele frequency p is a neutral equilibrium. But if the marble is pushed forward or backward up the side of the half-pipe, it will return once again to the corresponding rest position at the bottom of the pipe. For any particular allele

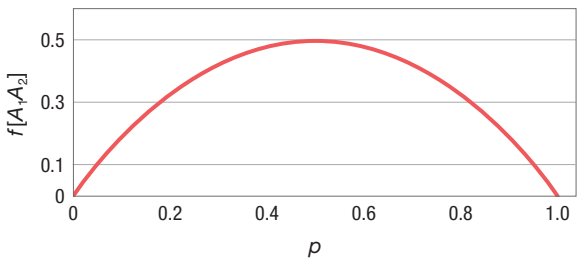


FIGURE 7.9 Heterozygote frequency at Hardy–Weinberg equilibrium. The Hardy–Weinberg equilibrium frequency $f[A_1A_2]$ of the heterozygote is a function of the allele frequency p of the A_1 allele: $f[A_1A_2] = 2pq = 2p(1 - p)$.

Thus, we see that the Hardy–Weinberg model settles down to equilibrium genotype frequencies of p^2, $2pq$, and q^2 after a single generation. And, provided that the assumptions of the model are met, genotype frequencies remain at these values indefinitely. **Box 7.3** expands on this point.

KEYCONCEPT QUESTION

7.1 You observe that the genotype frequencies in a population are $f[A_1A_1] = 0.3$, $f[A_1A_2] = 0.2$, $f[A_2A_2] = 0.5$. How many different explanations can you think of for why this population may not be in Hardy–Weinberg equilibrium?

An Example of Hardy–Weinberg Genotype Frequencies: The Myoglobin Protein

To see an example of Hardy–Weinberg genotype frequencies in a human population, we will consider polymorphism of the gene that codes for myoglobin. Myoglobin is a protein that supplies oxygen to the muscles when needed. Molecular genetic analysis reveals that human myoglobin alleles typically take one of two forms—let's call them A_1 and A_2—that differ by only two bases (Takata et al. 2002).

frequency p, the genotype frequency $f[A_1A_2] = 2pq$ is a stable equilibrium.

Returning to the story at the opening of this chapter, at last we can see where Yule and his colleagues went wrong in their intuitions about what Mendel's rules predicted for population-wide genotype frequencies. Yule and his colleagues expected that Mendel's rules predicted a stable equilibrium for both genotype frequencies *and* allele frequencies, with allele frequencies returning to an even 1:1 ratio (**Figure 7.10B**). But instead, as Hardy and Weinberg each showed, Mendel's rules predicted a mixed equilibrium. Genotype frequencies are stable for *given* allele frequencies, but allele frequencies themselves are at a neutral equilibrium.

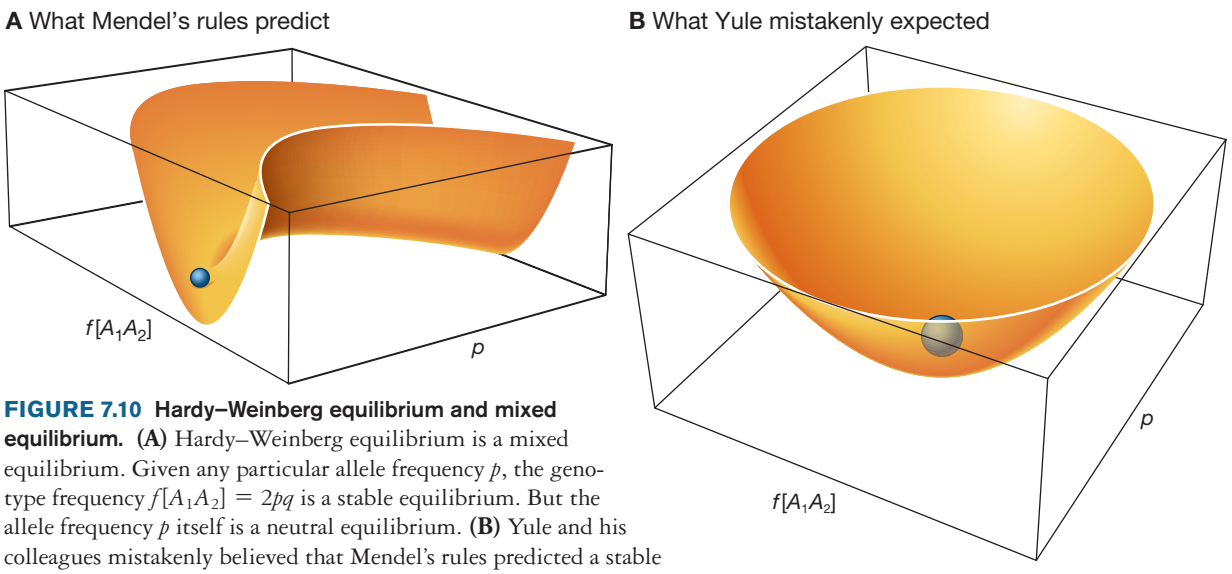


FIGURE 7.10 Hardy–Weinberg equilibrium and mixed equilibrium. **(A)** Hardy–Weinberg equilibrium is a mixed equilibrium. Given any particular allele frequency p, the genotype frequency $f[A_1A_2] = 2pq$ is a stable equilibrium. But the allele frequency p itself is a neutral equilibrium. **(B)** Yule and his colleagues mistakenly believed that Mendel's rules predicted a stable equilibrium both in allele frequencies and in genotype frequencies.

To study the distribution of these two alleles in a Japanese population, Tomoyo Takata and his colleagues collected blood samples from 100 Japanese volunteers. The researchers then used a form of polymerase chain reaction (PCR) to determine the genotype of each individual at the myoglobin locus. They found that among their subjects, the frequency of the A_1 allele was $p = 0.755$, while that of the A_2 allele was $q = 0.245$.

If no other evolutionary processes are in operation, the equilibrium frequencies of genotypes in this example can be predicted from allele frequencies by using the Hardy–Weinberg model. As we have seen, when the allele frequencies are p and q, we expect the genotype frequencies to be p^2, $2pq$, and q^2 at Hardy–Weinberg equilibrium. In this case, that means the expected Hardy–Weinberg genotype frequencies (f_{exp}) will be

$$f_{exp}[A_1A_1] = (0.755)^2 = 0.57$$
$$f_{exp}[A_1A_2] = 2 \times 0.755 \times 0.245 = 0.37$$
$$f_{exp}[A_2A_2] = (0.245)^2 = 0.06$$

Takata and his colleagues found that the *observed* genotype frequencies (f_{obs}) were as follows:

$$f_{obs}[A_1A_1] = 0.59$$
$$f_{obs}[A_1A_2] = 0.33$$
$$f_{obs}[A_2A_2] = 0.08$$

What can we conclude from these observations? The observed frequencies seem to be very close to the expected frequencies, close enough that any deviation could be due to chance. But is this correct? We need a statistical test to evaluate whether the observed frequencies deviate significantly from the expected. **Box 7.4** describes how one can evaluate whether a given set of genotype frequencies is in Hardy–Weinberg equilibrium proportions using a chi-square (χ^2) test. Applying the procedure described there, we find that the χ^2 value for Takata's data is 0.157. (You may want to practice by confirming that value yourself.) This is far lower than the critical value of 3.84, above which we would have a statistically significant deviation from Hardy–Weinberg equilibrium frequencies at the 5% level.

Takata's results are therefore consistent with the hypothesis that there are no evolutionary processes operating on the A_1 and A_2 alleles. There is good reason to think that this may be the case. We don't expect selection to be operating: the base pair differences that distinguish the A_1 and A_2 alleles are synonymous mutations, and therefore the myoglobin proteins produced by each allele are identical. We expect random mating in the absence of any phenotypic differences between the two alleles. Because it takes a pair of perfectly placed point mutations to convert A_1 to A_2 or vice versa, mutation rates between these two loci are low enough to be negligible. Migration is also negligible: Migration into the Japanese population has traditionally been low, presumably low enough that the frequencies at these alleles have been unaffected. Finally, the population studied is very large. There are more than 125 million people in Japan. Thus, we would expect that the large population assumption of the Hardy–Weinberg model has been satisfied as well.

While both our knowledge of the biology of these two alleles and the results of the Takata study are consistent with the Hardy–Weinberg model, the study does not definitively demonstrate that the Hardy–Weinberg assumptions are met for

BOX 7.4 Testing for Hardy–Weinberg Equilibrium

At Hardy–Weinberg equilibrium, both allele frequencies and genotype frequencies remain unchanged from generation to generation. Thus, if we observe a change in the allele or genotype frequencies in a population, we can safely infer that either (1) the population was not initially at Hardy–Weinberg equilibrium or (2) at least one of the five Hardy–Weinberg assumptions has been violated.

We also know that the Hardy–Weinberg model predicts that if a population is initially away from Hardy–Weinberg equilibrium, the equilibrium genotype frequencies will be reached in one generation—without any change in allele frequencies. Therefore, if we observe allele frequencies changing at all or if we observe genotype frequencies continuing to change over multiple generations, we can again conclude that at least one of the five Hardy–Weinberg assumptions has been violated.

But what if we observe a population in which neither the allele frequencies nor the genotype frequencies are changing? This still does not necessarily mean that the population is in Hardy–Weinberg equilibrium.

How can we tell? Using the model we have developed thus far, we can use the known genotype frequencies of a population to test whether that population is at or near Hardy–Weinberg equilibrium. For example, suppose that based on a sample of 20 individuals, we observe a population with the following genotype frequencies:

$$f[A_1A_1] = 0.5 \qquad f[A_1A_2] = 0.2 \qquad f[A_2A_2] = 0.3$$

We use these genotype frequencies to calculate the allele frequencies:

$$p = f[A_1A_1] + \frac{f[A_1A_2]}{2} = 0.6$$

$$q = f[A_2A_2] + \frac{f[A_1A_2]}{2} = 0.4$$

These are the *observed* allele frequencies in our population. Next, we calculate the *expected* Hardy–Weinberg genotype frequencies for a population with these allele frequencies. Call these expected Hardy–Weinberg frequencies $f_{exp}[A_1A_1]$, $f_{exp}[A_1A_2]$, and $f_{exp}[A_2A_2]$:

$$f_{exp}[A_1A_1] = p \times p = 0.36$$
$$f_{exp}[A_1A_2] = 2pq = 0.48$$
$$f_{exp}[A_2A_2] = q \times q = 0.16$$

These expected genotype frequencies seem to be considerably different from our observed genotype frequencies. But 20 individuals is a small size: How can we be sure this difference is not merely a consequence of sampling error? Researchers often use a statistical test known as Pearson's chi-square (χ^2) test. To conduct a chi-square test, one computes the value of the test statistic χ^2, a quantity that measures how far the observed values deviate from the expected values:

$$\chi^2 = \sum_i \frac{(O_i - E_i)^2}{E_i}$$

Here, the O_i term represents the number of individuals *observed* in each category (A_1A_1, A_1A_2, and A_2A_2), and the E_i term represents the number of individuals we would have *expected* to observe in each category under Hardy–Weinberg equilibrium. In our case, we would have expected to observe $20 \times 0.36 = 7.2$ A_1A_1 individuals, $20 \times 0.48 = 9.6$ A_1A_2 individuals, and $20 \times 0.16 = 3.2$ A_2A_2 individuals. (Note that the expected number of individuals need not be an integer.) The value of the χ^2 test statistic is then

$$\chi^2 = \frac{(10 - 7.2)^2}{7.2} + \frac{(4 - 9.6)^2}{9.6} + \frac{(6 - 3.2)^2}{3.2} = 6.81$$

To interpret this quantity, we need to compare it with a *critical value* from a chi-square table (which can readily be found online or in a statistics textbook). To use such a table, we first need to figure out how many *degrees of freedom* we have in our test. Basically, degrees of freedom measure the difference between the number of free parameters in our data and the number in the model we are testing (Good 1973). Our data have two free parameters: the number of A_1A_1 individuals in the population, and the number of A_1A_2 individuals in the population. (The number of A_2A_2 individuals is then constrained so that there are 20 individuals in total). The model we are testing, the Hardy–Weinberg model, has just one free parameter: the frequency p of the A_1 allele. (If we know p, then q is constrained so that p and q sum to 1). Therefore, we have $2 - 1 = 1$ degree of freedom.

We compare our χ^2 value, 6.81, to the critical value in a table for 1 degree of freedom at the desired level of significance—for example, 5%—which turns out to be 3.84. Our χ^2 value of 6.81 is larger than this; therefore, our result is significant at the 5% level. In other words, if all the assumptions of the Hardy–Weinberg model have been met, then there is less than a 5% chance that our results differ so greatly from Hardy–Weinberg equilibrium frequencies due to sampling error alone. We conclude that it is likely that one or more of the Hardy–Weinberg model's assumptions have been violated in this case.

Suppose that our population had instead been at or near Hardy–Weinberg equilibrium. Could we have then definitively concluded that all the Hardy–Weinberg assumptions had been met? While this *could* have been the case; the answer is no: A population may be in Hardy–Weinberg equilibrium even as one or more of the model's assumptions are violated. For example, ongoing mutation could occur without shifting the genotype frequencies away from Hardy–Weinberg proportions.

the myoglobin locus. For one thing, while we know that the genotype frequencies are currently in Hardy–Weinberg proportions, we do not yet know that they will remain there. Furthermore, even if we could show that the Hardy–Weinberg assumptions were met at this one locus, this would not mean that they would be met at *all* loci in the human genome. Indeed, we know that the assumptions are in fact violated: We have evidence that the evolutionary processes of natural selection, assortative mating, mutation, and drift all operate on human populations. Later in this chapter, we will look at an example in which two of these processes, mutation and selection, oppose one another in human populations.

7.3 Natural Selection

In the previous section, we examined what happens to genotype frequencies when the Hardy–Weinberg assumptions are met. In this section, we will extend our model to include the action of natural selection. Before doing so, let us begin by sketching out an example of natural selection that occurs in the wild. From there, we will use the data from this example to make predictions about allele frequency change.

Selection for Coat Color in Pocket Mice

As an example of natural selection in the wild, we return to the character of coat color in mice, which we discussed in depth in Chapter 3. Here, we will consider not the oldfield mouse but instead a related species, the rock pocket mouse (*Chaetodipus intermedius*), which Hopi Hoekstra studied with Michael Nachman and Susan D'Agostino (Nachman et al. 2003; Hoekstra et al. 2004; Nachman 2005). Pocket mice live in rocky areas at low elevations in the Sonoran and Chihuahuan deserts and are well adapted to desert life. Within the confines of the desert, *C. intermedius* lives in one of two very different types of habitat—either on light-colored rocks or on much darker rocks associated with lava flows. Mice that live on light-colored rocks tend to have a sandy, gray coat color, while mice that inhabit lava fields are darker (**Figure 7.11**) (Benson 1933; Dice and Blossom 1937). Just as with the oldfield mice in Chapter 3, coat color influences predation risk for pocket mice. Pocket mice whose coat colors match their environment are much less susceptible to predation than mice that stand out against the rocks they inhabit (Dice 1947). We would expect, then, that natural selection would favor individuals with coat colors that offer camouflage in their natural environment.

FIGURE 7.11 Pocket mice live in light and dark rock habitats. **(A)** Light-colored rock habitat and light- and dark-coated pocket mice on light rock. **(B)** Dark lava field habitat and light- and dark-coated pocket mice on dark rock.

The genetic control of coat color in rock pocket mice is also very similar to the genetic control seen in oldfield mice. In pocket mice, coat coloration is influenced by the same melanocortin-1 receptor (Mc1R) that we described for oldfield mice in Chapter 3. In the pocket mice, the *Mc1R* locus has two alleles that

we will call D and d. The D allele is associated with dark coloration, whereas the d allele is associated with light coloration (Nachman et al. 2003). D is dominant to d, so that DD and Dd individuals both display dark coloration, and only individuals with the dd genotype display light coloration.

Here, we have a system in which an important character—coloration—is associated with a single locus and clearly tied to survival. But just how beneficial is it for an individual to have the allele coding for a coat coloration that matches the background environment; that is, how advantageous is it for mice on the dark lava fields to be DD or Dd and for mice along the light-colored rocks to be dd?

To address this question, Nachman and his colleagues collected individuals at both lava sites and light-colored rock sites in an area along the border between Arizona and Mexico (Nachman et al. 2003; Hoekstra et al. 2004). Most individuals at the lava sites were dark-colored, and most individuals at the light-colored rock sites were light-colored. Each population, however, had a number of individuals that were "mismatched"; that is, individuals whose coats did not match their environment. From their data on survival and migration, the researchers were able to demonstrate that light-colored pocket mice living in the dark lava fields suffered higher rates of mortality. Their chances of survival ranged from 60% to 98% of the chances of survival of dark-colored mice on the dark lava fields. With these data in hand, we can now start to make specific predictions about how the frequencies of the D and d alleles should change as a result of natural selection. To do so, we must build a mathematical model of natural selection that we can then use to examine the pocket mouse example.

A Simple Model of Natural Selection

We begin with the Hardy–Weinberg model, but we will relax Hardy–Weinberg assumption number 1: We will now allow natural selection to operate on our population. To use the terminology we developed earlier in discussing the Hardy–Weinberg model, but also to allow us to link back to the pocket mouse example, let us again consider two alleles—allele A_1 (at frequency p) and allele A_2 (at frequency q). Think of A_1 as the D allele for dark coloration in our mouse example, and let A_2 represent the d allele for light coloration. Because A_1 is dominant to A_2, both the A_1A_1 and A_1A_2 genotypes display dark coloration. But against a dark lava field, only the A_2A_2 individuals stand out and suffer a reduced survival probability. On the lava fields, natural selection is thus acting against the A_2 allele.

To quantify the strength of natural selection against allele A_2, we use a parameter called the **selection coefficient**, labeled s, to describe the fitness reduction of the light phenotype relative to the dark phenotype. By convention, the fitness of one type—here the dark phenotype—is set to 1. The fitness of the other phenotype—here the light phenotype—is set to $1 - s$. The value $s = 0$ indicates no selection against an allele; $s = 0.25$ indicates a 25% reduction in fitness, $s = 0.50$ indicates a 50% reduction in fitness, and so forth. For light-colored mice in dark lava environments, Nachman and his team measured survival probabilities ranging from 98% to 60% of that experienced by the dark-colored mice, depending on the population examined. As a result, they estimated selection coefficients against light coloration ranging from 0.02 to 0.40. In our mathematical example, we will use a selection coefficient $s = 0.1$.

TABLE 7.1
Fitnesses for a Dominant Locus

A_1 DOMINANT TO A_2

Genotype	Fitness
A_1A_1	1
A_1A_2	1
A_2A_2	$1 - s$

Our goal now is to predict the change in allele and genotype frequencies over time as the result of natural selection, with intensity quantified by the selection coefficient s. We begin by constructing a table of genotypes and their corresponding fitness values (**Table 7.1**). In this table, fitness is a measure of the relative lifetime reproductive success of our three genotypes.

For example, imagine that before natural selection operates, we have 100 A_1A_1, 100 A_1A_2, and 100 A_2A_2 individuals in our population, but after selection, the numbers are reduced to 60 A_1A_1, 60 A_1A_2, and 54 A_2A_2. If we denote the fitnesses of A_1A_1 and A_1A_2 as 1, the relative fitness of A_2A_2 is $(54/100)/(60/100) = 54/60 = 0.9$. As such, $s = 0.1$. **Box 7.5** demonstrates how we can make detailed predictions regarding allele frequency change when natural selection is operating in the case of the pocket mouse.

For example, Box 7.5 demonstrates that when A_1 is dominant, the frequency of the A_1 allele should increase by $pq^2s/(1 - q^2s)$ in every generation. **Figure 7.12** uses this expression to plot the way that the allele frequency of A_1 would change over evolutionary time for three different values of s. In our rock pocket mouse example where $s = 0.1$, if the frequency of the dominant dark allele started at a frequency of just 0.005 in dark lava environments, we would expect that within 400 generations, it would increase to a frequency near 1.

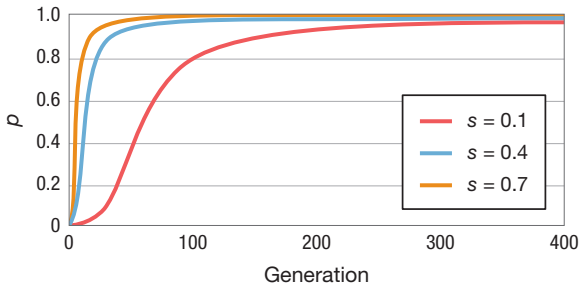


FIGURE 7.12 The consequences of natural selection favoring a dominant allele. The larger the selection coefficient, the stronger the action of natural selection. As a result, allele frequencies change faster and the A_1 allele approaches fixation earlier when $s = 0.7$ than when $s = 0.4$ or $s = 0.1$. Here, we plot the *trajectory*—the path over time—of the frequency p of the dominant A_1 allele for three different selection coefficients. The horizontal axis indicates time in generations, and the vertical axis, ranging from 0 to 1, indicates the frequency of the A_1 allele. The initial frequency of the A_1 allele is 0.005.

Modes of Frequency-Independent Selection

In the example we just considered, the genotypes producing the dark phenotype are favored over the genotypes producing the light phenotype, irrespective of the frequency of each type. Dark mice have the same fitness in a given environment, regardless of whether their coloration is rare or common. Our mouse example is an instance of **frequency-independent selection**, where the fitness associated with a trait is not directly dependent on the frequency of the trait in a population.

In general, there are a number of ways in which frequency-independent selection can operate. These differ in how the relative fitnesses of the A_1A_1, A_1A_2, and A_2A_2 genotypes vary in relation to one another.

Directional Selection

The most straightforward type of frequency-independent selection is known as **directional selection**. Under directional selection, one allele is consistently favored over the other allele. As a result, selection drives allele frequencies in a single direction, toward an increasing frequency of the favored allele. Eventually, the favored allele will become *fixed* in the population: It will replace all other alternative alleles at the same locus. When an allele becomes fixed, we say that it has reached **fixation**. (Strictly speaking, an allele will never reach complete fixation under the infinite population size assumption of the Hardy–Weinberg model, but it will get arbitrarily close. For simplicity of language, we will speak of this as fixation.)

In our basic model of directional selection, fixation of the favored allele A_1 is an equilibrium. Once A_1 is fixed, allele frequencies do not change further. Moreover,

BOX 7.5 Natural Selection Favoring a Dominant Allele

Here, we build a model in which natural selection acts on a trait controlled by the A locus, where the A_1 allele is dominant to the A_2 allele. As in Table 7.1, fitnesses are as follows:

Genotype	Fitness
A_1A_1	1
A_1A_2	1
A_2A_2	$1-s$

Genotype frequencies before and after selection are therefore as follows:

Genotype	A_1A_1	A_1A_2	A_2A_2
Frequency before selection	p^2	$2pq$	q^2
Frequency after selection	p^2	$2pq$	$q^2(1-s)$

We know that $p^2 + 2pq + q^2 = 1$, so $p^2 + 2pq + q^2(1-s)$ must be less than 1. Yet, the A_1A_1, A_1A_2, and A_2A_2 genotypes make up our entire population after selection, so their frequencies must sum to 1. To arrive at a sum of 1, we take the frequency of each genotype after selection and divide it by the sum of these frequencies:

A_1A_1	A_1A_2	A_2A_2
$\frac{p^2}{[p^2+2pq+q^2(1-s)]}$	$\frac{2pq}{[p^2+2pq+q^2(1-s)]}$	$\frac{q^2(1-s)}{[p^2+2pq+q^2(1-s)]}$

Expanding the denominator, we get $p^2 + 2pq + q^2 - q^2s$. If we replace $p^2 + 2pq + q^2$ with 1, our denominator equals $1 - q^2s$.

A_1A_1	A_1A_2	A_2A_2
$\frac{p^2}{1-q^2s}$	$\frac{2pq}{1-q^2s}$	$\frac{q^2(1-s)}{1-q^2s}$

Because $p = f[A_1A_1] + f[A_1A_2]/2$, it follows that p', the frequency of allele A_1 in the offspring, will be

$$p' = \frac{p^2 + pq}{1 - q^2s} = \frac{p}{1 - q^2s}$$

To see this, factor the numerator, $p^2 + pq$, to $p(p + q)$ and replace $p + q$ with 1.

We can use the fact that $p + q = 1$ (and so $q = 1 - p$) to write our last equation slightly differently, as

$$p' = \frac{p}{1 - s(1-p)^2}$$

This form of the equation for p' is called a recursion equation because it shows us what p' is in direct relation to p.

We can also write an expression for the change in allele frequency $p' - p$:

$$p' - p = \frac{p}{1 - q^2s} - p$$

To give both terms a common denominator, multiply the second term by

$$\frac{1 - q^2s}{1 - q^2s}$$

This gives us

$$\begin{aligned} p' - p &= \frac{p}{1 - q^2s} - \frac{p(1 - q^2s)}{1 - q^2s} \\ &= \frac{p}{1 - q^2s} - \frac{p - pq^2s}{1 - q^2s} \\ &= \frac{p - p + pq^2s}{1 - q^2s} \\ &= \frac{pq^2s}{1 - q^2s} \end{aligned}$$

This expression is useful for computing the expected change in allele frequencies from one generation to the next, given the fitnesses listed at the top of this box. For example, we used it to generate the results shown in Figure 7.12.

it is a *stable* equilibrium (see Box 7.1). If allele frequencies are perturbed slightly away from fixation of A_1—for example, a few A_2 individuals are introduced into the population—the population will quickly return to its original state: fixation of A_1. Technically, in this model, fixation of A_2 is also an equilibrium. If A_2 is fixed entirely, then there are no A_1 alleles to increase in frequency, and thus allele frequencies do not change either. But fixation of A_2 is an *unstable* equilibrium. If this system is perturbed slightly by the addition of a few A_1 alleles, the system will not return to fixation of A_2, but will instead go all the way to fixation of A_1 at the other extreme.

Figure 7.13 illustrates various ways in which directional selection could operate in favor of the A_1 allele. When A_1 is dominant to A_2, the genotypes A_1A_1 and A_1A_2 produce the same phenotype and have the same fitness, but A_2A_2 has a lower fitness. When A_1 and A_2 exhibit incomplete dominance, all three genotypes produce different phenotypes, with the A_1A_2 heterozygote presenting a phenotype intermediate to the two homozygote phenotypes. In this case, A_1A_1 has the highest fitness, A_1A_2 has an intermediate fitness, and A_2A_2 has the lowest fitness. When A_1 is recessive to A_2, the A_1A_1 genotype has the highest fitness, while A_1A_2 and A_2A_2 produce the same phenotype and share the same lower fitness value.

Rates of Fixation under Directional Selection

The plot of allele frequency trajectories in Figure 7.13 not only tells us that the A_1 allele eventually goes to fixation in all three cases, but also informs us about the rate at which this process occurs. Given the same fitness difference between the A_1A_1 and A_2A_2 homozygotes, the A_1 allele approaches fixation most rapidly in the case of incomplete dominance, somewhat less rapidly in the dominant case, and much more slowly in the recessive case.

We would like to explain two qualitative features of these trajectories. First, why does a rare A_1 allele quickly increase in frequency in the dominant and incompletely dominant cases, but not in the recessive case? Second, once A_1 is common, why does it take a long time to go to fixation in the dominant case but not in the incompletely dominant or recessive cases? We address these questions in turn.

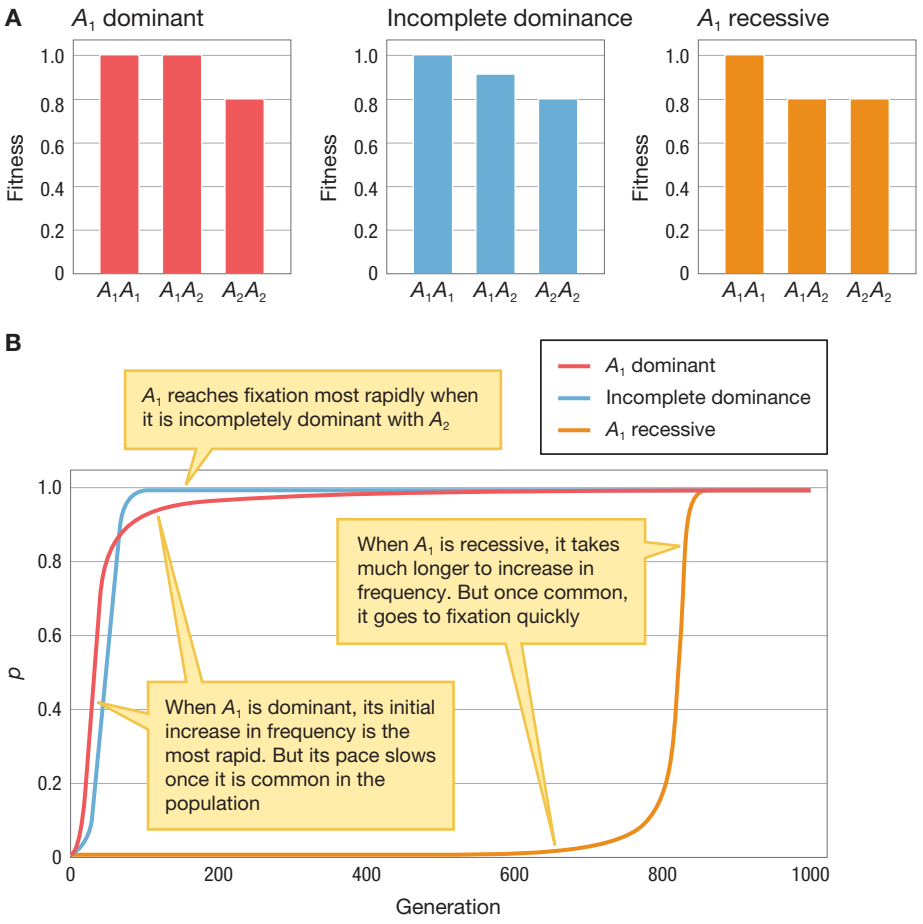


FIGURE 7.13 Directional selection at one locus with two alleles. **(A)** In directional selection, one allele A_1 is favored over another, A_2. This can occur in different ways: A_1 can be dominant (red), A_1 and A_2 can show incomplete dominance (blue), or A_1 can be recessive (orange). **(B)** The trajectories of p, the frequency of the A_1 allele, are illustrated from a starting value of $p = 0.005$.

We can understand why a rare A_1 allele quickly increases in frequency in the dominant and incompletely dominant cases but not in the recessive case by considering the genotypes in which A_1 and A_2 typically occur and at the average selective differences that result. Suppose that A_1 is initially rare, as shown in Figure 7.13. Then, initially, most copies of the A_1 allele appear in A_1A_2 heterozygotes. When A_1 is dominant to or incompletely dominant with A_2, these heterozygotes enjoy a selective advantage, and thus the frequency of the A_1 allele responds immediately with a sizeable increase. But when A_1 is recessive, the heterozygotes have the same fitness as the A_2A_2 homozygotes that make up the majority of the population. Selection increases the frequency of the allele A_1 only in the rare events in which A_1A_1 homozygotes—which see the fitness benefits—are produced.

Once the A_1 allele becomes more common in the population, it starts to occur in A_1A_1 homozygotes an appreciable fraction of the time, but the A_2 allele now typically appears in heterozygotes. When A_1 is dominant to A_2, this means that most A_2 alleles now appear in individuals with the same phenotype as A_1A_1 homozygotes. Because this is an advantageous phenotype, there is no longer strong selection against the A_2 allele. Selection slows down, and it takes a very long time to eliminate the A_2 allele entirely from the population. Rare recessive alleles mostly reside in heterozygotes where they suffer no fitness disadvantage. When A_1 and A_2 are incompletely dominant, however, there is no way for A_2 alleles to hide from the effects of selection. Thus, in the incompletely dominant case, selection against A_2 continues to be strong even once A_1 becomes very common. As a result, the A_2 allele is more quickly removed from the population.

KEYCONCEPT QUESTION

7.2 Two alleles at the *A* locus, A_1 and A_2, are under directional selection, with A_1 favored and A_2 disfavored. Each is currently at frequency 0.5 in the population. In which situation will A_1 be fixed more quickly: when A_1 is dominant or when A_1 is recessive? Explain.

Overdominance and Underdominance

There are two additional ways that frequency-independent selection can act on one locus with two alleles. In the case of **overdominance**, also known as **heterozygote advantage**, the A_1A_2 heterozygote has a higher fitness than either the A_1A_1 or the A_2A_2 homozygotes (**Figure 7.14**). In this case, the direction of natural selection depends on the current allele frequencies in the population. When A_1 is rare, it will usually occur in heterozygotes. As a result, the average fitness of individuals carrying the A_1 allele will be higher than the average fitness of all individuals in the population. But when it is common, the A_1 allele will usually occur in A_1A_1 homozygotes that have a lower fitness than the population average. As a result, A_1 increases in frequency when rare and decreases in frequency when common.

When overdominance occurs, natural selection leads to a **balanced polymorphism**, a stable equilibrium that is *polymorphic*; that is, in which both alleles are present. Because this is a stable equilibrium, allele frequencies will return to their equilibrium values after a perturbation away from the equilibrium. We refer to selection that leads to a balanced polymorphism as **balancing selection**. In this system, we also have an equilibrium in which A_1 is fixed and one

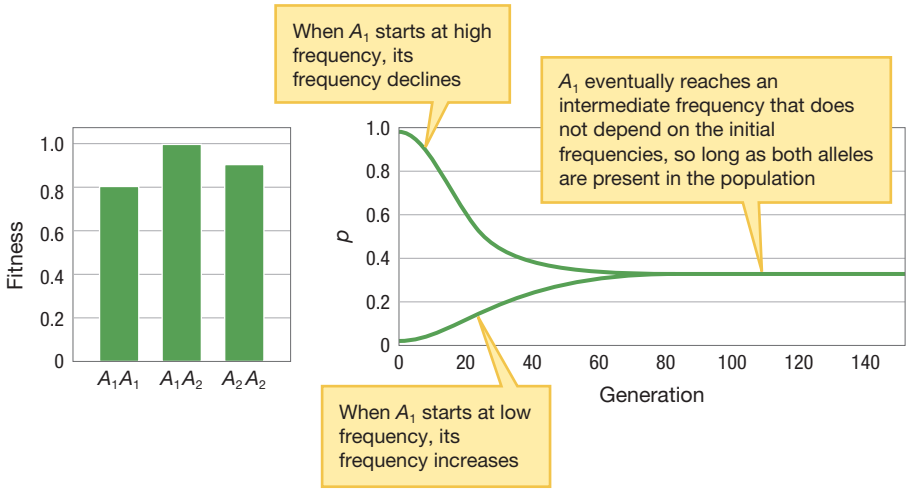


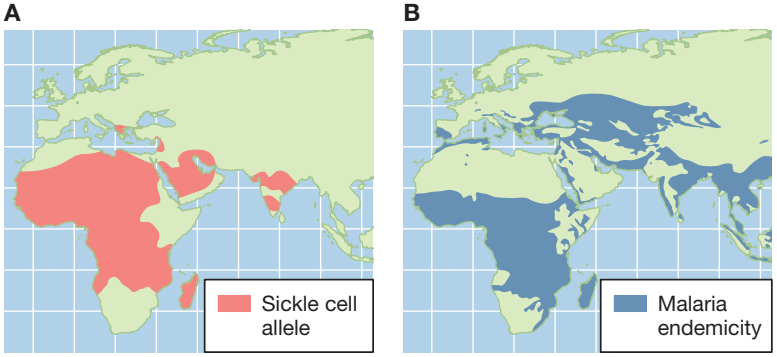
FIGURE 7.14 Overdominance. In the case of overdominance, the heterozygote has a higher fitness than either homozygote. Irrespective of the initial frequencies, so long as both alleles are present in the population, the resulting fitness trajectory leads to an intermediate frequency of the A_1 and A_2 alleles. Here, we show trajectories with random mating and initial frequencies $p = 0.025$ and $p = 0.975$.

in which A_2 is fixed, but these are unstable equilibria. As soon as the second allele is introduced, the system moves away from fixation of one allele and toward the stable equilibrium in which both alleles are present at intermediate frequencies.

The sickle cell mutation in the human hemoglobin gene is a classic example of overdominance. In its homozygous form, the sickle cell allele induces a change in the shape of red blood cells from a round disk to a "sickle" shape, hence the name. These sickle-shaped cells clump together, preventing blood from flowing smoothly through the circulatory system. As a result, affected individuals suffer numerous health problems including anemia, chronic pain, bacterial infection, organ damage, and ultimately reduced life expectancy. But in its heterozygous form, the sickle cell mutation does not cause significant pathology. Rather, this mutation inhibits the growth and spread of the malaria parasite within the bloodstream and thus is partially protective against malarial disease (Gong et al. 2013). As a result, sickle cell heterozygotes actually have a fitness advantage in areas where malaria is endemic. Consequently, the sickle cell allele has reached relatively high frequencies in populations that originated in these areas but is rare in other populations (**Figure 7.15**).

We should not let the elegance of the sickle cell example generate a misleading impression about the importance of overdominance as a mechanism of preserving genetic polymorphism. While a few such cases are well known—the HLA loci involved in immune recognition and the ABO locus that determines blood type are good examples—overdominance is probably quite rare in general. When we see a balanced polymorphism, we should not rush to conclude that it is the result of overdominance (Bubb et al. 2006).

FIGURE 7.15 The sickle cell allele is common where malaria is endemic and rare elsewhere. **(A)** Geographic range of the sickle cell allele in human populations. **(B)** Historical geographic distribution of endemic malaria. Adapted from Piel et al. (2010).



Underdominance is the reverse of overdominance. In underdominance, the A_1A_2 heterozygote has a lower fitness than either the A_1A_1 or A_2A_2 genotype. In this situation, fixation of either allele is a stable equilibrium. Natural selection will favor one allele over the other—but which allele becomes fixed in the population will depend on where the population starts (**Figure 7.16**). If A_1 is very rare, it will typically appear in A_1A_2 heterozygotes that have

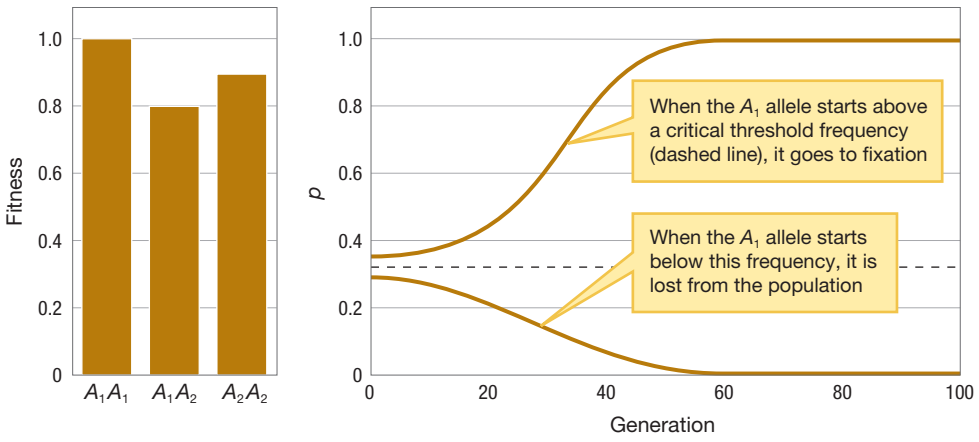


FIGURE 7.16 Underdominance. In the case of underdominance, the heterozygote has lower fitness than either homozygote. The resulting fitness trajectory leads to fixation of one allele or the other, depending on the starting allele frequencies. Above the threshold (dashed line) the A_1 allele goes to fixation. Below the unstable equilibrium, the A_1 allele is lost from the population. Here, we show trajectories for the frequency p of the A_1 allele, with random mating and initial frequencies $p = 0.30$ and $p = 0.36$.

lower-than-average fitness, so it will be lost. When A_1 is very common, the A_1 allele will typically appear in A_1A_1 homozygotes that have higher-than-average fitness, so it will go to fixation. The same holds true for the A_2 allele. As a result, in the case of underdominance, there is a threshold frequency of the A_1 allele, above which A_1 will be fixed and below which A_1 will be lost.

Real-world examples of underdominance are even harder to find than are real-world examples of overdominance. This makes sense: Polymorphisms responsible for underdominance will be lost as one allele or the other goes to fixation. One case of underdominance that can be observed occurs when a mouse population contains hybrid New Zealand Black (NZB)/New Zealand White (NZW) mice (Helyer and Howie 1963a,b; Theofilopoulos and Dixon 1985). NZW homozygotes are phenotypically normal, while NZB homozygotes exhibit a number of autoimmune defects. The NZB/NZW heterozygotes experience even more severe autoimmune disease, and they are used as a medical model of the human autoimmune disease lupus (**Figure 7.17**).

In both overdominance and underdominance, there is an intermediate equilibrium frequency of the A_1 allele at which the average fitnesses of the A_1 and A_2 alleles are the same, assuming random mating. In the case of overdominance, this equilibrium frequency is a *stable* equilibrium, and it is the allele frequency that we observe at the balanced polymorphism. In the case of underdominance, this equilibrium is an *unstable* equilibrium, and it represents the threshold allele frequency above which A_1 goes to fixation and below which A_2 goes to fixation. These equilibrium frequencies can be calculated as shown in **Box 7.6**.

In the cases of overdominance and underdominance, the direction of selection depends on the frequencies of the A_1 and A_2 alleles. So why do we classify overdominance and underdominance as forms of frequency-*independent* selection? The answer is that when we talk about frequency-independent selection or frequency-dependent selection, we are referring to the way that the fitnesses of *phenotypes* (or the genotypes that produce them) depend on the frequencies of *phenotypes*—not on the way that the average fitnesses of individuals carrying a given allele depend on the frequencies of those alleles. In both overdominance and underdominance, the fitness of each genotype, and its corresponding phenotype, is constant and independent of the frequencies of the genotypes in the population. The fitnesses of individuals carrying the A_1 and A_2 alleles vary according to the frequencies of those alleles only because these frequencies determine the chance that any given A_1 allele or any given A_2 allele ends up in a heterozygote instead of a homozygote.

FIGURE 7.17 Heterozygotes and autoimmune defects. The NZB/NZW hybrid mouse develops severe autoimmune disease and is used as a model system for the study of lupus. Because the heterozygote has a lower fitness than either homozygote, this is an example of underdominance.

BOX 7.6 Equilibrium Allele Frequencies in Overdominance and Underdominance

Both the balanced polymorphism due to overdominance and the critical threshold due to underdominance are equilibria—and in both cases, allele frequencies can be computed in the same way. The key is to recognize that at a polymorphic equilibrium, the average fitness of the A_1 allele is precisely equal to the average fitness of the A_2 allele. If we can find the frequency p of the A_1 allele such that the fitnesses of these two alleles are the same, we have found the equilibrium.

Let w_{11} be the fitness of the A_1A_1 homozygote, w_{12} be the fitness of the A_1A_2 heterozygote, and w_{22} be the fitness of the A_2A_2 homozygote. Assuming random mating, no mutation, no migration, and large population size, the frequencies of each genotype before selection are simply Hardy–Weinberg frequencies. Thus, before selection, a fraction p of the A_1 alleles are in homozygotes, and a fraction $1-p$ are in heterozygotes. As a result, the average fitness of the A_1 allele is $pw_{11} + (1-p)w_{12}$. By similar logic, a fraction $1-p$ of the A_2 alleles are in homozygotes and a fraction p are in heterozygotes, so the average fitness of the A_2 allele is $pw_{12} + (1-p)w_{22}$. At equilibrium, these average fitnesses are precisely equal, giving us the equation

$$pw_{11} + (1-p)w_{12} = pw_{12} + (1-p)w_{22}$$

Solving for p, we get

$$p = \frac{w_{22} - w_{12}}{w_{11} - 2w_{12} + w_{22}} = \frac{w_{22} - w_{12}}{(w_{11} - w_{12}) + (w_{22} - w_{12})}$$

This is the equilibrium frequency of A_1: the frequency of the balanced polymorphism in the overdominance case, and the frequency of the critical threshold in the underdominance case. From the latter form of this expression, we can see that the equilibrium frequency p will be closer to 0 when the heterozygote fitness w_{12} is closer to the A_2 homozygote fitness w_{22}, and it will be closer to 1 when w_{12} is closer to the A_1 homozygote fitness w_{11}.

Applying this equation to the overdominance example in Figure 7.14, we get

$$p = \frac{0.9 - 1}{(0.8 - 1) + (0.9 - 1)} = 1/3$$

Indeed, $p = 1/3$ is the frequency of A_1 at the balanced polymorphism that is reached in that example. Applying this same equation to the underdominance example in Figure 7.16, we get

$$p = \frac{0.9 - 0.8}{(1 - 0.8) + (1 - 0.9)} = 1/3$$

And, indeed, $p = 1/3$ is the frequency of A_1 at the critical threshold in that example.

Modes of Frequency-Dependent Selection

Frequency-dependent selection occurs when the costs and benefits associated with a trait depend on its frequency in the population. Frequency-dependent selection can be *positive* or *negative*; we will treat these two cases in turn. With positive frequency-dependent selection, the fitness associated with a trait *increases* as the frequency of the trait increases in a population (**Figure 7.18**). Thus, under positive frequency-dependent selection, each phenotype is favored once it becomes sufficiently common in the population. If the phenotypes are controlled by two alternative alleles at a single locus, one of the two alleles will eventually be fixed and the other will be lost—although which is fixed and which is lost depends on the initial allele frequencies.

For example, land snails have shells that either coil to the right or coil to the left. In the so-called flat snail species, individuals mate in a face-to-face position, and because of physical constraints, mating in these species can only take place between individuals whose shells coil in the same direction (**Figure 7.19**) (Asami et al. 1998). In such a situation, positive frequency-dependent selection operates on the direction of the shell's coil. The higher the frequency of either type of shell—right coil or left coil—the greater the mating success of that type, because

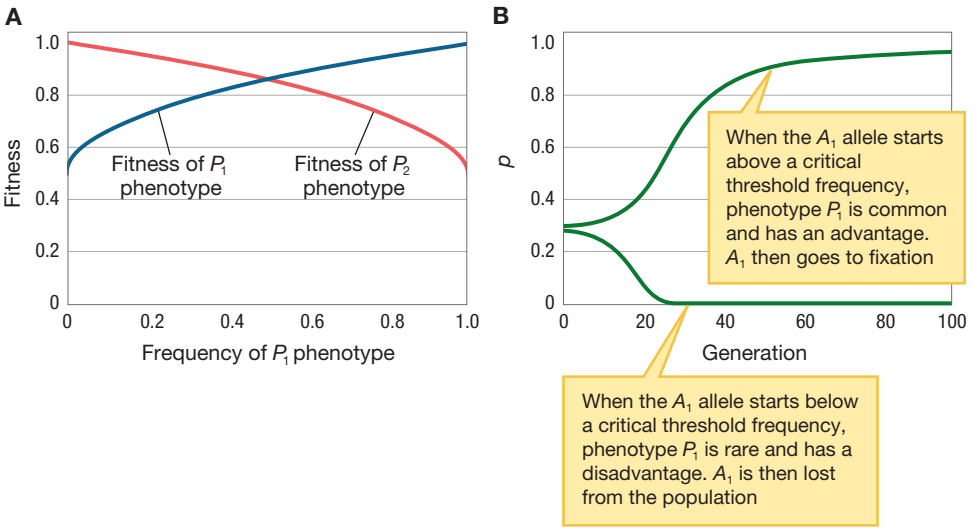


FIGURE 7.18 Positive frequency-dependent selection. The more frequent a phenotype, the higher its fitness. **(A)** The fitnesses of phenotypes P_1 and P_2 depend on the frequency of the P_1 phenotype. **(B)** Suppose that the phenotype in question is determined by a single locus A, with A_1 dominant to A_2. That is, the A_1A_1 and A_1A_2 genotypes exhibit phenotype P_1, while the A_2A_2 genotype exhibits phenotype P_2. The frequency p of the A_1 allele over time then depends on the starting frequency of A_1. If A_1 is sufficiently common, it will be fixed; otherwise, it will be lost.

more potential mates exist, and thus mates are easier to find. Notably, in so-called tall species of land snails, males mount females from above, and therefore snails with shells that coil in opposite directions can still mate. In this situation, we see much weaker frequency dependence than in flat snails.

With negative frequency-dependent selection, the fitness associated with a trait *decreases* as the frequency of the trait *increases* in a population. Thus, under negative frequency-dependent selection, each phenotype is favored when it is rare. If the phenotypes are controlled by two alternative alleles at a single locus, both alleles will be maintained in a balanced polymorphism (**Figure 7.20**). Thus, negative frequency-dependent selection, like overdominance, is a form of balancing selection.

Since the early 1980s, researchers have known that some aspects of foraging behavior in *Drosophila* larvae are under genetic control. Some larvae, known as "rovers," explore their environment widely even when food is present, whereas others, known as "sitters," exhibit limited movement as long as they have something to eat (Sokolowski 1980, 2001) (**Figure 7.21**). Both types are readily observed in natural populations of *Drosophila melanogaster*. Why is this polymorphism preserved? In other words, why hasn't one type or the other gone to fixation? To answer this question, we need to understand both the genetics involved and the ecological context in which selection acts on this behavior.

In a series of genetic studies, Maria Sokolowski and her colleagues demonstrated that the sitter/rover distinction is due to a single polymorphism at a locus called, appropriately enough, *forager*. Homozygotes for the recessive for^S (forager-sitter) allele display sitter behavior, whereas individuals with at least one copy of the dominant for^R (forager-rover) allele exhibit rover behavior (de Belle 1987, 1989; Sokolowski 2001).

To explain why both alleles persist in natural populations of *Drosophila*, Sokolowski and her colleagues hypothesized that there is negative frequency-dependent selection for foraging behavior. You can imagine why this could be the case: In a population where "sitting" is common, any local food patch would be quickly exhausted, whereas a "roving" strategy might reveal yet-undiscovered

FIGURE 7.19 Mating between two *Euhadra congenita*, a "flat" species of snail. Mating in this species can only take place between individuals whose shells coil in the same direction.

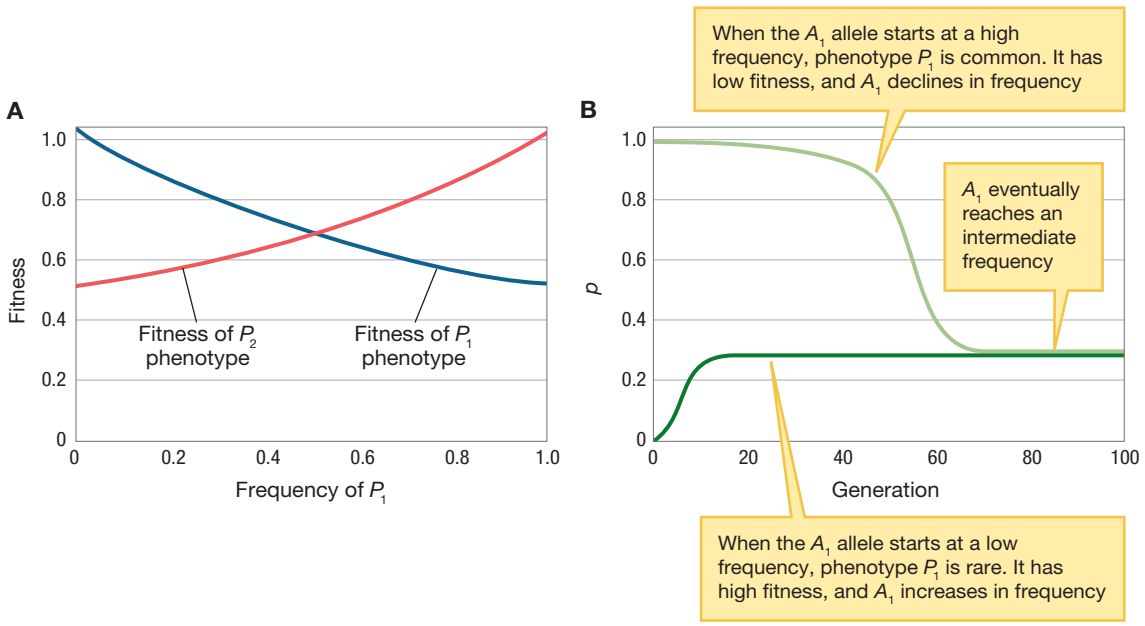


FIGURE 7.20 Negative frequency-dependent selection. The rarer a phenotype, the higher its fitness. **(A)** The fitnesses of phenotypes P_1 and P_2 depend on the frequency of the P_1 phenotype. **(B)** Suppose that the phenotype in question is determined by a single locus A, with A_1 dominant to A_2. That is, the A_1A_1 and A_1A_2 genotypes exhibit P_1, while the A_2A_2 genotype exhibits phenotype P_2. So long as both alleles are present from the start, the frequency p of the A_1 allele reaches an intermediate value regardless of the starting allele frequencies.

food sources. In a population where "roving" is common, "sitters" could reap the benefits of a food patch while other individuals went exploring. To test this hypothesis, the researchers allowed sitter and rover individuals to compete in a laboratory environment in groups composed of different phenotype frequencies (Fitzpatrick et al. 2007). Starting with newly hatched (first instar) individuals, they raised larvae of both types in a shared environment until the larvae pupated. They reasoned that if negative frequency-dependent selection were operating, each type would outperform its competitor when rare and be outperformed when common. As a measure of fitness, they recorded the fraction of each type that successfully reached pupation.

In the absence of resource competition among the larvae, Sokolowski and her colleagues did not find evidence for their hypothesis. Instead, they observed that when the larvae were raised in a nutrient-rich environment, the rover type had a fitness advantage irrespective of its frequency in the population (**Figure 7.22A**). If this were the whole story, we would expect the *for*^{R} allele to become fixed in wild populations (contrary to observations). But it is not the whole story. When the researchers raised groups of larvae in a nutrient-poor environment, where resource competition was an important factor, they observed precisely the pattern predicted by the negative frequency-dependence hypothesis. Sitters outperformed rovers when sitters were rare, but rovers outperformed sitters when rovers were rare (**Figure 7.22B**). These findings provide support for the hypothesis that

KEYCONCEPT QUESTION

7.3 A stable polymorphism between the B_1 and B_2 alleles is maintained over many generations in a large population cage of lab mice. You hypothesize that this is the result of either negative frequency-dependent selection or overdominance. Briefly describe how you would design an experiment to distinguish between these alternatives and how you would interpret the results. Assume that you can easily tell the genotypes of your mice, you can set up new population cages, and you can measure the reproductive success of any or all individuals.

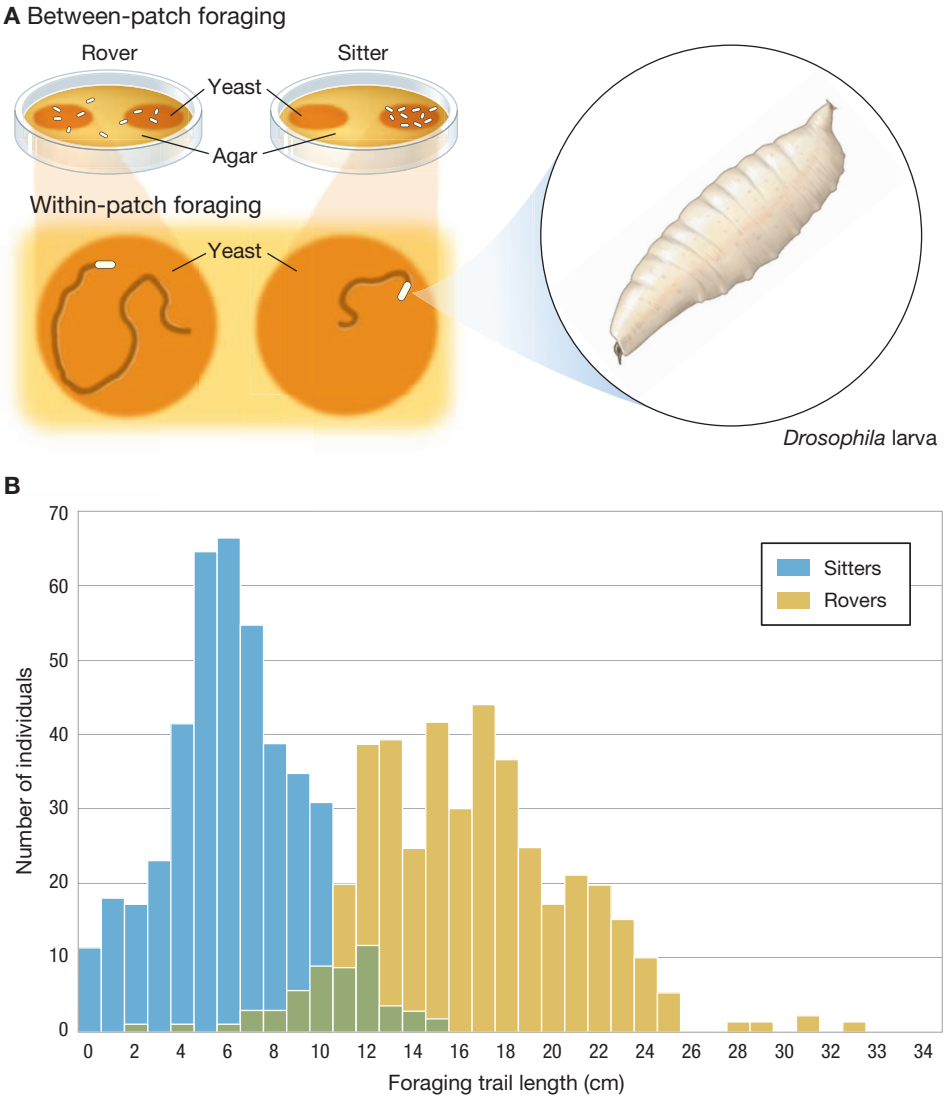


FIGURE 7.21 Two behavioral types of *Drosophila* larva. **(A)** The "sitter" phenotype stays put once it finds food, while the "rover" phenotype continues to explore even when food is present. **(B)** Even within a food patch, rovers travel greater distances than sitters. Adapted from Sokolowski (2001).

negative frequency-dependent selection is responsible for maintaining the for^R/for^S polymorphism in wild populations, where competition for resources is significant.

Viability Selection versus Fecundity Selection

Thus far in this chapter, most of our examples have involved viability selection: fitness differences that arise because of differences in rates of survival and mortality. But of course natural selection doesn't just favor survival, it favors individuals who leave the most surviving offspring. Thus, natural selection can operate on more than just survival probabilities; it can also act on the number of offspring produced, known as **fecundity**.

Recall that in Chapter 3, we defined fitness as expected reproductive success relative to other individuals in a population. In simple models (for example, those in which organisms are *semelparous*; that is, they reproduce only once at the end of their lives) it is straightforward to see how viability and fecundity differences combine to influence fitness. The expected reproductive success of an individual is equal to the probability that the individual survives to reproduce, multiplied by the number of offspring that are produced if the individual does survive. As

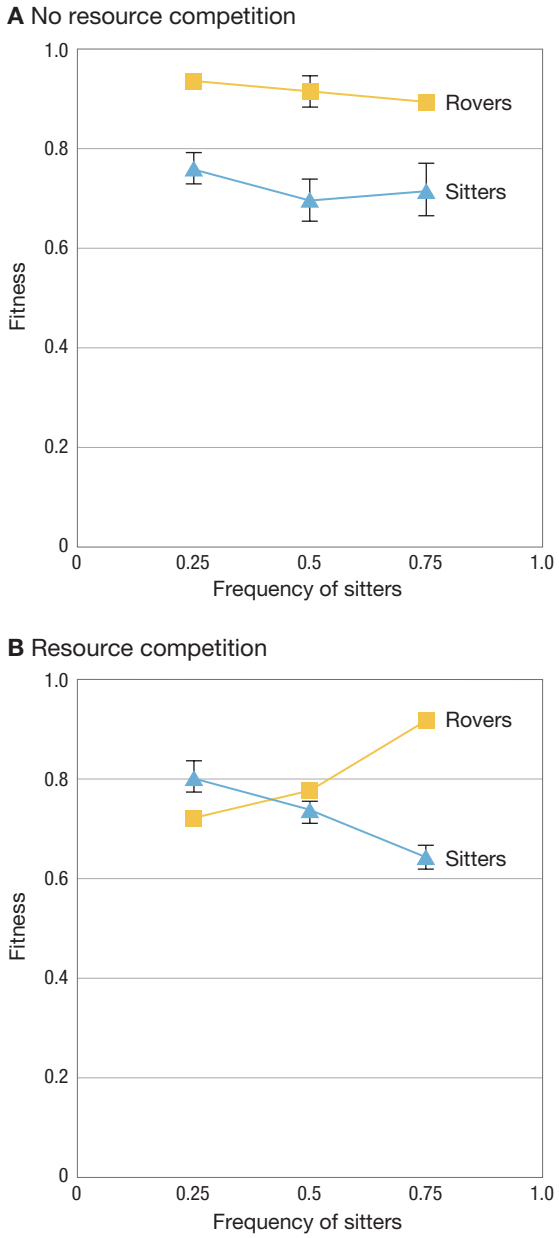


FIGURE 7.22 Negative frequency dependence under resource competition. **(A)** In the absence of resource competition, rovers had a higher fitness—measured as the probability of reaching pupation—than did sitters, irrespective of their frequency in the population. **(B)** Under resource competition, rovers had higher fitness when sitters were common, but lower fitness when sitters were rare. This provides evidence of negative frequency-dependent selection. Adapted from Fitzpatrick et al. (2007).

a consequence, viability and fecundity act equivalently in the simplest population genetic models. Halving the number of offspring produced or halving the probability of survival each halves an organism's fitness, and thus each has the same effect in these basic models.

A dramatic example of fecundity differences without viability differences comes from a study of sunflower hybrids formed when wild sunflowers (*Helianthus annuus*) are crossed with their domesticated relatives, the crop sunflower (also called *Helianthus annuus*) (**Figure 7.23**). From an applied perspective, crop scientists are highly concerned with what happens to these hybrids, because it is through such hybrids that novel genes from genetically modified crops could make their way into wild populations with potentially severe ecological consequences. Charity Cummings and her colleagues studied what happens to the genes from genetically modified crops after they were introduced into a wild sunflower population by crossing wild sunflowers with domesticated crop sunflowers to produce hybrids (Cummings et al. 2002).

To find out, the researchers set up three replicate study populations, each with 100 wild plants and 100 hybrid plants. They measured both the viability and fecundity components of each plant's fitness: survival rate and lifetime seed production. In terms of viability selection, there was little difference between hybrids and wild plants. But there were large fecundity differences: The hybrids suffered a striking reduction in lifetime seed production (**Table 7.2**). These data provided Cummings and her colleagues with fitness estimates for their experimental populations. From these fitness estimates, they were able to use a simple model of natural selection to predict the change in the frequency of crop sunflower alleles over the subsequent generation. In accord with their predictions, when they went back to measure strain frequencies after a single generation, they found that the frequency of crop sunflower alleles had dropped from 25% (half of their initial plants were hybrids, each with 50% crop sunflower alleles) in their initial plots to a mere 3% after one generation. Selection on fecundity, rather than on viability, rapidly eliminates these crop sunflower alleles from wild populations.

7.4 Mutation

As we saw in Chapter 6, genetic mutation generates the variation on which natural selection acts. In that chapter, we also saw that mutation is undirected. By this, we mean that mutations occur randomly with respect to their effects on the organism's fitness. Organisms may be able to alter the *rate* of mutation when under stress or in other circumstances (Bjedov et al. 2003), but to date there is no evidence whatsoever that they can affect the probability that a mutation will turn out to be favorable.

TABLE 7.2

Survival and Lifetime Seed Production for Hybrid and Wild Sunflowers[a]

Site	PLANT SURVIVAL (%)		TOTAL NUMBER OF VIABLE SEEDS	
	Hybrid (N = 100)	Wild (N = 100)	Hybrid	Wild
1	100	98	15,428	635,000
2	100	100	3026	274,453
3	99	100	11,960	671,200

[a]Hybrid plants do not suffer reduced viability, but their lifetime seed production is dramatically decreased. Adapted from Cummings et al. (2002).

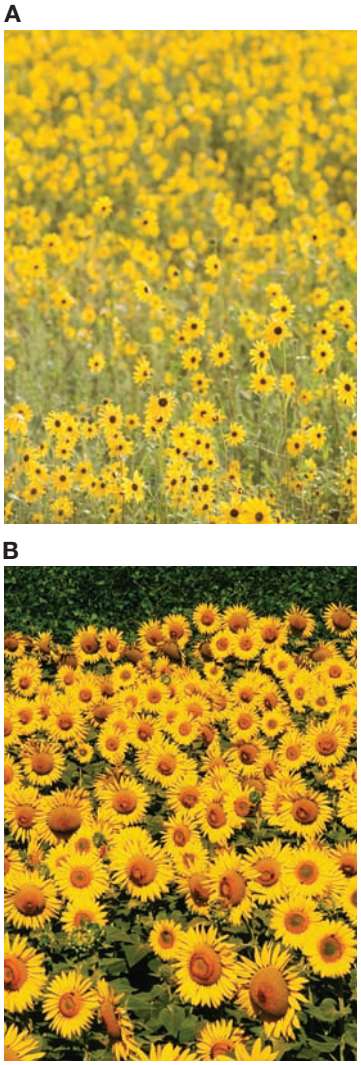


FIGURE 7.23 Wild and domestic sunflowers. (A) Wild sunflowers and **(B)** domestic sunflowers.

Mutation Can Change Allele Frequencies in a Population

Mutation, like natural selection, can influence allele frequencies in a population. As with the effects of natural selection, we can model this process mathematically. To understand how mutation rate alone can affect allele frequency, let us consider two alleles—A_1 and A_2. In the simplest case, imagine that allele A_1 mutates to allele A_2 with probability μ and that allele A_2 mutates to A_1 with probability ν (**Figure 7.24**).

Suppose that the frequency of the A_1 allele in the parental generation is p and the frequency of the A_2 allele is q. In **Box 7.7**, we show that when mutation is the only process operating to change allele frequencies, the equilibrium frequency of the A_1 allele, which we label p^*, is equal to $\nu/(\mu + \nu)$. Correspondingly, the equilibrium frequency of the A_2 allele is given by $q^* = \mu/(\mu + \nu)$. From this expression, we see that when μ is large relative to ν—that is, when A_1 mutates to A_2 much faster than vice versa—the A_2 allele will have a high frequency at equilibrium.

Figure 7.25 illustrates the change in allele frequencies in a population with mutation rates $\mu = 0.00010$/generation and $\nu = 0.00005$/generation. As expected, this population eventually approaches equilibrium when the A_1 allele is at frequency $p^* = 0.00005/(0.00005 + 0.00010) = 1/3$. Notice that this process typically operates far more slowly than does natural selection. Even with the exceptionally high mutation rate of $\mu = 0.00010$ that we have used in Figure 7.25, it takes tens of thousands of generations for the allele frequencies to approach equilibrium. Moreover, this is a stable equilibrium: If we perturb the allele frequencies slightly away from equilibrium in either direction, they will return to the equilibrium values.

We have now calculated what happens to allele frequencies as a result of mutation. But how does mutation affect *genotype* frequencies? Provided that the other Hardy–Weinberg assumptions are met (no selection, random mating, no migration, large population size), the genotype frequencies will always be in the Hardy–Weinberg proportions. Given frequencies p and q of alleles A_1 and A_2, the genotype frequencies for $A_1A_1 : A_1A_2 : A_2A_2$ will be $p^2 : 2pq : q^2$.

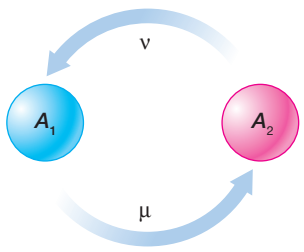


FIGURE 7.24 A model of mutation. The A_1 allele mutates to A_2 at rate μ, and A_2 mutates to A_1 at rate ν.

Mutation–Selection Balance

Thus far, we have looked at the consequences of selection without mutation and mutation without selection. In practice, both processes operate at the same

BOX 7.7 A Population Genetic Model of Mutation

Suppose that the frequency of the A_1 allele in the parental generation is p and the frequency of the A_2 allele is q. Then over the course of one generation, some A_1 alleles will convert to A_2 alleles by mutation (this happens with frequency $p\mu$), and some A_2 alleles will convert to A_1 alleles (with frequency $q\nu$). Thus, after one generation, the frequency p' of A_1 alleles in the population will be

$$p' = p(1-\mu) + q\nu = p(1-\mu) + (1-p)\nu$$

Correspondingly, the frequency q' of A_2 alleles in the population will be

$$q' = q(1-\nu) + p\mu = (1-p)(1-\nu) + p\mu$$

If mutation is the only process changing allele frequencies in such a population, then the frequency of the A_1 allele will eventually reach an equilibrium value, called p^*. At that equilibrium value, the allele frequency p' in one generation is unchanged from p in the previous generation: $p = p' = p^*$. Substituting p^* for both p and p' in the first equation above, we get $p^* = p^*(1-\mu) + (1-p^*)\nu$. With a little bit of algebra we can solve this equation for p^*, and when we do, we get $p^* = \nu/(\mu + \nu)$. Correspondingly, the equilibrium frequency of the A_2 allele is $q^* = \mu/(\mu + \nu)$.

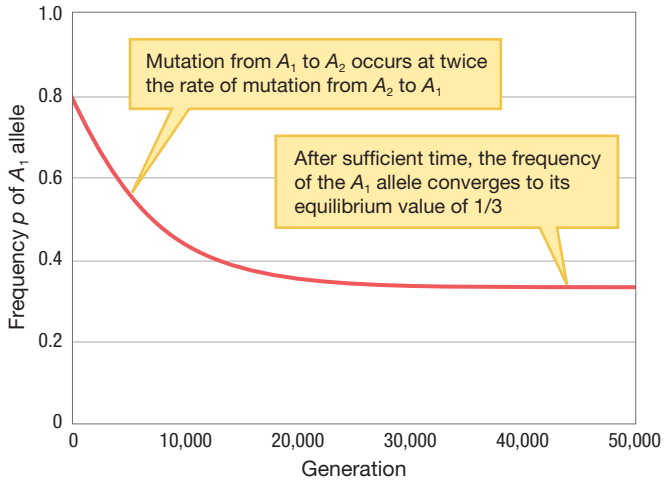


FIGURE 7.25 Mutation can cause changes in allele frequencies. Here, mutation from A_1 to A_2 occurs at twice the rate of mutation from A_2 to A_1. The frequency p of the A_1 allele eventually converges to a value of 1/3.

time—and their interaction is critically important to understanding why and at what frequency deleterious mutations remain present in populations. Using the same mathematical approach we have taken throughout this chapter, we can put both evolutionary processes together in a single model to get a picture of how natural selection can act to oppose deleterious mutations.

Suppose that we have two alleles, A_1 and A_2, at the A locus in a diploid population. Let A_1 be the normal or wild-type allele, and let A_2 be a deleterious recessive allele, such that the fitnesses for the three types A_1A_1, A_1A_2, and A_2A_2 are 1, 1, and $1 - s$, respectively. Further, let's assume that the A_1 allele mutates to the A_2 allele at rate μ. To keep the algebra simple, we assume that the rate of back mutation—mutation from A_2 back to A_1—is negligible. This is a reasonable assumption if we think of A_1 as coding for a functional form of a protein, while A_2 codes for a nonfunctional version. In such a case, there will be many ways to "break" the functional protein determined by A_1, so the mutation rate from functional to nonfunctional will be relatively high. By contrast, there will typically be only one way to fix any particular nonfunctional protein coded by A_2; namely, by reversing whatever specific change made it nonfunctional in the first place. As a result, the mutation rate from nonfunctional to functional will be relatively low.

In **Box 7.8**, we build our model in which selection and mutation operate in turn. When mutation and selection are the only two processes operating in our model, even though the A_1 allele is favored by selection, it will never be fixed in the population because A_2 alleles are continually being regenerated by the process of mutation, as illustrated in **Figure 7.26**. Eventually, the population will reach a steady state, a stable equilibrium frequency of the A_1 allele. The value of that steady state depends on whether the deleterious allele is recessive or dominant.

If the deleterious allele is recessive, that equilibrium occurs when the frequency of the wild-type allele is $p = 1 - \sqrt{\mu/s}$ and the frequency of the deleterious recessive allele is $q = \sqrt{\mu/s}$. Box 7.8 shows how these values can be derived. We call these frequencies the **mutation–selection balance**. At this equilibrium, the action of natural selection to increase the frequency of A_1 is exactly balanced by the action of mutation to produce new A_2 alleles. A similar set of calculations, not included here, reveals that if the deleterious allele is dominant instead of recessive, mutation–selection balance occurs when the frequency of the wild-type allele is approximately $p = (s - \mu)/s$ and the frequency of the deleterious dominant allele is approximately $q = \mu/s$.

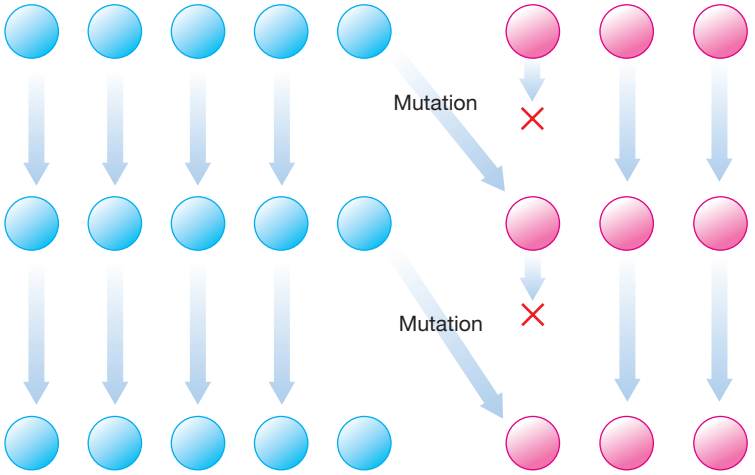


FIGURE 7.26 Mutation–selection balance. Deleterious mutation transforms A_1 alleles (blue) into selectively disfavored A_2 alleles (pink). As indicated by the red X's, natural selection eliminates some A_2 alleles from the population. At equilibrium, these two processes exactly balance one another.

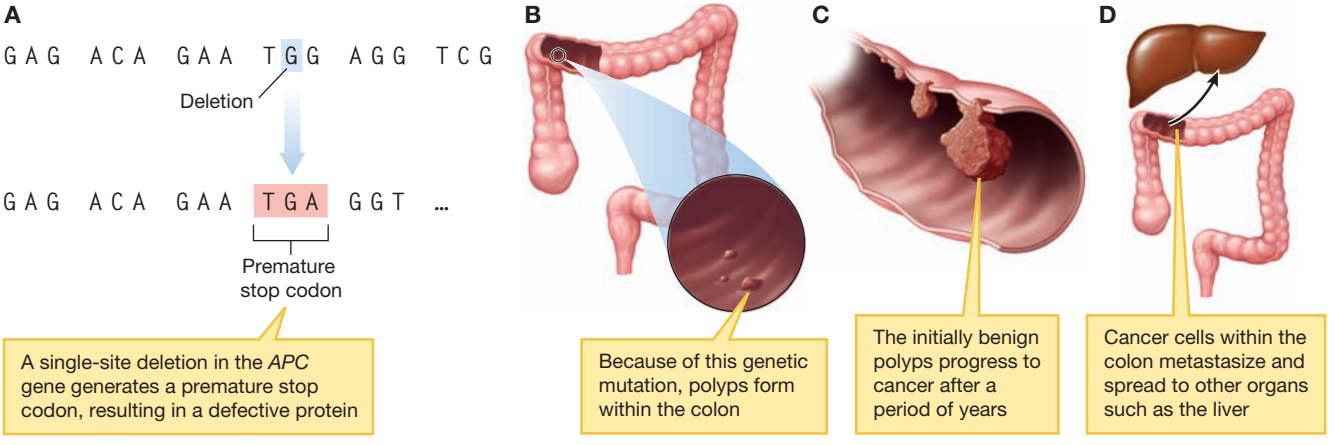
KEYCONCEPT QUESTION

7.4 Will a deleterious allele with selective disadvantage *s* be present at a higher frequency when it is dominant or when it is recessive? Give both an answer based on mathematical formulae and an intuitive explanation for this answer.

Familial Adenomatous Polyposis Is Maintained by Mutation–Selection Balance

Many genetic diseases of humans have negative fitness consequences. Why haven't the alleles responsible for such diseases been eliminated from the population by selection? One common answer is that many genetic diseases persist in mutation–selection balance. Familial adenomatous polyposis (FAP) is a common genetically inherited disorder, affecting approximately 1 in 8000 individuals (Bisgaard et al. 1994). Patients with FAP have large numbers of polyps that form in the colon. Left untreated, some of these initially benign polyps progress to malignant cancerous states when the patient is 35–40 years old, leading to cancer of the colon and other organs (**Figure 7.27**). As a result, untreated patients suffer a shortened life expectancy.

FIGURE 7.27 The development and progression of familial adenomatous polyposis. (A) A nonsense mutation occurs in the germ-line *APC* gene. In this particular example, we show a section of the *APC* gene. A single base deletion at position 41 creates a premature stop codon (TGA) and thus codes for a defective protein. **(B)** Polyps develop in the large intestine around the time of adolescence. These polyps are initially benign rather than cancerous. **(C)** The polyps progress to a cancerous stage, usually when the patient is 35–40 years of age. Colon cancer develops as a result. **(D)** Cancer cells metastasize to the liver and other organs.



BOX 7.8 Mutation–Selection Balance for a Deleterious Recessive Allele

Suppose we have two alleles A_1 and A_2 at the A locus. A_2 is a deleterious recessive allele, such that the fitnesses for the three types A_1A_1, A_1A_2, and A_2A_2 are 1, 1, and $1 - s$, respectively. A_1 mutates to the A_2 allele at rate μ, and we assume that the rate of back mutation from A_2 to A_1 is negligible.

To build our model, we will assume that selection and mutation operate in turn. Suppose that we begin with allele frequencies p and q for the A_1 and A_2 alleles, respectively. First, we write down the consequences of natural selection, as we did in Box 7.5. After natural selection operates, but before mutation, the frequency of the A_1 allele will be

$$\begin{aligned} p_{\text{after selection}} &= \frac{p^2 + pq}{p^2 + 2pq + q^2(1 - s)} \\ &= \frac{p(p + q)}{1 - q^2 s} \\ &= \frac{p}{1 - q^2 s} \end{aligned}$$

Then we allow mutation to operate:

$$p_{\text{after mutation and selection}} = (1 - \mu) p_{\text{after selection}}$$

Mutation and selection are the only two processes operating in our model, so now we can write the expression for p', the frequency of the A_1 allele after one generation. We do so by applying the two formulae above:

$$\begin{aligned} p' &= p_{\text{after mutation and selection}} \\ &= (1 - \mu)\, p_{\text{after selection}} \\ &= (1 - \mu) \frac{p}{1 - q^2 s} \end{aligned}$$

In this model, even though the A_1 allele is favored by selection, it will never be entirely fixed in the population, because A_2 alleles are continually being regenerated through mutation. But eventually the population will reach an equilibrium frequency of the A_1 allele. At this equilibrium, which we call the *mutation–selection balance*, the action of natural selection to decrease the frequency of A_2 is exactly balanced by the action of mutation to produce new A_2 alleles by mutation from A_1. This is a stable equilibrium: After a perturbation away from the equilibrium allele frequencies, the population returns to equilibrium.

We can find the frequency p of the A_1 allele at the mutation–selection balance by recognizing that at this equilibrium, p does not change from generation to generation. Thus, if we can isolate p in the equation

$$p = (1 - \mu) \frac{p}{1 - q^2 s}$$

we will obtain the equilibrium frequency at the mutation–selection balance.

Recognizing that $q = 1 - p$, our task is to solve the following equation for p:

$$p = (1 - \mu) \frac{p}{1 - (1 - p)^2 s}$$

To do so, we first divide each side by p:

$$1 = (1 - \mu) \frac{1}{1 - (1 - p)^2 s}$$

We then multiply through by the denominator:

$$1 - (1 - p)^2 s = (1 - \mu)$$

We next subtract 1 from each side and then multiply each side by $-1/s$:

$$(1 - p)^2 = \frac{\mu}{s}$$

Now solving for p, we get the pair of solutions $p = 1 \pm \sqrt{\mu/s}$. Of these, only $p = 1 - \sqrt{\mu/s}$ is in the range $[0, 1]$ that is necessary for an allele frequency. This is the mutation–selection balance: The equilibrium frequency of the favored allele A_1 is $1 - \sqrt{\mu/s}$, and the frequency of the deleterious recessive allele A_2 is $\sqrt{\mu/s}$.

One of the major causes of FAP is the occurrence of mutations to the *APC* tumor suppressor gene (Bodmer 1999). This locus, which is approximately 9000 base pairs in length, offers numerous mutational targets, any of which are sufficient to induce the FAP condition. These are dominant mutations; individuals with a single copy of a disease allele progress to disease. To remain consistent with our notation throughout this chapter, we will refer to the normal (nondisease) *APC* allele as A_1 and to any of the disease-causing alleles as A_2.

Population genetic analysis can inform our understanding of the causes and consequences of FAP. To learn more about the mutation process that generates FAP and the fitness consequences of the disease, Marie Bisgaard and her colleagues studied 154 individuals listed in an exhaustive Danish case registry (Bisgaard et al.

1994). To estimate the *penetrance* of the disease—the probability that an individual with a disease-causing allele develops the disease—they looked at the disease status of the offspring of the registered cases, and they found that the penetrance of FAP is near 100% at age 40.

To estimate the mutation rate μ from A_1 to A_2, the researchers used pedigree data to estimate that 39 of the 154 FAP cases in the registry were due to new, rather than inherited, mutations. During the same period, there were approximately 2,000,000 total surviving births in Denmark—and thus $2 \times 2{,}000{,}000$ new *APC* gene copies in the population. This allowed them to estimate the mutation rate from A_1 to A_2 to be 39/4,000,000; that is, approximately $\mu = 10^{-5}$.

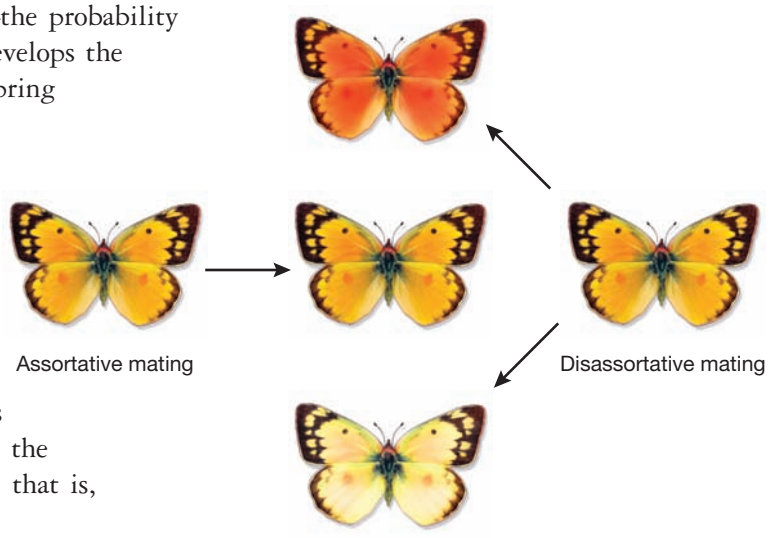


FIGURE 7.28 Assortative and disassortative mating. In assortative mating, like mates with like. In disassortative mating, individuals mate with phenotypes or genotypes different from their own.

By tallying up the number of surviving offspring of parents with FAP, Bisgaard and her colleagues were also able to estimate the fitness consequences and thus the selection coefficient s. The 154 affected individuals in the registry had a total of 297 children by the end of their lives or reproductive years; a normal cohort of 154 in Denmark at the same time would be expected to have 340 children. This leads to an estimated fitness of $297/340 = 0.87$. In other words, FAP imposed a selective cost of $s = 0.13$.

Now that we have estimates of the mutation rate μ and the selection coefficient s, we can check these by computing the expected equilibrium frequencies of the normal and disease-causing alleles A_1 and A_2 under mutation–selection balance. The *APC* variant causing FAP disease is a dominant allele, so its equilibrium frequency will be approximately $q = \mu/s$. Thus, we expect the frequency of the A_2 allele at mutation–selection balance to be approximately $q = 10^{-5}/0.13 = 1/13{,}000$. The frequency of A_1A_2 heterozygotes in the population would then be approximately $2pq = 1/6500$. This is a close match to the observed frequency of disease in the population.

7.5 Nonrandom Mating

One of the Hardy–Weinberg assumptions is that individuals choose their mates randomly with respect to their own genotypes. All of the mathematical models we have developed in this chapter thus far have made this assumption as well. But it can easily be violated, as we saw in the case of the flat snails that can only mate if the shells of both partners coil in the same direction. If individuals tend to mate with others of the same genotype or phenotype, we call this **assortative mating** (Figure 7.28). When individuals tend to mate with others of different genotypes or phenotypes, we call this **disassortative mating**. Here we consider examples of each in turn.

Inbreeding

Inbreeding, in which individuals mate with genetic relatives, is one very common type of assortative mating. Inbreeding is assortative because in an inbred population, gametes are not paired at random, but are instead preferentially paired with gametes from close relatives. Thus, in an inbred population, a pair of gene copies at the A locus may be **identical by descent**; that is, they may be identical because of shared

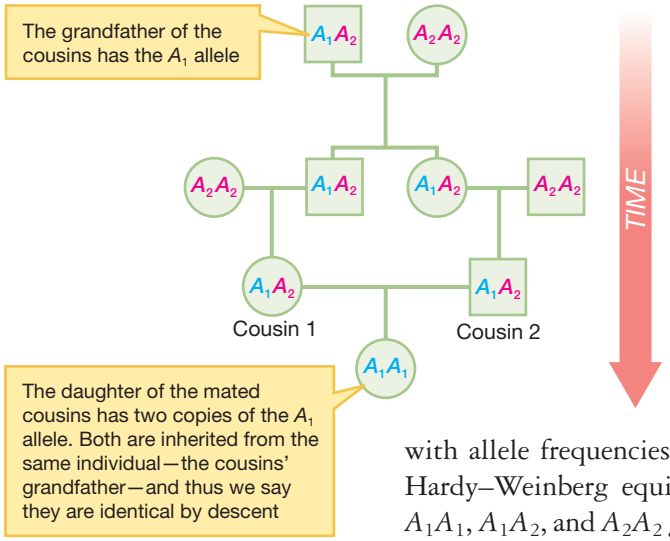


FIGURE 7.29 Identity by descent. Identity by descent is illustrated in a pedigree representing a mating between two full cousins. Each of the two full cousins has received an identical copy of the A_1 allele from their grandfather. Each passes this allele on to the daughter at the bottom of the figure. She therefore has two copies of the A_1 allele that are identical by descent.

descent through a recent ancestor (**Figure 7.29**). It is important to recognize that when we talk about identity by descent, we are making a claim about the history of two gene copies, not their genetic sequence. Two gene copies can be the same in terms of sequence (for example, both may be A_1), but if they do not have a shared ancestor, they are not considered identical by descent.

The most extreme type of inbreeding is self-fertilization, or *selfing*—when an individual fertilizes its own gametes. Selfing is common in flowering plants, and it provides a convenient example of the way that inbreeding affects genotype frequencies. Suppose that we have a population with allele frequencies $A_1 = 0.8$ and $A_2 = 0.2$. If the population is initially in Hardy–Weinberg equilibrium at the A locus, the genotype frequencies for the A_1A_1, A_1A_2, and A_2A_2 genotypes will be 0.64, 0.32, and 0.04, respectively. Suppose that the population now begins to reproduce exclusively by selfing. What offspring genotypes will be produced? A_1A_1 individuals produce only A_1 gametes, and thus they will produce only A_1A_1 offspring. Similarly, A_2A_2 individuals will produce only A_2A_2 offspring. The heterozygotes A_1A_2, however, do not produce only heterozygote offspring. Because they produce both A_1 and A_2 gametes, which then pair at random, these heterozygotes will produce A_1A_1, A_1A_2, and A_2A_2 offspring in a 1:2:1 ratio. As a result, after one generation of selfing, we will have more A_1A_1 and A_2A_2 homozygotes in the population and fewer A_1A_2 heterozygotes (**Figure 7.30**).

Note that although genotype frequencies change, *the allele frequencies remain constant over time*. This is generally true of inbreeding in the absence of other evolutionary processes. Because inbreeding does not add or remove alleles from the population and does not differentially pass one allele or another into the next generation, inbreeding acting alone does not cause changes in allele frequencies.

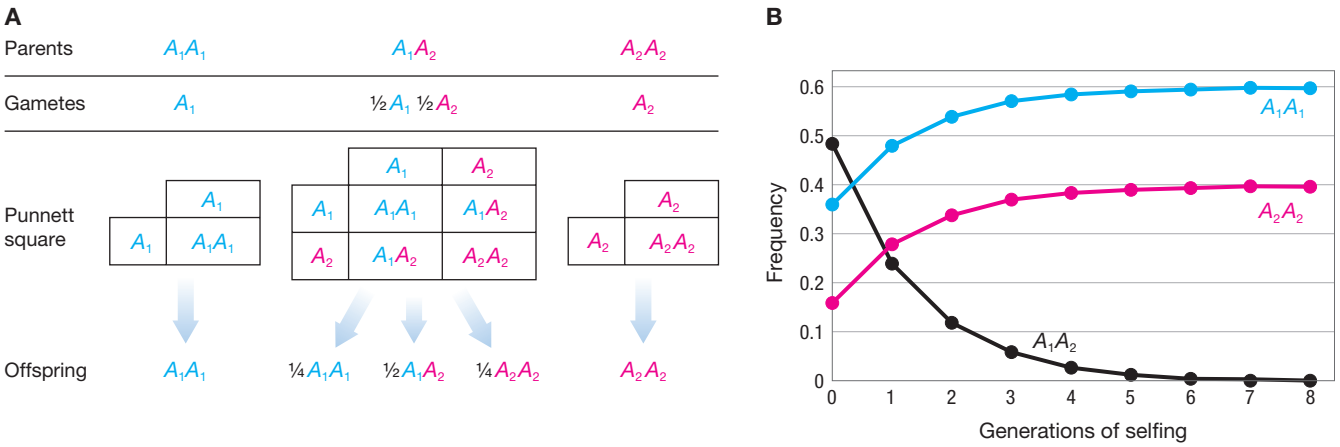


FIGURE 7.30 Reproduction by selfing. **(A)** A_1A_1 and A_2A_2 parents produce only A_1A_1 and A_2A_2 offspring, respectively, whereas A_1A_2 parents produce all three types of offspring. As a result, the number of homozygotes among the offspring increases and the number of heterozygotes decreases. **(B)** Selfing rapidly eliminates heterozygotes. Selfing over eight consecutive generations results in a population with very few heterozygotes. We begin with a Hardy–Weinberg population and track the frequencies of each genotype. Initially, allele frequencies of A_1 and A_2 alleles are $p = 0.6$ and $q = 0.4$, respectively. After eight generations of selfing, $f[A_1A_1]$ is nearly 0.6, $f[A_1A_2]$ is very close to 0, and $f[A_2A_2]$ is almost 0.4. Genotype frequencies have changed dramatically, but allele frequencies have not changed; p remains 0.6 and q remains 0.4.

The selfing example we just considered is an extreme form of inbreeding; in general, there is a broad continuum between selfing and purely random mating. In **Box 7.9**, we examine how population geneticists quantify this continuum using an approach known as *F*-statistics. The larger the value of the inbreeding coefficient *F*, the closer the population lies to the selfing end of the continuum.

Inbreeding Depression

Inbreeding depression occurs when the offspring of genetic relatives have reduced fitnesses. Overdominance is one possible cause of inbreeding depression: Because offspring of matings among relatives are more likely to be homozygous, heterozygotes, which have higher fitness, will be less common.

However, a more significant cause of inbreeding depression—probably the most significant cause—is the presence of numerous rare deleterious recessive alleles in populations (Carr and Dudash 2003; Charlesworth and Willis 2009). As we saw in our treatment of mutation–selection balance in Section 7.4, natural selection opposes deleterious mutations, and therefore in a large population we expect to find deleterious alleles at low frequency at most loci. Among these deleterious alleles, recessive alleles will be more common than dominant alleles because their effects are masked in heterozygotes, and so selection is less effective at removing them. When offspring are produced by outcrossing (that is, mating between unrelated or at least very distantly related individuals), most deleterious recessive alleles appear in heterozygotes and thus have little effect on fitness. When close relatives mate, however, the offspring tend to carry alleles at many loci that are identical by descent (see Figure 7.29). When the identical-by-descent alleles are deleterious recessives, their effects are revealed, and the fitness of the homozygous individual is diminished.

Inbreeding depression can be particularly important in conservation biology because threatened populations tend to be more inbred. As we will see in the next chapter, smaller populations lose genetic variation at a higher rate, and individuals in smaller populations have more loci with alleles that are identical by descent. Therefore, any time a population goes through a rapid decline in size, the amount of inbreeding increases. This remains true even if the population subsequently rebounds in size. Habitat fragmentation further exacerbates the problem. When a large population is subdivided into several small ones with limited movement between them, individuals end up mating with closer relatives.

Olof Liberg and his colleagues measured the impact of inbreeding depression in a study of gray wolves (*Canis lupus*) as they recolonized Sweden and Norway. By the mid-1960s, wolves had gone extinct on the Scandinavian Peninsula. But in 1978 a few individuals—probably migrants from Russia or eastern Finland—were observed in northern Sweden. In 1983, a single breeding pair established a small population along the Swedish–Norwegian border farther to the south. An additional migrant from the Finnish–Russian wolf population added further genetic variation in 1991 (Vila et al. 2003), and by 2011 the Scandinavian population had grown to about 300 wolves (**Figure 7.31**) (Wabakken et al. 2001; Liberg et al. 2005; Wabakken et al. 2011).

FIGURE 7.31 Recolonization of the Scandinavian Peninsula by the gray wolf. By the late 1960s, the gray wolf was extinct on the Scandinavian Peninsula. In 1983, a breeding pair of wolves—immigrants from the Finnish–Russian population, according to DNA evidence—established a new population in Sweden and Norway. As of 2011, this population has grown to around 300 individuals living as solitary breeding pairs (orange dots) or in family packs (black dots). Adapted from International Union for Conservation of Nature (IUCN) Red List (2015), Wabakken et al. (2001), and Wabakken et al. (2011).

BOX 7.9 Wright's *F*-statistic

Population geneticists often quantify the degree of inbreeding in a population using a measure introduced by the population geneticist Sewall Wright and known as **Wright's *F*-statistic**. The *F*-statistic measures the correlation between the two homologous alleles in a single individual; as we will see, this can serve as a measure of the extent to which inbreeding has occurred in a population. The *F*-statistic can either be computed from a known pedigree or estimated using information about genotype frequencies in a population.

In the idealized infinite populations that we are considering in this chapter, the easiest way to think about the *F*-statistic is to return to our gamete pool model (Section 7.2). Recall that in the random mating situation for which we originally developed the model, it is as if all of the gametes in the population were mixed together in one large gamete pool and then drawn out in pairs at random. In an inbred population, each gamete instead has some chance of being paired with a gamete that is identical by descent and some chance of being matched at random. This suggests a modification to our gamete pool model. Imagine that instead of forming one big gamete pool, the gametes are divided into two separate gamete pools. A fraction $(1 - F)$ goes into a *random mating pool* in which they are paired at random, and the remaining fraction F goes into an *inbred pool* in which they are paired with another gamete that is identical by descent. Thus, the offspring from the random mating pool may be homozygotes or heterozygotes, but the offspring from the inbred pool are always homozygotes (**Figure 7.32**).

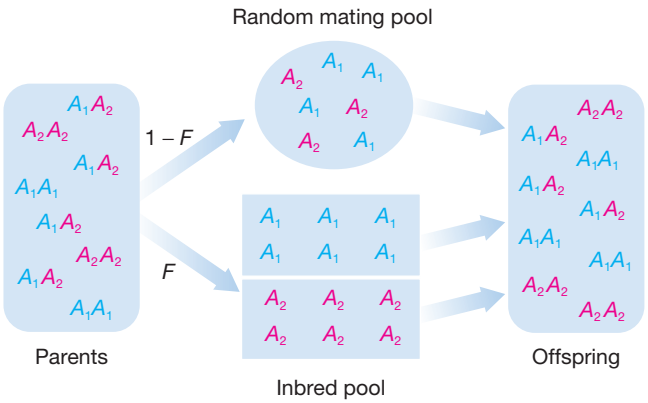


FIGURE 7.32 A gamete pool interpretation of *F*-statistics. We can understand *F*-statistics in terms of a mating model in which a fraction $1 - F$ of the gametes is paired randomly at fertilization (random mating pool), while the remaining fraction F of the gametes is paired with other gametes that are identical by descent (inbred pool).

We can view the results of inbreeding as if they were obtained from this model of two gamete pools. The *F*-statistic simply measures the fraction that goes into the inbred pool, and thus populations that are more inbred have higher values of F. The larger the value of F, the higher the fraction of homozygotes in the population.

Using the *F*-statistic, the expected genotype frequencies under inbreeding follow in straightforward fashion from this model of two gamete pools. Suppose the frequencies of the A_1 and A_2 alleles are p and q, respectively. A fraction F of the offspring are drawn from the inbred pool: All of these will be homozygotes with a fraction p of them being A_1A_1 and a fraction q being A_2A_2. In addition, some homozygotes are produced out of the random mating pool as well, when one A_1 happens to be paired with another A_1 by chance or when one A_2 is paired with another A_2 by chance. Of the fraction $(1 - F)$ of the offspring derived from the random mating pool, p^2 of them will be A_1A_1 homozygotes, q^2 will be A_2A_2 homozygotes, and the remaining $2pq$ will be A_1A_2 heterozygotes. Thus, the entire offspring population will be composed of a fraction $p^2(1 - F) + pF$ of A_1A_1 homozygotes, a fraction $q^2(1 - F) + qF$ of A_2A_2 homozygotes, and a fraction $2pq(1 - F)$ of A_1A_2 heterozygotes. These fractions are summarized in **Table 7.3**.

TABLE 7.3

Genotype Frequencies for an Inbred Population Depend on the Value of the *F*-statistic

Genotype	Genotype Frequency When Inbreeding Occurs
A_1A_1	$p^2(1 - F) + pF = p^2 + pqF$
A_1A_2	$2pq(1 - F)$
A_2A_2	$q^2(1 - F) + qF = q^2 + pqF$

To determine the wolf population's level of inbreeding, the team created a pedigree for the members of 24 breeding pairs between 1983 and 2002, based on genetic sequence data from samples of the tissue, blood, or scat of 163 wolves. This allowed them to calculate the inbreeding coefficient (Wright's *F*-statistic) for each litter of pups. The team also estimated the fitnesses of the offspring of each breeding pair by recording the survival rate of the pups until the first winter after

birth. They found a strong and statistically significant negative correlation between the inbreeding coefficient of each litter and the number of surviving pups in the litter (**Figure 7.33**). These results highlight the importance of genetic diversity to the success of a population, and highlight the impact that additional gene flow from the Finnish–Russian wolf population could have on the success of the Swedish–Norwegian population.

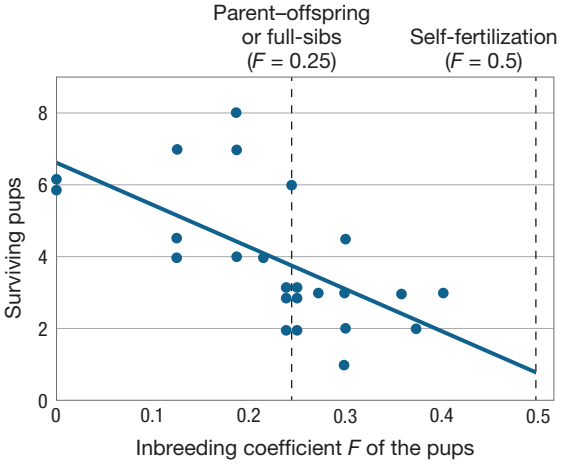


FIGURE 7.33 Inbreeding depression in a Scandinavian population of the gray wolf. For births between 1983 and 2002, the surviving number of pups in a litter was strongly and significantly correlated with the inbreeding coefficient *F* of the litter. More highly inbred litters had lower survival, indicating inbreeding depression. Adapted from Liberg et al. (2005).

Disassortative Mating

Disassortative mating occurs when individuals tend to mate with partners that differ from themselves with respect to a given locus or trait. As a result, disassortative mating tends to generate an excess of heterozygotes relative to Hardy–Weinberg equilibrium.

One straightforward cause of disassortative mating is disassortative preference: a preference for individuals that differ from oneself. For example, evidence suggests that many mammals prefer mates that differ from themselves at the *MHC* loci—a highly polymorphic set of loci associated with the immune system. While the mechanisms responsible are only partially understood, studies on mice indicate that olfactory cues are used to discriminate among potential mates by MHC type.

Disassortative mating need not be driven by preferences alone, nor does it require that all individuals prefer mates that are different from themselves. Anne Houtman and Bruce Falls uncovered an interesting case in white-throated sparrows (*Zonotrichia albicollis*) (Houtman and Falls 1994). White-throated sparrows have two differently colored morphs: a smaller, tan-striped form and a larger, more aggressive, white-striped form (**Figure 7.34**).

These two morphs are controlled by a polymorphism involving a chromosomal inversion—a region of DNA that has reversed direction and often has a low recombination rate. White-striped birds are heterozygous for the inversion, and tan-striped birds are homozygous for the normal chromosome.

In the wild, more than 90% of white-throated sparrows mate with individuals of the opposite morph. Houtman and Falls wanted to determine the mechanism responsible for this pattern of disassortative mating. To do so, they designed a series of mate preference experiments using captive birds (**Figure 7.35**). From these experiments, the researchers found that females of both morphs have a strong preference for tan-striped males, whereas males do not have a strong preference between the two female morphs. Thus, mate preferences by themselves seem unlikely to generate the observed patterns of disassortative mating.

If preferences alone are not driving the pattern of disassortative mating in the wild, what could be the source of this disassortative mating? To answer this, Houtman and Falls conducted competition experiments in which individuals were paired in a single cage with same-sex rivals of the opposite morph. They found that in males and females alike, white-striped individuals aggressively dominated tan-striped rivals.

Taken together with the preference observations, these results now suggest an explanation for the trend toward disassortative mating recorded in nature. White-striped females are able to dominate their tan-striped rivals, and thus they are able to mate with the desirable tan-striped males. Tan-striped females are unable to always obtain access to the tan-striped males, and they typically mate with white-striped males. Thus, the majority of matings involve birds of opposite morphs.

FIGURE 7.34 Two color morphs of the white-throated sparrow. The white-throated sparrow has two distinct color morphs that differ in the brow coloration: white-striped (upper bird) and tan-striped (lower bird). More than 90% of matings occur between individuals of opposite morphs.

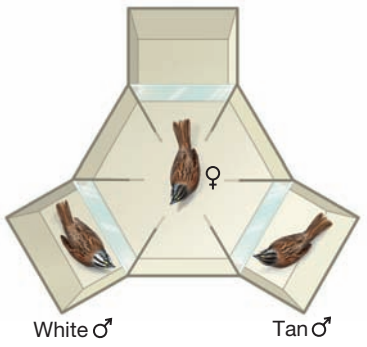


FIGURE 7.35 Houtman and Falls' experiment. Rivals of opposite morphs (males in this diagram) occupy two of the three chambers adjacent to the hexagonal arena that holds the subject (a female in this diagram). Dividers prevent the rivals from seeing one another and prevent the subject from interacting with more than one of the rivals at a time. Adapted from Houtman and Falls (1994).

7.6 Migration

While an idealized population in a mathematical model may be isolated from the rest of the world, real populations are often linked to other populations by a flow of migrants between them. As a result, allele frequencies change within populations. When individuals immigrate into a population, they may bring new or previously uncommon alleles with them. When individuals emigrate from a population, allele frequencies may change as well, if the emigrants are more likely than members of the population at large to carry a particular allele. We can extend our models of gene frequency change to include the possibility of migration. **Figure 7.36A** shows a mainland–island model of migration, in which we have different allele frequencies on the mainland and on a small island. Migrants occasionally reach the island from the mainland. Migrants from the island may reach the mainland as well, but we will ignore this on the grounds that they will have a negligible effect on allele frequencies in the vastly larger mainland population.

We can develop a simple mathematical model to show what will happen to genotype and allele frequencies on the island as a result of migration. Suppose that the initial frequencies of the A_1 and A_2 alleles on the island are given by p_i and q_i, and the allele frequencies on the mainland are given by p_m and q_m. We assume that migration is the only violation of the Hardy–Weinberg assumptions; that is, we have no natural selection at the A locus, we have random mating, we have no mutation, and we have a very large population that is not subject to chance fluctuations (genetic drift)—this latter assumption may be questionable for an island, but we maintain it here for simplicity. (In the next chapter, we will see what happens when genetic drift comes into play in a small island population.)

Let k be the fraction of the island population made up of new migrants from the mainland. Then, after migration occurs, genotype frequencies on the island will be

$$f[A_1A_1] = (1 - k)p_i^2 + kp_m^2$$
$$f[A_1A_2] = 2(1 - k)p_iq_i + 2kp_mq_m$$
$$f[A_2A_2] = (1 - k)q_i^2 + kq_m^2$$

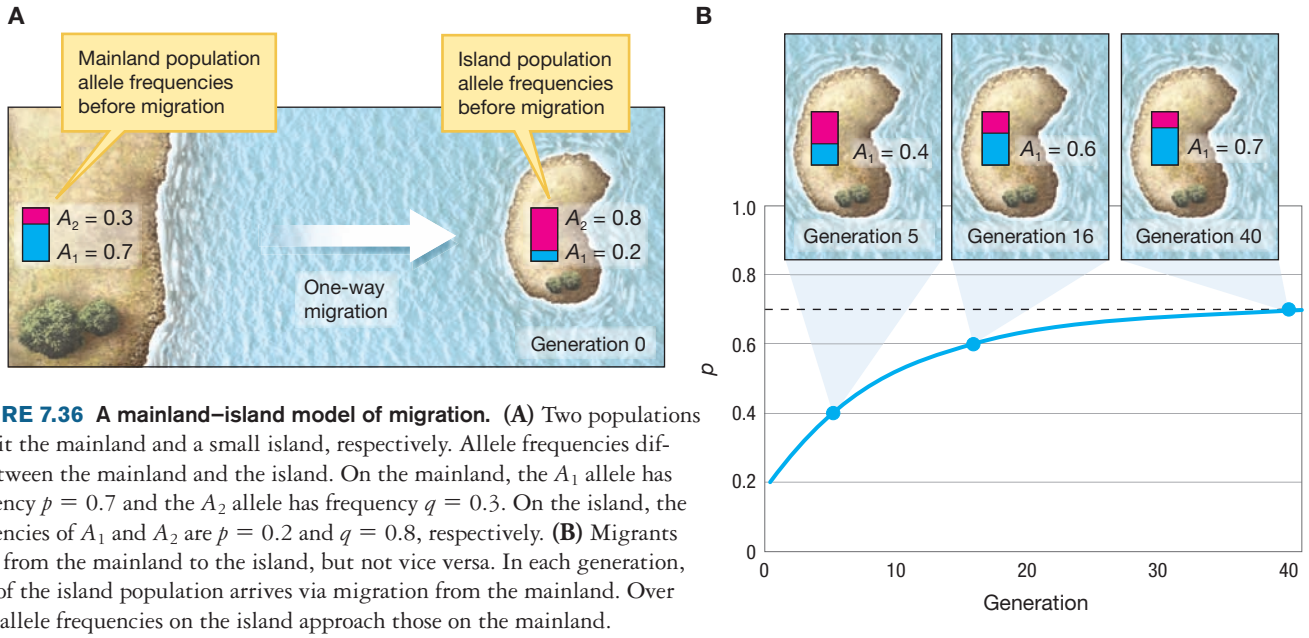


FIGURE 7.36 A mainland–island model of migration. (A) Two populations inhabit the mainland and a small island, respectively. Allele frequencies differ between the mainland and the island. On the mainland, the A_1 allele has frequency $p = 0.7$ and the A_2 allele has frequency $q = 0.3$. On the island, the frequencies of A_1 and A_2 are $p = 0.2$ and $q = 0.8$, respectively. **(B)** Migrants travel from the mainland to the island, but not vice versa. In each generation, 10% of the island population arrives via migration from the mainland. Over time, allele frequencies on the island approach those on the mainland.

Unless allele frequencies were initially the same on the island and the mainland ($p_i = p_m$), the new frequencies on the island will not be Hardy–Weinberg proportions.

What happens to allele frequencies as a consequence of migration? We can see the answer by calculating the *change* in allele frequencies on the island as a result of migration. Initially, before migration, the frequency of the A_1 allele on the island was p_i. The frequency of the A_1 allele after migration—call it p_i'—will be

$$p_i' = (1 - k)p_i + kp_m$$

Thus, the net change in allele frequencies will be

$$\Delta p_i = p_i' - p_i = k(p_m - p_i)$$

From this expression, we can also calculate the equilibrium allele frequencies on the island if migration continues. By definition, at equilibrium, the allele frequencies no longer change, so we set $\Delta p_i = 0$ in our expression above. For nonzero migration ($k > 0$), this gives us the solution $p_i = p_m$. This means that the system reaches equilibrium only once the allele frequencies on the island and mainland are the same.

Figure 7.36B provides an illustrative example of how migration changes allele frequencies over time. Initially, the frequency of the A_1 allele on the mainland is $p_m = 0.7$, and on the island it is $p_i = 0.2$. Over the course of 40 generations, with 10% of the island population arriving by migration each generation, the frequency of the A_1 allele on the island has nearly reached its equilibrium value of 0.7, the allele frequency on the mainland.

7.7 Consequences on Variation within and between Populations

In this chapter, we used the Hardy–Weinberg equilibrium to illustrate evolutionary models of how allele frequencies change at the population level in the absence of natural selection. We then relaxed a number of assumptions of the Hardy–Weinberg model to examine the effects on allele frequency change at the population level. This led to the development of models that include the processes of natural selection, mutation, non-random mating, and migration.

As we learned in Chapter 3, genetic variation is the fuel for natural selection. It is thus instructive to consider what effect each of these processes has on genetic variation within populations and between populations. The Hardy–Weinberg model demonstrates that in the absence of evolutionary processes, the ongoing process of Mendelian inheritance does not decrease (or increase) the amount of variation in a population, nor does it alter the amount of variation between populations.

Natural selection, by contrast, favors some variants and disfavors others. Selection will typically decrease the amount of variation in a population as disfavored alleles are lost and favored alleles go to fixation. Forms of balancing selection, such as overdominance and negative frequency dependence, act to preserve variation—but these tend to be uncommon in natural populations. Whether selection increases or decreases variation between two populations will depend on whether the populations experience similar selective conditions. If selective conditions are similar, as we might imagine for black lava populations of the rock pocket mouse we discussed earlier, selection will favor the same phenotype in both populations, and thus it will tend to decrease variation—at least in phenotype—between the populations. If selective conditions differ between the environments, as we see when comparing rock pocket mice in light rock and dark

TABLE 7.4

Effects of Population Genetic Processes

Evolutionary Process	Variation within Population	Variation between Populations
Natural selection	Decreases (except in cases of balancing selection)	Increases if selective conditions differ; decreases if conditions are the same
Mutation	Increases	Increases
Nonrandom mating	No effect on allele frequencies (in the absence of sexual selection)	No effect on allele frequencies (in the absence of sexual selection)
Migration	Increases	Decreases

lava populations, selection will tend to favor different phenotypes in each population, and it will tend to increase variation between the populations.

Mutation, as a source of new variation, increases the amount of variation within a population. Because different mutations may arise in different populations, mutation will also tend to increase variation between populations. In the absence of sexual selection (higher mating success for one genotype than another), nonrandom mating does not change allele frequencies on its own. Thus, it has comparably little effect on variation in this regard, although it is worth noting that assortative mating and inbreeding tend to decrease the frequency of heterozygotes in a population. Yet, in doing so they may increase the average differences between individuals within a single population. Disassortative mating increases the fraction of heterozygotes within a population, and it may help stabilize polymorphism, as we saw in the example of the white-throated sparrows.

Migration will tend to bring new alleles into a population, and, in this way, it will typically serve to increase the variation within a population. But, as we saw in the mainland–island model, the long-term effect of migration between populations is to equilibrate their allele frequencies—and thus migration decreases the variation between populations. **Table 7.4** summarizes these conclusions about the effects of natural selection, mutation, nonrandom mating, and migration on variation within and between populations.

In all of the models we have considered in this chapter, we have assumed that evolution is occurring in very large populations, such that chance fluctuations have a negligible effect on allele or genotype frequencies. But evolution often operates in small populations that are subject to chance fluctuations, and such populations have their own evolutionary dynamics. We will explore these dynamics in the next chapter.

SUMMARY

1. The field of population genetics provides a quantitative way of describing, modeling, and predicting how allele and genotype frequencies in populations change over time.
2. The Hardy–Weinberg model serves as a null model in population genetics, telling us what happens to allele frequencies and genotype frequencies when no evolutionary processes—natural selection, mutation, nonrandom mating, migration, and genetic drift—are operating.
3. When none of these five evolutionary processes are operating, the Hardy–Weinberg model makes three predictions: (a) allele frequencies will not change over time, (b) genotype frequencies will be at the so-called Hardy–Weinberg equilibrium frequencies, and (c) a population with genotype frequencies away from Hardy–Weinberg equilibrium frequencies will return to these frequencies in a single generation.

4. For a locus with two alleles, A_1 and A_2, at frequencies p and q respectively, the Hardy–Weinberg genotype frequencies are as follows: $f[A_1A_1] = p^2$, $f[A_1A_2] = 2pq$, $f[A_2A_2] = q^2$.
5. Natural selection, mutation, nonrandom mating, and migration can each drive changes in genotype frequencies in a population.
6. Natural selection can take on various forms. Directional selection, overdominance, and underdominance are types of frequency-independent selection in which the fitness of a genotype is independent of its frequency in the population. These contrast with positive and negative frequency-dependent selection, in which the fitness of a genotype depends on the genotype frequencies in the population.
7. Mutation–selection balance can maintain deleterious alleles at low frequency in a population.
8. Assortative mating, in which individuals tend to mate with similar individuals, increases the frequency of homozygotes in a population. Disassortative mating, in which individuals mate with dissimilar individuals, increases the frequency of heterozygotes.
9. Migration between populations brings their allele frequencies closer to one another.
10. The evolutionary processes considered in this chapter have diverse but predictable effects on variation within and between populations.

KEY TERMS

assortative mating (p. 245)
balanced polymorphism (p. 233)
balancing selection (p. 233)
directional selection (p. 230)
disassortative mating (p. 245)
fecundity (p. 239)
fixation (p. 230)
frequency-dependent selection (p. 236)
frequency-independent selection (p. 230)
Hardy–Weinberg equilibrium (p. 220)
Hardy–Weinberg model (p. 220)
heterozygote advantage (p. 233)
identical by descent (p. 245)
inbreeding (p. 245)
inbreeding depression (p. 247)
mutation–selection balance (p. 243)
overdominance (p. 233)
population genetics (p. 218)
selection coefficient (p. 229)
underdominance (p. 234)
Wright's F-statistic (p. 248)

REVIEW QUESTIONS

1. The Hardy–Weinberg equilibrium at a single locus is a mixed equilibrium.
   a. At this equilibrium, do allele frequencies represent a neutral, stable, or unstable equilibrium?
   b. If we assume that allele frequencies are fixed, do genotype frequencies represent a neutral, stable, or unstable equilibrium?
2. Enumerate the assumptions of the Hardy–Weinberg model.
3. List three predictions of the Hardy–Weinberg model.
4. Which of the following modes of selection result in a balanced polymorphism: directional selection, overdominance, underdominance, positive frequency dependence, negative frequency dependence?
5. Two genotypes of sunflower have different fitnesses despite having the same probability of survival. How can this be?
6. Which evolutionary process does not change allele frequencies over time but causes a deviation from Hardy–Weinberg proportions?
7. What factors determine the frequency of a deleterious allele at mutation–selection balance?
8. Which evolutionary process changes allele frequencies over time but causes no deviation from Hardy–Weinberg proportions?
9. How can nonrandom mating lead to an excess in heterozygotes above that expected under the Hardy–Weinberg model?
10. What evolutionary process or processes tend to decrease the variation between separate populations?

KEY CONCEPT APPLICATION QUESTIONS

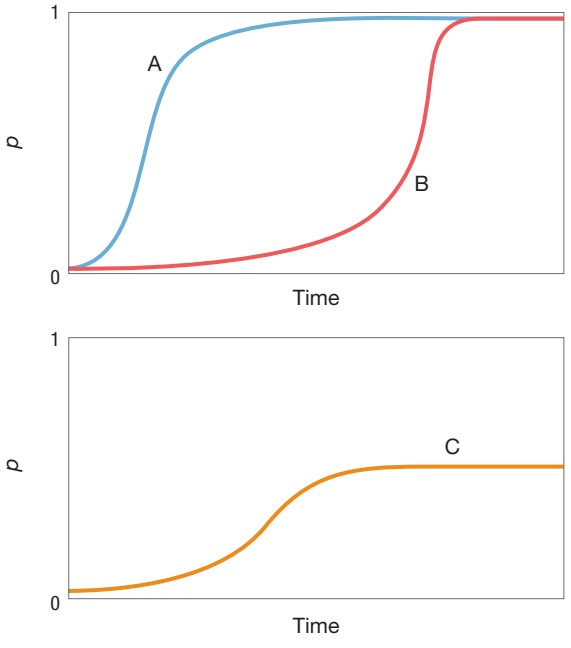
11. Thomas keeps a very large number of socks unpaired in his sock drawer.
    a. A fraction p of them are black, and a fraction $q = 1 - p$ are blue. If Thomas were to pair up all of his socks in the dark so he could not distinguish them by color, what fraction of the pairs do you expect would be mismatches, with one blue and one black sock?
    b. Suppose that Thomas were to match each pair properly, wear them, wash them all (as singletons), and then rematch them again in the dark. Assuming that black and blue socks wear out or are lost at the same rate, assuming no new socks are added, and assuming that socks don't change color in the laundry, what fraction of the pairs will be mismatches this second time around? Explain how your answer relates to the time it takes to reach Hardy–Weinberg equilibrium.
12. Red-green color blindness is a recessive trait on the X chromosome: males with a single copy of the responsible allele and females with two copies display the trait. The frequency of red-green color blindness in males in the United States is approximately 7%. Knowing this, estimate the frequency of the trait in U.S. females. What assumptions did you have to make in order to make that estimate?
13. A biologist studies a genetic locus A, with alleles A_1 and A_2, in two adjacent populations of blue jays. She samples from each population, and she finds the following genotype frequencies:

	A_1A_1	A_1A_2	A_2A_2
Population 1	0.09	0.42	0.49
Population 2	0.64	0.32	0.04

    a. Is the A locus at Hardy–Weinberg equilibrium frequencies in population 1? How about in population 2?
    b. She then combines all of her data from two populations to get the following genotype frequencies:

	A_1A_1	A_1A_2	A_2A_2
Pooled Data	0.365	0.37	0.265

    This population is not at Hardy–Weinberg equilibrium. Why not? Explain which of the assumptions needed for Hardy–Weinberg equilibrium have been violated by combining the population data.
14. A squirrel population exhibits two different alleles, R and r, at the R locus. By genotyping members of this population, a researcher finds genotype frequencies for the RR, Rr, and rr genotypes of 0.2, 0.3, and 0.5, respectively.
    a. What are the allele frequencies in this population?
    b. Under the Hardy–Weinberg model, what genotype frequencies would be observed given these allele frequencies?
    c. Suppose the genotype frequencies reported above were based on a sample of 20 individuals. Use a chi-square test to determine whether the observed frequencies differ significantly from those expected at Hardy–Weinberg equilibrium. (With 1 degree of freedom, the 5% critical value of the χ^2 statistic is 3.84.)
    d. Suppose instead the genotype frequencies were based on a sample of 70 individuals. Would these frequencies then differ significantly from the Hardy–Weinberg proportions?
15. Under the Hardy–Weinberg model, a population will reach Hardy–Weinberg frequencies in a single generation. This model assumes that genotype frequencies are the same in both sexes: Consider a situation where this is not the case. A researcher creates a new *Drosophila* population by selecting 2000 males from a stock population fixed for the dominant A allele at a neutral locus A and 2000 females from a different stock population fixed for the recessive a allele at the A locus. The A allele is located on an autosome, not a sex chromosome. These individuals then mate among themselves.
    a. Assuming that there is no mutation, what genotype frequencies will be observed in the first offspring generation?
    b. How long will it take this population to reach Hardy–Weinberg proportions?
    c. Explain why this population took two generations instead of one to reach Hardy–Weinberg equilibrium.
16. The three trajectories below indicate how the frequency p of the A_1 allele changes over time, after starting with $p = 0.01$. For each set of fitness values below, indicate which trajectory—A, B, or C—is the best match.



	A_1A_1	A_1A_2	A_2A_2	
i.	1.0	0.8	0.8	*Best match:* _____
ii.	0.8	1.0	0.8	*Best match:* _____
iii.	1.0	1.0	0.8	*Best match:* _____

17. In Box 7.5, we derived a model for how allele frequencies change because of natural selection when the favored allele A_1 is dominant to the alternative allele A_2. In particular, we found the change in the frequency of the A_1 allele from one generation to the next is equal to

$$\frac{pq^2s}{1 - q^2s}$$

Derive an analogous model for the case in which the favored allele A_1 is recessive. In this case, what is the expression for the change in frequency of the A_1 allele from one generation to the next?

18. In a large random-mating population of lab mice, the A_1 allele is dominant and confers a 25% fitness advantage over the A_2A_2 wild type. Initially, the allele frequencies for A_1 and A_2 are $p = 0.4$ and $q = 0.6$, respectively.
    a. After one generation, what will the new frequency of the A_1 allele be?
    b. After three generations, what will the allele frequencies be?

19. A scientist studies a species of pea whose plants produce flowers with one of three colors: red, pink, or white. She discovers that the colors are produced by combinations of two alleles, *R*1 and *R*2, at a single locus. *R*1 is responsible for red pigmentation, so R_1R_1 individuals have red flowers, R_2R_2 individuals have white flowers, and the pink phenotype is found in R_1R_2 heterozygotes. One season, 250 plants grow and flower. Of these plants, 50 have red flowers, 100 have white flowers, and 100 have pink flowers.
    a. What are the allele frequencies of *R*1 and *R*2 in this population?
    b. What are the expected proportions of the three phenotypes at Hardy–Weinberg equilibrium?
    c. The plants are susceptible to wilting and dying under dry conditions. This season, none of the pink-flowered plants die, but half of the red-flowered plants and half of the white-flowered plants die. Assuming equal reproductive success among surviving plants, estimate the fitnesses of each genotype.
    d. Assuming equal reproductive success among the surviving plants, what was the average fitness of all individuals in the population this year, relative to the fitness of the pink-flowered plants?
    e. Assuming equal reproductive success, what are the resulting allele frequencies of *R*1 and *R*2?

20. In a given population, the wild-type A_1 allele is dominant to the recessive A_2 and A_3 alleles. Fitnesses of the A_1A_1, A_2A_2, and A_3A_3 genotypes are 1.0, 0.9, and 0.8, respectively. Mutation from A_1 to A_2 occurs at rate r, and mutation from A_1 to A_3 occurs at rate $4r$. Ignoring back mutation from A_2 and A_3 to A_1, which allele will be more common at mutation–selection balance, A_2 or A_3? Explain.

21. Suppose two alleles, A_1 and A_2, exhibit overdominance, with fitnesses $w_{11} = 0.8$, $w_{12} = 1.0$, and $w_{22} = 0.9$. Under random mating, we would expect to observe a balanced polymorphism between A_1 and A_2. What should we expect to observe if instead the population is strictly selfing, with hermaphroditic parents that self-fertilize exclusively? What would happen if all members of the population reproduce *apomictically*; that is, asexual parents produce offspring that are genetically identical to themselves?

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8

Evolution in Finite Populations

◀ A male southern elephant seal (*Mirounga leonina*) roars from a South Georgia Island beach.

In the middle of the Irish Sea, between Britain and Ireland, lies a small island known as the Isle of Man. The island is home to an unusual breed of cat, the Manx, easily recognized by its shortened or missing tail (**Figure 8.1**). Manx cats have reportedly been found on this island for several hundred years. One local legend has it that they arrived in 1588 aboard a ship from the Spanish Armada that was wrecked on the sea cliffs at Spanish Head at the southwestern tip of the island.

An even more curious story for the origin of these cats appears in Joseph Train's 1845 history of the Isle of Man:

> My observations on the structure and habits of the specimen in my possession, leave little doubt on my mind of its being a . . . cross, between the female cat and the buck rabbit. In August, 1837, I procured a female [Manx] kitten, direct from the Island. Both in its appearance and habits it differs much from the common house cat: the head is smaller in proportion, and the body is short; a fud or brush like that of a rabbit, about an inch in length, extending from the lower vertebra, is the only indication it has of a tail. The hind legs are considerably longer than those of the common cat, and, in comparison with the

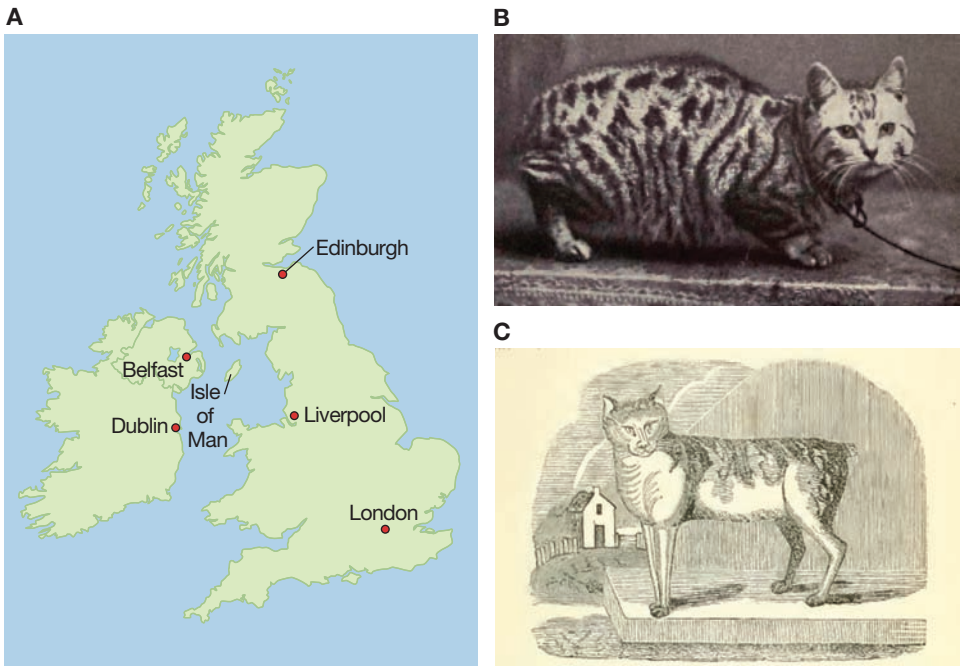


FIGURE 8.1 The Isle of Man, home to the Manx cat. **(A)** Map showing the location of the Isle of Man. **(B)** In this 1902 photograph of a Manx cat, we can see the long hind legs and the absence of a tail. **(C)** Joseph Train's illustration of a Manx cat in his 1845 *A Historical and Statistical Account of the Isle of Man*. Train incorrectly speculated that the breed resulted from a cross between a cat and a rabbit.

> fore legs, bear a marked similarity in proportion to those of the rabbit. Like this animal too, when about to fight, it springs from the ground and strikes with its fore and hind feet at the same time. The common cat strikes only with its fore paws, standing on its hind legs. The [Manx] discharges its urine in a standing posture, like a rabbit, and can be carried by the ears apparently without pain. (Train 1845, p. 2)

But in actuality, the Manx cat is not a cat–rabbit hybrid, but rather an ordinary domestic cat carrying an unusual genetic mutation. The primary genetic determinant of the *Manx phenotype,* which includes both the reduced or absent tail and longer hind legs than forelegs, is a single autosomal locus *M* (for *Manx*). The *M* allele is dominant, conferring the Manx phenotype in *Mm* heterozygotes. In the *MM* homozygous form it is lethal, with most *MM* individuals aborted prenatally (Robinson 1993). Because homozygote lethality generates strong natural selection against the *M* allele, one might be surprised that the *M* allele, virtually unknown elsewhere, should have become common in the Isle of Man cat population. Indeed, this would be a surprising outcome in a very large population. But on a small island, natural selection is not the only process that influences allele frequencies. Allele frequencies can change because of random effects associated with low population size. Moreover, chance variation in the initial allele frequencies in a founding population may lead to dramatically different allele frequencies on an isolated island compared to those on a mainland. This phenomenon, known as the *founder effect*, is most likely responsible for the prevalence of the *M* allele in the Manx cats on the Isle of Man. We will see how it works and consider additional examples of the founder effect in Section 8.3.

In this chapter, we address the following questions:

- How does the process of evolution in small populations differ from what is seen in large populations?
- How does genetic drift work, and what are its consequences?

- How do gene copies spread through populations, and how do coalescent trees help us to understand this process?
- How do demographic processes such as population bottlenecks and the founder effect contribute to evolutionary change?
- What happens when genetic drift interacts with mutation and selection?
- What does the neutral theory of molecular evolution predict about the nature of genetic variation, and to what degree is the neutral theory supported by contemporary evidence?

8.1 Random Change and Genetic Drift

In the previous chapter, we developed simple mathematical models of how gene frequencies change with and without the action of natural selection. In those simple models of evolution, we assumed that populations were large—so large, in fact, that in every generation the law of large numbers applied to changes in gene frequencies. The law of large numbers states that as the size of a random sample increases, the *realized frequencies*—those frequencies that we actually observe—usually will be very close to the expected frequencies. But when sample sizes are small, the realized frequencies will not always be close to the expected frequencies.

By way of illustration, suppose that you tossed a fair coin 1000 times. At the end of the coin tosses, the odds are that you would observe something very close to a 1:1 ratio of heads to tails. If instead you only tossed your coin 10 times, you might get 5 heads and 5 tails for another 1:1 ratio. But more than 75% of the time, you'd get some other combination: 4 heads and 6 tails, or 6 heads and 4 tails, or 3 heads and 7 tails, and so on. *In experiments with small sample sizes, realized frequencies are not always very close to the expected frequencies*. The same thing happens in populations. In very large populations, the realized genotype frequencies will be very close to the expected genotype frequencies. For this reason, in the previous chapter we assumed that the genotype frequencies of the offspring were always exactly those expected, given the genotype frequencies and the relative fitnesses of the parents.

In a small population, the realized genotype frequencies often may deviate substantially from the expected genotype frequencies for any number of reasons. By chance, in any given generation, some mating pairs may form more or less often than expected; certain genotypes may produce more or fewer offspring than expected; other genotypes may survive more or less often than expected. All of these factors will make it less likely that the actual genotype frequencies in our population will match the expected frequencies.

If we want to think about evolution in small populations in a quantitative way, we need a model of evolution in such populations. The **Wright–Fisher model**, named after its creators Sewall Wright and R. A. Fisher, is one of the simplest such models, and it is used widely in population genetics. Loosely speaking, the basic Wright–Fisher model is a small-population version of the Hardy–Weinberg model that we developed in the previous chapter. Because we will make reference to Wright–Fisher populations throughout this chapter, we present the model in further detail in **Box 8.1**.

BOX 8.1 The Wright–Fisher Model

In Chapter 7, we examined the Hardy–Weinberg model, which provides an idealized picture of how genotype frequencies change over time in a very large population. Using the Hardy–Weinberg model, we were able to see what happens in a large population in the absence of such evolutionary processes as selection, migration, mutation, and nonrandom mating. We also saw how to relax some of the assumptions of the Hardy–Weinberg model in order to study the evolutionary consequences of selection and other processes.

The Wright–Fisher model can be seen as a counterpart to the Hardy–Weinberg model, *for small populations* (**Figure 8.2**). Again, it provides us with a baseline for how genotype frequencies are expected to change over time in the absence of selection, migration, mutation, and nonrandom mating. As in the Hardy–Weinberg model, the Wright–Fisher model assumes a population of diploid sexual organisms that reproduce in discrete, non-overlapping generations. As in the Hardy–Weinberg model, the most basic form of the Wright–Fisher model assumes:

1. Natural selection is *not* operating on the trait or traits affected by the locus in question.
2. Mating in the population is random with respect to the locus in question.
3. No mutation is occurring.
4. There is no migration into the population from other populations.

And just as with the Hardy–Weinberg model, with the Wright–Fisher model we can relax each of these assumptions to see how various evolutionary processes affect genotype frequencies over time. But unlike in the Hardy–Weinberg model, in the Wright–Fisher model we assume that the population size is small instead of very large. In doing so, we take account of chance events that influence allele frequencies in a small population.

The basic idea behind the Wright–Fisher model is to consider a population of N diploid organisms, each of which produces a large number of gametes that go into a common pool. Because the gamete pool is very large, allele frequencies in the gamete pool exactly reflect those in the parental generation. But then we draw $2N$ gametes at random from this pool. As a result of random chance, allele frequencies in this small sample of $2N$ gametes may not be exactly the same as the frequencies in the large gamete pool. This is where the model differs from the Hardy–Weinberg model. These gametes are then paired up at random to produce N new diploid offspring for the next generation. Figure 8.2 shows an example with $N = 10$. There, the frequency of the A_1 allele is 0.5 in the parental generation, but 15 of the 20 gametes drawn from the gamete pool happen to carry the A_1 allele, so the frequency of the A_1 allele in the offspring generation is now 0.75.

Because we have only one gamete pool, instead of having separate pools for gametes from male parents and gametes from female parents, this version of the Wright–Fisher model is sometimes described as modeling a *hermaphroditic* or *monoecious* species, such as many flowering plants, in which each parent produces both male and female gametes. A model with two separate sexes in each generation and two separate gamete pools for eggs and sperm, although somewhat more complicated, is conceptually similar and has similar mathematical properties.

Most of the theoretical results presented in this chapter will be based on the Wright–Fisher model of population genetics. This will allow us to explore how drift interacts with other evolutionary processes.

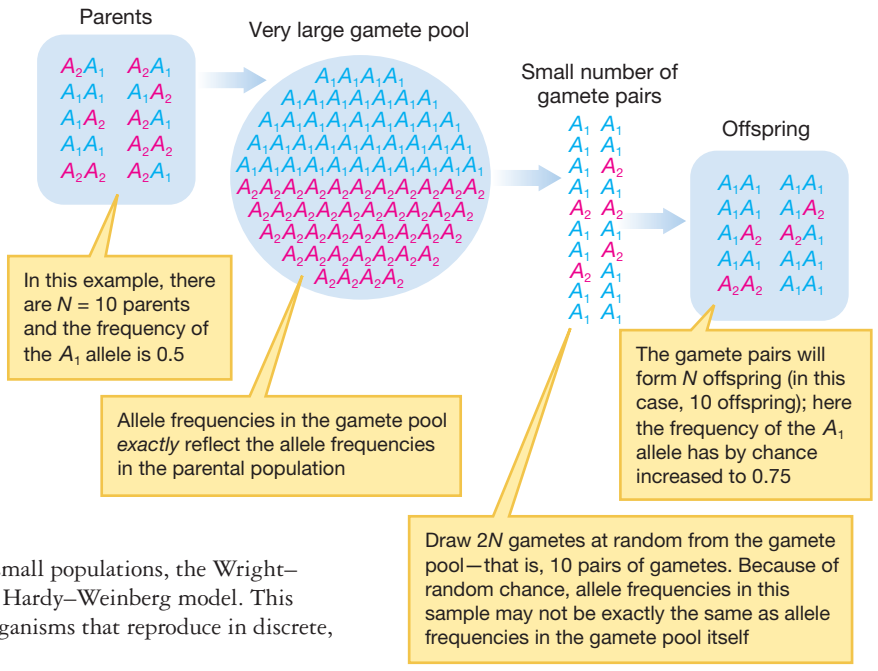


FIGURE 8.2 The Wright–Fisher model. For small populations, the Wright–Fisher model can be seen as a counterpart to the Hardy–Weinberg model. This model assumes a population of diploid sexual organisms that reproduce in discrete, non-overlapping generations.

Genetic drift is the process of random fluctuation in allele frequencies due to sampling effects in finite populations. There are three general consequences of genetic drift:

1. In a finite population, allele frequencies fluctuate over time, even in the absence of natural selection.
2. Some alleles are fixed, others are lost, and the fraction of heterozygotes in the population decreases over time.
3. Separate populations diverge in their allele frequencies and in terms of which alleles are present.

We will consider these three points in turn.

Genetic Drift Causes Allele Frequencies to Fluctuate over Time

The fundamental effect of genetic drift is to cause fluctuations in allele frequencies in a population, even in the absence of natural selection or other evolutionary processes. The *rate* at which allele frequencies fluctuate because of drift depends on the size of the population. Drift acts more powerfully in small populations than in large populations, and thus drift causes larger allele frequency fluctuations in small populations.

Figure 8.3 illustrates the result of genetic drift in populations of size 10, 100, and 1000 individuals. All three populations start with two alleles, A_1 and A_2, each at a frequency of 50%. These alleles are **selectively neutral**; that is, there is no fitness difference between them. As a result, natural selection does not act on the frequencies of these alleles. But because of genetic drift, allele frequencies change nevertheless. Over time, random fluctuations lead to rapid changes in the allele frequencies in the smallest population, modest changes in allele frequencies in the intermediate population, and small changes in allele frequencies in the large population.

As a result of genetic drift, one particular allele may reach a frequency of 100% in a given population, while the other alleles at that locus are lost. Recall from Chapter 7 that when this happens, we say that the remaining allele has been fixed, or has reached fixation, in the population. Because drift acts more strongly in small populations, fixation occurs more quickly in small populations than in large ones. We see this happening in Figure 8.3: All of the populations of size 10 reach fixation, but none of the populations of size 100 or size 1000 do. However, if we were to run these simulations for more and more generations, then each and every finite-sized population, no matter how large, would *eventually* become fixed for one or the other of the two alleles. In general, the average time to fixation of a neutral allele scales with the size of population (Kimura and Ohta 1969).

FIGURE 8.3 Genetic drift is stronger in smaller populations. The three graphs show simulations of genetic drift in diploid populations of size 10, 100, and 1000, respectively, each starting with the neutral A_1 and A_2 alleles at equal frequency, under random mating with no mutation or migration. Each graph shows 10 different runs of the simulation, with one highlighted in blue for visibility. In each case, drift causes allele frequencies to fluctuate over time, but the fluctuations are far more dramatic in the smaller populations. In each population of size 10, one allele or the other goes to fixation—a frequency of 1.0—within 100 generations.

Genetic drift is a random process. Therefore, while it is certain that some allele will eventually be fixed in each population in this model, it is not certain *which* allele will become fixed in which population. In some of the populations plotted in Figure 8.3, the A_1 allele is fixed; in others, the A_2 allele is fixed. It turns out that, at a given time, the probability that an allele at a neutral locus will eventually be fixed is equal to the frequency of that allele in the population at that time.

The easiest way to see this is to recognize that in a finite population, sooner or later every allele is either fixed or lost because of drift. Thus, in a population of N diploid individuals, there are $2N$ gene copies at any given locus. If the locus is neutral, each of these $2N$ gene copies is equally likely to be fixed, and so an allele that is present in only a single copy has a $1/2N$ chance of being fixed. If instead there are k copies of a given allele, each of these copies has a $1/2N$ chance of being fixed, for a total probability $k/2N$ that this particular allele is fixed.

Why are the random fluctuations that result from genetic drift important in the evolutionary process? For one thing, they cause allele frequencies to shift—and thus cause evolutionary change—in the absence of natural selection. As we saw in Chapter 3, natural selection lacks foresight, and so evolution might get stuck at a locally optimal but globally suboptimal phenotype if natural selection were the only process operating. But drift can also cause shifts in allele frequencies in the opposite direction of what would be favored by natural selection. Additionally, drift has important effects on the amount of variation present in populations and on divergence between populations: We consider these consequences in the subsections that follow.

Genetic Drift Causes Heterozygosity to Decrease within a Population over Time

Another important consequence of drift is that it tends to reduce variation within populations. There are at least two different ways to see this intuitively. First, we could simply notice that in the absence of natural selection, genetic drift causes alleles to go to fixation in a finite population over evolutionary time. When alleles are fixed, variation is lost. Second, we could think about finite population size as a sort of inbreeding because in a finite population, there is a nonzero chance that individuals mate with genetic relatives. As we learned in Chapter 7, inbreeding leads to the loss of genetic variation. And as we illustrate below, we can measure this loss of variation in the wild.

Population geneticists often use quantities known as *observed heterozygosity* and *expected heterozygosity* to measure the amount of variation in a population. The **observed heterozygosity**, H_o, at a given locus is defined as the fraction of individuals in the population that are heterozygous at the given locus. For example, suppose we have three alleles, A_1, A_2, and A_3, at the A locus. If the genotype frequencies of the three homozygotes are $f[A_1A_1] = 0.2$, $f[A_2A_2] = 0.2$, and $f[A_3A_3] = 0.1$, the remaining fraction (0.5) of the individuals in the population will be heterozygotes, and the observed heterozygosity will be $H_o = 0.5$. In general, the observed heterozygosity is 1 minus the frequency of homozygotes in the population, expressed as

$$H_o = 1 - \sum_{i=1}^{n} f[A_iA_i]$$

The **expected heterozygosity**, H_e, is the fraction of heterozygotes expected under the Hardy–Weinberg model, given the allele frequencies in the population. According to the Hardy–Weinberg model (Chapter 7), if the frequency of the ith allele is p_i, the fraction of homozygotes for allele i will be p_i^2. Thus, the expected frequency of the heterozygotes will be

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

KEYCONCEPT QUESTION

8.1 Researchers measure genotype frequencies in a wild population of mice, and they find that the observed heterozygosity is significantly lower than the expected heterozygosity for this population. Propose a hypothesis for the evolutionary process that could be responsible for this observation.

Expected heterozygosity is often easier to measure than observed heterozygosity, especially if there are many alleles at the locus in question, because one does not need to know the frequencies of all genotypes, only the frequencies of all alleles.

In a Wright–Fisher population, expected heterozygosity decreases by an average factor of $1/2N$ in each generation (**Box 8.2**). When N is very large, $1/2N$ is very small, and we see little decrease in heterozygosity due to drift. When N is small, however, $1/2N$ is relatively large, and we see substantial loss of heterozygosity due to drift. Looking back at our simulations of genetic drift in Figure 8.3, we can see this happening. In the small populations, allele frequencies rapidly diverge from 0.5 (where heterozygosity is maximal) and eventually reach fixation or loss of A_1 (where heterozygosity is zero).

We see the same process in natural populations. Where human activities such as overfishing reduce population size, they may have evolutionary consequences as well as ecological ones; that is, they may contribute to genetic drift. To see whether drift had occurred in a heavily exploited New Zealand snapper fishery in Tasman Bay, Lorenz Hauser and his colleagues studied DNA sequences from snapper scales collected there over the period 1950–1986 and from fresh samples collected in 1998 (Hauser et al. 2002). Heavy commercial fishing began in this area in 1950, so the earliest samples reflected levels of heterozygosity prior to fishing, whereas the later samples revealed heterozygosity levels after extensive commercial fishing. Hauser and his colleagues reasoned that if commercial fishing were causing genetic drift in this snapper population, they should see a decline in heterozygosity over time, because drift reduces population heterozygosity. **Figure 8.4** shows their findings.

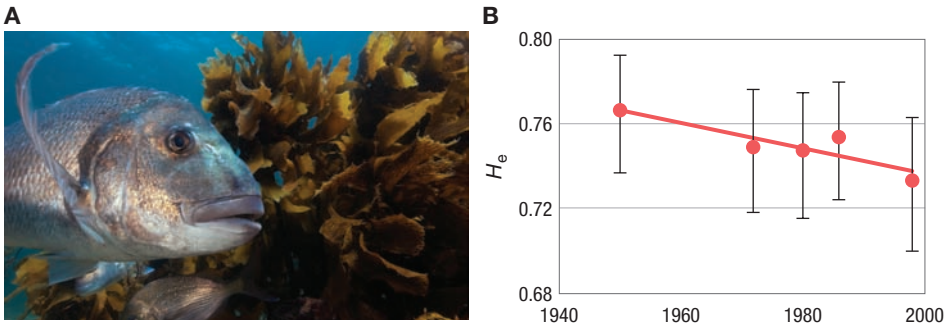


FIGURE 8.4 Loss of heterozygosity. **(A)** Genetic drift is a likely explanation for the loss of expected heterozygosity over time in an overfished population of New Zealand snapper (*Pagrus auratus*) in Tasman Bay, New Zealand. **(B)** The graph plots the expected heterozygosity of the New Zealand snapper over time. Panel B from Hauser et al. (2002).

BOX 8.2 Quantifying the Effects of Genetic Drift on Variation

Wright's F-statistic, which we introduced in Box 7.9, provides an alternative to H_o and H_e for measuring the effects of drift on variation in a population. Recall that F quantifies the correlation between the two gene copies at a locus. We can think of F as the probability that the two gene copies at a locus in a single individual are identical by descent.

The conceptual difference between the heterozygosity approach and the F-statistic approach is that the former quantifies allelic similarity, whereas the latter focuses on the probability of identity by descent and thus on history irrespective of allelic state. Recall that two gene copies can be the same in terms of genetic sequence (for example, both may be the A_1 allele), but if they did not come from a shared ancestor, they are not considered identical by descent. The F-statistic approach provides an elegant mathematical formulation of how drift reduces variation over time.

In an idealized population of infinite size with random mating, all parents will be unrelated, and therefore the two gene copies at a locus in any individual will never be identical by descent. But in a finite population, things work differently.

To see how this process increases the probability of identity by descent and thus the value of F, a thought experiment is helpful. Imagine that, at some arbitrary time in the past, we define all gene copies in the population as distinct—that is, not identical by descent—irrespective of their genetic sequence (**Figure 8.5**). At this time, the probability that any two gene copies in a newly formed offspring are identical by descent is zero. By definition, $F = 0$ at this point. As time proceeds forward, however, some of the gene copies in the population will be lost by drift. Of those that are not lost, many will be present in multiple copies. Some of the gene copies present in multiple copies will end up paired in offspring of the next generation. In those individuals, the two alleles at our given locus will be identical by descent. The value of F in the population will now be greater than zero.

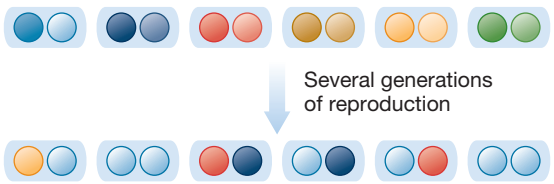


FIGURE 8.5 Genetic drift increases the probability of identity by descent over time. Diploid individuals (shown by blue shaded boxes) in a population each have two gene copies (indicated by colored circles) at a given locus. Initially, we label all gene copies as distinct—here indicated by color in the top row—irrespective of their allelic state. After many generations, some of the gene copies have left no descendants, while others have left multiple descendant copies. Thus, some of the individuals pictured in the bottom row—the second and the sixth from left in this illustration—have gene copies that are identical by descent.

Using the gamete pool approach that we first presented in Chapter 7, we can derive a mathematical expression for how F changes over time in a finite-sized Wright–Fisher population. (Here, as in Box 8.1, we consider the case where a single individual can produce both types of gametes necessary for fertilization; this greatly simplifies the derivation and closely approximates what happens with two sexes.) Imagine that each parent contributes a large number of gametes to the common gamete pool, as shown in **Figure 8.6**. Offspring are then formed by drawing pairs of gametes at random from the gamete pool.

Suppose that the value of F in a parental population of size N is F_{parental}. There are $2N$ different sources of gametes; namely, each of the $2N$ gene copies in the parental generation. Therefore, with probability $1/2N$, the two gene copies in an offspring will come from the same gene copy in the parental generation. In this case, the probability of identity by descent is 1. With probability $1 - 1/2N$, the two gene copies in an offspring will correspond to two different gene copies in the parental generation. In this case, the probability of identity by descent is F_{parental}. Putting these two cases together, the overall probability of identity by descent is

$$F_{\text{offspring}} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_{\text{parental}} \qquad (8.1)$$

The value of F is always in the range [0, 1] inclusive, so this equation ensures that $F_{\text{offspring}}$ will be greater than or equal to F_{parental} (with equality only in the case when $F_{\text{parental}} = 1$). This derivation shows that F will increase over time in a finite population (Hartl and Clark 2007). If F is equal to zero at time 0

At the set of genetic loci that they sequenced, the expected heterozygosity (H_e) in this fishery showed a statistically significant decline between 1950 and 1998.

From their results, the authors concluded that genetic drift was operating strongly in the population. This result might be somewhat surprising, given that this fishery is estimated to contain at least 3 million individuals—a population

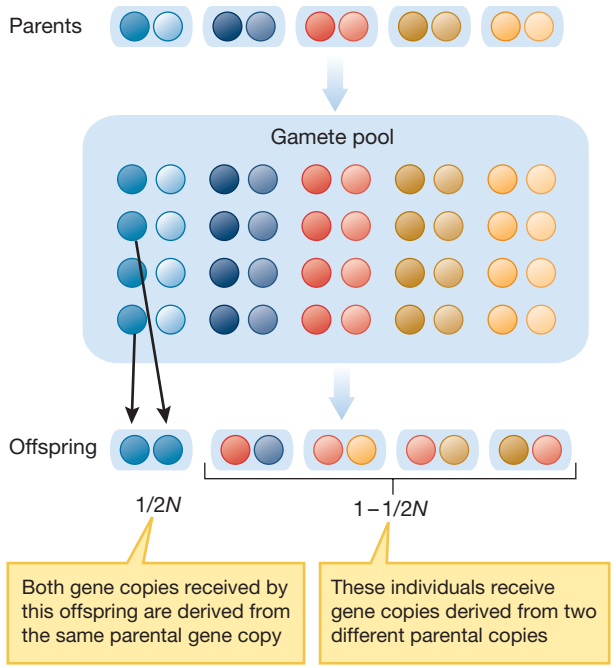


FIGURE 8.6 A gamete-pool approach to calculating how *F* changes over time in a population. With probability $1/2N$, both gene copies in an offspring derive from the same gene copy in a parent, and thus they are identical by descent with probability 1. With probability $1 - 1/2N$, the gene copies in an offspring derive from two different gene copies in the parent, and thus they are identical by descent with the same probability as were gene copies in the parental generation.

(that is, $F_0 = 0$), by applying Equation 8.1 repeatedly, we find that

$$F_t = 1 - \left(1 - \frac{1}{2N}\right)^t \tag{8.2}$$

Shortly, we will use this expression to quantify the effect of drift on expected heterozygosity in a finite population.

In a Hardy–Weinberg population (that is, infinite size with no inbreeding), Wright's F-statistic is $F = 0$. There the expected fraction of heterozygotes in the population will be $2pq$, while the expected fraction of homozygotes will be $1 - 2pq$. If instead $F > 0$, the expected fraction of heterozygotes will be

$$H_e = 2pq(1 - F) \tag{8.3}$$

We can now compare H_e values for a parental generation (call it H_{parental}) with H_e values for the offspring generation (call it $H_{\text{offspring}}$). Using Equation 8.1, we find that

$$\frac{H_{\text{offspring}}}{H_{\text{parental}}} = \frac{2p_{\text{offspring}}\, q_{\text{offspring}}(1 - F_{\text{offspring}})}{2p_{\text{parental}}\, q_{\text{parental}}(1 - F_{\text{parental}})}$$

Yet, we know that the expected values of p and q do not change from the parental generation to the offspring because of drift alone, so these cancel in the expression above, and we can write

$$\frac{H_{\text{offspring}}}{H_{\text{parental}}} = \frac{1 - F_{\text{offspring}}}{1 - F_{\text{parental}}}$$

Rearranging Equation 8.1 for how F changes over time, we get

$$1 - F_{\text{offspring}} = \left(1 - \frac{1}{2N}\right)(1 - F_{\text{parental}})$$

and therefore

$$\frac{H_{\text{offspring}}}{H_{\text{parental}}} = \left(1 - \frac{1}{2N}\right)$$

or equivalently

$$H_{\text{offspring}} = \left(1 - \frac{1}{2N}\right)H_{\text{parental}}$$

The expected heterozygosity decreases by a factor of $1/2N$ each generation because of drift in a finite population.

It is important to recognize that although drift causes heterozygosity to decrease *on average*, heterozygosity can increase in *particular* instances. Sometimes, drift may increase the frequency of a rare allele in a population and thus increase heterozygosity, at least for a while. But if we were to assess the effects of drift on 1000 independent populations, for example, we would see that drift reduces heterozygosity more often than drift increases it.

so large that drift might be expected to have only minimal effects. Nonetheless, drift had a measurable effect because populations of pelagic fish—that is, fish that live in open-water areas—commonly have relatively few individuals in each generation produce most of the offspring in the next generation. Thus, despite the large absolute population size, the population experienced rates of drift that

BOX 8.3 Effective Population Size

In populations in the real world, genetic drift does not proceed exactly as we would expect in an idealized Wright–Fisher population. The actual or "census" population size—the number of individuals we can count—will vary from generation to generation, and this influences the rate of drift (Wright 1931, 1938, 1969). In addition, individuals in real populations contribute unequally to future generations, due to differential reproductive success, differential mortality, or other factors. To account for these differences in the rate of drift, population geneticists commonly use the concept of *effective population size* as a tool with which to understand how key population parameters, such as expected heterozygosity (H_e) or Wright's F-statistic, change over time (Charlesworth 2009). Here, we will concentrate on the most commonly used of these statistics, the *inbreeding effective population size* (N_e), which we use to quantify change in the value of Wright's F-statistic.

In a Wright–Fisher population, the rate at which F changes because of drift is given by Equation 8.1 in Box 8.2, which is

$$F_{\text{offspring}} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right)F_{\text{parental}}$$

In an actual population, drift may operate differently for a number of reasons, and thus F may change at a different rate. Using the statistic for inbreeding effective population size, we can quantify how drift causes F to change in a non-Wright–Fisher population. *The inbreeding effective population size* (N_e) *is defined as the size of a Wright–Fisher population that would undergo an equivalent change in the value of* F. The value of N_e is defined by the equation

$$F_{\text{offspring}} = \frac{1}{2N_e} + \left(1 - \frac{1}{2N_e}\right)F_{\text{parental}} \qquad (8.4)$$

This is simply Equation 8.1, with N replaced by N_e. Using a bit of algebra, we can rearrange Equation 8.4 into a direct expression for N_e, as shown by the equation

$$N_e = \frac{1 - F_{\text{parental}}}{2(F_{\text{offspring}} - F_{\text{parental}})}$$

When we start with an outbred population ($F_{\text{parental}} = 0$), this expression further simplifies to

$$N_e = \frac{1}{2F_{\text{offspring}}}$$

To understand how drift operates in populations that do not meet all of the assumptions of the Wright–Fisher model, population geneticists have a set of formulas that can be used to approximate the effective population size of various non-Wright–Fisher populations. Below we consider two such examples.

Fluctuating Population Size

Suppose we have a population that fluctuates in size from generation to generation, with N_1 individuals in the first generation, N_2 in the second, N_3 in the third, and so on, and N_k in the kth generation. What is its effective population size over these k generations? It turns out that the effective population size is closely approximated by what is known as the *harmonic mean* of the population sizes in each generation:

$$N_e \approx \frac{k}{\frac{1}{N_1} + \frac{1}{N_2} + \cdots + \frac{1}{N_k}} \qquad (8.5)$$

The harmonic mean heavily weights the smallest values, so that the harmonic mean of a set of numbers is typically much closer to the smallest value than to the arithmetic mean or

might be expected in a population of fewer than 200 individuals. We therefore say that although the census population is large, of the order 3 million fish, the **effective population size** of the fishery is very small, probably fewer than 200 fish. In **Box 8.3**, we explore the concept of effective population size further, and we consider the sorts of demographic considerations that can cause the effective population size to be substantially less than the census population size.

KEYCONCEPT QUESTIONS

8.2 In Box 8.2, we showed that in a Wright–Fisher population, the expected heterozygosity decreases by an average factor of 1/2N in each generation as a result

average of those numbers. As a result, effective population size will be greatly diminished when a population spends even small amounts of time at low population numbers.

For example, suppose that over a 100-year period, an annual population spends 95 years at size 100,000 and 5 years at size 50. Its effective population size is then given by the equation

$$N_e \approx \frac{100}{\frac{1}{100{,}000} + \frac{1}{100{,}000} + \cdots + \frac{1}{50} + \frac{1}{50}} = \frac{100}{\frac{95}{100{,}000} + \frac{5}{10}} \approx 991$$

The effective population size over the 100 years, 991, is much closer to the smallest population sizes experienced than to the largest ones, even though the years with small population size are relatively rare. We will explore this effect further in Section 8.3, when we discuss population bottlenecks.

Uneven Sex Ratio

Fluctuating population size is not the only factor that influences effective population size. If the members of a population contribute unequally to future generations (and hence to the subsequent genetic variability in those future generations), effective population size is reduced. This happens whenever a population features an uneven sex ratio. In a sexually reproducing species, if we let N_m equal the number of reproductive males in a population, and let N_f equal the number of reproductive females, the effective population size is approximately

$$N_e \approx \frac{4N_mN_f}{N_m + N_f}$$

For example, loggerhead turtles (*Caretta caretta*) exhibit a strong sex ratio skew, with many more females than males present at hatching (Mrosovsky and Provancha 1992), among juveniles (Wibbels et al. 1991), and although the difference is less dramatic, among adults (Freedburg and Wade 2001; Casale et al. 2005) (**Figure 8.7**). Suppose that we have a breeding population of 10,000 loggerhead turtles, of which 8000 are female and 2000 are male. While the total population size is 10,000, the effective population size is

$$N_e \approx \frac{4 \times 2000 \times 8000}{2000 + 8000} = 6400$$

Thus, the skewed sex ratio reduces the effective population size of these turtles to less than two-thirds of the actual population size. This means that drift will act more strongly, and heterozygosity will be lost more quickly, in this population than it would be in a population of the same size with an even sex ratio.

Overall, effective population sizes tend to be substantially smaller than census population sizes. In a wide-ranging meta-analysis of nearly 200 studies of effective population size, Richard Frankham found that across a range of taxa, effective population size (N_e) averages only one-tenth of the census population size (N), and that it can drop considerably lower in some species such as marine invertebrates (Frankham 1995).

FIGURE 8.7 Skewed sex ratio. Loggerhead turtles (*Caretta caretta*) exhibit skewed sex ratios. Females make up more than 80% of some loggerhead populations, and this reduces the effective population size.

of drift. Does this mean that over time, we would expect to see fewer heterozygotes than predicted by Hardy–Weinberg proportions? Why or why not?

8.3 In natural populations, the effective population size, N_e, is typically less than the census population size, N. Is there any way that N_e could exceed N? Why or why not?

Genetic Drift Causes Divergence between Populations over Time

To get a better understanding of how drift affects populations, it can be useful to consider more than one population at a time. Let us begin with a thought experiment, and then we can move to an empirical example.

A Thought Experiment

Imagine that we have an archipelago of small islands, each able to maintain a constant-size population of 10 diploid individuals (Christiansen 2008). Moreover, suppose that each island is spaced far enough from the others that there is no migration between islands. Also assume that there is no natural selection, mutation, or assortative mating. Thus, drift is the only evolutionary process in operation (**Figure 8.8**).

Suppose that we seed each island with 10 A_1A_2 heterozygotes, so that each island receives 10 copies of the A_1 gene and 10 copies of the A_2 gene. Because genetic drift is a random process, we know that different things will happen on different islands. On some islands, the A_1 allele will eventually become fixed; on others, the A_2 allele will eventually become fixed. On some islands, fixation will occur quickly; on others, it will take a long time to reach fixation.

Instead of looking at the frequencies of *different types of individuals* within a population, here we are focusing on the frequencies of *different types of populations*. The bar graphs in Figure 8.8 show the frequency of islands that have populations with 0, 1, 2, and so on, copies of the A_1 allele, in the original founding population ($t = 0$), and then the expected frequencies at subsequent times ($t = 1$, 2, 4, 8, 16, and 32) under the Wright–Fisher model we outlined in Box 8.1. For example, at $t = 1$, about 17% of the islands have populations with unchanged allele frequencies—10 copies of the A_1 allele and 10 copies of the A_2 allele—but on all of the other islands, the frequency of the A_1 allele has already drifted away from 0.5. As time goes on, drift continues. By $t = 8$, an appreciable number of islands have populations that have already fixed either the A_1 allele or the A_2 allele. By $t = 32$, few of the islands have populations that remain polymorphic.

From this example, we see that genetic drift leads to divergence—differences in allele frequencies and ultimately the fixation of different alleles—among the populations on the islands in our hypothetical archipelago. In the next subsection, we will see that something similar happens in real archipelagos.

Drift and Divergence in the Galápagos Archipelago

Galápagos lava lizards (*Microlophus albemarlensis*) are moderately sized (17–25 centimeters in length) insectivorous lizards that inhabit dry rocky areas of numerous islands of the Galápagos archipelago (**Figure 8.9**). They are thought to disperse between islands only rarely, and they form a set of independent populations on the large island of Santa Cruz and its surrounding islets.

These lizard populations have not always been separate, however (**Figure 8.10**). During much of the Late Pleistocene—as recently as 12,000 years ago—large volumes of water were trapped in kilometer-thick glacial ice sheets covering northern North America and Eurasia. As a result, sea levels around the world were substantially lower than at present. During this period, Isla Santa Cruz was connected to many surrounding islands and islets by land, and overseas distances to the other islands were considerably smaller. At that time, populations of lava lizards presumably were able to mix more readily. Once the glaciers receded and the sea rose to its current level, the populations were separated, and migration between populations was eliminated or severely curtailed.

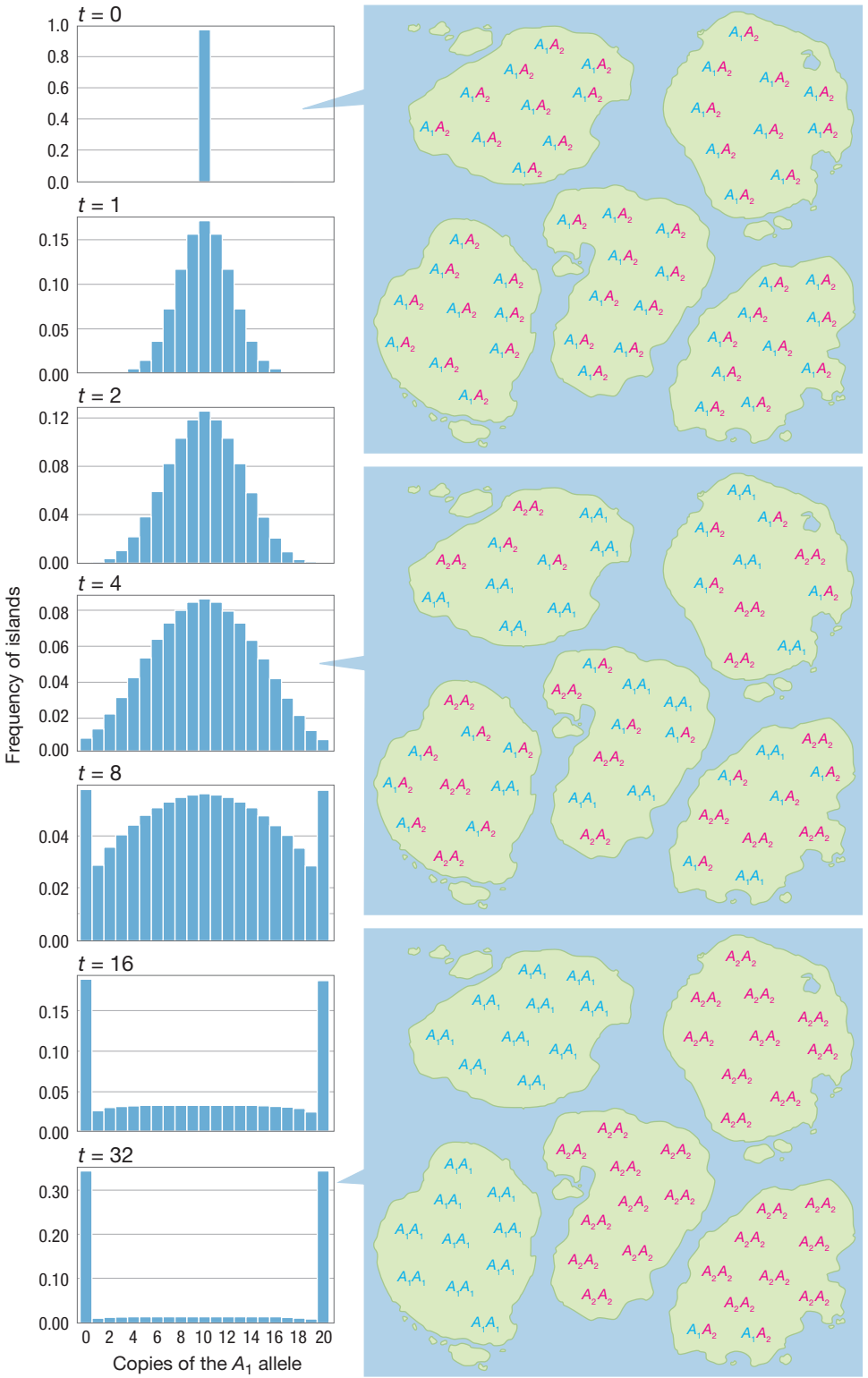


FIGURE 8.8 Genetic drift in island populations. A thought experiment illustrates how drift leads to divergence between populations. We envision a large number of islands, each with 10 diploid inhabitants. At time 0, the islands are founded by A_1A_2 heterozygotes at the neutral A locus. The inhabitants then mate randomly, and there is no mutation or migration. The bar graphs at the left show the frequency of *islands* with 0, 1, 2, and so on, copies of the A_1 allele at times $t = 0$ through $t = 32$. Note that the vertical scale varies from graph to graph. Over time, most islands become fixed either for the A_1 allele or for the A_2 allele. At the right are shown groups of five islands from the $t = 0$, $t = 4$, and $t = 32$ distributions given at the left. ▶

FIGURE 8.9 Galápagos lava lizard. Genetic drift and divergence have been studied in the Galápagos lava lizards (*Microlophus albemarlensis*).

This leaves us with a situation very similar to that of the hypothetical archipelago from our previous thought experiment. To explore the consequences of genetic drift on these recently separated populations, Mark Jordan and Howard Snell assessed the genetic diversity of 17 populations by sequencing 11 different

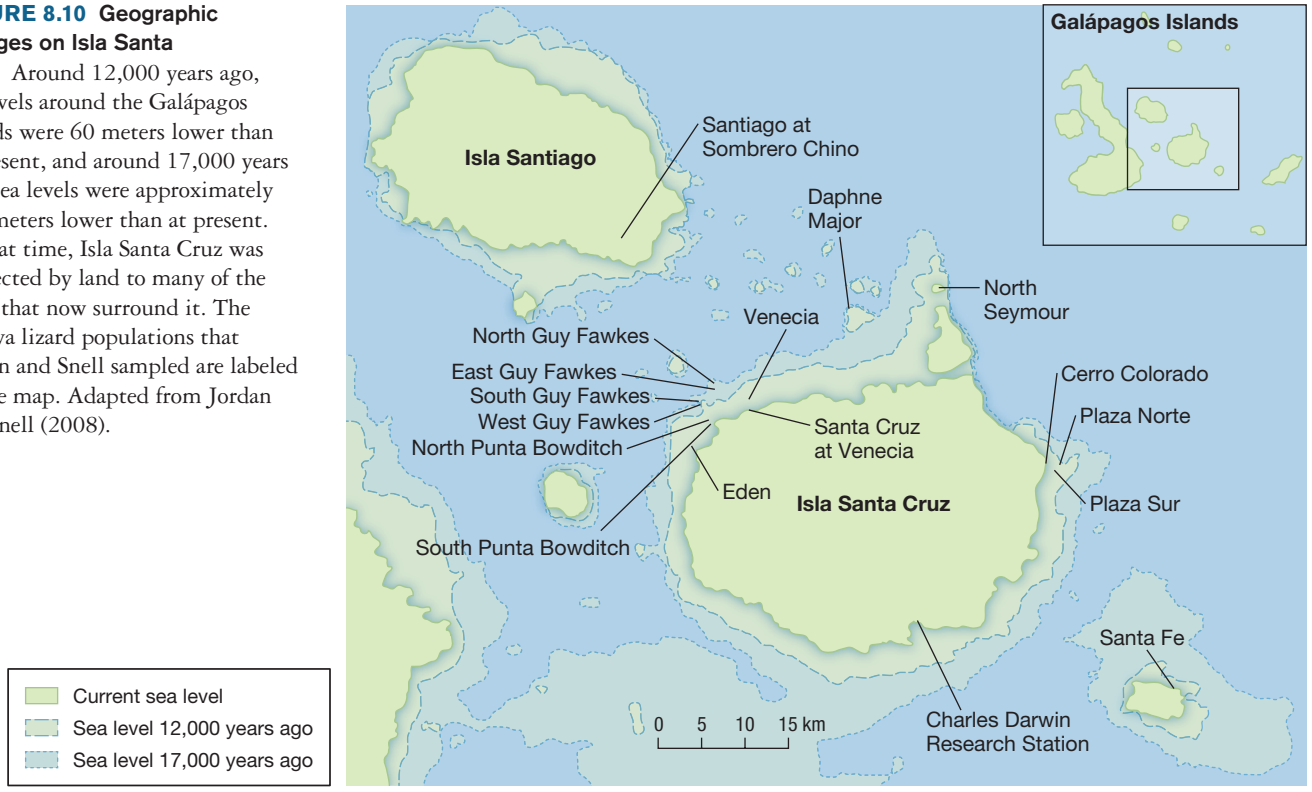


FIGURE 8.10 Geographic changes on Isla Santa Cruz. Around 12,000 years ago, sea levels around the Galápagos Islands were 60 meters lower than at present, and around 17,000 years ago, sea levels were approximately 130 meters lower than at present. At that time, Isla Santa Cruz was connected by land to many of the islets that now surround it. The 17 lava lizard populations that Jordan and Snell sampled are labeled on the map. Adapted from Jordan and Snell (2008).

microsatellite markers in a sample of individuals from these populations (Jordan et al. 2002; Jordan and Snell 2008). Microsatellites are short stretches of DNA sequence in which a brief sequence—for example, CAG—is repeated several times. Microsatellites tend to make very good genetic markers for studying relatively short periods of evolutionary time. First, they are typically selectively neutral. Second, they tend to be highly variable in length because copy number changes readily by a type of mutation known as slippage-induced mutation.

Jordan and Snell reasoned that in the absence of gene flow between populations, genetic drift should strongly influence the patterns of diversity at these microsatellite loci. This allowed them to make a number of predictions. First, drift is expected to operate more strongly—and cause the loss of more variation—in smaller populations. Thus, the smaller lizard populations on smaller islands would be expected to have fewer microsatellite alleles than would larger populations on larger islands. As illustrated in **Figure 8.11**, this is exactly what Jordan and Snell found.

Jordan and Snell also found strong evidence of genetic drift in the patterns of genetic divergence *between* lizard populations on the various islands, with different islands revealing very different alleles and allele frequencies. Here, we see *population subdivision*, in which there is limited or no gene flow between subpopulations of a larger population, along with genetic drift leading to divergence among subpopulations of the lava lizards on the Galápagos.

In both our thought experiment and our example from the Galápagos, we considered genetic drift and differentiation on the islands of archipelagos. Island populations of terrestrial species make convenient systems for studying drift, because gene flow between populations on different islands is kept to a minimum.

It is important to stress that genetic drift occurs not only on islands, but in every population. Moreover, population subdivision can occur without physical barriers as obvious as those imposed by the stretches of open ocean between islands. More subtle geographic barriers, or even behavioral differences, can likewise restrict gene flow and thus create population subdivision, leading to accelerated genetic drift and possible divergence among subpopulations.

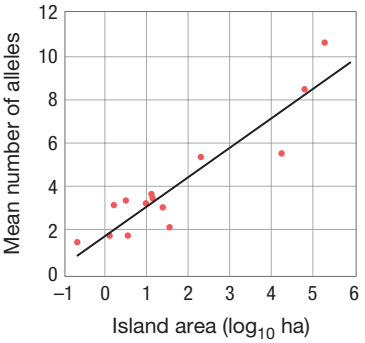


FIGURE 8.11 Lizard populations on smaller islands have lower diversity in microsatellite alleles than that of populations on larger islands. Here, we plot the area of the island for each sample population (horizontal axis) against the mean number of alleles per microsatellite locus (vertical axis). The former serves as a measure of population size; the latter as a measure of genetic diversity. The statistically significant relationship between island size and genetic diversity, indicated by the solid line, suggests that genetic drift has been operating more strongly in smaller populations, as predicted. From Jordan and Snell (2008).

8.2 Coalescent Theory and the Genealogy of Genes

To develop a deeper understanding of how drift operates and how it influences variation in a population, we can look at the genealogical relationships in that population. It will be particularly useful to examine these genealogical relationships one locus at a time. By doing so, we will be able to see how gene copies spread through a finite population over generations. We can see the process of drift in action, as gene copies increase or decrease in number due to chance, and we can observe the process by which alleles reach fixation over time. This is the fundamental idea behind an area of population genetics known as **coalescent theory** (Kingman 1982; Hudson 1990; Wakeley 2008).

From Species Trees to Gene Trees

Thus far, the phylogenetic trees we have drawn have typically been *species trees* or *population trees*; that is, they represent historical patterns of branching descent for a group of species or populations. We can also draw trees known as *gene trees*, which represent these genealogical relationships for a single locus. We have actually seen this approach already: When we build a phylogenetic tree using sequence data from a single genetic locus, we are not reconstructing the species tree directly, but rather we are inferring the pattern of descent with modification at this one specific locus. Such a phylogeny *is* a gene tree, in that strictly speaking it tells us about the history of that gene, not the history of the populations in which that gene appears. Although a gene tree often provides a good approximation for a species tree, gene trees for different loci will not necessarily agree with one another or with the species tree. Most of the phylogenetic methods that we examined in Chapter 5 work by finding the species tree that is most consistent with the various gene trees for multiple loci.

So now, in the spirit of thinking about gene trees and using them to understand the process of genetic drift in small populations, we will shift our attention to understanding the genealogical pattern of ancestry among gene copies in a population of diploid organisms. By way of illustration, **Figure 8.12A** shows a genealogical diagram—a depiction of which gene copy derived from which ancestral copy—for a neutral locus in a population of five diploid organisms over a period of 11 generations. In each generation, some gene copies manage to replicate themselves and contribute to the next generation; other gene copies fail to replicate and are lost. Because we are only interested in the genealogy of genes, not the genealogy of individuals, we can ignore which gene copies are in which individual, as shown in **Figure 8.12B**, and then "untangle" the genealogical graph to provide a clean picture with no crossing lines, as in **Figure 8.12C**.

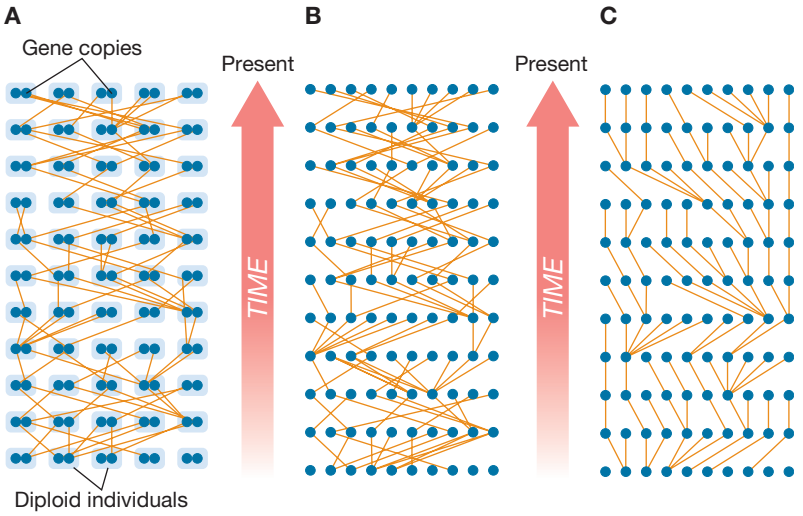


FIGURE 8.12 Gene genealogies for a diploid population. **(A)** This figure shows a simulated genealogy of gene copies (blue circles) at a neutral locus in a population of five diploid individuals (five shaded boxes) over 11 generations. Orange lines indicate ancestry. Yet, even for this small a population and this short a time period, the graph is complex and difficult to interpret, with many crossing lines. **(B)** Because we are only interested in the gene genealogy and not in the diploid individuals, we can ignore the identity of the individuals in which each gene copy resides. **(C)** If we do not require that gene copies in the same diploid individual be placed adjacent to one another in the diagram, we can "unscramble" the graph, generating a genealogical diagram with no crossing lines. This form, which is much easier to interpret at a glance, summarizes the genealogical relationships among the gene copies present in the population. Adapted from Felsenstein (2004).

Suppose we are interested in the genealogical relationships among some set of gene copies in the current population. If we know the genealogical graph for the population, we can trace the ancestry of these gene copies backward in time, as illustrated in **Figure 8.13A**. What we find, as we trace back in time from the present, is that gene copies coalesce; that is, two or more distinct gene copies at some point in time are all descended from the same ancestral gene copy. For example, in Figure 8.13A, gene copies ii and iii coalesce after a single generation. Three generations later, their lineage coalesces with the lineage leading to gene copy i, as indicated by the red circle in the figure. This circle is the **coalescent point** for gene copies i, ii, and iii; in other words, it is the gene copy that is the most recent common ancestor of i, ii, and iii.

We can also consider the coalescent process for the entire population. **Figure 8.13B** shows what happens as we trace back in time from *all* of the gene copies in the population at the present. We have to go back further, but eventually we reach a coalescent point, indicated by the green circle in the figure, for these as well. This coalescent point is the gene copy that is the common ancestor to all gene copies in the population at the present time.

Furthermore, notice that by tracing the genealogy backward, we have created a tree structure: This *coalescent tree* shows the branching pattern of relatedness among the gene copies in the population.

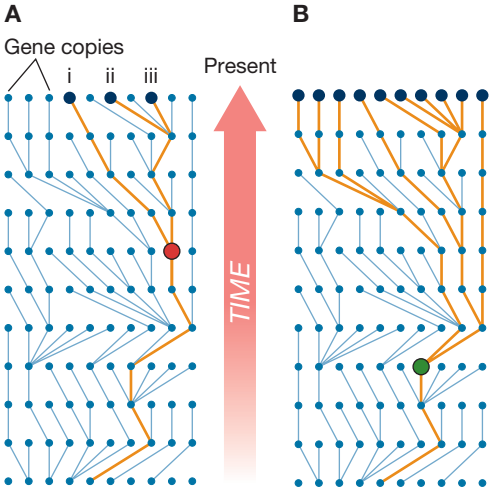


FIGURE 8.13 Tracing back the ancestry of specific gene copies. **(A)** The genealogical history of the three highlighted gene copies is indicated. The three gene copies are all derived from a single gene copy four generations back (red circle). We say that these gene copies *coalesce* at the red circle. **(B)** The genealogical history of all gene copies in the population at the present time is traced back. In this case, all of the gene copies in the population are derived from a single gene copy seven generations back (green circle).

Dynamics of the Coalescent Process

One of the major advantages of taking a coalescent approach is that this way of thinking is particularly amenable to mathematical treatment. The basic idea in mathematically modeling the coalescent process is to think of a genealogy as a stochastic process running *backward in time.* Suppose that we sample k gene copies from a population of N diploid individuals. At the present, which we will call time t, these k gene copies are all distinct. Now imagine that we take a step backward to time $t - 1$, and look at the previous generation. With some probability, any two or more of our k gene copies may come from the same gene copy

BOX 8.4 A Mathematical Treatment of the Coalescent Process

Following Kingman (1982), we can write down an elegant mathematical model that provides a close approximation to the neutral coalescent process we have discussed here. We will follow Felsenstein's simplified derivation (Felsenstein 2004).

Consider k gene copies in a much larger Wright–Fisher population of N diploid individuals. Each of the k gene copies is descended from a random ancestral gene copy, so the chance that any particular pair shares a common ancestor in the previous generation is $1/2N$. But our k gene copies form a total of $k(k-1)/2$ different pairs, ignoring the order of the pairing. If we assume that N is large and that $k \ll N$, the chance that more than two gene copies come from the same copy in the previous generation is very small, as is the probability that more than one pair will coalesce at the same time. Thus, the probability that a coalescent event occurs in a single generation is approximately $k(k-1)/4N$. The waiting time until the first coalescent event is then approximately geometrically distributed with rate $k(k-1)/4N$ and average waiting time $4N/(k[k-1])$.

After the first coalescent event occurs, there are now $k-1$ distinct lineages. Again, the probability that any pair of these lineages coalesces in the previous generation is $1/2N$. These $k-1$ lineages form $(k-1)(k-2)/2$ unordered pairs, so the probability that a coalescent event in a single generation occurs is now approximately $(k-1)(k-2)/4N$, and the average waiting time until this occurs is $4N/[(k-1)(k-2)]$.

How long will it take until all k lineages have coalesced? Because each coalescent event is approximately independent, we can simply sum the average waiting times for each successive coalescent event, from the first, when there are k lineages, until the last, when there are only two lineages. This gives us

$$\sum_{i=2}^{k} \frac{4N}{i(i-1)} = 4N\left(1 - \frac{1}{k}\right)$$

This equation provides us with the results described in the text. When k is relatively large, this quantity is closely approximated by $4N$; hence, the average coalescent time for k gene copies in a large population is approximately $4N$. The final coalescent event occurs between two lineages that can be paired in only one way. In each generation, there is $1/2N$ probability that they will coalesce. Thus, the expected time for the last event to occur is $2N$, fully half of the total coalescent time for all k lineages.

at $t-1$. If that occurs, we call it a coalescent event. It turns out that, for a neutral locus, we can formulate an elegant mathematical model of this process. This model tells us the distribution of times until coalescence and also the distribution of gene tree topologies that arise at a neutral locus. We explore this model in **Box 8.4**.

For a neutral locus in a diploid Wright–Fisher population of size N, the average time to coalescence for any randomly chosen *pair* of gene copies turns out to be $2N$ generations (Hudson 1990). For a larger group of gene copies, the average time to coalescence of all of these copies is approximately $4N$ generations.

In the coalescent process for a neutral locus, much of the action happens only shortly before the present. Because in coalescent theory we envision a process running backward in time from the present to the past, we refer to these events as "early." Thus, most of the coalescent events between pairs of gene copies are expected to occur early on. We see this in **Figure 8.14**, which

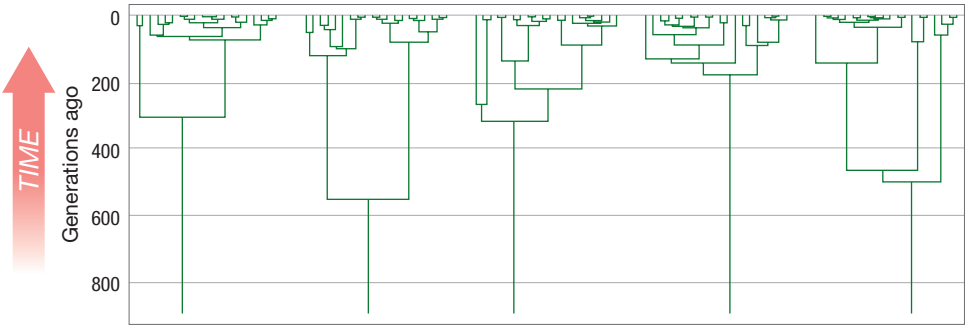


FIGURE 8.14 Coalescent trees vary in shape. Here are five simulated coalescent trees for a sample of $k = 20$ gene copies in a population of $N = 100$ diploid individuals. Adapted from Wolfram Demonstrations Project (2011).

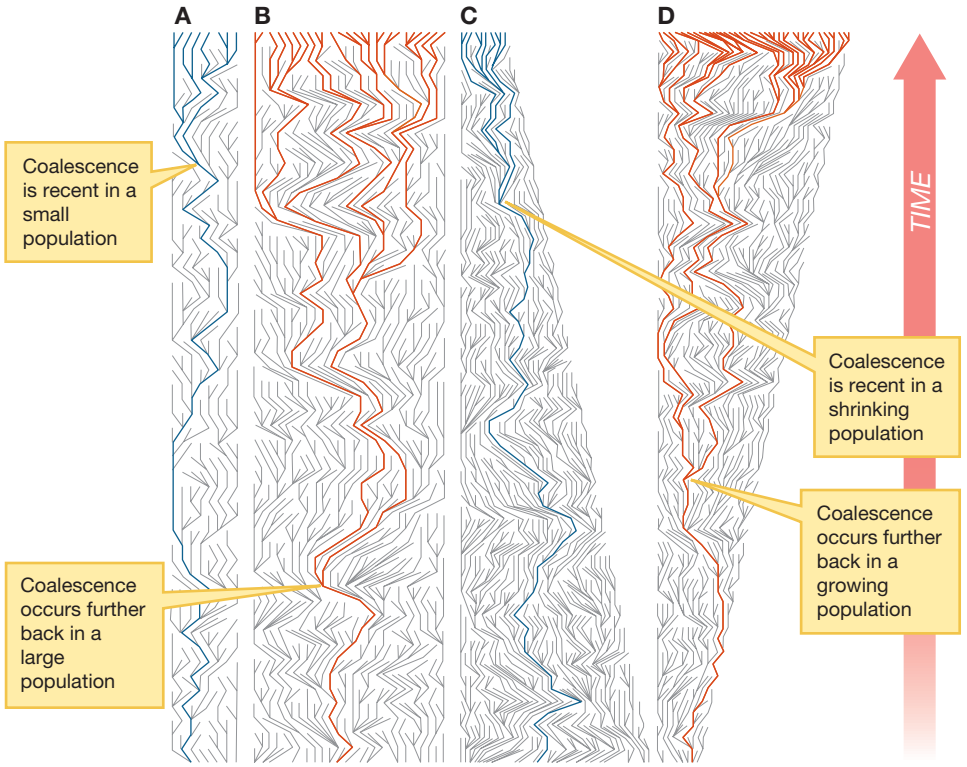


FIGURE 8.15 The effect of demography on coalescence. Gene genealogies, with the coalescent tree highlighted, in **(A)** a small population of constant size, **(B)** a large population of constant size, **(C)** a declining population, and **(D)** an expanding population.

shows five different simulated coalescent trees for 20 gene copies at a neutral locus. In each of the five trees, the majority of coalescent events occur very early, fewer than N generations back from the present. In fact, the expected time for the population to coalesce down to just two parental lineages is only $2N$ generations. But the final coalescent event typically takes a very long time. Even once we are down to two lineages, it takes on average another $2N$ generations for the final two lineages to coalesce.

It is important to recognize that these results about coalescent times refer to expected times or averages; there is substantial variation around the mean. As a result, different loci in the same population may have very different coalescent times. We see this in Figure 8.14 as well. Although all five trees result from simulating the same random process, the time until coalescence varies from less than 200 generations to more than 500 generations.

Coalescent times depend strongly on the demography of a population. In a Wright–Fisher population of constant size, we have seen that the coalescent time of any pair of alleles is $2N$, and the average coalescent time of a sample of k alleles is approximately $4N$. Therefore, in a small population with small N, coalescence occurs in less time than in a large population with large N (**Figure 8.15**).

KEYCONCEPT QUESTION

8.4 Consider a neutral locus in a constant-size population of 500 diploid individuals. Which is expected to take longer: coalescence of the 1000 gene copies at this locus down to 10 ancestral copies or coalescence of those 10 ancestral copies down to a single ancestral copy?

Bugs in a Box

How can we develop an intuitive understanding of these results? Coalescent trees can be hard to think about because it is not easy to envision a process running backward in time. To get around this difficulty, population geneticist Joe Felsenstein has proposed a delightful metaphor for thinking about coalescence as a stochastic process that runs *forward* in time. Felsenstein envisions a box full of voracious and cannibalistic bugs (**Figure 8.16**). The bugs wander around the box at random, and any time two bugs encounter each other, one eats the other. The process continues until the box contains only a single surviving bug. Mathematically, Felsenstein's bugs-in-a-box metaphor is identical to the coalescent process for a neutral locus, but with time running forward instead of backward. In Felsenstein's metaphor, the bugs represent gene copies. When one bug eats another, this represents a coalescent event. When only one bug is left in the box, the entire population has coalesced.

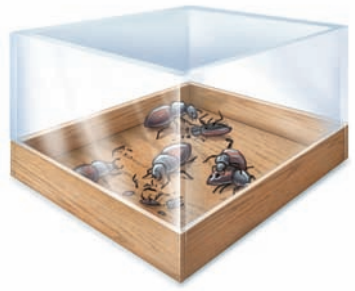


FIGURE 8.16 Bugs in a box. The coalescent process is mathematically analogous to a process in which hungry bugs run around inside a box and one eats another any time two meet.

Thinking about what would happen in a box of bugs like this, we can get an intuitive feel for many of the results we have observed for the coalescent process. Early on, the box is full of many bugs, and they run into each other often. Thus, cannibalism events occur at a rapid pace early in the process, just as coalescent events occur rapidly early in the coalescent process. Later, as the number of bugs in the box declines, contact among bugs occurs less often, and the rate of cannibalism slows. But eventually the box will contain three bugs, then two, and ultimately, perhaps after a long wait, only one. Sometimes, the remaining two bugs will encounter each other after a short period; other times they will wander extensively before colliding. As a result, the time until we are left with only a single bug varies widely from one instance of the process to the next.

The Coalescent Process and Genetic Variation

How does the coalescent process influence the amount of variation we see in populations, particularly in small populations? Thus far in this section, we have focused on the genealogy of gene copies, irrespective of their allelic state. To understand patterns of genetic variation, we now need to add allelic differences to our coalescent model.

Figure 8.17 illustrates a simulated coalescent tree with allele states shown on the tree. This figure highlights the fundamental observation that links coalescent trees with genetic variation: *Any allelic differences among a set of gene copies at the same locus must have arisen by mutation subsequent to the coalescent point for this set of gene copies*. Thus, if we know the shape of the coalescent tree and the places where mutations arose after the coalescent point, we know everything about the variation in the current population.

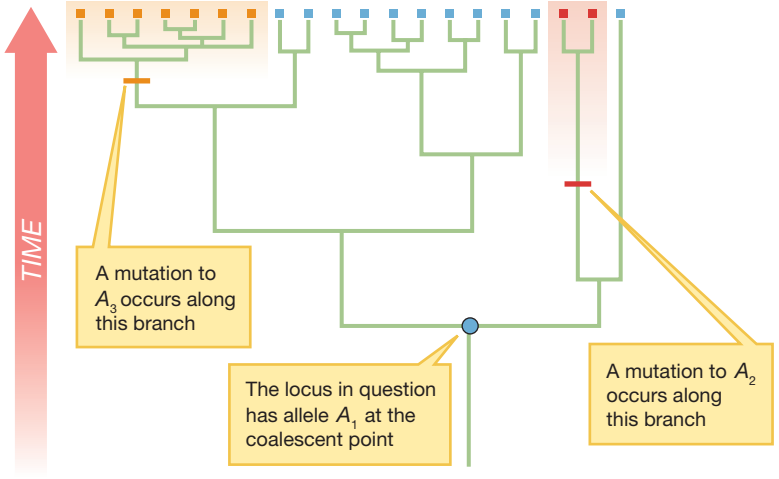


FIGURE 8.17 A coalescent tree with allelic states shown. Mutations generate new alleles, shown in orange and red. Notice that all of the variation at this locus has arisen subsequent to the coalescent point.

The structure of coalescent trees in a population tells us a great deal about the amount of variation we should expect to see. If all of the gene copies in a population coalesce only seven generations back, then any variation present in the population must have arisen by

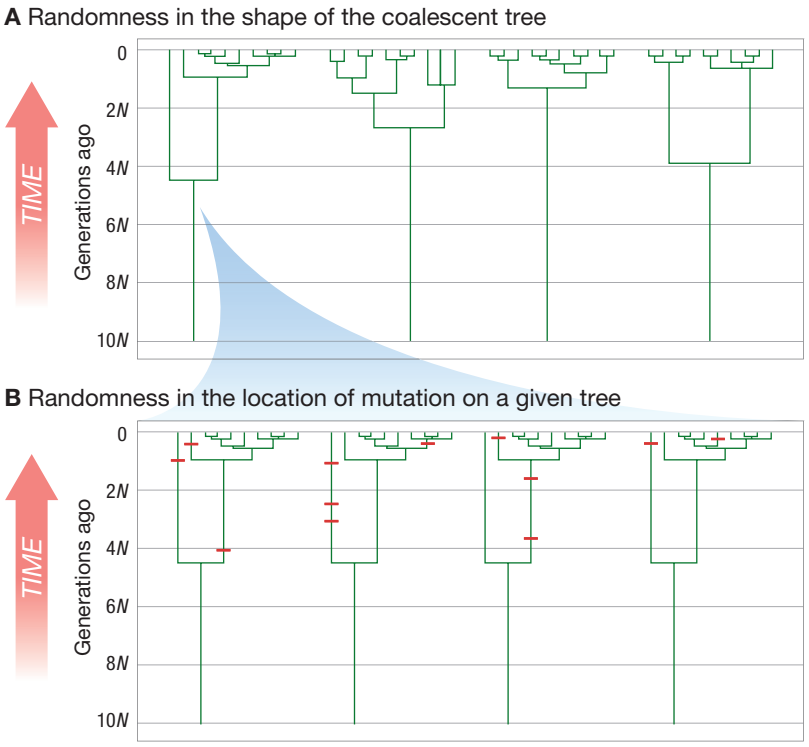


FIGURE 8.18 Separating genealogy and mutation. The distribution of variation at a neutral locus depends on two separate processes: **(A)** the random process by which the shape of the coalescent tree is determined, and **(B)** the random process of mutation events (shown by the red bars) along the branches of this coalescent tree. Notice that in a neutral model, the locations of the mutations have no effect on the shape of the tree, which is determined simply by the demographic history of the population. For example, any of the mutational histories shown in panel **B** — or any other mutational history — could underlie the coalescent tree at left in panel **A**. Adapted from Wolfram Demonstrations Project (2011).

mutation at some time in the past seven generations. If instead the population does not coalesce until 70 generations back, there will have been much more time for variation to arise by mutation. With all else equal, we expect the total number of mutations differentiating any two gene copies to be proportional to the total branch length from these two gene copies back to the point at which they coalesce. Thus, the deeper the coalescent point, the more variation we expect to see in the population. We will illustrate this by exploring what the coalescent tree at a neutral locus tells us about the process of genetic drift.

The coalescent process is particularly elegant for a neutral locus. For such loci, we can separate the genealogical history of the locus from the mutational process that takes place at that locus (Hudson 1990; Nordborg 2007). Thus, we can think of the process by which variation arises at the locus as the result of two separate processes: (1) the genealogical process by which a coalescent tree is formed, and (2) the mutation process by which variation arises along the coalescent tree (**Figure 8.18**). We can separate these processes because, at a neutral locus, all gene copies are equally likely to leave descendants, irrespective of their allelic state. Thus, the mutation process and the allelic states of gene copies have no effect on the genealogical process and the resulting shape of the coalescent tree.

In this case, the coalescent process tells us about the strength of genetic drift to eliminate genetic variation. In Figure 8.15A, we showed a simulated coalescent tree for a small population; in Figure 8.15B, we showed a simulated coalescent tree for a larger population. As we noted, the small population has a much more recent coalescent time; thus, we expect that less variation will have been generated since coalescence in the small population. This is consistent with the finding we discussed in Section 8.1: that drift will act more strongly to reduce heterozygosity in a small population than in a large one.

The pattern of variation that we see at a neutral locus is therefore the result of two sources of randomness superimposed on one another: (1) the randomness associated with which particular genealogical history happens to occur (that is, the coalescent tree of the current population), and (2) the randomness associated with where mutations arise along this coalescent tree (Felsenstein 2004; Nordborg 2007).

Let us focus on a Wright–Fisher population of constant size with no selection, assortative mating, or migration. If two randomly selected alleles are separated by on average $4N$ generations, and the mutation rate is μ per locus per generation, we expect two randomly selected alleles to differ by an average of $4N\mu$ mutations. But there are two sources of randomness that cause variation around this average number of differences: (1) genealogical history is a

random process, so the two alleles may be separated by considerably more or less than $4N$ generations; and (2) the mutation process varies, so if the two alleles are separated by, say, 1000 generations, we may see more or less than 1000μ mutations distinguishing them.

The mathematical relationship between the coalescent process and the amount of variation present in the population provides a tool for inferring details of population history. Typically, the history of the population is unknown, but researchers can readily assess current levels of variation. Using a coalescent model, researchers can infer coalescent times from patterns of genetic variation and thereby estimate historical demographic parameters such as population size over time (Emerson et al. 2001).

We conclude this section by noting that selective processes also have a substantial influence on the shape of coalescent trees. Selection drives alleles quickly to fixation, leading to a more recent coalescent time. **Figure 8.19** illustrates the gene genealogy for new mutants that are either neutral, positively selected, or subject to balancing selection. A conventional gene genealogy for a neutral locus is shown in Figure 8.19A. Here, a new neutral allele arises by mutation as indicated. In this particular example, the new allele drifts, by chance, to fixation. Note, however, that most newly arisen neutral alleles will be lost, rather than fixed, by drift.

Alleles under **positive selection**—that is, alleles that are selectively favored relative to others at the same locus—do not have to rely on drift alone to reach fixation. In Figure 8.19B, the new allele is positively selected and, because of selection, it quickly replaces all other alleles in the population. As a result, the population has a more recent coalescent point than that in the neutral example. This is a useful observation. Because a recent selective event results in a more recent coalescent point, we expect to find less neutral variation—that is, fewer synonymous or *silent* substitutions—at the locus under selection.

As we learned in Chapter 7, forms of balancing selection such as overdominance or negative frequency dependence can maintain balanced polymorphisms of two or more alleles. In Figure 8.19C, a new allele arises by mutation that is under balancing selection with the ancestral allele. Because balancing selection favors the new allele when it is rare, but favors the ancestral allele when the new allele is common, neither allele is easily able to go to fixation. As a result, both remain in the population for an extended period of time, and the coalescent point for this locus occurs further in the past than it did for the neutral and positively selected cases. Because the population is finite, we expect one allele will eventually replace the other by chance despite balancing selection. But this may take a very long time to occur, and in the meantime we observe a balanced polymorphism with a coalescent point far from the present.

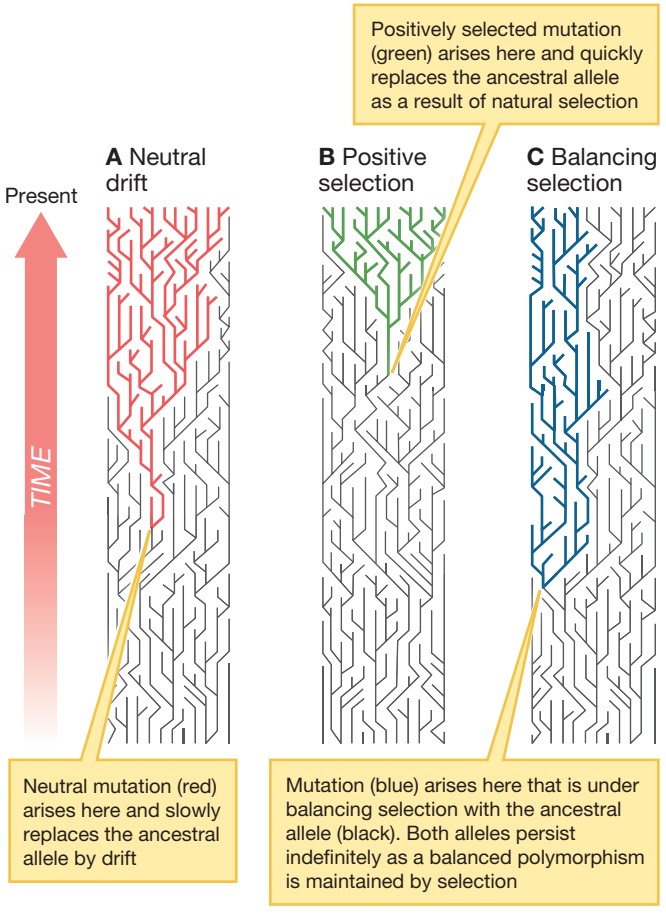


FIGURE 8.19 Gene genealogies and selection. **(A)** Gene genealogy for a new allele subject to neutral drift. In this particular case, the gene shown drifts to fixation. **(B)** Gene genealogy for a new allele subject to positive selection. Here, natural selection quickly drives the new allele to fixation. **(C)** Gene genealogy for an allele under balancing selection. Here, the two alleles both persist indefinitely in a balanced polymorphism. Adapted from Bamshad and Wooding (2003).

8.3 Demography, Biogeography, and Drift

We have seen that genetic drift operates most powerfully when population sizes are very small. Although many natural populations tend to be large most of the time, certain demographic and biogeographic processes can reduce their size considerably. Even a brief reduction in population size can cause drift to operate strongly. In the language of effective population size (see Box 8.3), even a short period of having a small census population size can massively reduce the effective population size. In this section, we will consider two particularly important processes of this type: (1) population bottlenecks and (2) the founder effect.

Population Bottlenecks

We have learned that genetic drift can be an important evolutionary process in small populations. But what happens in large populations, especially those without significant subdivision? Are they protected from the operation of drift? Not entirely, because natural populations inevitably fluctuate in size over time. Even very large populations can go through rough periods where population size becomes small. And when populations become very small, even for a short time, allele frequencies can change dramatically. This is because of the sampling that occurs during the reduction of population size and because of the accelerated pace of genetic drift in the small population. This process is so important in natural populations that population geneticists have a specific name for it: A brief period of small population size is called a **population bottleneck** (Figure 8.20).

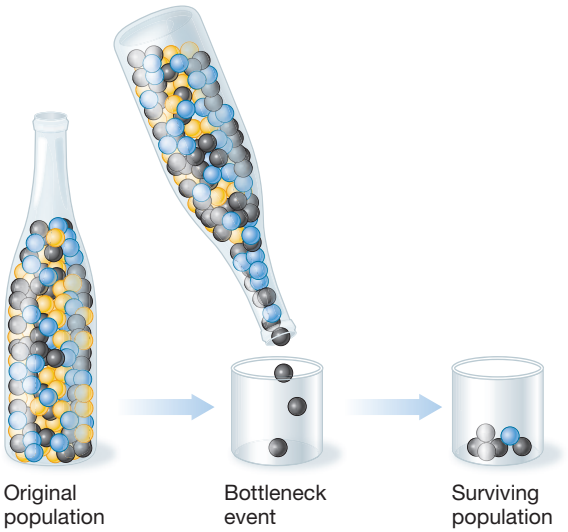


FIGURE 8.20 The population bottleneck concept. In the original population, there are three different alleles, represented here by blue, black, and yellow balls. A bottleneck cuts population size dramatically, leading to shifts in allele frequency simply by chance. Compare the frequency of black and blue balls before and after the bottleneck. Bottlenecks can even result in the loss of certain alleles. The yellow allele is lost in this example.

A Simulation of the Effects of a Bottleneck

In Figure 8.21, we show the results from a simulation of 10 replicate populations of size 1000 going through a brief population bottleneck. Notice that the biggest changes in allele frequency come during the bottleneck. Even though the population consists of 1000 diploid individuals for most of the period shown, the bottleneck has a considerable effect on allele frequencies, and alleles even go to fixation in two of the replicate populations.

We can infer the effects of a bottleneck on the rate of genetic drift from the equation for effective population size that we developed in Box 8.3. There, we saw that the effective population size of a population that varies in size from generation to generation is given by the harmonic mean of the population sizes in each generation. In the case of a bottleneck, the population size is large for much of the time, only briefly becomes small, and again grows back to its usual large size. How does this affect the effective population size? The harmonic mean of these population sizes will tend to be close to the smallest population size; that is, to the size of the population during the tightest part of the bottleneck. Because the effective population size is small, we expect the rate of drift to be high—exactly as we have seen is the case when a bottleneck occurs.

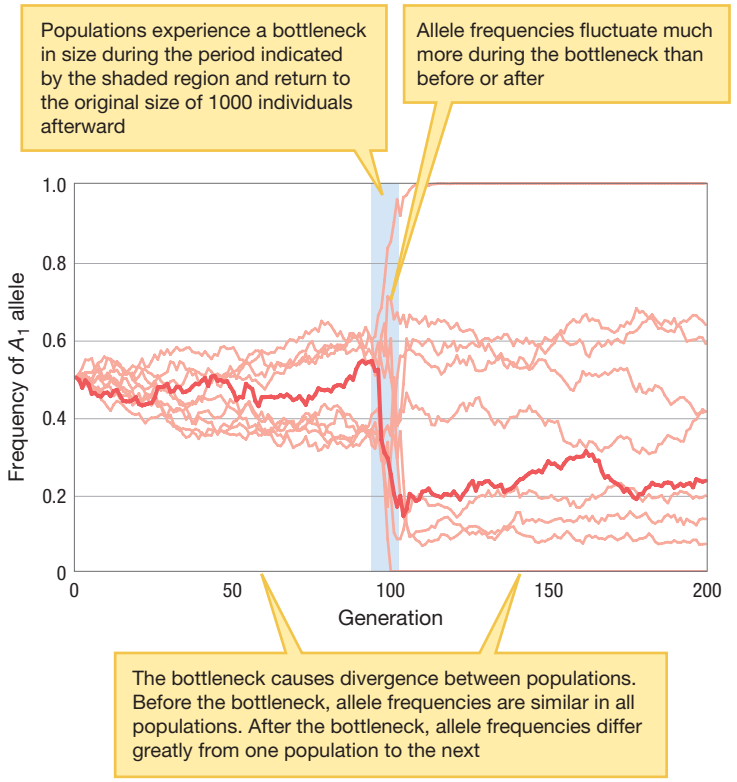


FIGURE 8.21 A bottleneck causes a drastic shift in allele frequency. Here, we see the results from simulations of 10 replicate populations, each with 1000 diploid individuals, going through a brief population bottleneck. Population size decreases rapidly during the bottleneck, reaching a low point of 10 individuals before quickly rebounding. Each population starts with the A_1 and A_2 alleles each at frequency 50%. One sample trajectory is highlighted for emphasis. Allele frequencies drift gradually until the population bottleneck, at which point the drift accelerates dramatically, causing large changes in allele frequency. As the populations are restored to their original sizes, the rate of allele frequency fluctuation slows.

A Strong Bottleneck Reduced the Heterozygosity of Elephant Seals

The effects of a population bottleneck are illustrated by one of the most remarkable recoveries from near-extinction yet observed: that of the northern elephant seal (*Mirounga angustirostris*) (**Figure 8.22**). This species, which breeds on the beaches of California and Baja California, was hunted to the very edge of extinction in the nineteenth century. Although the commercial harvest ceased as the seal population declined, museum collectors killed many of the remaining animals. In 1892, eight individuals, thought to be the last of the northern elephant seals, were discovered on Guadalupe Island off the west coast of Mexico (Hoelzel 1999). These were promptly killed for museum specimens!

FIGURE 8.22 Bottlenecks in a natural population. Male northern elephant seals at San Simeon, California.

Fortunately, these were not the last members of the species. Roughly 10–20 individuals had been missed by hunters, and from these few individuals the population began its recovery. After vigorous protection efforts, the northern elephant seal population rebounded, and it now numbers well over 100,000 individuals.

As we have seen, population genetics predicts that a bottleneck should cause a dramatic reduction in heterozygosity in the northern elephant seal population. To test this prediction, Michael Bonnell and Robert Selander took blood samples from 159 individuals at five different breeding locations (Bonnell and Selander 1974). They used a technique known as *enzyme electrophoresis* to look for molecular variation in the structure of 21 different proteins—and by this assay found no variation whatsoever. In a 1993 follow-up study, A. Rus Hoelzel and his colleagues surveyed 41 additional proteins using similar methods and again found zero variation

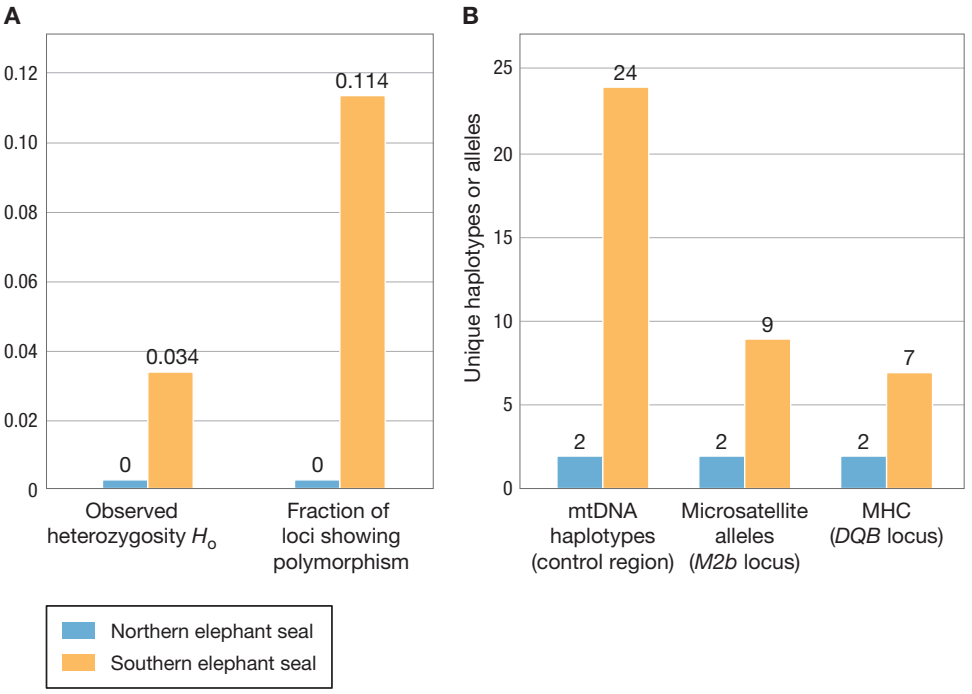


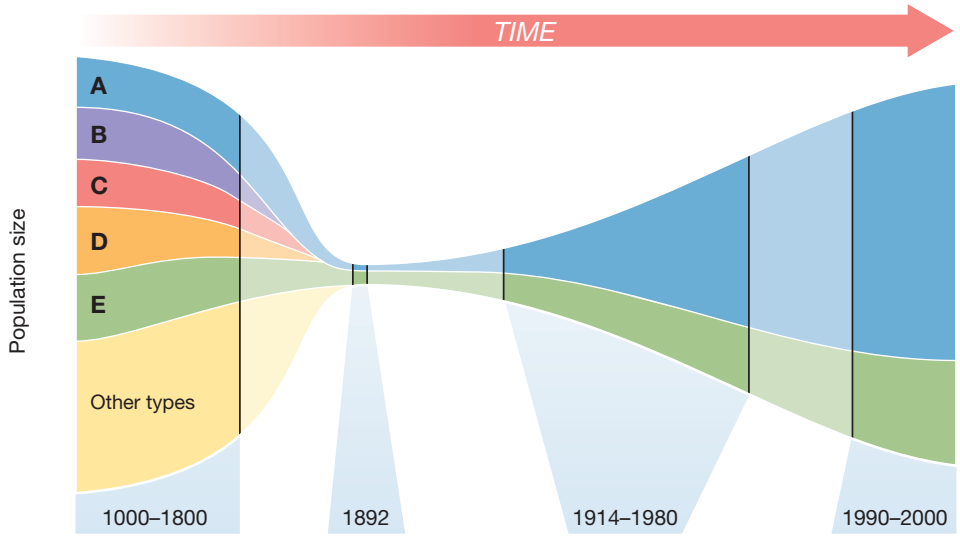
FIGURE 8.23 Low variation in northern elephant seals. **(A)** Based on enzyme electrophoresis data, no molecular variation is observed in any of the 62 northern elephant seal proteins surveyed by enzyme electrophoresis. By contrast, enzyme electrophoretic studies on southern elephant seals reveal significant molecular variation. **(B)** DNA sequence studies do reveal some genetic variation in northern elephant seals, but it is extremely limited compared to that found in southern elephant seals. Adapted from Hoelzel (1999) and Hoelzel et al. (1999b).

(Hoelzel et al. 1993). As summarized in **Figure 8.23A**, this lack of variation was in marked contrast to observations of considerable molecular variation in the proteins of the southern elephant seal (*Mirounga leonina*). This species, which is the northern elephant seal's closest relative, did not experience a comparable population bottleneck. These findings strongly support the theoretical prediction that a tight bottleneck should greatly reduce the genetic variation within a population.

Enzyme electrophoresis—while the best approach available in 1974 when Bonnell and Selander conducted their study—is a relatively coarse-grained tool for surveying the extent of molecular variation. Some protein structure variants will not be detected by this method. Moreover, because enzyme electrophoresis operates at the level of protein product rather than at the level of DNA, it is unable to detect silent substitutions in the DNA sequence. More recent studies have used DNA sequencing to take a finer-grained look at the extent of molecular variation in northern elephant seal populations. These studies compared the DNA sequences in several highly variable regions of DNA such as the control loop region of the mitochondrial DNA, the *M2b* microsatellite locus, and several major histocompatibility complex (MHC) loci (Hoelzel et al. 1993, 1999a,b; Weber et al. 2004). In each case, variation is extremely limited in the northern elephant seal and more abundant in its southern relative (**Figure 8.23B**).

Yet, none of this work decisively shows that the population bottleneck caused the low level of heterozygosity among northern elephant seals. Perhaps this population had unusually low levels of variation even before the bottleneck. There are other reasons why we might expect low heterozygosity in elephant seals, including the highly skewed distribution of reproductive success in this species, where a dominant male mates with many different females. To demonstrate definitively that the reduction in heterozygosity occurred coincident with the bottleneck, researchers would have to take genetic samples from individual seals that lived before the bottleneck.

Fortunately, museum samples make this possible. Diana Weber and her colleagues did exactly this in a study published in 2000 (Weber et al. 2000).



Number of Individuals

mtDNA Type	1000–1800	1892	1914–1980	1990–2000
A	1	0	4	107
B	2	0	0	0
C	1	0	0	0
D	1	0	0	0
E	0	2	2	42

FIGURE 8.24 Loss of genetic diversity through a bottleneck. Weber and colleagues compared mitochondrial DNA (mtDNA) diversity of northern elephant seals over time. From museum specimens, they sequenced individuals that lived before, around, and some time after the time of the bottleneck. From currently living animals, they and another group sequenced numerous mtDNA samples. Though the pre-bottleneck sample size was only five individuals, they found four different mtDNA types, suggesting a population with far more diversity than the current population, which revealed two mtDNA types in 149 samples.

They extracted mitochondrial DNA from bone and dried skin of animal samples taken before, during, and after the tightest part of the bottleneck. In 149 samples taken from post–bottleneck specimens by these and other investigators, only two genotypes were found. Samples from the late nineteenth century reveal that both extant genotypes date at least as far back as the tightest portion of the bottleneck. By contrast, in the five bone samples from before the bottleneck, the researchers found four distinct genotypes (**Figure 8.24**). This strongly indicates greater diversity prior to the bottleneck and establishes that the bottleneck was coincident with, and presumably the cause of, the severe reduction in heterozygosity that we observe in the current elephant seal population.

Founder Effect

We began this chapter with the story of the *Manx* (*M*) mutation in the cats on the Isle of Man. The high prevalence of this mutation there, and its comparative rarity elsewhere, is probably a result of a phenomenon known as the **founder effect**. The founder effect refers to the change in allele frequencies that results from the sampling effects that occur when a small number of individuals from a large population initially colonize a new area and *found* a new population. For example, islands often draw their initial inhabitants, or founders, from large mainland populations nearby. This sampling process introduces random change. Genes in founders usually represent only a subset of the genes present in the mainland population, and so the allele frequencies in the founders may deviate by chance from those in the large population. Moreover, alleles that are extremely rare on the mainland, such as the *Manx* allele, may become common on the island if carried by one of the founders of the island population.

Founder Effect in an Island Population

Darwin pointed out that many plants "migrate" to small islands by drifting on water currents or by having their seeds transported in the mud stuck to a bird's foot. Such a scenario offers ample opportunity for founder effects to influence allele frequencies in island populations. By way of example, some plant species are polymorphic for the direction that their flowers tilt relative to the floral axis. In the plant *Heteranthera multiflora*, this tilting trait is controlled by a single locus with two alleles, labeled *R* for right leaning and *r* for left leaning (Jesson and Barrett 2002). The *R* allele is dominant, so that *RR* and *Rr* individuals have right-leaning flowers, and *rr* individuals have left-leaning flowers. Imagine that the frequency of *R* is 0.3 on the mainland so that, at Hardy–Weinberg equilibrium, the frequencies of each phenotype are approximately the same—right-leaning flowers at 51% and left-leaning flowers at 49%. Five migrants move from the mainland to the island (**Figure 8.25A**). These five migrants, being diploid, carry with them 10 gene copies at the *R* locus. There is only about a 27% chance that our founding island population will have the same allele frequencies as our mainland population (**Figure 8.25B**); that is, random fluctuations create a 73% chance that the founders of our island population will have different allele frequencies for the tilting trait than were found on the mainland.

Genetic drift affects not only the gene frequencies in the founding population on the island but also the *long-term frequencies* of genes in future generations of offspring. If natural selection is not acting on alleles *R* and *r*, then over the long run our island population will become fixed for one of the two alleles—sooner or later a string of chance events will cause the loss of one of the alleles, and hence the fixation of the other. Moreover, if the island population is smaller than the mainland population, this process of genetic drift will proceed more quickly, as we saw in Figure 8.3.

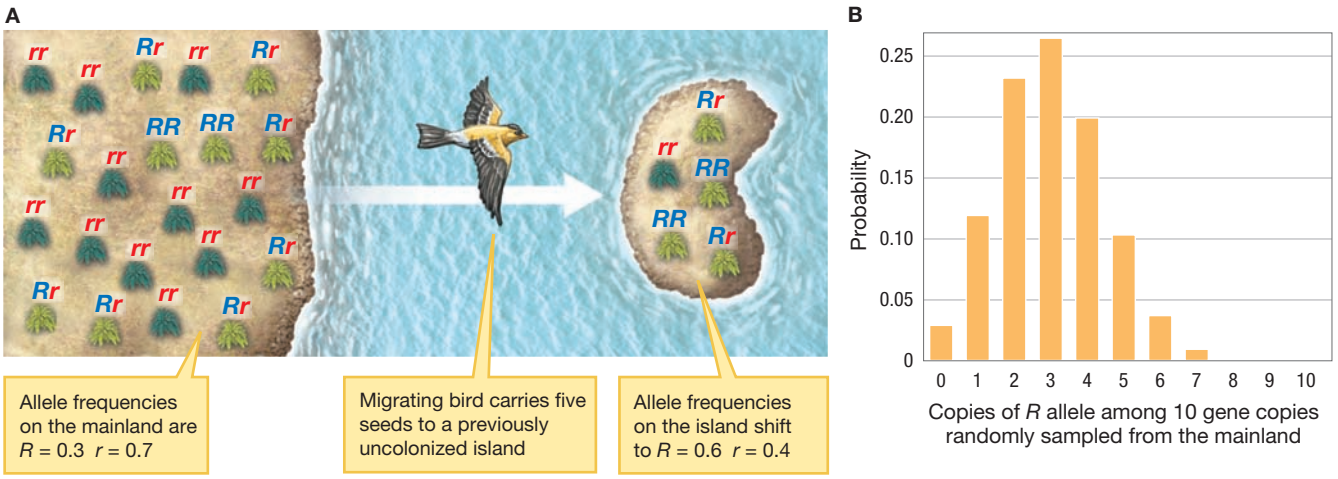


FIGURE 8.25 The founder effect. **(A)** Five plants from a larger mainland colonize a smaller island. **(B)** If the founders are sampled randomly from the mainland population, they may carry anywhere from 0 to 10 copies of the *R* allele, although it is unlikely that they would carry more than 7 copies. The probability that the five founders of the island population carry exactly 3 copies of the *R* allele—and thus have the same allele frequencies on the island as on the mainland—is less than 0.27.

As we showed in Section 8.1, the probability that a particular allele will become fixed over the long run is equal to its initial frequency on the island. In Figure 8.25A, the founder population consists of six copies of the *R* allele and four copies of the *r* allele. In the absence of selection, the probability that our island population would become fixed for *R* is therefore 0.6, and the probability that it would become fixed for *r* is 0.4.

Founder effects can occur even without a sharp geographic separation such as that between a mainland and an island. As an example, let us consider work on founder effects in the black spruce tree.

Founder Effects, Mitochondrial DNA, and Black Spruce

Consider what happens when glaciers recede after an ice age, and a species moves back into the once-glaciated areas. Those individuals that colonize the newly uncovered land are not randomly sampled from the species at large, but rather tend to come from the so-called *leading edge subpopulations* near the previous limit of the species range during the ice age. This process of colonization from the populations nearest the previous range limits is known as a **leading edge expansion** (**Figure 8.26**). Like the founder effects associated with island colonization, leading edge expansions result in reduced genetic diversity in the newly colonized region.

The genetic consequences of leading edge expansions after the recent ice ages can be observed widely throughout the Northern Hemisphere in plant and animal species alike (Hewitt 1996, 2000). Isabelle Gamache and her colleagues studied such founder effects in the black spruce (*Picea mariana*) growing in the subarctic forest tundra of the eastern coast of Canada's Hudson Bay (**Figure 8.27**) (Gamache et al. 2003). Glaciers disappeared from this area about 6000 years ago, and it was recolonized by tree species such as the black spruce, which eventually reached its northernmost latitude about 1500 years ago.

The genetics of dispersal and recolonization are particularly interesting in plants. While seeds have to be dispersed into a new area for the initial colonization to take place, seed dispersal is not the only source of genetic variation for an established population: Pollen from other populations can blow in on the wind and fertilize the plants that have become established there (**Figure 8.28**).

Thus, in the process of colonization, genetic material is carried by two different sources that differ dramatically in their mobility. Seeds, whether they are carried by animals or are able to float on the wind, tend to disperse across limited distances. In contrast, pollen is much lighter and can travel much farther by wind, covering greater distances in much greater volume. We can tease apart the patterns of seed dispersal and pollen dispersal because not *all* genetic material travels in pollen. Mitochondrial DNA is maternally inherited, and thus it is passed on only through seeds; it

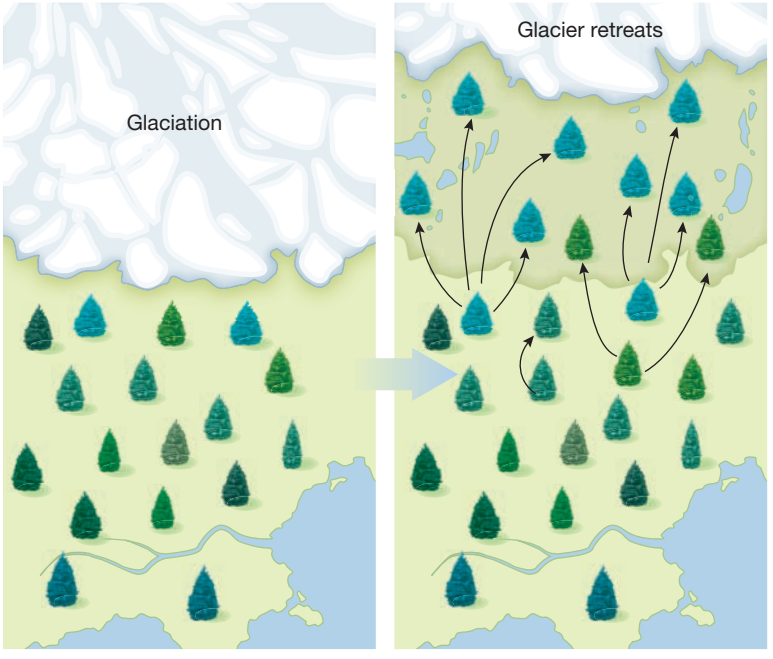


FIGURE 8.26 Leading edge expansion. A land mass is half covered by glaciation during an ice age. South of the ice sheet, the uncovered land provides a refuge for a number of populations (genetic diversity is indicated by foliage color). When the ice sheet recedes at the end of the ice age, the uncovered terrain is colonized by individuals from the leading edge subpopulations—here, the populations adjacent to the former glacier. The populations farther from the leading edge contribute relatively little to the colonization. The consequence is a sort of founder effect in which we observe reduced genetic diversity in the recently colonized area.

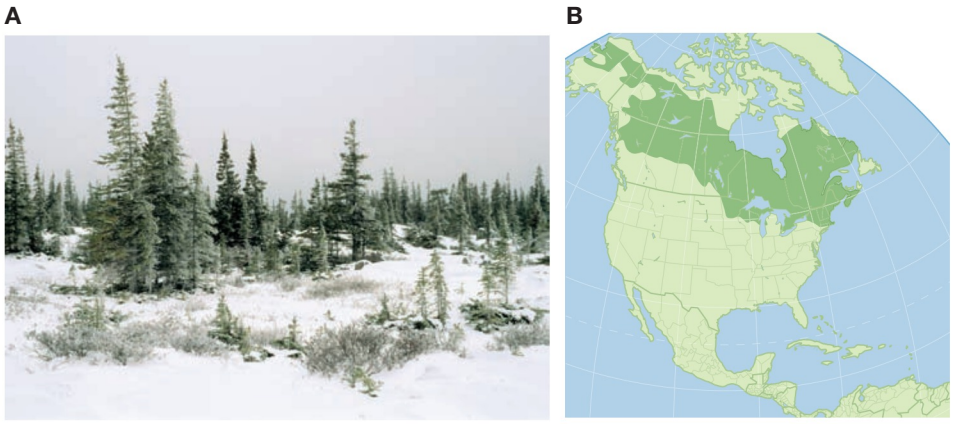


FIGURE 8.27 Leading edge expansion of black spruce. **(A)** A forest of black spruce in Canada. **(B)** The current distribution of black spruce in North America is shown in dark green. Panel B from Viereck and Johnston (1990).

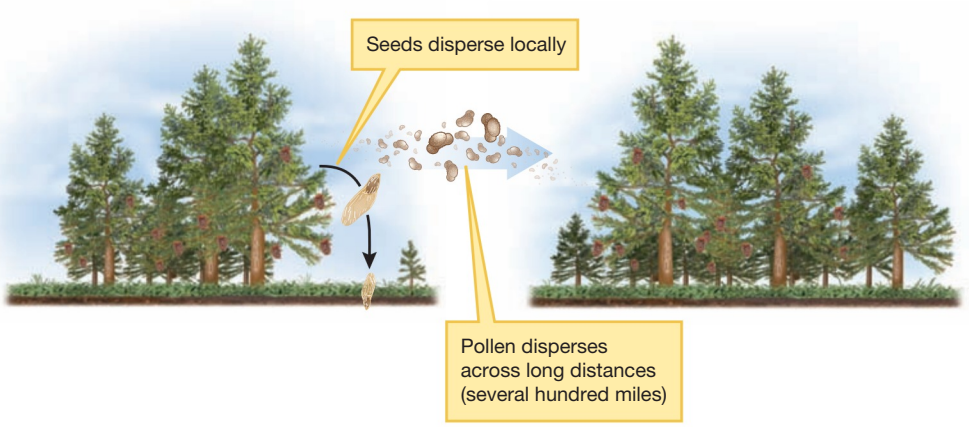


FIGURE 8.28 Differing dispersal distances. Seeds disperse across short distances. Pollen disperses across long distances on the wind.

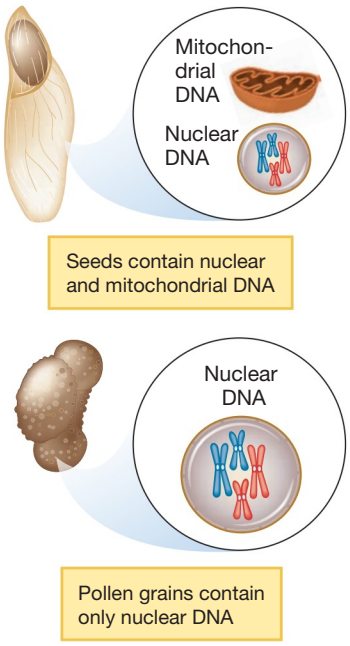


FIGURE 8.29 Seeds carry additional genetic material. Seeds contain both nuclear DNA and mitochondrial DNA, whereas pollen grains contain only nuclear DNA.

is absent from pollen (**Figure 8.29**). We might therefore expect the geographic distribution of mitochondrial DNA variants to reflect only patterns of seed dispersal, whereas nuclear DNA variants will reflect patterns of both pollen and seed dispersal.

In an early study of black spruce in the Hudson Bay area, researchers studying nuclear DNA found no reduction of genetic diversity in post–ice-age populations of black spruce, and thus no evidence of founder effects (Desponts and Simon 1987). This is perhaps unsurprising given that wind-dispersed pollen need not travel only from the leading edge populations during a recolonization event: Vast quantities of such pollen can move long distances, minimizing any possible founder effects.

In addition to using nuclear DNA to study the movement of pollen, Gamache and her colleagues also examined the effect of migration via wind-dispersed seeds by using the DNA found in mitochondria, the energy-producing organelles of cells (Gamache et al. 2003). Wind-dispersed seeds occur in much smaller numbers than wind-dispersed pollen, and hence we might expect to find genetic drift affecting mitochondrial gene frequencies. To compare nuclear and mitochondrial DNA, these researchers took foliage samples from about 30 trees in each of nine populations along a 1000-kilometer transect of forest. This transect included populations at the northernmost distribution of black spruce, as well as much larger populations to the south, and the diversity of both nuclear and mitochondrial DNA was calculated for each population.

Gamache and her team found that the migration of mitochondrial DNA (mtDNA) via wind-dispersed seeds was much more restricted and localized than

the migration of nuclear DNA via pollen dispersal. There were two lines of evidence for this. First, all the different types of nuclear DNA found in large parent populations were represented in northern subpopulations. When it came to mitochondrial DNA, however, although the southern populations contained four different types of mtDNA, every one of the northern subpopulations had one and only one type of mtDNA, called mitotype I (**Figure 8.30**). This suggests that, by chance, either mitotype I was able to move north into a single subpopulation and then spread even farther north through time or that a single long-distance migration event involving mitotype I occurred. Both are consistent with the idea of founder effects.

A second line of evidence for founder effects in black spruce was that, when both southern and northern populations were compared, *between-population* variability in mitochondrial DNA was 10 times greater than between-population measures of nuclear DNA variability. In other words, northern and southern populations were very *similar* with respect to nuclear DNA, but very *different* with respect to mitochondrial DNA. Fixation for a single genetic type within a population, combined with high between-population variation, is a hallmark of genetic drift. Indeed, Gamache and her colleagues were able to use their estimates of genetic diversity to calculate the effective number of mitochondrial DNA seed "migrants" and nuclear DNA pollen "migrants" entering populations in each generation. As expected, the average number of mitochondrial DNA migrants per generation was almost 10 times lower than the average number of nuclear DNA migrants (Gamache et al. 2003).

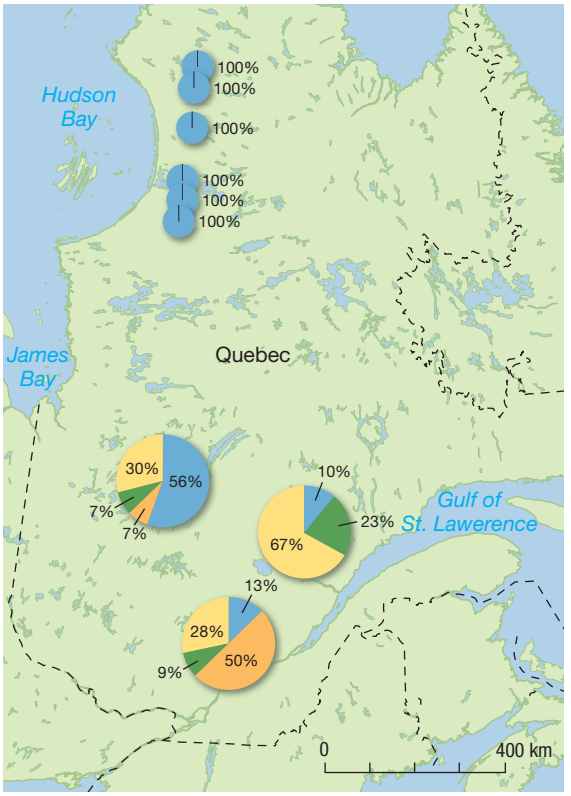


FIGURE 8.30 Limited mitochondrial diversity in leading edge expansion. Nine black spruce populations in Quebec, Canada, were sampled to determine the frequencies of mitotypes within each population. The pie charts indicate the mitotypes in each population. The six northern subpopulations of black spruce were all fixed for a single type of mtDNA, called mitotype I (shown in blue). The southern populations contained three or four mitotypes, indicated by different colors in the pie charts. Adapted from Gamache et al. (2003).

8.4 The Interplay of Drift, Mutation, and Natural Selection

As we have seen, genetic drift increases the homozygosity of a population. Indeed, if drift were the only evolutionary process operating, any finite population would eventually become entirely homozygous. In practice, however, populations do not become entirely homozygous because mutation provides a continual supply of new genetic variation. This leads to a balance or steady state in which the loss of heterozygosity due to drift is balanced by the gain in heterozygosity due to mutation. In **Box 8.5**, we develop a simple model that predicts the amount of variation that we expect to find at a neutral locus in a Wright–Fisher population at steady state.

The Mathematics of Selection and Drift

In our discussion of selection in Chapter 7, we looked at large populations in which drift was not operating. In our treatment of drift thus far in this chapter, we have primarily looked at neutral loci in which selection is not operating. But selection and drift are not mutually exclusive modes of evolutionary change. Both can, and usually do, operate simultaneously in natural populations. Having seen how each

BOX 8.5 Wright's *F*-statistic at a Neutral Locus with Mutation

Neutral variation is produced by mutation, and it is lost due to drift. At steady state, how much variation do we expect to see at a neutral locus subject to mutation? We can derive a mathematical expression for the expected value of Wright's *F*-statistic at a neutral locus in a Wright–Fisher population at the mutation–drift equilibrium. To do so, we revisit Equation 8.1 from Box 8.2. This equation specifies the change in Wright's *F*-statistic over a single generation:

$$F_{\text{offspring}} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right)F_{\text{parental}}$$

Recall that F is simply the probability that two gene copies are identical by descent (IBD) in the absence of mutation. As we have seen, this equation accounts for the probability that both have the same ancestor in the parental generation or in some prior generation. But now we want to incorporate mutation, which provides another way for gene copies to fail to be IBD. If either gene copy undergoes a mutation from the parental to the offspring generation, two gene copies that otherwise would have been IBD now are not. If the mutation rate is μ per locus per generation, there is a $(1 - \mu)$ chance that a specific single gene copy in the offspring has not mutated since the parental generation, and a $(1 - \mu)^2$ chance that neither gene copy at a given locus has mutated since the parental generation. Thus, the chance that two gene copies are IBD in the presence of mutation is obtained by multiplying the right-hand side of Equation 8.1 by $(1 - \mu)^2$, so that we get

$$F_{\text{offspring}} = \left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right)F_{\text{parental}}\right](1 - \mu)^2 \quad (8.6)$$

We can find the equilibrium or steady-state value of F by setting $F_{\text{offspring}} = F_{\text{parental}}$ in Equation 8.6 and solving the resulting equation to get

$$F_{\text{equilibrium}} = \frac{(1 - \mu)^2}{2N - (1 - \mu)^2(2N - 1)}$$

Because the mutation rate μ is typically small, both μ and μ^2 will be small and can be ignored in an approximation of the equilibrium value of F. But we cannot ignore the $N\mu$ term, because N can be large. This gives us the following approximation:

$$F_{\text{equilibrium}} \approx \frac{1}{4N\mu + 1}$$

In Box 8.2, we saw that heterozygosity tends to decrease with increasing values of F. This means that, as intuition would suggest, heterozygosity will tend to be lower when (1) population size is small and (2) mutation rate is low.

acts alone, we are now in a position to think about how these processes interact with one another.

Even alleles that are favored by natural selection are not guaranteed to become fixed in a population. The early population geneticist J. B. S. Haldane looked at a simple model in which a new, slightly beneficial allele with a fitness of $1 + s$ arises in a large population and competes with the wild type that has a fitness of 1 (Haldane 1927). Even though the population size is large, the new mutation is surprisingly unlikely to be fixed. Haldane found that the fixation probability is approximately $2s$. This means that a new beneficial mutation that confers a 1% fitness advantage has only a 1 in 50 chance of being fixed in a large population! Haldane's approximation pertains to a dominant mutation: A newly arisen recessive mutation provides little selective benefit while rare and thus has a much lower chance of fixation.

The reason that drift matters here even in a large population is that we are now looking at what happens to the *initial* mutant allele. In large populations, allele frequencies fluctuate less because of drift, but a new allele begins at a lower frequency. Think about a new allele arising in a haploid population of size 100 or size 1,000,000. In a population of 100, drift can cause substantial fluctuations in allele frequencies, but the new allele begins at a frequency of 1 in 100; relatively

speaking, it doesn't have all that far to go to reach fixation. In a population of 1,000,000, drift has less effect on allele frequencies overall, but the new allele begins at a frequency of only 1 in 1,000,000; it has a really long way to go if it is to reach fixation. In Haldane's model, these effects cancel out, and the probability of fixation is independent of population size.

While the population size term dropped out of Haldane's expression for the fixation probability of the *initial* mutation, if we look at an allele present at some intermediate frequency—say, 1% or 10% or 50%—the population size matters considerably. Broadly, the interplay between selection and drift depends on the strength of the selection and the population size. When selection is strong and population size is large, selection largely determines the change in allele frequencies. When selection is weak and population size is small, drift largely determines allele frequency change. To quantify this, the population geneticist Motoo Kimura (1924–1994)—best known as the architect of the neutral theory of molecular evolution (Section 8.5)—proposed a rule of thumb for when selection is effective and when drift dominates (Kimura 1983). In a diploid population, selection dominates when the selective advantage $s \gg 1/2N_e$; drift dominates when $s \ll 1/2N_e$. When s and $1/2N_e$ are of similar magnitude, both selection and drift are important. Thus, selection can operate effectively on an allele with a fitness advantage of $s = 0.001$ in a population of 10,000 individuals, but not in a population of 100 individuals.

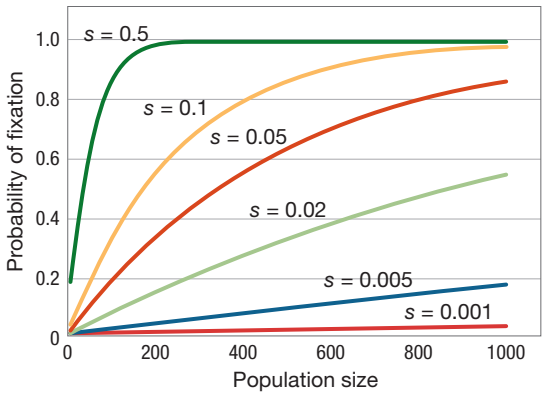


FIGURE 8.31 Selection versus drift. Here, we plot the approximate probability that a selectively favored allele A_2, initially present at a frequency of 1%, goes to fixation. The fitness effects of the A_2 allele are *multiplicative*: fitnesses are 1 for the A_1A_1 genotype, $1 + s$ for the A_1A_2 genotype, and $(1 + s)^2$ for the A_2A_2 genotype. The horizontal axis indicates the population size; the vertical axis indicates the probability of fixation. The effectiveness of natural selection at fixing a favored allele depends on population size and the strength of selection.

Figure 8.31 illustrates the effectiveness of natural selection. In this graph, we show the approximate probability that a rare but selectively favored allele, A_2, initially present at frequency 1%, goes to fixation in a Wright–Fisher population. We see that when selection is very strong (for example, $s = 0.5$), the favored allele A_2 goes to fixation with high probability even in a relatively small population. By contrast, when selection is weaker (for example, $s = 0.005$), the favored A_2 allele is more likely than not to be lost even in a population of size 1000.

8.5 The Neutral Theory of Molecular Evolution

Having covered the molecular basis of mutation (Chapter 6) and the process of genetic drift (this chapter), we can now explore the process of evolutionary change at the molecular scale. In the study of molecular evolution, biologists look not at phenotype directly, but rather at how DNA or RNA sequences change over time and how the amino acid sequences that compose proteins change over time. This approach provides a fine-scale view of how the minimal units of heredity—nucleic acid sequences—change over time and in turn generate changes at the phenotypic level.

The Ubiquity of Molecular Variation

In the mid-1960s, the development of enzyme electrophoresis provided researchers with a ready way of uncovering *cryptic molecular variation*—differences in amino acid sequence that do not manifest themselves in phenotypic differences. Richard Lewontin and Jack Hubby examined a number of loci in a population of *Drosophila*

pseudoobscura, and to the great surprise of most population geneticists, they found that approximately one-third of these were polymorphic, with a remarkably high heterozygosity of 12% (Lewontin and Hubby 1966). Harry Harris carried out a similar study on humans: Of the 10 loci he studied, 3 were polymorphic with a heterozygosity of 6% (Harris 1966).

From these and other studies that followed, population geneticists were forced to conclude that molecular variation is far more common in populations than they had previously imagined. That conclusion posed a major problem. At the time, most explanations for the presence and/or maintenance of variation in a population required strong natural selection. Concurrently, it was thought that when variation was observed at a locus, either it was being maintained by balancing selection or natural selection was in the process of replacing one allele with another. But with so much variation present, researchers realized that natural selection could not be the whole story (Kimura 1968). Selection is costly in that it requires either differential survival or differential reproductive success, and researchers had found ways to quantify the "cost of natural selection" and relate it to the amount of variation in a population (Haldane 1957; Kimura 1961). There was simply not enough natural selection going on to account for this much variation. There had to be some other explanation.

The Neutral Theory Proposes That Most Substitutions Are Selectively Neutral

Perhaps the most straightforward explanation is that selection may not be acting on this variation at all. Although most heritable phenotypic differences result in fitness differences and thus are subject to natural selection, the same might not be true of molecular differences. To account for the extensive molecular variation observed in populations, Kimura proposed the **neutral theory** of molecular evolution in 1968 (Kimura 1968, 1977, 1983, 1993; Jukes and Kimura 1984; Dietrich 1994; Nei et al. 2010). The neutral theory proposes that at the molecular level of DNA sequence or amino acid sequence:

1. Most of the variation present within a population is selectively neutral.
2. Most of the changes in DNA or amino acid sequence over time—and thus many of the molecular differences between related species—are selectively neutral.

According to the neutral theory, most of the genetic variation within a population is neutral and thus not subject to natural selection. Therefore, when a DNA sequence does change over time, some process other than selection is usually responsible. The neutral theory argues that the critical process is genetic drift.

When studying molecular evolution, we will often be concerned with allelic **substitutions**. A substitution occurs when a new allele arises by mutation and is subsequently fixed in the population. The substitution rate, usually measured in terms of substitutions per generation, is defined as the rate at which new alleles become fixed in the population.

It is important to understand that the neutral theory proposes that most *substitutions* are neutral, not that most *mutations* are neutral. Proponents and critics of the neutral theory universally agree that most mutations are deleterious and

will be purged from the population by natural selection. But of the remaining mutations that are not purged, the neutral theory proposes that many may be neutral. Similarly, the neutral theory does not propose that most loci are selectively irrelevant in the sense that fitness doesn't depend on the DNA sequence at that locus. It only proposes that when there are alternative alleles present at appreciable frequency, these alternative alleles are often neutral with respect to one another. The so-called *neutralist–selectionist debate* is not a dispute about the effects of typical mutations; it is a dispute about whether drift or selection is the primary driver of evolutionary change in the subset of mutations that reach a high frequency in populations.

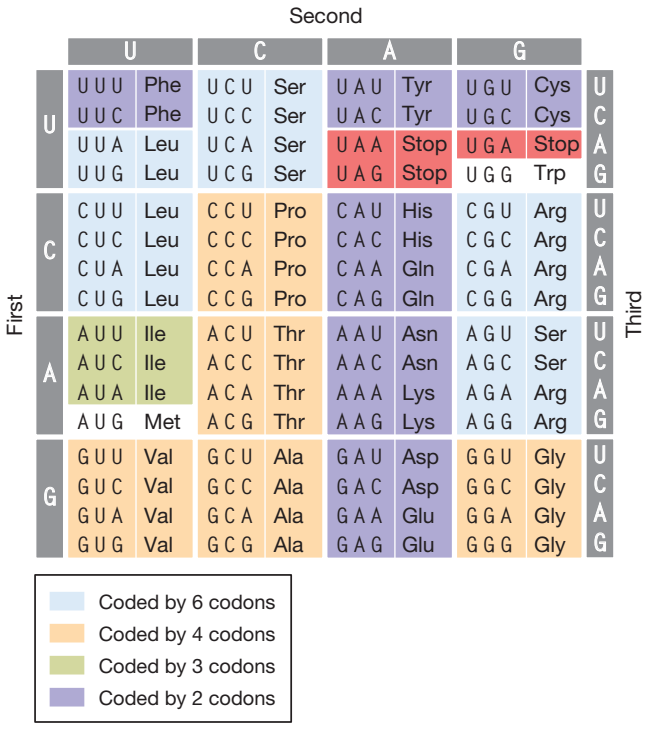
Reasons for Selective Neutrality

The neutral theory suggests that many alternative alleles may be selectively neutral, but why should this be? There are a number of biological reasons why allelic differences might have no fitness consequences; we will explore them here.

Synonymous Substitutions

One of the predominant reasons that molecular variation may be neutral is that many molecular changes do not cause changes in phenotype. First and foremost, the degeneracy of the genetic code means that many changes in protein-coding DNA sequences do not cause changes in the amino acid sequence of the corresponding protein. Because 64 possible nucleotide triplets (codons) are used to code for only 20 amino acids (plus three stop codons), there is redundancy, and most amino acids are coded for by several different codons. Typically, codons that code for the same amino acid differ in the third position (**Figure 8.32**). Thus, many nucleotide changes—particularly those in the third position—do not change the amino acid specified. As we discussed in Chapter 6, mutations that do not result in a changed amino acid are known as synonymous or silent mutations. Because such changes do not alter the sequence of the protein that they encode, they will typically be neutral or very close to neutral.

FIGURE 8.32 Degeneracy in the genetic code. The genetic code exhibits degeneracy, such that DNA base changes—especially in the third codon—do not always change the amino acid specified. Many amino acids are coded by six (blue), four (orange), three (green), or two (purple) different codons. Adapted from Agris (2008).



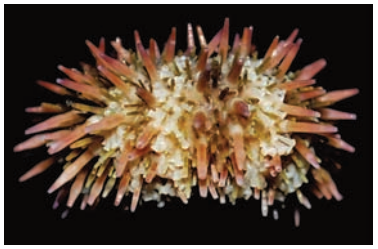
In an influential 1977 article, Kimura compared the sequences of messenger RNA (mRNA) across species to test his idea that many of the genetic differences that we see when comparing the same gene across two different species are in fact neutral substitutions (Kimura 1977). Using data on the sequence of mRNA from both human and rabbit hemoglobin (Salser et al. 1976), Kimura noted that of 53 nucleotide positions that can be compared across humans and rabbits, there were differences in six base pairs. Only one of these changes, however, led to a difference in amino acid coding; the other five (83%) were synonymous mutations. By contrast, Kimura calculated that if mutations occurred and accumulated at random, we would expect only 24% to be synonymous.

Kimura found support in similar results from Michael Grunstein's work on the rate of molecular evolution of the

FIGURE 8.33 Molecular differences between sea urchin species. The sea urchins **(A)** *Strongylocentrotus purpuratus* and **(B)** *Lytechinus pictus*. **(C)** In a comparison of the histone H4 protein sequences of these two species at codon sites 24 to 34, Grunstein's team found five changes at the third base pair of codons. All five are silent, nonfunctional changes. Panel C adapted from Grunstein et al. (1976) and Kimura (1979).

A

B



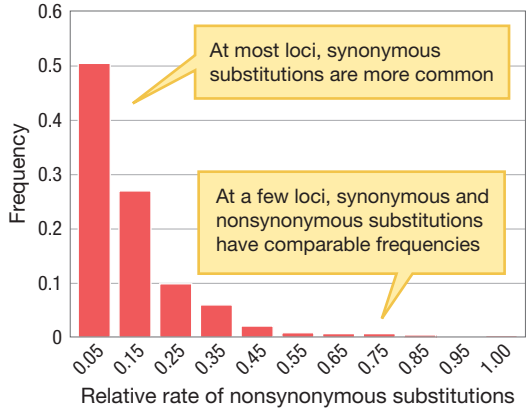
C

Codon site	24	25	26	27	28	29	30	31	32	33	34
L. pictus messenger RNA	GAU	AAC	AUC	CAA	GGA	AUA	ACU	AAA	CCG	GCA	AUC
S. purpuratus messenger RNA	GAC	AAC	AUC	CAA	GGU	AUC	ACG			GCU	AUC
Histone H4 amino acid sequence	Asp	Asn	Ile	Gln	Gly	Ile	Thr	Lys	Pro	Ala	Ile

histone H4 protein found in two species of sea urchins, *Strongylocentrotus purpuratus* and *Lytechinus pictus* (Grunstein et al. 1976) (**Figure 8.33**). Grunstein and his coworkers had found that of the 84 nucleotides in the mRNA segment that they compared across these two sea urchin species, 9 of the 10 base pair differences found were synonymous.

More recent work shows that this pattern is very common. When we compare genetic sequences in two or more related species, we see an excess of synonymous substitutions over nonsynonymous substitutions in many, though not all, protein-coding genes. **Figure 8.34** shows, for 835 genes compared between mice and rats, the relative rates of synonymous versus nonsynonymous substitution. The vast majority of these genes show a great excess of synonymous substitutions, indicating that substitutions have been more common at silent sites than at nonsilent sites.

FIGURE 8.34 Most genes show higher rates of synonymous substitution. The substitution rate at nonsynonymous sites relative to the substitution rate at synonymous sites for 835 mouse–rat gene pairs. Nonsynonymous sites tend to evolve much less rapidly than synonymous sites. Adapted from Hurst (2002).



Nonsynonymous Substitutions with Little Effect on Function

In contrast to synonymous mutations, nonsynonymous mutations *do* change the amino acid sequence. Many nonsynonymous mutations are not neutral because they change the way that a protein functions, and such changes have fitness consequences. While many nonsynonymous sites may be under selection, some nonsynonymous mutations may have minimal fitness effects. For example, changes to amino acids that are distant from the binding site of a protein often have weaker consequences on protein function than those of changes at the binding site of a protein.

As an example, birds and mammals sense temperature using proteins called transient receptor potential vanilloid (TRPV) channels. One domain of these proteins binds adenosine triphosphate (ATP), which in turn modulates the receptor's response to temperature. To understand better the ATP-binding function of these channels, Christopher Phelps and his colleagues compared the DNA sequence of three closely related TRPV channels—$TRPV_1$, $TRPV_3$, and $TRPV_4$—across three species: humans, rats,

and chickens (Phelps et al. 2010). They found that while the structure of the ATP-binding site was highly conserved, other regions of the protein were far more variable (**Figure 8.35**). This indicates that changes to areas other than the binding site may have smaller functional consequences than those of changes to the binding site; some of these changes may have no effect on function, and thus they may be selectively neutral.

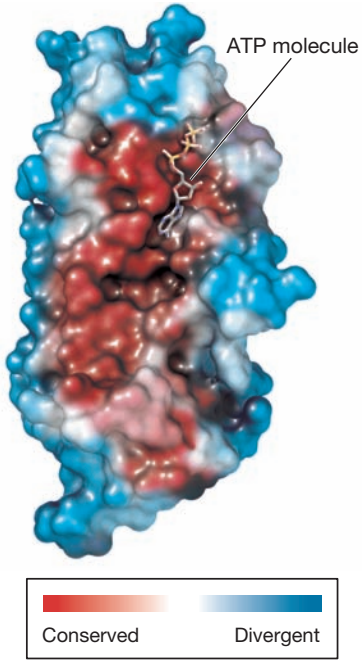


FIGURE 8.35 Conserved and divergent sites in a channel protein. In this representation of the ATP-binding domain of the $TRPV_1$ protein (bound to an ATP molecule), highly conserved amino acids are indicated in red and divergent ones in blue. The binding site of this molecule is the most highly conserved region. This suggests that amino acid sequence changes that alter this binding site will have greater effects on function than those that alter other parts of the molecule. At least some amino acid sequence changes in the most divergent regions may be selectively neutral or nearly so. From Phelps et al. (2010).

Noncoding Regions

In most eukaryotes, only a small fraction of the genome encodes the sequence of proteins. The rest of the genome is *untranslated*. This is not to say that it necessarily lacks any function; untranslated sections of DNA may have important regulatory functions, for example. But it is likely that many mutations in noncoding regions of the genome will have very minor effects or even no effect on function and fitness. **Pseudogenes**—nonfunctional and typically untranslated segments of DNA that arise from previously functional genes—are often particularly informative about evolutionary history, as they are derived from known homologous genes and subject to neutral drift.

Because pseudogenes do not affect function, mutations in pseudogenes tend to be neutral, and they accumulate rapidly over evolutionary time. Pseudogenes can arise through a number of processes. In the process of *gene duplication*, a second copy of the gene is inserted into the genome during DNA replication. As such a copy is a duplicate of another functional gene, mutations that prevent expression may not be selected against. In the process of *retroposition*, mRNA from a functional gene is reverse-transcribed by a mobile genetic element known as a *retrotransposon* and inserted into the genome. Because it lacks the appropriate promoter structure, it will tend not to be expressed and thus forms a pseudogene. More rarely, through a process of *deactivation*, genes become pseudogenes without leaving behind a functional copy. In this process, mutation disables an active gene: If the gene is not strongly selected, the deactivated form can be lost as a result of drift. We humans appear to owe our susceptibility to scurvy to such a deactivation event. The primate lineage, of which we are members, arose as fructivores—fruit eaters. Because fruit is rich in vitamin C, early primates would have initially faced minimal selection costs from the loss of the L-gulono-γ-lactone oxidase gene used to synthesize that vitamin. But it is because of the loss of this gene that humans suffer from scurvy if they lack a dietary source of vitamin C.

We will investigate the structure of the genome in further detail in Chapter 10 and consider other reasons why genes may be untranslated or nonfunctional, but for now it will be sufficient to note that mutations in noncoding regions do not change the sequence of proteins, and thus they may be neutral, at least if they do not disrupt gene regulation.

Effective Neutrality

As we saw in Section 8.4, in finite populations, natural selection cannot operate effectively on mutations that have extremely small fitness consequences. The random change in allele frequencies due to drift overwhelms any effects due to natural selection. Thus, even when alternative alleles do have an effect on function and fitness, they can be *effectively neutral* if these effects are sufficiently small. As a rule of thumb, an allele will be effectively neutral under the same conditions that

favor drift over selection: when the selective coefficient s is much smaller than $1/2N_e$, where N_e is the effective population size.

Neutral Theory as a Null Model

Of course, much has happened since Kimura first championed the neutral theory. Not only have evolutionary biologists made important empirical and theoretical advances, but also the molecular genetics tools available to test the predictions of the neutral theory have improved dramatically. Indeed, recent work in evolutionary genomics now provides researchers with the ability to undertake *genome-wide* assessments of mutation rates in some species (Lynch et al. 2008). Such powerful techniques, when fully employed, will allow biologists to test better many questions regarding mutation rates and the neutral theory.

Some of the basic insights of the neutral theory have withstood the test of time. As we have seen across a wide range of organisms, sites that are expected to have a minimal effect on phenotype—synonymous sites, as well as sites within pseudogenes, introns, and untranslated regions—evolve at a substantially higher rate than that of nonsynonymous sites within coding regions.

But genome-scale analysis is beginning to reveal that positive selection has also been extremely important in driving molecular evolutionary divergence among species. For example, a series of genomic studies on *Drosophila* species has estimated that positive selection is responsible for 40% to 70% of the nonsynonymous substitutions that have occurred in these species (Welch 2006). Even in noncoding regions, a large fraction of substitutions appear to have been driven by positive selection (Andolfatto 2005). Similar results have been obtained for numerous bacterial and viral taxa as well. Curiously, when comparable methods are applied to the genomes of humans and great apes, the fraction of adaptive substitutions within this clade appears to be dramatically lower than that in other taxa (Eyre-Walker 2006).

While much early work by researchers had aimed to demonstrate the plausibility of the neutral theory and the importance of genetic drift as an evolutionary process, one of the most important contemporary functions of the neutral theory is that it serves as a *null model* against which we can test for the operation of selection or other evolutionary processes. The basic idea is straightforward: The neutral theory makes predictions about the amount of variation expected in a population, the relative rates of synonymous and nonsynonymous substitution, and other population genetic quantities. If we wish to determine whether selection is acting on a locus, we can investigate whether these quantities are consistent with what we would expect under a neutral model. If they are not, we might expect that some other process, possibly natural selection, is operating. In the subsections that follow, we illustrate how this is done.

Ratio of Nonsynonymous to Synonymous Changes

Because of the degeneracy of the genetic code, not all nucleotide substitutions in a protein-coding region change the amino acid sequence of the protein specified. This fact proves useful in determining the nature of selection on that locus.

The basic approach is to compare the pattern of substitutions actually observed with the pattern that would be expected if the variation at a particular gene were selectively

neutral. That is, we compare what we actually observe with a neutral model of evolution in which the variation would not be under selection. Under a neutral model, nonsynonymous mutations that change the amino acid sequence of a protein would be just as likely to go to fixation by genetic drift as would synonymous mutations that do not change the amino acid sequence. Thus, if the variation at a protein-coding locus is selectively neutral, we would expect to see as many nonsynonymous substitutions as synonymous substitutions. When making this comparison, we need to correct for the fraction of mutations that give rise to nonsynonymous and synonymous substitutions; the former are about 3 times as frequent as the latter, although this can vary given biases in mutation rates and other factors.

One major advantage of this approach is that—unlike many other tests of selection—comparisons of nonsynonymous and synonymous changes tend not to be affected by demographic events such as population bottlenecks or expansions. To compare nonsynonymous and synonymous substitutions, researchers align protein-coding sequences for two or more species and then compute the ratio K_a/K_s, where K_a is defined as the number of nonsynonymous substitutions per nonsynonymous site, and K_s is defined as the number of synonymous substitutions per synonymous site. If the variation at a protein-coding gene is selectively neutral, we expect the same substitution rate at nonsynonymous sites as at synonymous sites, and thus we expect that the K_a/K_s ratio will be approximately 1. If a locus is under positive selection, we expect that nonsynonymous substitutions will occur as a result of selection more rapidly than synonymous substitutions will occur as a result of drift, and thus the K_a/K_s ratio will exceed 1. If a locus is under **purifying selection**—selection to maintain the currently common allele despite occasional deleterious mutations—we expect the opposite. Under purifying selection, nonsynonymous substitutions will be rare relative to synonymous substitutions, and thus the K_a/K_s ratio will be less than 1 (**Table 8.1**) (Nei and Gojobori 1986). **Figure 8.36** illustrates a hypothetical situation in which K_a is substantially less than K_s.

In some published papers, the quantities K_a and K_s are replaced with the similar quantities *dN* and *dS* instead. In those papers *dN* and *dS* often represent *rates* of non-synonymous and synonymous substitutions, rather than tallies.

TABLE 8.1

Interpreting the K_a/K_s Ratio

Nature of Selection	K_a/K_s Ratio
Purifying selection	$K_a/K_s < 1$
Near neutrality	$K_a/K_s \approx 1$
Positive selection	$K_a/K_s > 1$

Positive Selection Associated with a Recent Adaptive Radiation

In an elegant study, Marianne Barrier and her colleagues used a K_a/K_s comparison to test the hypothesis that changes in regulatory genes play an important role during evolution in novel environments (Barrier et al. 2001). The Hawaiian silversword alliance is a group of plants that underwent a recent evolutionary radiation—a rapid burst of speciation—on the Hawaiian Islands about 5 million years ago. These species evolved numerous adaptations for living and reproducing in a wide range of ecosystems, from bogs to forests to the harsh, high-altitude barrens of several Hawaiian volcanoes (Robichaux et al. 1990) (**Figure 8.37**).

The silversword alliance provided Barrier and her colleagues with a way of testing the hypothesis that regulatory gene evolution has been important in this adaptive radiation. If changes in regulatory genes have influenced the radiation of the silversword alliance on the Hawaiian Islands, we would expect species in the

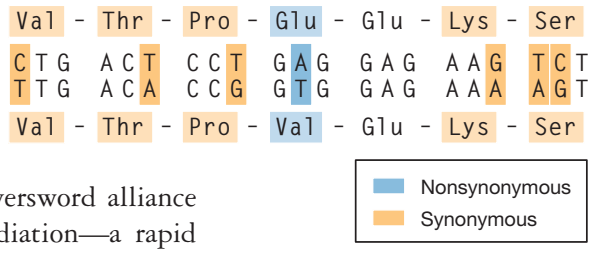


FIGURE 8.36 Comparing K_a and K_s. The two sequences differ by six synonymous substitutions and one nonsynonymous substitution. Because the rate of synonymous substitution has been higher than that of nonsynonymous substitution, this suggests that purifying selection has been operating on the gene.

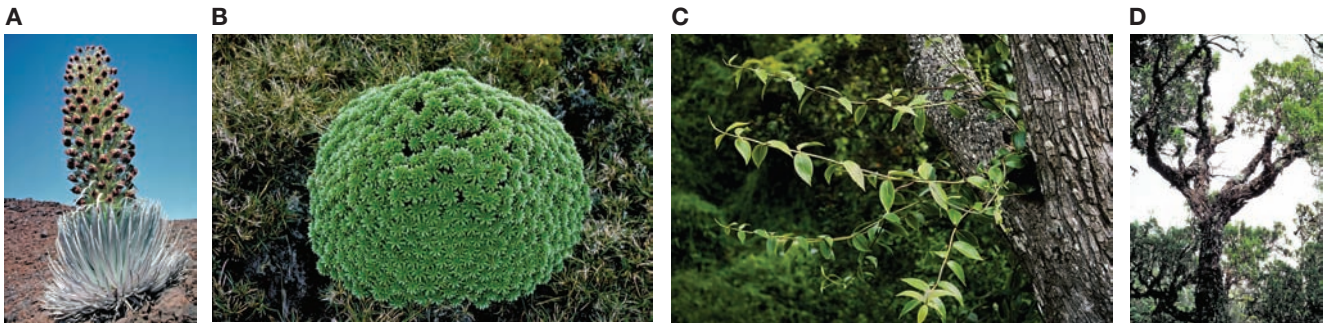


FIGURE 8.37 Adaptive radiation and adaptations. Because of their evolutionary radiation about 5 million years ago, species in the silversword plant alliance grow in a range of habitats and forms, including **(A)** cactuslike rosettes (*Argyroxiphium sandwicense*, ssp. *macrocephalum*), **(B)** "cushion plants" (*Dubautia waialealae*), **(C)** climbing vines (*Dubautia latifolia*), and **(D)** treelike shrubs (*Dubautia reticulata*).

alliance to exhibit more evidence of positive selection on regulatory genes than do closely related mainland species. To test this, Barrier and her colleagues sequenced two regulatory genes involved in floral development. They computed K_a/K_s ratios for the two regulatory genes in the silversword alliance species, and then they compared them to the K_a/K_s ratios for regulatory genes in a set of closely related mainland species known as North American tarweeds. Their results are shown in **Figure 8.38**. The North American tarweed species, which have not undergone a recent adaptive radiation, have low K_a/K_s ratios at both regulatory loci, indicating that the loci have been under purifying selection; that is, that selection has opposed changes at these loci. The silversword alliance species reveal a different pattern. The K_a/K_s ratios for these genes exceed 1.0 in many of the silverswords, indicating a history of positive selection. These results suggest that the adaptive radiation of the silversword alliance species was facilitated by natural selection favoring changes in the regulatory loci, which in turn caused the changes in phenotype that have allowed these species to diversify into the many niches that they now inhabit.

Mapping Selection within a Single Protein

By comparing the rates of nonsynonymous and synonymous substitutions, evolutionary biologists can resolve the effects of selection down to the scale of individual amino acids within a protein. To understand how selection operates on sialidase, a key protein product of the avian pathogens *Mycoplasma synoviae* and *Mycoplasma gallisepticum*, microbiologists Meghan May and Daniel Brown computed K_a/K_s ratios for each codon of the sialidase gene across 20 strains of the two pathogen species. By mapping the K_a/K_s ratios onto a physical model of the protein as bound to its substrate (**Figure 8.39**), they were able to reveal regions under positive selection (gold in the figure) and regions under purifying selection (magenta in the figure). They found that the binding site and the regions involved in catalytic activity were under strong purifying selection ($K_a/K_s < 1$), presumably to maintain the protein's basic function (May and Brown 2009). But they found that in *M. synoviae*, some sites on the external surface of the protein were under strong positive selection ($K_a/K_s > 1$), for reasons that are not yet fully understood.

Comparing Variation within a Population to Divergence between Populations

Although the criterion $K_a/K_s > 1$ is a good indicator of positive selection, it is an extremely demanding standard because it implicitly requires that all sites within the tested region are under positive selection. But some parts of a protein

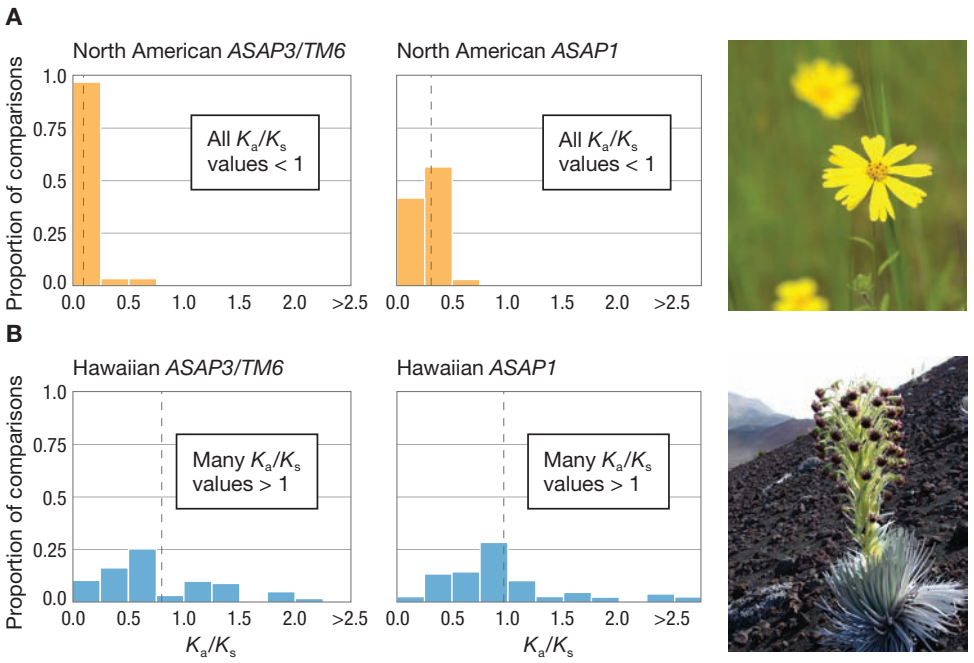


FIGURE 8.38 Positive selection in the Hawaiian silversword group. The bar graphs indicate the K_a/K_s ratios for two regulatory loci (*ASAP3/TM6* and *ASAP1*) in pairs of species in **(A)** North American tarweeds and **(B)** Hawaiian silverswords. The dashed lines are the mean K_a/K_s ratios. North American tarweeds have K_a/K_s ratios that are well below 1.0, indicating that the genes in this group have been under purifying selection. We see a very different pattern in the Hawaiian silverswords, where K_a/K_s ratios commonly exceed 1.0, indicating that the genes in this group have been under positive selection, presumably associated with the adaptive radiation and physiological divergence that the Hawaiian silverswords have undergone over the past 5 million years. Adapted from Barrier et al. (2001).

may be under positive selection while others are under purifying selection: many legitimate cases of positive selection have K_a/K_s ratios well below 1 (Kreitman 2000). For this reason, it would be very useful to have a way of detecting positive selection even in these cases. The McDonald–Kreitman test provides such a method by extending the basic approach of comparing the rates of synonymous and nonsynonymous changes (McDonald and Kreitman 1991; Egea et al. 2008). Instead of looking at only allele substitutions *between species*, as we did in the previous subsection, the McDonald–Kreitman test compares the pattern of allele substitutions *between species* to the pattern of allelic polymorphisms *within species*.

The McDonald–Kreitman test compares ratios of nonsynonymous to synonymous change across two different timescales: a short timescale represented by polymorphism within a species, and a long timescale represented by sequence divergence between species. This provides a powerful tool for detecting positive selection. The basic logic is as follows: Under a neutral model of evolution, selection neither acts on variation at the nonsynonymous sites nor acts on variation at the synonymous sites. Thus, under the neutral model of evolution, the ratio of nonsynonymous to synonymous polymorphism within a population (sometimes called *pN*/*pS*) should be the same as the ratio of nonsynonymous to synonymous substitutions between populations (in the McDonald–Kreitman test, this ratio is typically called *dN*/*dS* instead of K_a/K_s). If a locus is under purifying selection, deleterious mutations will create some level of polymorphism within a population:

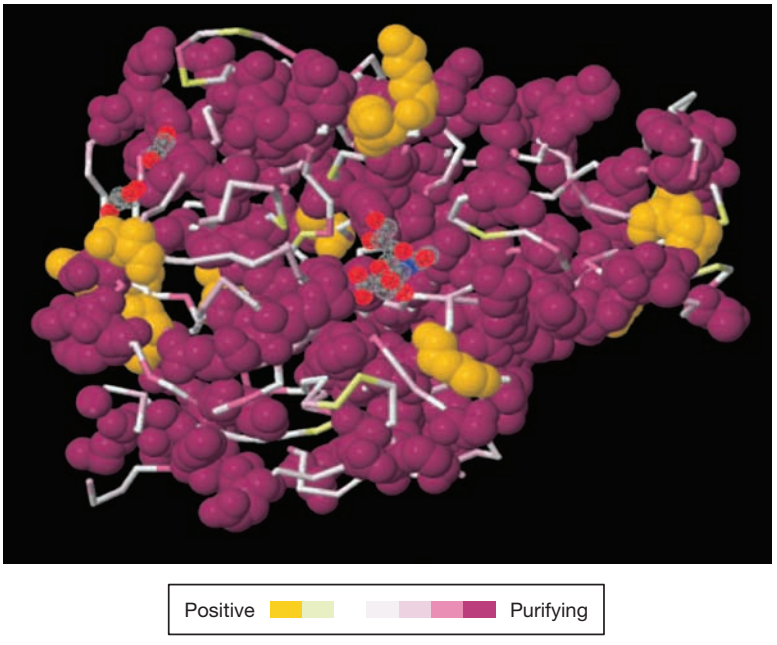


FIGURE 8.39 Positive selection within a single protein. In the sialidase protein of two *Mycoplasma* species, some of the surface regions have undergone positive selection (gold), whereas much of the internal structure, especially around the binding site (gray and red substrate shown bound) is under strong purifying selection (magenta).

TABLE 8.2

Interpreting the Results of the McDonald–Kreitman Test

Nature of Selection	Comparing Ratios
Purifying selection	$pN/pS > dN/dS$
Near neutrality	$pN/pS \approx dN/dS$
Positive selection	$pN/pS < dN/dS$

These deleterious variants are unlikely to be fixed, however, and therefore they will contribute very little to differences between populations. Thus, under purifying selection, we expect the pN/pS ratio to exceed the dN/dS ratio ($pN/pS > dN/dS$). If a locus is instead under positive selection for different traits in the different populations, beneficial mutations will go to fixation relatively quickly, leading to low levels of polymorphism within populations but high levels of divergence between populations: the dN/dS ratio will exceed the pN/pS ratio ($pN/pS < dN/dS$) (**Table 8.2**).

The McDonald–Kreitman test can be used at a genome-wide scale to characterize the types of selection operating on populations. In one notable example, Carlos Bustamante and his colleagues had access to an extraordinary data set: the genomic sequences of more than 20,000 loci from 39 different humans, obtained from Celera Genomics. What could the data tell them about human evolution? They realized that when they augmented the data by including the genomic sequence from a chimpanzee, they could use the McDonald–Kreitman approach to explore what types of selection have been operating on the human genome, and even to identify genes that have been under particularly strong positive or purifying selection (Bustamante et al. 2005).

The researchers narrowed down the data to 11,000 protein-coding loci that could be properly aligned with the chimpanzee sequence, and they compared the diversity among the 39 human samples to the divergence between humans and chimpanzees (**Figure 8.40**). Within humans, they found that 0.169% of the nonsynonymous sites and 0.470% of the synonymous sites were polymorphic, for a pN/pS ratio of 0.360. Comparing human and chimpanzee sequences, they found that the two species differed at 0.242% of the nonsynonymous sites and 1.02% of the synonymous sites, for a dN/dS ratio of 0.237. Because the pN/pS ratio was significantly larger than the dN/dS ratio, they were able to conclude that much of the nonsynonymous variation seen in humans is under purifying selection and thus due to mildly deleterious mutations.

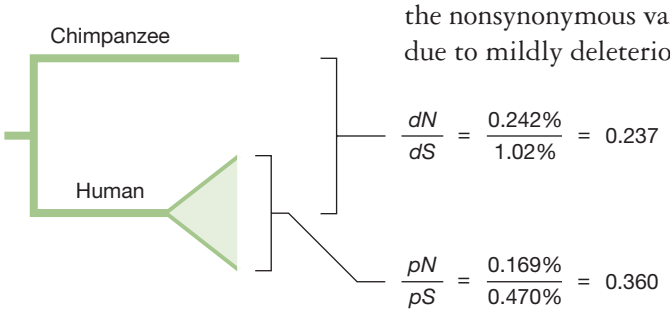


FIGURE 8.40 Using the McDonald–Kreitman test to find human loci under selection. The McDonald–Kreitman test identifies loci under selection by comparing the ratio of nonsynonymous to synonymous variation within a species (pN/pS) to the ratio of nonsynonymous to synonymous substitutions between species (dN/dS). Bustamante and his colleagues compared variation within humans to divergence between humans and chimpanzees.

This genome-wide approach also helps us to understand the nature of the evolutionary differences between humans and chimpanzees by homing in on the adaptive significance of the genetic substitutions that have occurred since these species diverged from their common ancestor. Using a conceptually similar approach, Bustamante and his co-workers singled out the genes that have been under positive or purifying selection. They found a relationship between the functional roles of these genes and the types of selection that they had experienced. Their findings hint at the important selection processes that have driven the divergence between humans and chimpanzees. For example, the team found that many transcription factors have been under positive selection; therefore, positive selection on gene regulation appears to have been important in the recent evolutionary history of these species. Similarly, they found evidence for positive selection at a number of loci associated with immune function, the formation of gametes, and sensory function. By contrast, selection on basic structural and metabolic functions does not appear to have been particularly important in driving the divergence between humans and chimpanzees. Bustamante and his colleagues found that most of the genes involved in cellular structure and biosynthetic

function show evidence of purifying selection, and thus, relative to transcription factors, such genes tend to be highly conserved between humans and chimpanzees (Bustamante et al. 2005).

The Distribution of Allele Frequencies Reveals Past Selective Events

Comparing the ratio of nonsynonymous to synonymous changes is only one way to test for selection. Another approach involves looking at the distribution of allele frequencies at a single locus. If the variation at a locus is selectively neutral, we expect to see a few common alleles and a larger number of less common alleles. It turns out that the allele frequency distribution in a neutral model depends only on the product of effective population size and mutation rate, a quantity that can be easily estimated from population genetic data. Selection causes deviations from this distribution, and thus, if we have population data on the distribution of allele frequencies, we can use the allele frequency distribution to tell us about the selective history of that locus in the population.

If a population has a number of common alleles with similar frequencies, this indicates the presence of balancing selection (selection for polymorphism) or ongoing positive selection. If, instead, the most common allele is more frequent than expected and the less common alleles are rarer than expected, this suggests purifying selection against deleterious variants or, alternatively, that a recent selective sweep has occurred. A number of statistical approaches test for natural selection by examining the deviations from the distribution of allele frequencies that would be expected under a neutral model (Tajima 1989; Simonsen et al. 1995). The disadvantage of many of these approaches is that they do not readily distinguish between selection and other demographic events such as gene flow or population expansions.

Fixation Probability and Substitution Rate for Neutral Alleles

The neutral theory of molecular evolution makes strong mathematical predictions about rates of evolutionary change. For example, Kimura showed that we can find simple expressions for the probability that a neutral allele is fixed in the population and for the rate at which novel substitutions occur. As we saw in Section 8.1, the probability that a neutral allele is fixed is simply its frequency in the population.

Once we know that the probability of fixation of a neutral allele is equal to its frequency in the population, we are ready to calculate the rate of substitution of neutral alleles in a population. Surprisingly, this turns out to be independent of the population size. Suppose that in a diploid population of size N, there are k neutral loci in the genome, and the mutation rate at each of these loci is ν. Then in each generation, we expect $2Nk\nu$ neutral mutations to arise in the population. Each new mutation will be at frequency $1/2N$ at the time that it arises, and thus each will have a fixation probability of $1/2N$. The rate at which neutral substitutions occur is simply the rate at which neutral mutations arise multiplied by the probability that each is fixed, as shown by the equation

$$\text{Substitution rate} = 2Nk\nu \times 1/2N = k\nu$$

The population-size terms N cancel out; thus, the substitution rate of neutral alleles in the population is simply the rate at which neutral mutations occur within a single (haploid) genome, irrespective of the population size. This is an astonishing result: Neutral substitutions occur in the *population* at the rate that neutral mutations arise in an *individual*.

This result allows researchers to estimate mutation rates in individuals by comparing DNA sequences across species. Michael Nachman and Susan Crowell (2000) used this approach to estimate the mutation rate in the human genome. Nachman and Crowell began by sequencing a set of pseudogenes shared by humans and our closest living relatives, chimpanzees (*Pan troglodytes*). Because pseudogenes are not expressed, substitutions within them should be neutral. By the result we just derived, the substitution rate along a single phylogenetic branch from the common ancestor should be equal to the mutation rate. Because there are two such branches—one leading to chimpanzees and one to humans—the sequences of two species are expected to diverge from one another at twice the mutation rate.

Nachman and Crowell tallied the number of substitutions between humans and chimpanzees in each of 11 autosomal pseudogenes and found that about 1.33% of the base pairs differ between these two species. If we assume that humans and chimps diverged 5 million years ago and that the generation time is 20 years, we get the following expression, which we can solve for ν:

$$2\nu = \frac{0.013 \text{ mutations/site}}{5{,}000{,}000 \text{ years} \times \text{generation}/20 \text{ years}}$$

$$\nu = 2.6 \times 10^{-8} \text{ mutations per site per generation}$$

(To improve their estimate, Nachman and Crowell used a correction to account for the time required for a variable site to coalesce within the ancestral population; assuming a reasonable effective population size of the order 10,000 individuals, the correction makes very little difference.) Because the diploid human genome contains approximately 7×10^9 base pairs, each individual human should carry about 180 new mutations. This figure is very similar to estimates obtained by other methods.

Kimura's powerful result equating the mutation rate and the neutral substitution rate has also contributed to the foundational logic of the so-called **molecular clock**. Because substitution rates at neutral loci do not depend on population size or other demographic parameters, proponents of the neutral theory suggest that selectively neutral mutations arise at similar rates in different taxa, and that they should also be fixed at similar rates. If this is indeed the case, the substitution rate gives us a way to measure time using genetic data. We explore this in the next subsection.

KEYCONCEPT QUESTION

8.5 Mutations at the *A* locus occur in approximately one individual out of 2000, but comparisons with closely related species suggest that substitutions at the *A* locus occur approximately once every 20,000 generations. Based on this information, has the *A* locus been neutral or under selection? Explain.

The Molecular Clock Concept

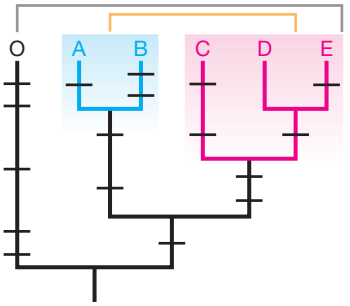
In the 1960s, biochemists studying the amino acid sequences of various proteins noticed an interesting pattern in the way that these sequences differed between species. Emil Zuckerkandl and Linus Pauling observed that for any two species, the number of amino acid differences in their hemoglobin molecules was approximately proportional to the time since they diverged on the phylogenetic tree (Zuckerkandl and Pauling 1962). Thus, closely related species have few differences, whereas more distantly related species have a larger number of differences. To account for these observations, they hypothesized that molecular evolution proceeds in a clocklike manner, with amino acid sequences changing at a constant rate over time, and at the same rate in different lineages.

Emanuel Margoliash found a similar pattern when looking at between-species differences in the amino acid sequence of the cytochrome *c* molecule (Margoliash 1963). These findings led Margoliash to propose the **genetic equidistance principle**: If molecular evolution proceeds at the same constant rate over time in different lineages, all members of a clade should be genetically equidistant from an outgroup to the clade (**Figure 8.41**). Margoliash gave an example: Because fish are an outgroup to the tetrapod vertebrates, we can expect the cytochrome *c* molecules in bird, mammal, and reptile species to all be about the same distance from the cytochrome *c* molecule in a fish species, where distance is measured as the number of DNA or amino acid sequence differences.

It is this principle of genetic equidistance that makes it possible to infer phylogeny from DNA or amino acid sequence data. When the principle breaks down, and evolution proceeds at different rates along different branches of the phylogenetic tree, phylogenetic inference methods run into problems such as the long-branch attraction problem described in Chapter 5.

Where we have a reasonable approximation to a molecular clock, we can use molecular data to estimate not only the phylogenetic relationships among species, but also the dates of evolutionary events. If the rate of mutation is known and is approximately the same across lineages, we can use such data to predict the point in time when groups diverged from one another. This prediction can be checked against other estimates of divergence, such as those that might be obtained through the fossil record (Donoghue and Benton 2007). The larger the number of selectively neutral alleles that differ between two groups, the further back in history we must go to find the point in time when the groups diverged (Kumar 2006).

In a dramatic early application of this approach, in 1967 Allan Wilson and Vincent Sarich used the molecular clock to date the divergence time of humans and chimpanzees (Sarich and Wilson 1967). To assess divergence, they used the serum albumin molecule—a very common protein in blood plasma. DNA sequencing technology had not yet been developed, so they needed a way of assessing the degree of similarity between versions of proteins in different species. They used *immunological cross-reactivity*—the strength of an immune reaction, specific to one protein, when confronted with another—as a measure of distance. The principle is that if molecular evolution operates in a clocklike fashion, then as species diverge, molecular changes in the structure of albumin should reduce the degree of cross-reactivity at an approximately constant rate. Using this approach, Sarich and Wilson estimated that humans and chimpanzees had diverged only 5 million years



	O	A	B	C	D	E
O	—	9	10	10	9	10
A		—	3	7	6	7
B			—	8	7	8
C				—	3	4
D					—	1
E						—

FIGURE 8.41 The genetic equidistance principle. The genetic equidistance principle suggests that if molecular changes occur at a constant rate across lineages, the members of any given clade should be equidistant from the members of an outgroup. In the phylogenetic tree shown, bars indicate substitution events. The table shows the genetic distance between pairs of species. All species A–E are approximately equidistant from the outgroup O. Moreover, species in the clade C–E are approximately equidistant from species in the clade A–B.

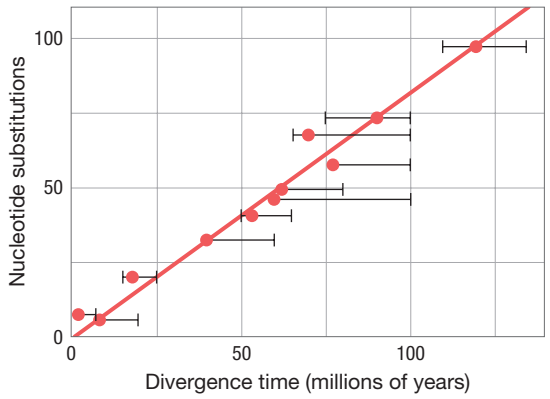


FIGURE 8.42 Nucleotide substitution rate appears to be approximately constant in mammals. Each point reflects a pair of mammalian species: The horizontal axis indicates their divergence time as estimated from fossil data, and the vertical axis indicates the estimated number of nucleotide differences in seven proteins compared across each species pair. Adapted from Wilson et al. (1977).

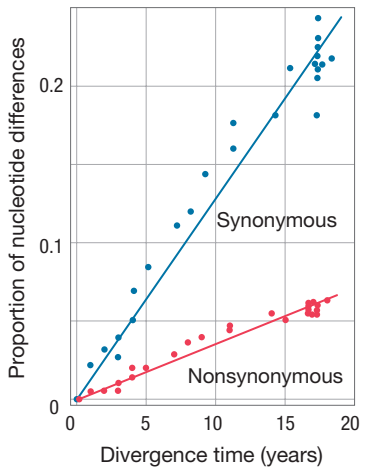


FIGURE 8.43 Clocklike molecular evolution in influenza A. The proportion of nucleotide differences as a function of divergence time between a 1968 strain of the influenza A virus and strains collected in subsequent years. Blue points denote synonymous differences, and red points denote nonsynonymous differences. Adapted from Gojobori et al. (1990).

ago, far more recently than the 30-million-year estimate that other researchers had derived from paleontological data. This estimate was extremely controversial when first published. But, as we will see in Chapter 19, it is now widely accepted and is closely in accord with more recent data based on genome-scale analysis (Hobolth et al. 2007).

In the late 1970s and early 1980s, there was considerable hope that most molecular evolution would turn out to be clocklike, with nearly constant rates of change across sites and along different evolutionary lineages. Wilson and his colleagues, for example, found that rates of amino acid sequence changes in a number of proteins were approximately constant across the mammalian clade (Wilson et al. 1977) (**Figure 8.42**).

By the late 1980s, however, this hope began to dim. One early indication of problems came from Vawter and Brown's comparison of substitution rates across lineages and genome regions (Vawter and Brown 1986). While the earliest studies of the molecular clock had relied on protein structure assayed via electrophoresis, immunological cross-reactivity, or other techniques, Vawter and Brown used a technique known as restriction typing to assess the differences between DNA sequences directly. They compared rates of substitution in mitochondrial DNA to rates of substitution in nuclear DNA for pairs of primate species and pairs of sea urchin species. They found that in primates, mitochondrial DNA evolved 5 to 10 times faster than nuclear DNA (Vawter and Brown 1986). Thus, different regions of the genome evolved at different rates. Worse still for the molecular clock hypothesis, they found that in sea urchins, mitochondrial and nuclear DNA evolved at approximately the same rate. From this, they could conclude that the rates of molecular evolution differ across species and across genomic regions. The clock turns at different rates in different lineages.

To salvage the molecular clock approach, researchers tried restricting their studies to a single genomic region within a single clade. If this worked, molecular clocks could still be useful for dating evolutionary events. To test this hypothesis, Takashi Gojobori and colleagues examined molecular evolution in the influenza A virus, and Thomas Leitner and Jan Albert studied molecular evolution in the human immunodeficiency virus (HIV) (Gojobori et al. 1990; Leitner and Albert 1999). Viruses are particularly useful organisms for testing this hypothesis because for known strains of a given virus, we do not have to estimate the dates of evolutionary events from fossil data or other sources of information. Rather, the viruses evolve so rapidly and are sampled so intensively by medical researchers that we can often very closely determine divergence dates from epidemiological information. For viruses such as influenza and HIV, parts of the viral genome have been mapped in numerous strains at many points over time and in many populations, allowing evolutionary biologists to construct phylogenies and test ideas about the neutral theory.

Gojobori and colleagues looked at the hemagglutinin H3 gene encoding a surface protein of the influenza A virus. They compared the H3 sequence from a strain sampled in 1968 to the H3 sequences from strains sampled in subsequent years and plotted the number of synonymous and nonsynonymous substitutions that had occurred as a function of the time elapsed between samples (**Figure 8.43**). The researchers found that substitutions were more common at synonymous sites

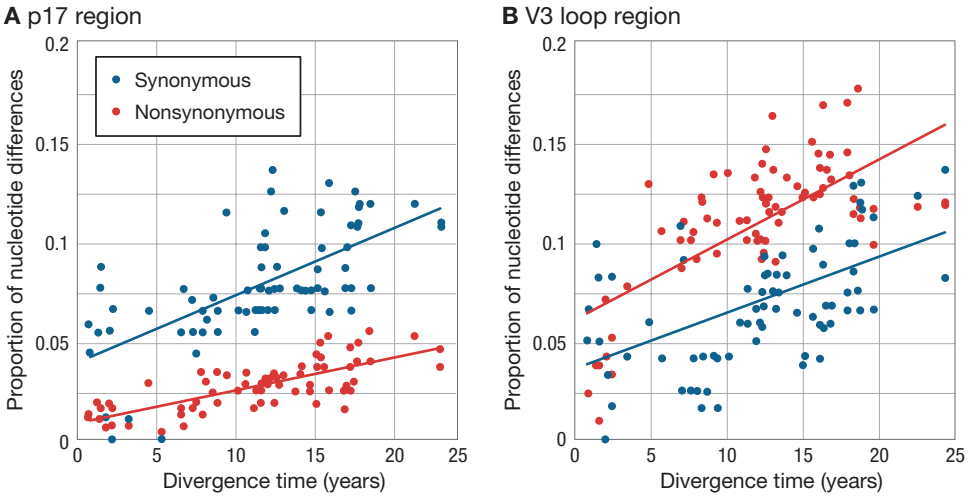


FIGURE 8.44 Clocklike molecular evolution in HIV. Proportion of nucleotide differences between pairs of HIV-1 strains in **(A)** the p17 region and **(B)** the V3 loop region of the HIV-1 genome, plotted against the divergence times of each pair. Synonymous differences are more common in the p17 region, but nonsynonymous differences are more common in the V3 loop region. Adapted from Leitner and Albert (1999).

than at nonsynonymous sites. Moreover, as predicted by the neutral theory, the substitution rate was constant across different strains of influenza A over the 20-year time interval examined by the researchers (Gojobori et al. 1990).

Leitner and Albert carried out a similar study on the HIV-1 virus based on a set of strains that had been closely tracked in Sweden. Their samples covered a shorter time period than that covered by the influenza virus samples in the Gojobori study, but they knew the exact chain of transmission by which their HIV isolates were related. As a result, they could calculate the number of years of evolution separating any two strains in their study. At a gene encoding an internal structural protein known as p17, Leitner and Albert found results similar to those found in the study by Gojobori and colleagues: The number of substitutions increased approximately linearly with time, and synonymous substitutions were more common than nonsynonymous substitutions (**Figure 8.44A**). But at a gene encoding an external protein involved in interactions with immune cells, known as the V3 loop of the envelope glycoprotein 120, they found a different pattern. While substitutions again accrued linearly over time, at this locus nonsynonymous changes were more frequent than synonymous changes (**Figure 8.44B**) (Leitner and Albert 1999). This suggests that at least some portion of the V3 loop has been under strong positive selection, presumably to escape immune recognition.

Another inherent limitation of molecular clock methods is that for any particular gene, the number of substitutional differences between two lineages will not increase indefinitely with time. As two lineages begin to diverge, most substitutions will occur at sites that were previously identical in the two species. During this period, differences will tend to accumulate at an approximately constant pace, and it is during this period that divergence will accumulate in a clocklike manner. But after two lineages have diverged substantially, further substitutions may occur at sites that already differ. Such substitutions do not contribute to increased divergence between the two lineages, and as a result the observed rate at which divergence increases with time begins to slow down. Once this happens, differences cease to accumulate in a clocklike fashion (**Figure 8.45**). This phenomenon is known as *saturation* because the sequence has become saturated with substitutions, and further substitutions will not be detected by comparison with an ancestral sequence.

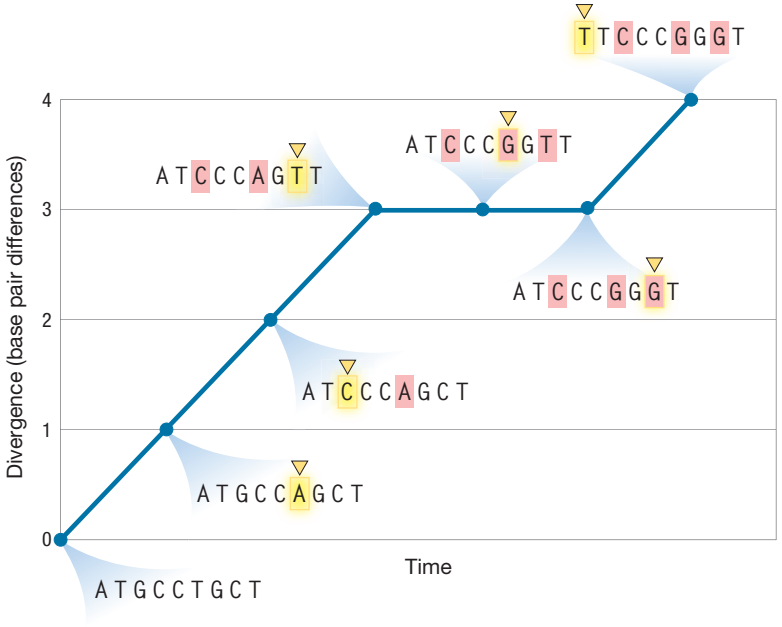


FIGURE 8.45 Saturation. Early on, after the divergence of two lineages, most new substitutions—indicated by the yellow triangles—occur at sites that were previously unaltered, and thus the divergence rate increases approximately linearly with time. Once substitutional differences become common between the two species, many new substitutions occur at previously substituted sites, indicated in pink, and the divergence rate slows. This phenomenon is known as saturation.

Statistical methods can be used to correct some of the effects of saturation, but eventually the number of sequence differences between two lineages reaches a steady state and provides no further information about the divergence time. Thus, there is a natural timescale for molecular clocks. Clocks based on sites that change rapidly, such as the silent, third codon positions, are useful for looking at short time periods. They accumulate changes quickly, and so they can be used to estimate recent evolutionary events, but they also saturate relatively quickly, and thus they are useless for inferring ancient events. As an extreme example, Jaume Jorba and colleagues measured the divergence rate of third codon sites in the rapidly evolving poliovirus. While divergence initially accumulates in clocklike fashion, after only a decade the effects of saturation become important and the rate of further divergence slows (Jorba et al. 2008) (**Figure 8.46**). Clocks based on sites that change very slowly, such as nonsynonymous sites in highly conserved genes, do not accumulate enough differences to be useful in dating recent events, but they also are slow to saturate, and thus they can be used to date ancient events. For example, the 16S ribosomal RNA sequence is useful for dating very old evolutionary events: It takes approximately 50 million years to accumulate 1% sequence divergence at this locus.

Over the past two decades, a great volume of work has used DNA sequence data to quantify rates of molecular evolution. These studies have collectively affirmed that different parts of the genome evolve at different rates. Synonymous sites—which tend to be neutral or very nearly neutral—accumulate substitutions more rapidly than do nonsynonymous sites, which tend to be under purifying selection. Noncoding regions tend to change more rapidly than coding regions, although some noncoding regions also appear to be under purifying selection, presumably because of their functional roles in gene regulation. "Housekeeping" genes that perform essential core functions tend to change less rapidly than do genes with more limited or specialized function. These differences are unsurprising; in all cases, the general pattern is that the stronger the action of purifying selection, the slower the substitution rate. These differences are also useful. The fact that different loci change at different molecular clock rates allows researchers to pick loci that change at a rate appropriate for answering the questions of interest. To study a recent evolutionary divergence, one might choose to look at rapidly changing sites; to study ancient evolutionary events, more highly conserved sites would be more useful.

These studies have also revealed that, as Vawter and Brown suspected, evolutionary rates differ along different lineages. This creates further problems for the use of molecular clocks. But this does not mean that molecular information is

useless for dating evolutionary events. Population geneticists have developed an ensemble of statistical methods, collectively known as *relaxed clock methods*, to partially compensate for the difficulties introduced by differing evolutionary rates (Welch and Bromham 2005; Battistuzzi et al. 2010). Dating based on clocklike methods remains an important tool in evolutionary biology, and how best to estimate such divergence dates from genomic information remains an active area of research.

Generation Time and the Rate of Neutral Substitution

We conclude this chapter with a puzzle and a likely solution. The puzzle is this: For species with similar mutation rates, the neutral theory predicts a constant rate of substitution per generation. This is known as the *generation time hypothesis*. But some empirical data suggest that the rate of substitution is approximately constant *per year*, despite the fact that generation times across taxa differ dramatically. The generation time for a rat, for example, is much shorter than the generation time for an elephant. Over absolute time, then, organisms with faster generation times would produce many more generations of offspring than their slower counterparts. How can we explain why, for pairs of species such as rats and elephants, the rates of molecular evolution are so similar?

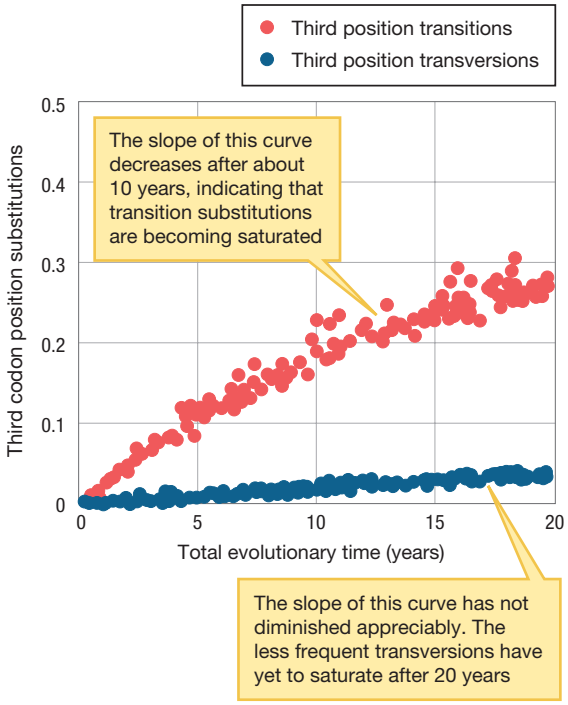


FIGURE 8.46 Rapid saturation of sequence divergence. Changes in the third codon position accumulate rapidly in poliovirus. Within a decade, frequently occurring transitions at the third position (red) have started to saturate. By contrast, transversions (blue) occur at a lower rate, and thus even after 20 years, transversion substitutions continue to accumulate in a clocklike manner. Adapted from Jorba et al. (2008).

In the 1970s, using an approach known as the *relative rates test*, Allan Wilson and his colleagues found that the annual rate of molecular change in short-generation-time and long-generation-time mammalian lineages was approximately equal (Wilson et al. 1977). This suggests that generation time—at least in mammals—should not strongly bias a molecular evolutionary clock. Although it must be acknowledged that some studies have found that generation time influences the rate of molecular evolution (Laird et al. 1969; Wu and Li 1985; Li et al. 1996; Thomas et al. 2010), others have supported the absence of a generation time effect. For example, using data on 17,208 genetic sequences from more than 300 species of placental mammals that varied from short generation times (rodents) to long generation times (primates), Sudhir Kumar and Sankar Subramanian estimated a fairly constant mutation rate of approximately 2×10^{-9} substitutions per base pair *per year* and found that neutral mutations accumulate at the same rate in short- and long-lived mammals (Kumar and Subramanian 2002). They found this to be the case both when the divergence time between mammalian species was estimated from fossil data and when it was estimated from molecular genetic data (**Figure 8.47**).

The finding that the mutation rate per base pair per year is often similar in long-generation and short-generation species makes molecular clocks more useful for estimating divergence times, because we do not automatically expect different rates of evolution along branches with different generation times. But *why* should it be the case that the mutation rate per base pair per year is similar in long-generation and short-generation species?

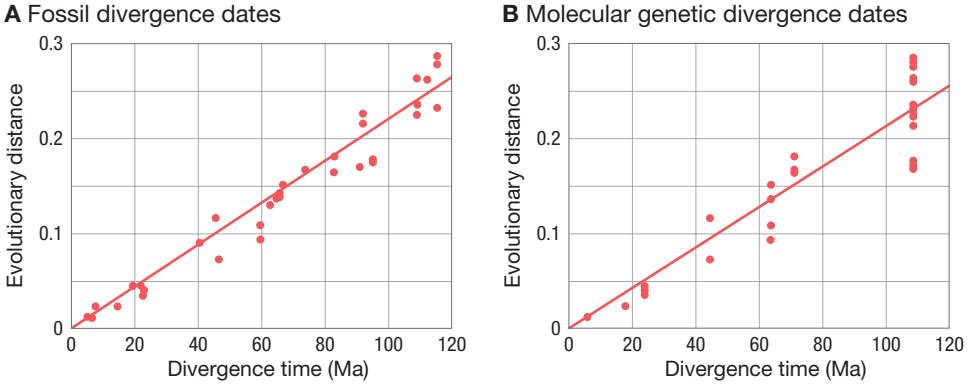


FIGURE 8.47 Accumulation of neutral substitutions in mammals. Each point represents a pair of mammalian species. The vertical axis—evolutionary distance—is a measure of the number of genetic differences between species. The horizontal axis—divergence time in millions of years (Ma)—is determined from **(A)** fossil evidence and **(B)** molecular genetic data. Both dating methods reveal a nearly constant substitution rate in placental mammals, independent of generation time. Adapted from Kumar and Subramanian (2002).

Tomoko Ohta provided an answer to this puzzle by modifying the neutral theory to account for the prevalence of mildly deleterious mutations. The **nearly neutral theory** of molecular evolution posits that most substitutions are, if not exactly neutral, only mildly deleterious (Ohta 1992). Their fate is consequently determined by the interplay between selection and drift as discussed in Section 8.4. Population size then plays a critical role in determining the balance between drift and selection. Whereas the neutral theory predicts that the substitution rate is independent of population size, the nearly neutral theory predicts that the substitution rate is higher in smaller populations, where mildly deleterious alleles can drift to fixation. This provides a possible resolution to the puzzle described earlier. Under the neutral theory, we would expect species with longer generation times to have lower annual substitution rates. But generation time is highly correlated with population size, so species with larger populations tend to have shorter generation times (**Figure 8.48**). These factors at least partly cancel each other's effects, leading to an approximately constant annual rate at which nearly neutral mutations are fixed across a wide range of generation times.

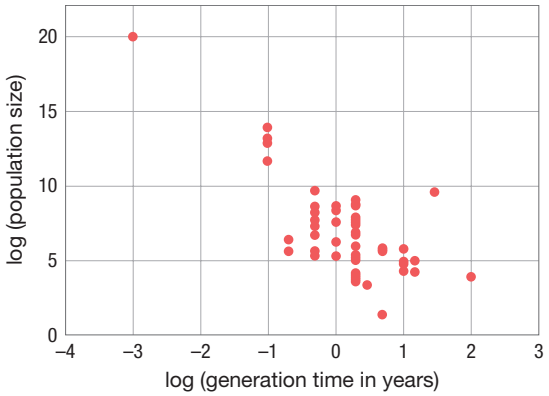


FIGURE 8.48 Population size and generation time. Species with larger populations tend to have shorter generation times. Based on data from 77 species; logarithms are base 10. From Chao and Carr (1993).

Over the past two chapters, we have explored the processes by which allele frequencies change in large and small populations. In the next chapter, we will explore what happens when allele frequencies are changing simultaneously at more than one locus. It is there—in the interplay between alleles at different loci—that we will find much of the action that makes evolutionary biology so interesting.

SUMMARY

1. In finite populations, allele frequencies will fluctuate as a result of random sampling effects. This process is known as genetic drift.
2. Genetic drift operates more strongly in smaller populations than in large populations.
3. Genetic drift reduces the heterozygosity—the fraction of individuals who are heterozygous at a given locus—within a population by causing alleles to be fixed or lost even in the absence of natural selection.
4. Because genetic drift is a random process, it causes divergence between populations over evolutionary time.
5. We can trace the genealogy of individual gene copies through a population. For any sample of gene copies at a single locus, somewhere in the past there is an ancestral gene copy from which all copies in our sample are descended.
6. Tracing this genealogy of gene copies back in time, we derive the coalescent tree. In a sexual population, every locus has a different coalescent tree.
7. Population bottlenecks, in which populations are temporarily reduced to a small number of individuals, accelerate genetic drift and can cause substantial changes in allele frequencies.
8. Allele frequencies in peripheral and island populations can differ greatly from allele frequencies in the populations from which they were derived because of the founder effect.
9. Drift reduces heterozygosity in a population, but mutation creates new variation. The mutation–drift balance represents a steady state between these two processes. Drift also interacts with natural selection and can reduce the ability of selection to fix favorable alleles.
10. The neutral theory of molecular evolution proposes that most variation in a population is neutral and most substitutions that occur over evolutionary time are neutral substitutions. If so, it follows that genetic drift plays a major role in the evolutionary process.
11. We can use the neutral model as a null model of molecular evolution against which to test for evidence of selection at given loci.
12. Under the neutral model, the fixation rate in a population is equal to the mutation rate in an individual in that population.
13. At many loci, molecular changes occur at an approximately constant rate over time. The behavior of this molecular clock makes it possible to assign dates to the branch points on a phylogeny using DNA sequences.
14. The nearly neutral model accounts for the independence of substitution rate and generation time by positing that most variation is mildly deleterious rather than precisely neutral. While long-lived species generate mutations at a lower rate, they also fix more of these mildly deleterious mutations because of their smaller population sizes. These effects cancel out, and substitution rate is roughly independent of generation time.

KEY TERMS

coalescent point (p. 272)
coalescent theory (p. 271)
effective population size (p. 266)
expected heterozygosity (p. 263)
founder effect (p. 281)
genetic drift (p. 261)
genetic equidistance principle (p. 299)
leading edge expansion (p. 283)
molecular clock (p. 298)
nearly neutral theory (p. 304)
neutral theory (p. 288)
observed heterozygosity (p. 262)
population bottleneck (p. 278)
positive selection (p. 277)
pseudogene (p. 291)
purifying selection (p. 293)
selectively neutral (p. 261)
substitutions (p. 288)
Wright–Fisher model (p. 259)

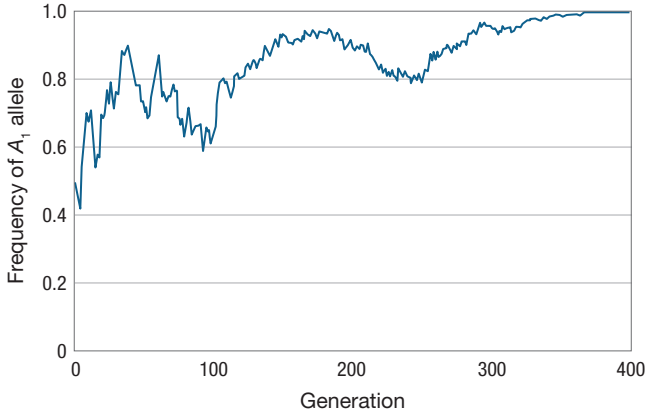
REVIEW QUESTIONS

1. How does population size affect the magnitude of allele frequency changes due to genetic drift?
2. On average, what effect does genetic drift have on the heterozygosity of a population?
3. On average, what effect does genetic drift have on the differences between populations?
4. What does it mean for gene copies to coalesce?

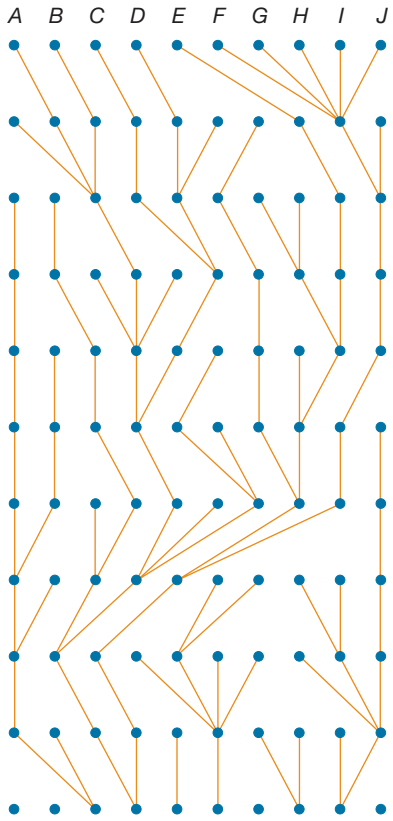
5. What is a population bottleneck, and how does it affect genetic diversity?
6. In a leading edge expansion, individuals from a source population move out to colonize a previous unoccupied region. When this happens, where is genetic diversity expected to be the greatest?
7. What is the probability that a dominant mutation with a small selective advantage s goes to fixation? How does this depend on population size, N?
8. Which one or more of the following are neutral under the neutral theory: (1) most new mutations; (2) most variation present within a population at any given time; (3) most allelic substitutions over time?
9. List three reasons why a mutation may be neutral.
10. What evolutionary puzzle can be resolved by the nearly neutral theory?

KEY CONCEPT APPLICATION QUESTIONS

11. A researcher sets up 100 replicate population cages. Each is founded with 50 *Drosophila melanogaster* individuals, drawn from a population that is polymorphic for the L_1 and L_2 alleles at neutral pseudogene locus L. After many months, the L_1 allele is fixed in 11 of the 100 cages, and the L_2 allele is fixed in 89 of the cages. Estimate the frequencies of the L_1 and L_2 alleles in the original population from which these cages were founded.
12. In many polygynous songbird species, such as wrens or red-winged blackbirds, a single male holds a territory and mates with several females in that territory. In monogamous species, such as cardinals and blue jays, mated pairs typically hold a territory, and males mate with only one female. In comparably sized populations, do you expect drift to have a stronger effect in a polygynous species or in a monogamous species? Explain.
13. Suppose we were to determine that the majority of mutations that change amino acid sequence in *Drosophila* were deleterious. Would this observation be inconsistent with the neutral theory of molecular evolution? Why or why not?
14. The following figure plots the frequency of the A_1 allele over time at a neutral locus in an isolated population. After 400 generations, this allele has become fixed in the population. Based on the graph, do you think this population has been growing, declining, or staying at a constant size?



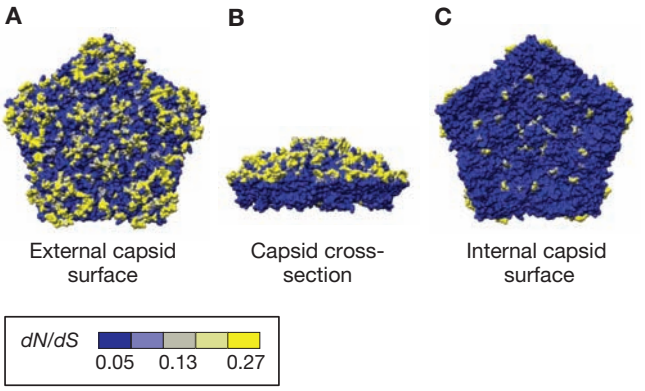
15. The figure below illustrates a gene genealogy for 10 different gene copies, A–J, at a neutral locus.



   a. Add an arrow to indicate the direction of time.
   b. Find the coalescent point for gene copies A, B, and D.
   c. Find the coalescent point for the group of all 10 gene copies A–J.
   d. Which two gene copies are more likely to be identical in sequence: E and F or G and H? Explain your reasoning in one sentence.
16. In a 2008 study, geneticist Asher Cutter wanted to estimate how long ago the fruit fly *Drosophila melanogaster* diverged from a closely related species, *Drosophila simulans* (Cutter 2008). He was able to do so using the result from

the neutral theory that, at neutral loci, the mutation rate is equal to the substitution rate. By sequencing *D. melanogaster*, *D. simulans*, and several other *Drosophila* species, he estimated that *D. melanogaster* had diverged from its common ancestor with *D. simulans* at 6.8% of its synonymous sites. (Note that the 6.8% difference is between *D. melanogaster* and the common ancestor: The difference between *D. melanogaster* and *D. simulans* would presumably be larger because evolution has occurred along the branch leading to *simulans* as well.) If the mutation rate in *Drosophila* is 5.8×10^{-9} mutations per site per generation and the generation time is approximately 1/10 of a year, how long ago did *D. melanogaster* and *D. simulans* diverge?

17. The human rhinovirus is responsible for many cases of the common cold. Its surface is covered with a number of protein-based pentamer subunits, as illustrated here.



To create this image, Amy Kistler and her colleagues sequenced a number of rhinovirus genomes and computed *dN*/*dS* ratios for each amino acid that makes up the pentamer (Kistler et al. 2007). The *dN*/*dS* values are indicated by color in the figure. Where on the capsid is purifying selection the strongest? Where is positive selection the strongest? Propose a hypothesis for why this pattern is observed.

18. Suppose that when comparing the DNA sequences at a given locus across various *Drosophila* species, you observed that the nonsynonymous-to-synonymous substitution ratio (K_a/K_s) was approximately 0.5. What would you tentatively conclude about the history of selection at the locus within the *Drosophila* clade?

19. If you then observed that within a single species, *Drosophila melanogaster*, the ratio of nonsynonymous to synonymous polymorphism (*pN*/*pS*) was 0.2, how would you revise your previous conclusion?

SUGGESTED READINGS

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9

Evolution at Multiple Loci

In the first chapter of this book, we described how the use of antibiotics selects for the evolution of antibiotic-resistant strains of bacteria. Such strains represent a major public health threat and annually are responsible for tens of thousands of deaths in the United States alone. Many of these strains have resistance mutations that modify the chemical composition of an antibiotic's target: a membrane protein, a ribosomal RNA, a component of the cell wall, or some other cellular structure. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) features a modified membrane protein that no longer binds methicillin. Such modifications are highly beneficial in the presence of antibiotics, but they can have substantial fitness costs to the bacterial cell in the absence of antibiotics because they hinder or eliminate the function of highly adapted elements of the cellular machinery.

Because bacteria suffer fitness costs to resistance in the absence of antibiotic use, we might expect that resistance evolution would be "reversible": In the presence of antibiotics, resistant strains would increase in frequency due to the benefits conferred under those conditions, while

◀ Sand dunes twist across Namibia's Namib–Naukluft National Park

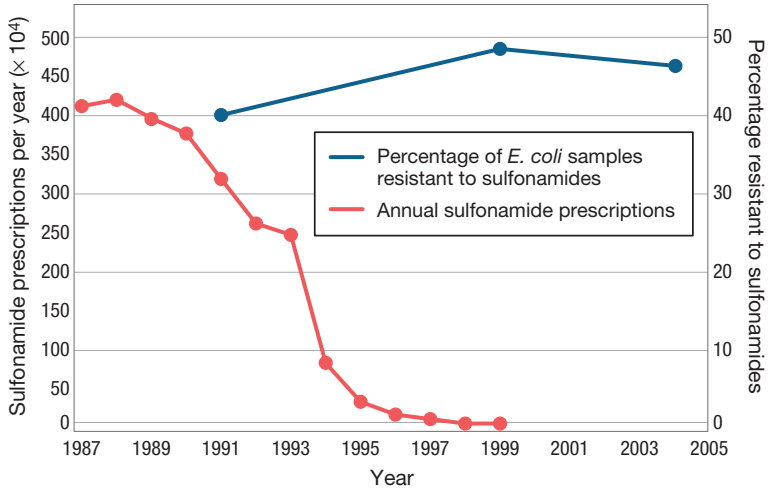


FIGURE 9.1 Sulfonamide use and sulfonamide resistance. This graph shows the estimated number of prescriptions for sulfonamide-containing drugs in the United Kingdom in red and the fraction of *E. coli* samples that exhibited sulfonamide resistance in blue. Curtailing sulfonamide use did not reduce the frequency of sulfonamide resistance. Adapted from Enne et al. (2001) and Bean et al. (2005).

in the absence of antibiotics, resistant strains would decrease in frequency and eventually be lost due to the costs of resistance when no antibiotics are present. Sometimes this is exactly what happens. In the early 1990s, Finland reduced its use of the antibiotic erythromycin almost by half. In response, the frequency of erythromycin resistance in streptococcal bacteria dropped sharply over the next 5 years (Seppälä et al. 1997).

Unfortunately, such drops do not always occur. Terminating antibiotic use is not necessarily sufficient to reverse the evolution of resistance, either within an individual patient (Sjölund et al. 2003, 2005) or at the level of the community at large (Chiew et al. 1998; Sundqvist et al. 2010). One of the most striking examples involves a class of antibiotics known as the sulfonamides, commonly used to treat urinary tract infections caused by *Escherichia coli* and other bacteria. After concerns about possible side effects of these antibiotics arose in the late 1980s, their use in the United Kingdom started to decline, and in 1995 the British government recommended against the use of these drugs under most circumstances. Use of sulfonamides in Britain promptly dropped to less than 3% of pre-1995 totals. This set up a natural experiment: What would happen to the frequency of sulfonamide resistance in the *E. coli* population once the use of the drugs was reduced more than 40-fold on a nationwide scale? The results were striking—and discouraging (Enne et al. 2001; Bean et al. 2005). Over 9 years, from 1995 to 2004, sulfonamide resistance did not decline at all in Britain despite the cessation of drug use (**Figure 9.1**).

How do we explain this? Why weren't the alleles for sulfonamide resistance lost after sulfonamide use was halted? If we were to think about evolution one locus at a time—in this case, focusing on the locus conferring antibiotic resistance—this would be hard to explain. But if we recognize that evolution occurs simultaneously at multiple loci, and that the loci interact to determine the course of evolution, then the long-term persistence of antibiotic-resistance alleles becomes easier to understand. There are a number of reasons why even when antibiotic use is halted, antibiotic resistance does not always disappear as quickly as one might expect given its initial fitness consequences. Among them, the most straightforward is that *compensatory mutations* arise at other loci in resistant bacterial strains. These compensatory mutations do not reduce the degree of resistance but do reduce or eliminate the fitness costs associated with the resistant phenotype. Compensatory mutations have been documented in a wide range of bacterial species for resistance to a wide range of antibiotics (Andersson and Hughes 2010).

To understand how natural selection operates on resistance and compensatory mutations, it helps to think in terms of the underlying genetics. Recall that bacteria are haploid, with only one gene copy at each locus. In the simplest cases, a mutation at one locus confers antibiotic resistance, and a compensatory mutation at a second locus reduces the fitness cost of resistance (Schrag and Perrot 1996). Call the resistance locus R, with alleles r (sensitive; that is, susceptible to antibiotics)

and *R* (resistant). Call the compensatory locus *C*, with alleles *c* (uncompensated) and *C* (compensated). The antibiotic-sensitive wild-type strain is drug sensitive and uncompensated; that is, it has the genotype *rc*. In the absence of antibiotics, the antibiotic-sensitive wild type has high fitness, but when antibiotics are in use, the fitness of the wild type is very low. Resistance arises by mutation at the *R* locus, from *r* to *R*. This gives rise to resistant, uncompensated individuals with genotype *Rc*, which can now grow even in the presence of antibiotics, albeit with appreciable fitness costs. Next, suppose that a compensatory mutation arises at the *C* locus, from *c* to *C*. This results in resistant, compensated individuals with the *RC* genotype (**Figure 9.2A**). Not only can they grow in the presence of antibiotics, but also the cost of resistance has been reduced dramatically by the compensatory mutation, and the *RC* individuals now have almost as high a fitness as the wild type even in the absence of antibiotics (**Figure 9.2B**).

In the presence of antibiotics, both the initial *R* mutation and the subsequent *C* mutation lead to substantial fitness increases, so each of the mutations is likely to increase rapidly in frequency during a period of antibiotic use. When antibiotic use is halted, the *RC* individuals have only a very slight fitness disadvantage relative to the *rc* wild type, so resistance will not decline at anything like the rate at which it arose. Once we start thinking about both the resistance locus and the compensatory locus at the same time, we can understand why antibiotic-resistant bacterial strains increase rapidly in frequency when drugs are used but disappear slowly when drugs are withdrawn.

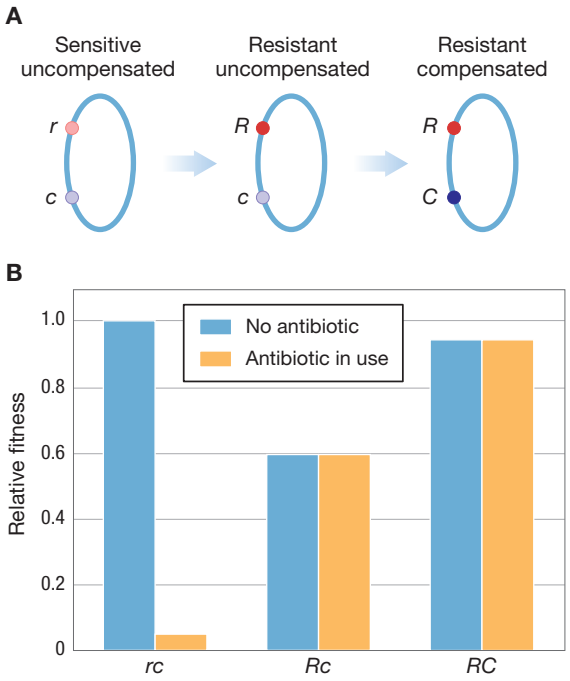


FIGURE 9.2 Evolution and fitness effects of resistance and compensation. **(A)** The resistant allele *R* replaces the sensitive allele *r*, and then the compensated allele *C* replaces the wild type *c*. **(B)** In the absence of antibiotics, the wild type *rc* is the most fit. The resistant allele *R* imposes a significant fitness cost that is largely ameliorated by the compensated allele *C*. In the presence of antibiotics, the wild type has very low fitness. Under these conditions, the *R* allele provides a large fitness benefit, and the *C* allele then provides an additional fitness advantage.

We will return to this example to see some of the additional complexities of evolution at multiple loci that are illustrated by the evolution of antibiotic resistance. For now, however, this example simply shows that to understand the evolution of a phenotype, we often need to understand how evolutionary processes operate on multiple interacting loci. Here we have considered haploid organisms: bacteria. Further complexities arise when we start to consider diploid organisms such as ourselves. In Chapters 7 and 8, we developed the basic mathematical machinery to describe, one locus at a time, how allele frequencies change in large and small populations. But we also need to be able to think about how allele frequencies change at *multiple loci*—and how the process of change at one locus influences what happens at another. And so, in this chapter, we will address the following questions:

- How do multiple genes interact to determine phenotypes?
- What is linkage disequilibrium, how does it arise, and how does it change over evolutionary time?
- How does the physical arrangement of genes within the genome influence the evolutionary process?
- What are adaptive landscapes, and how can they help us understand the course of evolution?
- How can we use quantitative genetic models to predict evolutionary change in traits even when we do not know the specific genetic basis for these traits?

9.1 Polygenic Traits and the Nature of Heredity

As we discussed in Chapter 2, Darwin crafted his theory of evolution by natural selection without having even a rudimentary understanding of the mechanisms of inheritance. Without that understanding, it was not possible to construct detailed, quantitative models of the process of natural selection. Then, in the late nineteenth and early twentieth centuries, researchers began to uncover the rules of heredity. They rediscovered Mendel's work, and they developed a detailed picture of how genetic inheritance operates. Initially, it was not at all clear that the new understanding of how genes are transmitted was consistent with Darwin's view of evolution by natural selection. One of the most important scientific accomplishments of the first half of the twentieth century was the reconciliation of the two major components of biology—Darwin's theory of natural selection and Mendel's discoveries about genetic transmission—leading to the founding of the field known today as population genetics. We now examine how this occurred.

Continuous versus Discontinuous Variation

The rediscovery of Mendel's work eventually solved a number of problems for Darwin's theory but, in the short term, it posed new challenges to his ideas about natural selection. Even before researchers revisited Mendel's work on the nature of heredity, an active debate raged over the nature of the changes by which evolution proceeded. Darwin argued that natural selection primarily acted on continuous variation—very small, gradual changes in form, such as the elongation of a bone, or a gradual shift in the color of an animal's fur—but even many of Darwin's closest allies disagreed.

Mendel's findings only exacerbated the controversy. Over the first decade of the twentieth century, researchers amassed an impressive body of experimental evidence in support of Mendelian inheritance, but this view of inheritance was seen as incompatible with Darwinian gradualism by most biologists of the time. The problem was that the Mendelian traits studied by geneticists involved large, discrete variations rather than the finely graded continuous characters that Darwin took to be essential for his theory of natural selection. This left researchers with a number of questions: Could Darwin's mechanism operate on Mendelian characters? Is Mendelian inheritance compatible with the sort of small, graded variation often observed for traits such as size or height? To what degree are small, graded variations heritable at all? These issues were resolved through the joint efforts of theoreticians and experimentalists.

Polygenic Traits Can Exhibit Nearly Continuous Variation

On the theoretical side, the recognition that many traits are **polygenic**—that is, affected by many genes simultaneously—was a first step in reconciling Darwinian natural selection with Mendelian inheritance. George Yule and R. A. Fisher independently developed mathematical models demonstrating that Mendelian inheritance was compatible with small, graded variations provided that multiple genes, each of relatively small effect, were involved (Yule 1902; Fisher 1918). This was a suggestion that Mendel himself had made based on observations of

flower color in the bean plant *Phaseolus vulgaris*, but the idea was largely overlooked after the rediscovery of his work. The patterns of multifactorial inheritance—that is, inheritance of a polygenic trait—are more complicated than simple Mendelian inheritance for a trait controlled by a single locus, but they are still predictable in a Mendelian framework.

On the experimental side, geneticist Herman Nilsson-Ehle observed a nearly continuous gradation of kernel colors in crosses between red-kernel and white-kernel variants of winter wheat when kernel color was polygenic—in this case, involving three genes (**Figure 9.3**) (Nilsson-Ehle 1908). The genes in Nilsson-Ehle's kernel color system interacted in a particularly straightforward way: They had **additive genetic effects**, meaning that the phenotype of any individual could be worked out simply by summing the effects of each allele that it carried. The more alleles for dark red color that an individual carried, the darker the phenotype.

Edward East found comparable results on polygenic traits and continuous variation in his cross-breeding experiments with maize (Emerson and East 1913). Thomas Hunt Morgan, working with the fruit fly *Drosophila*, likewise was able to isolate a number of Mendelian factors of very small effect (Morgan et al. 1925). Collectively, these theoretical and empirical observations established that the small, graded variations that were so important to Darwin's view of evolution were compatible with the Mendelian picture of inheritance.

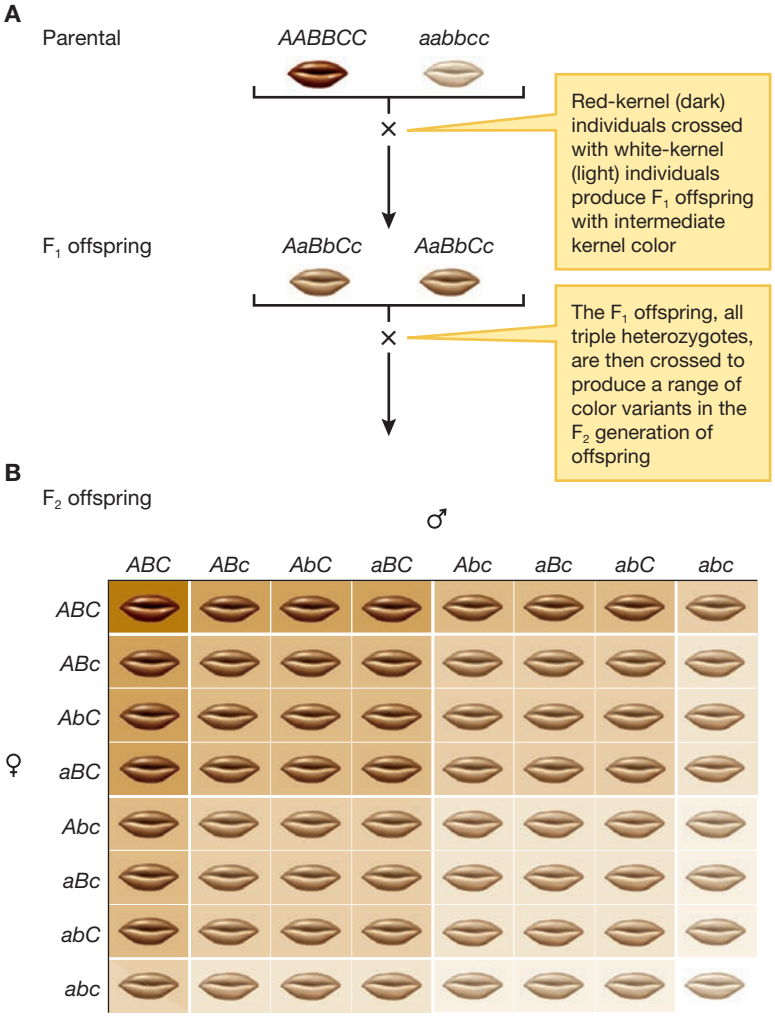


FIGURE 9.3 Multifactorial inheritance generates near-continuous variation. Grain color in winter wheat is controlled by three loci, here labeled *A*, *B*, and *C*. **(A)** Nilsson-Ehle crossed red-kernel parents with white-kernel parents to produce F_1 progeny of intermediate grain color. He then crossed the F_1 progeny to produce offspring with a range of grain colors in the ratios illustrated in **(B)**. Each box is shaded to emphasize the color of the wheat kernel therein.

The Importance of Latent Variation

Empirical work in the early twentieth century demonstrated that polygenic traits produced the variation necessary for natural selection to operate, but it also showed that new types, not seen in a parent population, could appear in the offspring produced by that population. Where could these new types have come from? Could they all have been the result of new mutations? Or could there have been other possible "variance-generating" engines that had been overlooked?

Consider this problem: It is relatively easy to see how a variational sorting process like the one we described in Chapter 2 could act on a population with heights ranging from 5′0″ to 5′10″ and generate offspring with heights in the range of 5′5″ to 5′10″ (**Figure 9.4A**). But natural selection doesn't create variation; it reduces variation—by favoring some forms over others. So why should new variants—for example, offspring in the range of 5′10″ to 6′4″ (**Figure 9.4B**)—arise in a population selected for greater height?

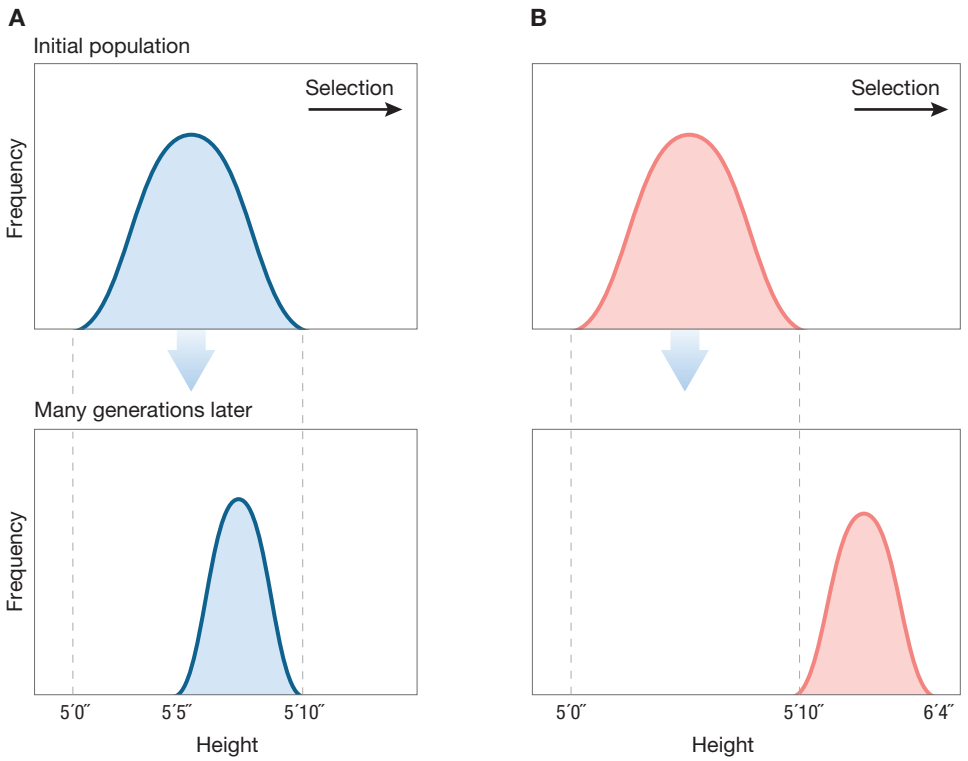


FIGURE 9.4 Sorting on existing variation and extending the range of variation. It is easy to see how natural selection can sort on pre-existing variation to shift the distribution of phenotypes within its current range, as illustrated in **(A)**. Initially, the population consists of a broad, bell-shaped distribution of heights. Selection for increased height sorts upon this distribution, narrowing the distribution and increasing the average height of individuals many generations later. But natural selection can also shift the phenotypes in a population beyond the range currently observed, as illustrated in **(B)**. Here, the distribution of heights after many generations shifts beyond the range observed initially. But where has the new variation come from? This question was resolved in the early twentieth century through the synthesis of Mendelian inheritance with Darwin's theory of natural selection.

The answer emerged through the synthesis of Mendelian heredity with Darwinian evolution. As we have seen, when multiple genetic factors are involved in determining a phenotype, variation at a relatively small number of genetic loci can potentially generate an enormous number of possible phenotypes. But these sorts of polygenic characters can also shed light on how we can see variants in generation 2 that we did not observe in generation 1, as in our height example. For example, Nilsson-Ehle noted that under certain conditions, the presence of two different variants at each of 10 loci would be sufficient to generate nearly 60,000 different phenotypes. Many natural populations might be too small to manifest all of these possible phenotypes. When a new phenotype is observed in subsequent generations, it need not be the result of a new mutation; it could simply be a new assortment of previously occurring Mendelian variation.

Herein is the answer to how natural selection could drive a population beyond its original range of variation without having new mutations arise. Because natural selection changes the allele frequencies in a population, it also changes the probabilities that various allele combinations will be realized. Over time, allele combinations that might have been highly unlikely to occur in a modestly sized population under the initial allele frequencies may become much more likely to occur under the shifted allele frequencies resulting from the operation of natural selection. **Box 9.1** provides a concrete illustration of such a case.

Thus, population geneticists came to recognize that under Mendelian inheritance, populations contained **latent variation**; that is, there was so much Mendelian variation within populations that not all possible genotypes could be

BOX 9.1 A Numerical Example of How Selection and Reassortment Can Generate New Phenotypes

Suppose we are studying a diploid population in which a trait such as cell volume is controlled by 10 unlinked loci labeled A–J, each of which contributes additively to the phenotype in question. At each locus, there are two alleles that we label A_1 and A_2, B_1 and B_2, . . . , J_1 and J_2. Suppose that for each allele X_1 that an individual possesses, the trait value is increased by 1 unit, and for each copy of X_2, the trait value increases by 0 units. The phenotype is then determined simply by the number of X_1 alleles and the number of X_2 alleles that an individual carries. If an individual has only X_2 alleles, that individual has a phenotype of 0. If an individual has only X_1 alleles, that individual has a phenotype of 20. Now suppose we start with a population in which each allele is at a frequency of 50% at each locus, as illustrated in the top panel of **Figure 9.5A**. A population of 100 individuals might have the distribution of phenotypes shown in the top panel of **Figure 9.5B**. In this particular population, the range of phenotypes goes from 4 to 15. Now suppose that natural selection operates on this population, so that only individuals with phenotypes of 11 or higher survive. The distribution of survivors is then as shown in the middle panel of Figure 9.5B.

Among the surviving members of the population, the allele frequencies have now shifted as a result of selection (and sampling effects), as shown in the bottom panel of Figure 9.5A. When these individuals produce new offspring, the offspring will have the shifted set of phenotypes shown in the bottom panel of Figure 9.5B.

In this example, in the course of a single generation of selection, three new phenotypes (16, 17, and 18) have arisen—not through mutation, but through reassortment of the latent variation that was present all along in the population. Prior to selection, when X_1 allele frequencies were 50%, the chances of producing an offspring with 16 or more X_1 alleles were very low. After selection, the X_1 allele frequencies increased substantially—and the chances of producing offspring with 16, 17, or even 18 X_1 alleles became sufficiently high that such offspring were observed in the next generation.

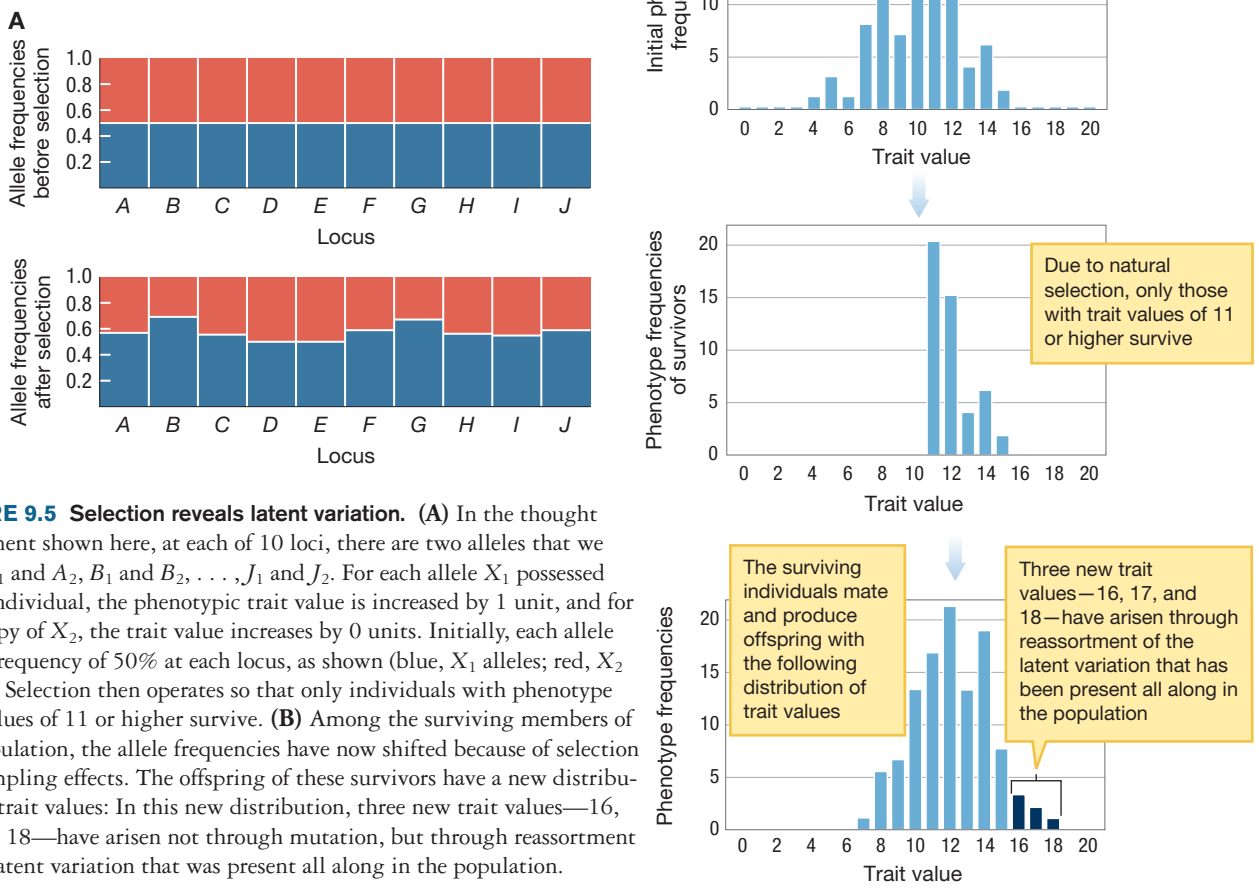


FIGURE 9.5 Selection reveals latent variation. **(A)** In the thought experiment shown here, at each of 10 loci, there are two alleles that we label A_1 and A_2, B_1 and B_2, . . . , J_1 and J_2. For each allele X_1 possessed by an individual, the phenotypic trait value is increased by 1 unit, and for each copy of X_2, the trait value increases by 0 units. Initially, each allele is at a frequency of 50% at each locus, as shown (blue, X_1 alleles; red, X_2 alleles). Selection then operates so that only individuals with phenotype trait values of 11 or higher survive. **(B)** Among the surviving members of the population, the allele frequencies have now shifted because of selection and sampling effects. The offspring of these survivors have a new distribution of trait values: In this new distribution, three new trait values—16, 17, and 18—have arisen not through mutation, but through reassortment of the latent variation that was present all along in the population.

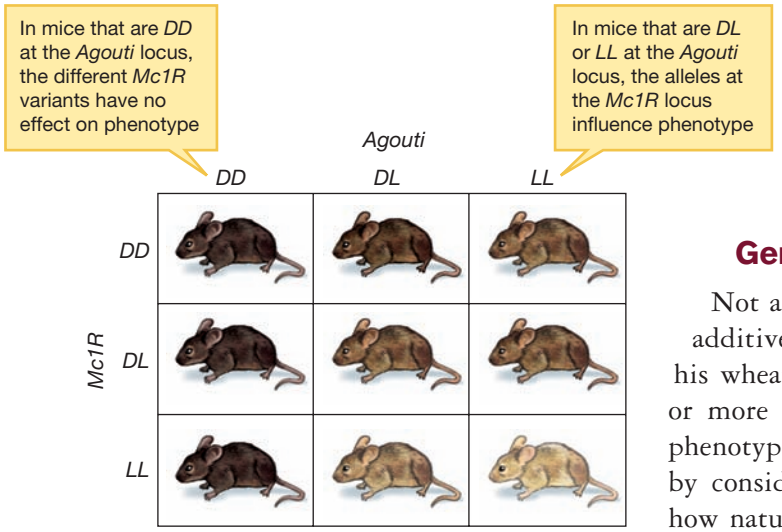


FIGURE 9.6 Epistasis between the *Mc1R* and *Agouti* loci. When both of the alleles at the *Agouti* locus are *D* (dark), different alleles at the *Mc1R* locus have no effect. In mice with at least one *L* (light) allele at the *Agouti* locus, the alleles at the *Mc1R* locus influence coat color. Because the effect of alleles at the *Mc1R* locus depends on their context—namely, which alleles are present at the *Agouti* locus—we say that there is epistasis between the *Mc1R* and *Agouti* loci. Adapted from Steiner et al. (2007).

represented. As a result, selection could shift allele frequencies, and genetic reassortment could then draw out new phenotypes from the preexisting variation, even in the absence of further mutation.

Gene Interactions

Not all genes interact to produce the straightforward additive genetic effects that Nilsson-Ehle observed in his wheat kernel color system. When the alleles at two or more loci interact in *nonadditive* ways to determine phenotype, we refer to this as **epistasis**. Let us begin by considering an example. In Chapter 3, we explored how natural selection operates on coat color variation in populations of the oldfield mouse, *Peromyscus polionotus.* There, we described two loci that influence coat color in this species: the *Mc1R* locus and the *Agouti* locus. The effects of alleles at the two loci do not combine additively, but rather the loci exhibit epistatic interactions; that is, the effect of an allele at the *Mc1R* locus depends on which alleles are present at the *Agouti* locus (Steiner et al. 2007).

In mice that are homozygous for the dark *Agouti* allele (*D*), the effects of the *Mc1R* locus are entirely masked—irrespective of the genotype at *Mc1R*, the mice have fully dark coloration. But when at least one copy of the light *Agouti* allele (*L*) is expressed, the effects of the *Mc1R* locus are revealed (**Figure 9.6**).

Because of epistasis, the phenotypic effects of these loci are *context dependent*; the phenotypic effects of alleles at one locus depend on the context that is set by the alleles at another locus. Natural selection then operates on allele combinations that determine particular phenotypes. Some allele combinations increase in frequency, while others may be eliminated from the population.

9.2 Population Genetics of Multiple Loci

Our goal in this section is to extend the models we discussed in Chapters 7 and 8 to deal with cases in which we are concerned with more than one locus at a time. We will aim to work out the rules—and write down the mathematical equations—for how allele frequencies at two or more loci jointly change.

Allele Frequencies and Haplotype Frequencies

To treat the population genetics of multiple loci, it is not enough simply to track the frequencies of the alleles at these loci. Rather, we need to track the frequencies of **haplotypes**. A haplotype is defined as a set of alleles, one at each locus under consideration. If, for example, we are interested in the *A*, *B*, and *C* loci of a diploid organism, *ABc* or *aBC* would be haplotypes, whereas *Aa BB Cc* would be a genotype. Often when population geneticists talk about a haplotype, they are

referring to a set of gene copies that tend to be inherited together because they are arranged along one particular chromosome. In organisms with haploid gametes (such as ourselves), we can also talk about the haplotype of a gamete.

By way of an example, suppose we are interested in tracking or modeling evolutionary dynamics at two loci—call them *A* and *B*—each of which has two alleles: *A* and *a*, *B* and *b*. (In this chapter, we will avoid the proliferation of subscripts in our notation by using *A* and *a* instead of A_1 and A_2; this does not imply that *A* is dominant and *a* recessive.)

To understand the population genetics of both loci together, we have to keep track not only of how many *A* and *a* alleles there are and how many *B* and *b* alleles there are, but also of which alleles at the *A* locus are associated with which alleles at the *B* locus. Why do we need to do this? The answer is that the allele frequencies do not uniquely determine the haplotype frequencies. For example, imagine a population with haplotype frequencies 33% *AB*, 17% *Ab*, 0% *aB*, and 50% *ab*. The allele frequencies in this population are 50% *A*, 50% *a*, 33% *B*, and 67% *b*. But if we knew only these allele frequencies and not the haplotype frequencies, we would be unable to tell that the *a* allele never co-occurs with the *B* allele. For example, given the same allele frequencies, the haplotype frequencies might instead have been 16.5% *AB*, 33.5% *Ab*, 16.5% *aB*, and 33.5% *ab*.

Similarly, to predict what sorts of offspring will be produced in a population, we need to consider not only the allele frequencies of the parents, but also the haplotype frequencies. In doing so, it will be helpful to recall how loci are physically positioned within the genome. In diploids, two loci *A* and *B* may be located either on separate chromosomes or on the same chromosome. In the former case, the alleles at these loci will segregate independently according to Mendel's laws. An *AaBb* parent will produce four types of gametes, *AB*, *Ab*, *aB*, and *ab*, with equal frequency (**Figure 9.7**, top). In the latter case, where the two loci are on the same chromosome, we say that there is **physical linkage** between the two loci. In the absence of recombination, physically linked loci segregate together. A parent with one *AB* chromosome and one *ab* chromosome—which we denote *AB*|*ab*—will produce only *AB* and *ab* gametes in the absence of recombination (Figure 9.7, bottom). Similarly, a parent with one *Ab* chromosome and one *aB* chromosome—denoted *Ab*|*aB*—will produce only *Ab* and *aB* gametes in the absence of recombination.

However, if recombination occurs, then alleles at loci on the same chromosome can be reassorted to form new combinations. *AB*|*ab* parents can produce *Ab* and *aB* gametes, and *Ab*|*aB* parents can produce *AB* and *ab* gametes. The rate of recombination, and thus the proportion of gametes of each type that are produced, depends on the physical distance between the *A* and *B* loci on the chromosome. If the two loci are located very close to one another, crossover

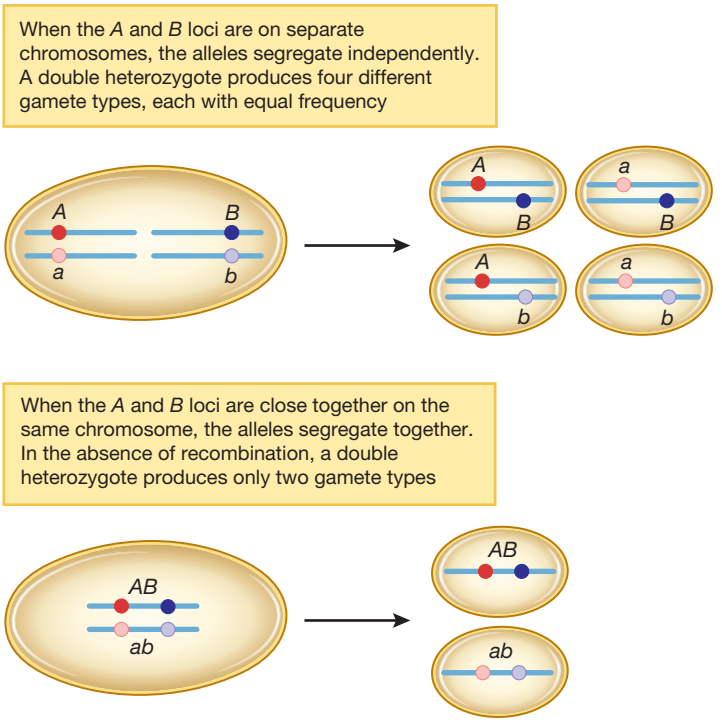


FIGURE 9.7 Location of two loci. Two loci can be located on different chromosomes or on the same chromosome (and hence physically linked).

between the two loci will occur only rarely, and the recombination rate will be low. If, instead, the two loci are far apart on the chromosome, there will be a high probability of crossing over between the two loci and a higher recombination rate.

With this in mind, we can now extend the Hardy–Weinberg model to two loci. To keep the algebra simple, we will consider the case in which the two loci are on the same chromosome and there is no recombination between them.

Hardy–Weinberg Proportions for Two Loci

Recall from Chapter 7 how to compute Hardy–Weinberg proportions for a single locus. If we have one locus A with alleles A and a at frequencies p and q in a very large population, the Hardy–Weinberg proportions reflect the probability that each genotype is formed by random mating with no selection, mutation, or migration. These probabilities are illustrated graphically in **Figure 9.8A**. In this figure, the height of each box represents the allele frequencies in the population; that is, the height p corresponds to the frequency of the A allele within the population, and the height q corresponds to the frequency of the a allele within the population. The *area* of each box—that is, the product of the allele frequencies—is then proportional to the Hardy–Weinberg frequency of the corresponding genotype. This is because the Hardy–Weinberg frequencies of each genotype are equal to the chance that two gametes that are drawn randomly from the population compose that genotype. The chance is simply the product of the allele frequencies of the two alleles that make up that genotype. Ignoring the order of the two alleles, Aa and aA are indistinguishable; each arises with frequency pq, for a total heterozygote frequency of $2pq$.

We can take the same approach when dealing with two loci, but we have to consider haplotypes rather than alleles. This is because we need to distinguish between two different kinds of double heterozygote ($Aa|Bb$) parents if we are to correctly predict the genotypes of the offspring. In the absence of recombination, $AB|ab$ parents will produce only AB and ab gametes, whereas $Ab|aB$ parents will produce only Ab and aB gametes.

Let the frequencies of the AB, Ab, aB, and ab haplotypes be s, t, u, and v, respectively. Under the Hardy–Weinberg assumptions, we can calculate the frequencies of

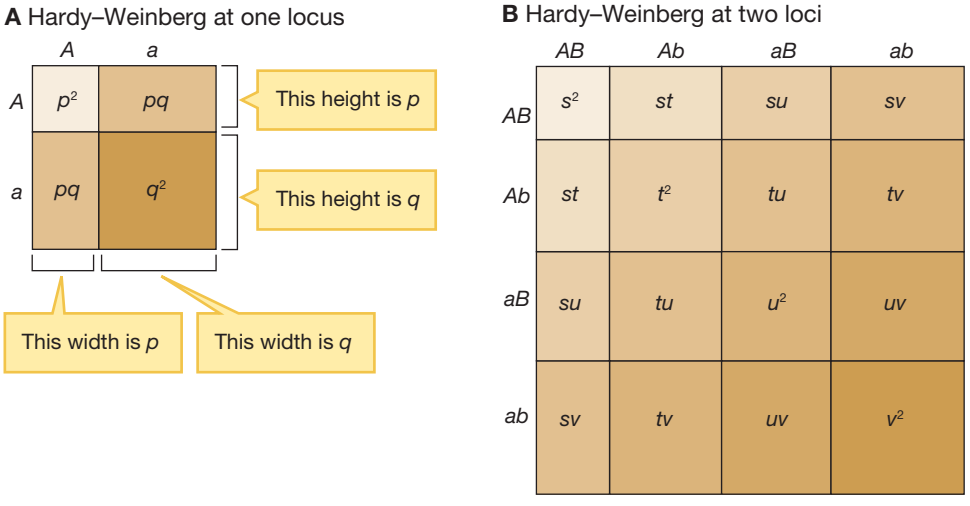


FIGURE 9.8 Hardy–Weinberg frequencies at one and two loci. **(A)** For one locus, the allele frequencies of A and a are p and q, respectively. The Hardy–Weinberg proportions are given by the products of the allele frequencies: p^2 for AA; pq and qp for Aa and aA, giving a total of $2pq$ heterozygotes; and q^2 for aa. **(B)** For two loci on the same chromosome, in the absence of recombination, the haplotype frequencies in the gametes of AB, Ab, aB, and ab are s, t, u, and v, respectively. The Hardy–Weinberg frequencies are given by the products of the haplotype frequencies, as shown in the figure.

offspring of each genotype by imagining that all parents contribute their gametes to a hypothetical gene pool from which gametes are paired at random to produce offspring.

Taking this approach, the Hardy–Weinberg frequencies of a particular genotype are given by the product of the corresponding haplotype frequencies in the gene pool (**Figure 9.8B**). For example, the frequency of individuals with the $AB|AB$ genotype is s^2. Notice that any genotype composed of two different haplotypes appears twice in Fig 9.8B, just as the Aa heterozygote appears twice in Figure 9.8A. This occurs because these genotypes can be formed in two different ways. For example, $Ab|aB$ can be formed by drawing Ab first and aB second, or vice versa. Each way occurs with a frequency tu, for a total Hardy–Weinberg frequency $2tu$ of the $Ab|aB$ genotype. Remember that the Hardy–Weinberg frequencies shown here assume no recombination. Shortly, we will consider what happens when recombination occurs.

Statistical Associations between Loci

In the one-locus Hardy–Weinberg model, there are no statistical associations between one gene copy at a locus and the other gene copy at the same locus. At the Hardy–Weinberg equilibrium, knowing that an individual received the A allele from its mother provides us with no new information about whether it received the A allele or the a allele from its father. This would not be true if we violated the random mating assumption of the Hardy–Weinberg model by introducing assortative mating, for example. If individuals preferentially chose similar mates, an offspring who received an A from its mother would be more likely than the population at large to have received an A from its father as well. But when all Hardy–Weinberg assumptions are met, the genotype frequencies are simply equal to the products of the allele frequencies, as illustrated in Figure 9.8A.

In the two-locus Hardy–Weinberg model, the same is true *of gene copies at each single locus considered separately.* Knowing that an individual received the A allele from its mother tells us nothing about whether it received the A or a allele from its father, and knowing that an individual received the B allele from its mother tells us nothing about whether it received the B or b allele from its father. This is why the genotype frequencies in Figure 9.8B are simply equal to the products of the haplotype frequencies.

Even though there are no statistical associations between the two gene copies at one locus in the Hardy–Weinberg model, *there can be associations between gene copies at two different loci*. How can this be? An illustration is useful. Suppose there are no ab haplotypes in the population, and therefore all b alleles are in Ab haplotypes. In this case, if we know that an individual has the b allele, we also know for certain that this individual has at least one A allele. When statistical associations are present between the alleles at the A locus and the B locus, we say that there is **linkage disequilibrium** between these alleles in the population. In the next subsection, we will see how to quantify the amount of linkage disequilibrium in a population.

Why do we care about linkage disequilibrium? For one thing, we have to account for statistical associations between loci if we want to track the changes in haplotype frequencies in a population over time. So if we aim to model evolution at multiple loci, we will need to take linkage disequilibrium into consideration.

We will need to understand how linkage disequilibrium arises due to evolutionary processes: mutation, selection, nonrandom mating, migration, and drift. And we will need to understand how it declines as a consequence of recombination and independent segregation.

Even if we only want to model how allele frequencies change at a single locus, linkage disequilibrium can be very important. Suppose that we want to track evolutionary change at the *A* locus. To do so, do we need to also worry about selection operating at other loci (*B*, *C*, *D*, and so forth)? The answer is that sometimes we do—but only if the *A* locus is in linkage disequilibrium with one of these other loci. For example, a neutral allele at the *A* locus can rapidly increase in frequency when it is in linkage disequilibrium with a selectively favored allele at a separate *B* locus. This process of genetic hitchhiking and related phenomena are important drivers of evolutionary change, as we will see later in this section.

Linkage disequilibrium is also important in the evolution and maintenance of sex. A prominent explanation for the evolutionary benefit of sex, known as the Fisher–Muller hypothesis, posits that the main function of sex is to accelerate evolution by breaking down linkage disequilibrium, and other hypotheses for sex can be framed similarly. Linkage disequilibrium also underlies an important process of sexual selection. In the Fisher process, linkage disequilibrium forms between alleles for female preference and alleles for male traits, driving the evolution of elaborate displays. We will consider both of these topics in Chapter 16.

Finally, the presence of linkage disequilibrium between loci can tell us a great deal about the history of selection on a population. We will see a striking example later in this chapter, when we show how researchers can use the extent of linkage disequilibrium around individual loci to identify loci that have been under natural selection in the recent past.

Quantifying Linkage Disequilibrium

To measure the associations between allele frequencies at two loci *A* and *B*, we need to know the haplotype frequencies at these loci. Let f_A, f_a, f_B, and f_b be the frequencies of the *A*, *a*, *B*, and *b* alleles, respectively, and let h_{AB}, h_{Ab}, h_{aB}, and h_{ab} be the frequencies of the *AB*, *Ab*, *aB*, and *ab* haplotypes, respectively. If the allele at the *A* locus occurs independently of the allele at the *B* locus, the haplotype frequencies will be equal to the product of the corresponding allele frequencies: $h_{AB} = f_A f_B$, $h_{Ab} = f_A f_b$, and so forth. But if there is a statistical association between the alleles at the *A* and *B* loci, these equalities will not hold.

We define the **coefficient of linkage disequilibrium *(D)*** as the difference between the actual frequency of the *AB* haplotype (h_{AB}) and the expected frequency ($f_A f_B$) of the same haplotype if the loci were independent; that is, if there were no association between the allele at one locus and the allele at the other. Mathematically, this is written as follows:

$$D = h_{AB} - f_A f_B \tag{9.1}$$

When the alleles at each locus occur independently, these terms will be equal, and the coefficient of linkage disequilibrium *D* will be zero.

When the alleles at each locus occur nonindependently, the linkage disequilibrium is nonzero. Suppose that the *A* allele is more likely to occur in combination with the *B* allele, and that the *a* allele is more likely to occur in combination with the *b* allele. Then by our mathematical definition (Equation 9.1), the coefficient of linkage disequilibrium *D* will take on a positive value. Conversely, if *A* is more likely to occur with *b*, and *a* is more likely to occur with *B*, the coefficient of linkage disequilibrium *D* will have a negative value. In the classical population genetics literature, researchers often used the terms **coupling** and **repulsion** to refer to these associations. We will use these terms here because they provide a convenient language for talking about these types of associations between alleles. When *A* tends to occur with *B* and *a* tends to occur with *b*, we call this coupling. This is because the "like" alleles represented by the capital letters tend to be coupled in the haplotypes of the population, as are the like alleles represented by the lowercase letters. In contrast, when *A* tends to occur with *b* and *a* tends to occur with *B*, we call this repulsion. This is because uppercase and lowercase alleles generally tend *not* to occur together. The *A* allele seems to repel *B* in favor of *b*, and the *a* allele seems to repel *b* in favor of *B* (**Figure 9.9**). Thus, we can view linkage disequilibrium as a measure of whether we have excess coupling haplotypes, in which case the value of *D* is positive, or excess repulsion haplotypes, in which case *D* is negative.

FIGURE 9.9 Coupling and repulsion haplotypes. When "like" alleles (*A* and *B*, or *a* and *b*) appear together, we call this coupling. We call the converse case (*A* with *b*, or *a* with *B*) repulsion.

Notice that the terms *coupling* and *repulsion* reflect nothing more than our choice of nomenclature for the loci in question. Suppose we had named the alleles differently; for example, suppose that we had called *B* by the name *c*, and *b* by the name *C*. Then the coupling pair *AB* would be written as *Ac* and would be considered a repulsion pair, while the repulsion pair *Ab* would be written as *AC* and would be considered a coupling pair. Thus, it is arbitrary whether we call a given haplotype a coupling haplotype or a repulsion haplotype, but this kind of arbitrariness is an inevitable if unfortunate consequence of how the coefficient of linkage disequilibrium *D* is defined. The sign of *D* depends on the notation we choose for our alleles in the first place.

Using the mathematical definition of *D* (Equation 9.1), we can express the frequency of each haplotype as a function of allele frequencies and linkage disequilibrium as follows:

$$h_{AB} = f_A f_B + D \quad (9.2a)$$
$$h_{Ab} = f_A f_b - D \quad (9.2b)$$
$$h_{aB} = f_a f_B - D \quad (9.2c)$$
$$h_{ab} = f_a f_b + D \quad (9.2d)$$

The value of the coefficient of linkage disequilibrium *D* depends not only on how the alleles at each locus are associated with one another, but also on the frequencies of each allele. In our two-locus, two-allele example, *D* takes on its maximum value when the frequency of each allele is 0.5 (and, as described earlier, reaching this maximum requires that *A* always co-occurs with *B* and *a* always co-occurs with *b*). In this case, $f_A = 0.5$, $f_B = 0.5$, and $h_{AB} = 0.5$. Applying Equation 9.2a, we see that $0.5 = (0.5 \times 0.5) + D$, and therefore $D = 0.25$. Similarly, *D* can achieve a minimum of $D = -0.25$ when each frequency is 0.5 and *A* never occurs with *B*. Thus, the coefficient of linkage disequilibrium ranges from −0.25 to 0.25. When

the associations among loci are not absolute or the allele frequencies deviate from 0.5, D will take on smaller absolute values.

Evolutionary Processes Create Linkage Disequilibrium

We have seen that linkage disequilibrium measures the statistical association between alleles at different loci. But how does this association arise, and what happens to it over time? We turn now to these questions.

Linkage Disequilibrium via Mutation

Linkage disequilibrium can arise from many of the evolutionary processes we have studied, including mutation, selection, drift, migration, and nonrandom mating. One of the simplest sources of linkage disequilibrium is the spread of a new mutation, as shown in **Figure 9.10**. Suppose a population is initially polymorphic at the A locus, with both A and a alleles present, but is monomorphic at an adjacent locus B on the same chromosome, with only B alleles present. Because only one of these two loci is polymorphic, there is no linkage disequilibrium. (We can also see this from Equation 9.1: if $f_B = 1$, then $f_A = h_{AB}$, and $D = 0$.)

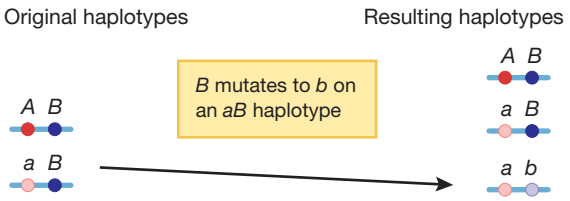


FIGURE 9.10 Mutation can create linkage disequilibrium. In this example, the b allele arises by mutation on a chromosome that carries the a allele at the A locus. As a result, a new coupling haplotype ab is created, but the corresponding repulsion haplotype Ab is not yet present in the population. The result is a positive coefficient of linkage disequilibrium D.

Now suppose that on an individual chromosome, a new allele b is formed by mutation at the B locus. This b allele will be adjacent to some allele at the A locus—for the purpose of this example, suppose that b arises adjacent to an a allele, as in Fig 9.10.

Prior to the mutation, only the AB and aB haplotypes were present in the population. Subsequent to the mutation, an additional haplotype, ab, has been formed. In this population, there is now a statistical association between alleles at the A and B loci. Most notably, the presence of the b allele at the B locus guarantees the presence of the a allele at the A locus. This means that the A and B loci are now in linkage disequilibrium in this population. Over time, this linkage disequilibrium may break down because of recombination, but we will discuss that process later. For now, the important point is that simple evolutionary history—the mutations that occur and the genetic background on which the mutations happen to arise—generates linkage disequilibrium among loci.

Linkage Disequilibrium via Natural Selection

Natural selection is another very important source of linkage disequilibrium. We will first provide an example of how natural selection can generate linkage disequilibrium, and then we will discuss how selection only generates linkage disequilibrium between alleles with epistatic interactions.

Consider the example in **Figure 9.11**. Either of two biosynthetic pathways is sufficient to produce an essential molecular product from precursor raw materials. Each pathway is controlled by a single locus, and the functional wild-type alleles (A, B) are dominant to the nonfunctional disease alleles (a, b). In this case, only $aabb$ individuals are unable to produce this molecule, and thus only these $aabb$ individuals manifest the disease phenotype. As a result, selection operates against the a allele, but only when it is part of an ab haplotype—and even then only if that ab haplotype is paired with a second ab haplotype. Similarly, selection operates against the b allele only when it is part of an ab haplotype. All other haplotypes

can produce the needed molecular product from precursor raw materials, so no other haplotype is selected against. In this example, there will be a dearth of *ab* haplotypes relative to what would be expected given the frequencies of the *a* and *b* alleles. Natural selection has created a statistical association between the alleles at the two loci; that is, selection has generated linkage disequilibrium between the loci.

Notice that in the example above, the selective consequence of carrying the *a* allele is contingent on the presence of the *b* allele. In other words, this example featured epistasis between the *A* and *B* loci. When there is no epistasis between loci, selection *will not* generate linkage disequilibrium (Felsenstein 1965). Without epistasis, the selective consequences of carrying each allele would be independent of the allele carried at the other locus, and there would be no statistical association between the alleles when we examined the consequences of selection.

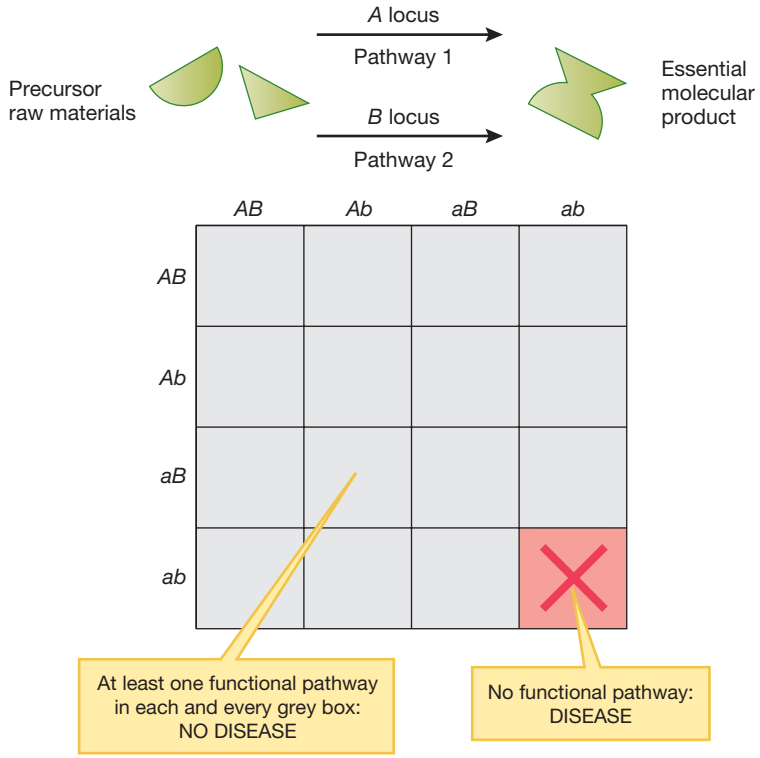


FIGURE 9.11 Selection can generate linkage disequilibrium. In this example, an *A* allele or a *B* allele—but not both—is needed to produce an essential molecular product from precursor raw materials. Natural selection disfavors only the *ab* haplotype, and even this haplotype is disfavored only when paired with another *ab* haplotype, resulting in *aabb* individuals who suffer disease because they are unable to produce the essential molecule. Thus, only the *ab* haplotype will be less common than expected among surviving adults given the allele frequencies in the population.

Linkage Disequilibrium via Nonrandom Mating

Nonrandom mating can also generate linkage disequilibrium. An example illustrates: Imagine a locus *P* that determines mate preference in female wrens and a locus *T* that determines tail size in male wrens. Females with a dominant *P* allele display a choosy phenotype, preferentially selecting long-tailed males, while *pp* females mate at random with respect to tail length. Males with a dominant *T* allele have long tails while *tt* males have short tails. Because females carrying *P* alleles mate only with males carrying *T* alleles, a nonrandom association builds up between the *P* and *T* alleles in the population. They are more likely to be paired together in a gamete—even if they are on different chromosomes. In other words, linkage disequilibrium arises between the *P* and *T* loci.

As we will see in Chapter 16, some models of sexual selection rely on linkage disequilibrium of this sort. In these models, linkage disequilibrium between loci for female preferences and loci for male traits drives the evolution of both elaborate male ornaments and strong female preferences for them.

Linkage Disequilibrium via Migration

Migration is another source of linkage disequilibrium. For example, suppose the *a* and *b* alleles are fixed in a mainland population of lizards, and the *A* and *B* alleles are initially fixed in an island population of the same species. All the haplotypes on the mainland are the coupling haplotypes *ab*, and all the haplotypes on the island are the coupling haplotypes *AB*. Considered separately, there is no linkage disequilibrium in either population, because each population is monomorphic. If a few individuals then migrate from the mainland to the island, however, *ab* haplotypes will be introduced to the island, and on the island there will now be a

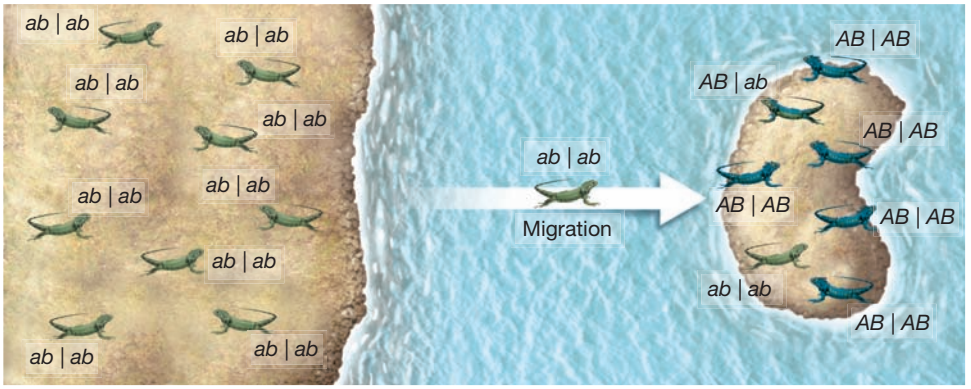


FIGURE 9.12 Migration creates linkage disequilibrium. In this example, the *a* and *b* loci are fixed on the mainland, while the *A* and *B* loci were previously fixed on the island. When *ab* haplotype migrants reach the island by migration, there will be a statistical association between alleles on the island; that is, there will be linkage disequilibrium.

statistical association between the *A* and *B* loci (**Figure 9.12**). In particular, even though both loci are polymorphic, all haplotypes on the island initially will be coupling haplotypes, so the coefficient of linkage disequilibrium *D* will be positive.

Linkage Disequilibrium via Genetic Drift

Drift can also generate linkage disequilibrium. We have already seen that drift can lead to the loss of alleles; it can do the same thing to haplotypes. For example, imagine a small population with four haplotypes (*AB*, *Ab*, *aB*, and *ab*) and a very low recombination rate between the *A* and *B* loci. Just as drift can lead to the loss of an allele, say *B*, it can also (and, in fact, more easily) lead to the loss of a haplotype, say *AB*. If this were to happen, the population would be left with only three haplotypes—*Ab*, *aB*, and *ab*—and thus with a nonrandom association between the *A* and *B* loci.

More generally, drift need not entirely eliminate any haplotype in order to generate linkage disequilibrium. Simply by causing random fluctuations in haplotype frequencies, drift can generate statistical associations between alleles at different loci and thus create linkage disequilibrium. Imagine a small population without any linkage disequilibrium. If, by chance, fewer coupling haplotypes than repulsion haplotypes are passed on to the next generation, negative linkage disequilibrium will result.

Recombination Breaks Down Linkage Disequilibrium

Once linkage disequilibrium is present in a population, we want to understand what happens to it. The short answer is that in the absence of other evolutionary processes, it is broken down by the process of recombination, and eventually it disappears. In this subsection, we will explore how this takes place.

Recombination occurs between haplotype pairs. Thus, to understand the effects of recombination, it will be useful for us to track the diploid genotypes in a population (**Figure 9.13A**). Returning to our two-locus model of a Hardy–Weinberg population, recall that there are 16 different ordered genotypes as shown by the 16 sections of the box in **Figure 9.13B**. In 12 of these, recombination will have no effect on the haplotypes produced. For example, recombination between an *AB* haplotype and an *Ab* haplotype will produce *AB* and *Ab* gametes—just as if recombination had not occurred at all. The genotypes for which recombination has no effect on the resulting haplotypes are represented by the 12 blue boxes in

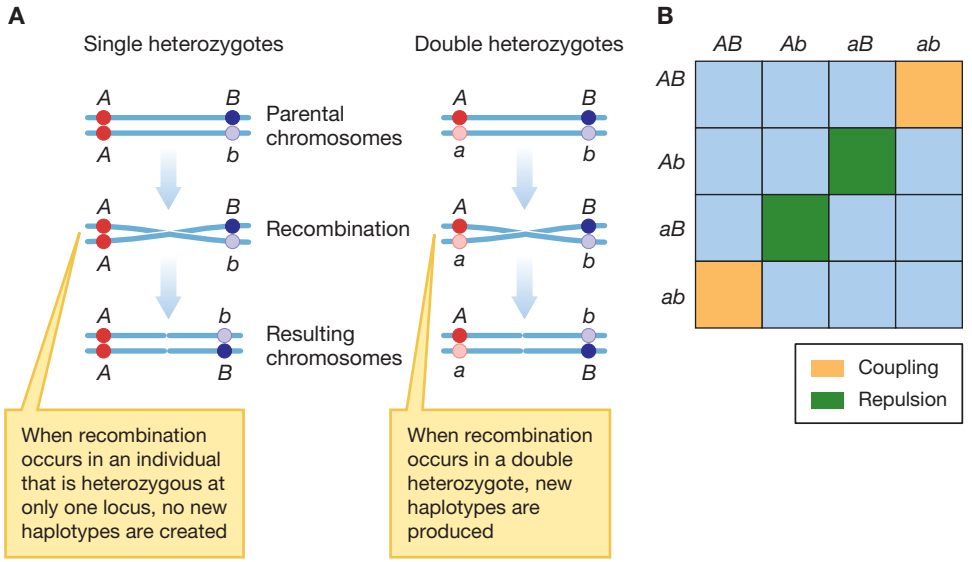


FIGURE 9.13 Recombination creates new haplotypes only in double heterozygotes. **(A)** When recombination between the *A* and *B* loci occurs in single heterozygotes (for example, *AB* and *Ab*), no new haplotypes are produced. When recombination occurs in double heterozygotes, new haplotypes are produced. **(B)** For the double homozygote and single heterozygote genotypes indicated by the blue squares, recombination does not alter the haplotypes produced. For the double heterozygotes, shown along the highlighted diagonal, recombination generates new haplotypes.

Fig 9.13B. But recombination does change the haplotypes produced by the four *double heterozygote* genotypes. These genotypes are represented by the green and gold boxes in Figure 9.13B. If recombination occurs between haplotypes *AB* and *ab*, the new haplotypes *Ab* and *aB* are produced. Similarly, recombination between haplotypes *Ab* and *aB* produces *AB* and *ab* haplotypes.

There is a useful mathematical relationship between the coefficient of linkage disequilibrium *D* and the genotype frequencies in the two-locus Hardy–Weinberg model. From Equations 9.2a–d, we can derive an alternative expression for the coefficient of linkage disequilibrium *D* as follows:

$$D = h_{AB}h_{ab} - h_{aB}h_{Ab} \tag{9.3}$$

The first term of this expression, $h_{AB}h_{ab}$, is one-half of the frequency of coupling double heterozygotes in a Hardy–Weinberg population. The second term, $h_{aB}h_{Ab}$, is one-half of the frequency of repulsion double heterozygotes in a Hardy–Weinberg population. Recall that the size of each region in our geometric picture of two-locus Hardy–Weinberg frequencies (see Figure 9.8B and 9.13B) is equal to the frequency of that genotype in the population. Thus, the coefficient of linkage disequilibrium *D* is simply one-half the difference between the size of the gold coupling regions and the size of the green repulsion regions. When the coupling regions are larger than the repulsion regions, the coefficient of linkage disequilibrium is positive; when the repulsion regions are larger, the coefficient of linkage disequilibrium is negative (**Figure 9.14**).

In our two-locus Hardy–Weinberg model, linkage disequilibrium does not change in the absence of recombination. Mathematically, we can see this from Equation 9.3, which states that linkage disequilibrium depends only on the frequencies of the four haplotypes. Because

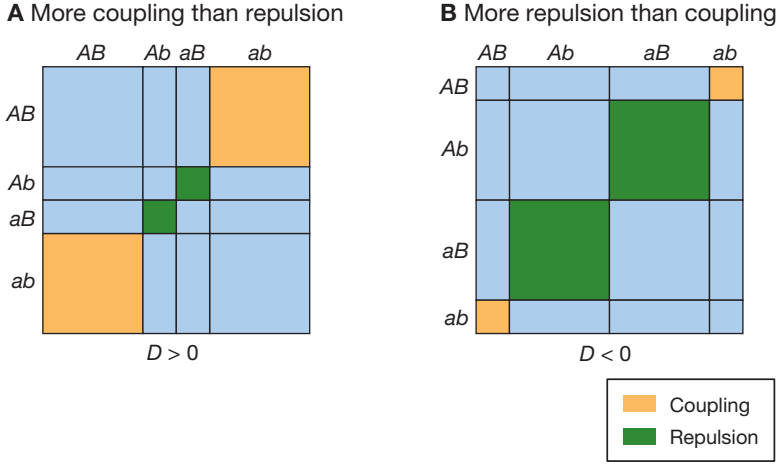


FIGURE 9.14 Another interpretation of the coefficient of linkage disequilibrium *D*. Based on Equation 9.3, we can view the coefficient of linkage disequilibrium as a measure of the difference between the frequency of coupling double heterozygotes and the frequency of repulsion double heterozygotes in a two-locus Hardy–Weinberg model. **(A)** A case with more coupling than repulsion; **(B)** a case with more repulsion than coupling.

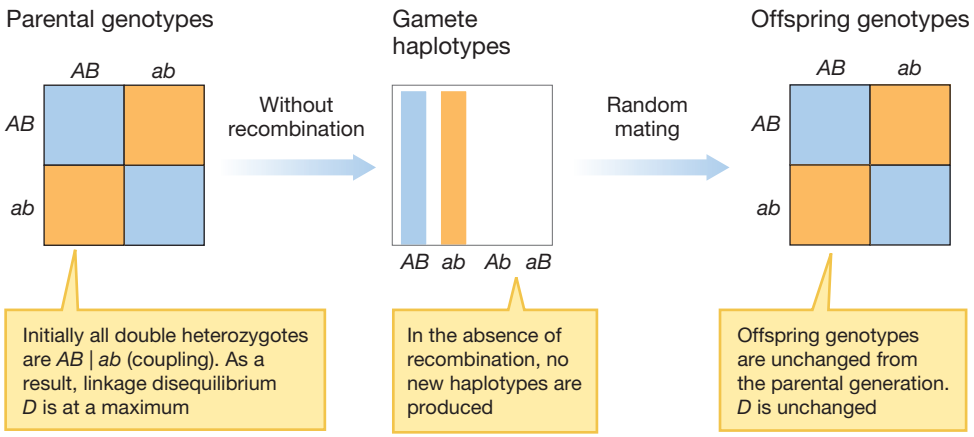


FIGURE 9.15 In the absence of recombination, linkage disequilibrium does not change in the Hardy–Weinberg model. In this example, only coupling haplotypes are present in the parental generation, and allele frequencies are (0.5, 0.5) at each locus. As a result, linkage disequilibrium is at its maximum of $D = 0.25$. Without recombination, the process of producing gametes and forming offspring from these gametes does not change the magnitude of the coefficient of linkage disequilibrium.

haplotype frequencies in the two-locus Hardy–Weinberg model do not change in the absence of recombination, linkage disequilibrium does not change either. **Figure 9.15** illustrates this by considering the simple case in which linkage disequilibrium is at a maximum in the parental generation. Gametes are produced, and then offspring genotypes are formed according to the Hardy–Weinberg model. The offspring genotype frequencies end up the same as the parental genotype frequencies, with the same coefficient of linkage disequilibrium.

Recombination, when it does occur, breaks down linkage disequilibrium; that is, unless the coefficient of linkage disequilibrium D is zero, D always decreases in absolute value as a consequence of recombination. Why? To see the answer, it helps to think in terms of what recombination does to the various genotypes. Suppose some of the haplotype pairs undergo recombination between the A and B loci. For the double homozygote and single heterozygote genotypes, these recombination events don't change the haplotypes that are produced. But for the double heterozygotes, coupling pairs that recombine will produce pairs of repulsion gametes, and repulsion pairs that recombine will produce pairs of coupling gametes. Thus, when D is positive and there are excess coupling double heterozygotes, there will be more new repulsion gametes produced by recombination among coupling pairs than there will be new coupling gametes produced by recombination among repulsion pairs. The frequency of coupling haplotypes relative to repulsion haplotypes therefore drops, causing a decline in the absolute value of D. An analogous argument holds in the case in which D is negative.

Figure 9.16 illustrates this process. The population starts at a maximum level of linkage disequilibrium, just as in Figure 9.15. But in this case, recombination does occur, and gametes with new repulsion haplotypes are produced as a result. When these gametes are paired to form offspring, some of those individuals are repulsion double heterozygotes, and thus the difference between the frequency of coupling heterozygotes and repulsion heterozygotes declines.

We can write a mathematical expression for how fast linkage disequilibrium declines because of recombination in a Hardy–Weinberg population. In **Box 9.2**, we show that if we denote r as the rate of recombination between the A and B loci, then the rate of change of the coefficient of linkage disequilibrium (ΔD) is given by

$$\Delta D = -rD$$

(where the equation above is derived as Equation 9.6 in Box 9.2). In other words, in a Hardy–Weinberg population, in each generation, the coefficient of linkage

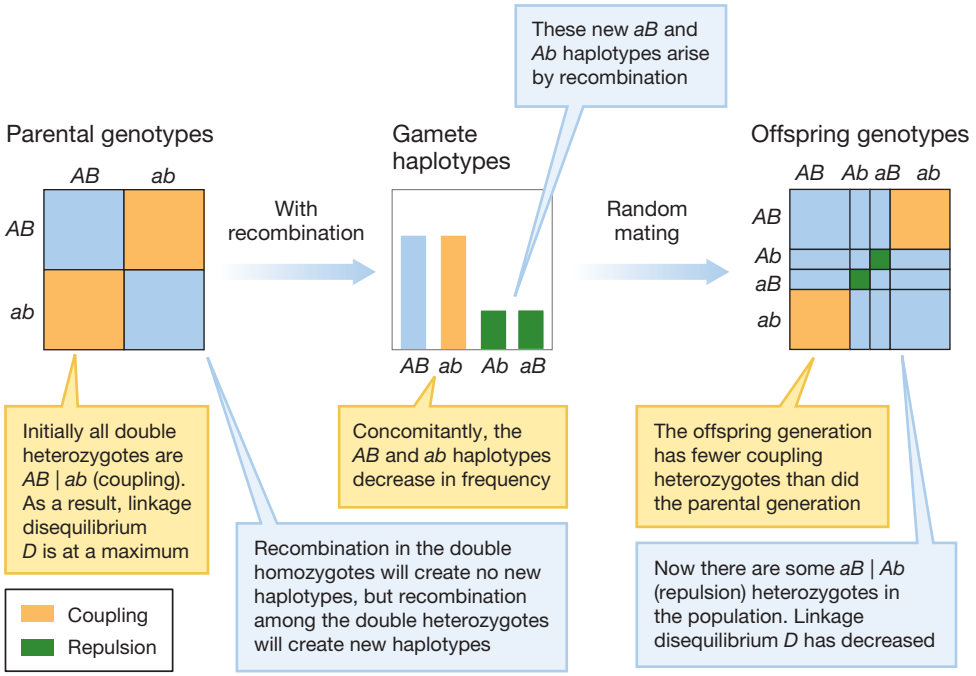


FIGURE 9.16 Recombination breaks down linkage disequilibrium. As in Figure 9.15, only coupling haplotypes are present in the parental generation, and allele frequencies are (0.5, 0.5) at each locus. As a result, linkage disequilibrium is at its maximum of $D = 0.25$. Recombination among coupling double heterozygotes forms new repulsion haplotypes. Some of these then pair together to form repulsion double heterozygotes in the offspring generation; the value of D has declined.

BOX 9.2 How the Coefficient of Linkage Disequilibrium Changes over Time in the Hardy–Weinberg Model

As we have done throughout this chapter, let f represent allele frequencies, h represent haplotype frequencies, and D represent the coefficient of linkage disequilibrium, but now make them functions of time t so that we can track how each changes over time. We will denote the change in D as $\Delta D = D(t+1) - D(t)$. As we noted in Equation 9.2a:

$$h_{AB}(t) = f_A(t)f_B(t) + D(t)$$

and

$$h_{AB}(t+1) = f_A(t+1)f_B(t+1) + D(t+1)$$

We can rearrange these two equations to solve for $D(t)$ and $D(t+1)$ as follows:

$$D(t) = h_{AB}(t) - f_A(t)f_B(t)$$

and

$$D(t+1) = h_{AB}(t+1) - f_A(t+1)f_B(t+1)$$

We can now calculate ΔD as follows:

$$\Delta D = f_A(t)f_B(t) - f_A(t+1)f_B(t+1) + h_{AB}(t+1) - h_{AB}(t) \quad (9.4)$$

But, as we learned in Chapter 7, allele frequencies do not change in a Hardy–Weinberg population. Therefore, $f_A(t) = f_A(t+1)$ and $f_B(t) = f_B(t+1)$. This means that we can simplify Equation 9.4 as follows:

$$\Delta D = h_{AB}(t+1) - h_{AB}(t) \quad (9.5)$$

So, to know how the linkage disequilibrium changes over time in this population, we only have to figure out how the frequency of the AB haplotype changes over time.

AB haplotypes are only created in two ways: either (1) from parents with AB haplotypes that do not recombine or (2) from parents with genotype $A*|*B$ that do recombine (where the asterisk [*] indicates that either allele may be present).

Let r be the recombination frequency between the A and B loci. The fraction of nonrecombining parental AB haplotypes is $(1-r)h_{AB}(t)$. The fraction of parents with one $A*$ haplotype and one $*B$ haplotype is simply $2f_A(t)f_B(t)$. Of these, a fraction r does recombine, and half of the gametes thus produced contain the A and B alleles from the $A*|*B$ pair. Thus, the total fraction of new h_{AB} haplotypes at time $t+1$ is as follows:

$$h_{AB}(t+1) = (1-r)h_{AB}(t) + rf_A(t)f_B(t)$$

We can substitute this expression into Equation 9.5, and with a little bit of algebra, we find that

$$\Delta D = -rD \quad (9.6)$$

This means that in each generation, the coefficient of linkage disequilibrium D decreases in absolute value by a rate equal to the recombination frequency (Robbins 1918).

disequilibrium D between two loci decreases in absolute value at the rate of recombination between the loci. Over time, the coefficient of linkage disequilibrium between these two loci will converge to zero. The higher the recombination rate, the faster this happens. Note that if the A and B loci are on different chromosomes, they segregate independently, and $r = 0.5$.

Even in populations that don't satisfy all of the Hardy–Weinberg assumptions, linkage disequilibrium will tend to be broken down unless it is maintained by selection or other processes. Moreover, the rate at which disequilibrium breaks down between two loci is proportional to the distance between them along the chromosome. This is the fundamental principle underlying the process of association mapping, a technique by which loci responsible for disease or other traits are located. Researchers measure the statistical association between disease state and the alleles present at a set of variable *marker loci* on a given chromosome or across the entire genome. The idea is that when the disease-related mutation arose in the population, it did so in one particular haplotype, and thus linkage disequilibrium was created between the disease allele and other polymorphic alleles in the genome. Over time, linkage disequilibrium will break down throughout the genome, but it will break down more slowly for the marker loci closest to the disease locus. In an association study, we aim to find statistical associations between marker loci and the disease state. The strengths of these associations, as we move from marker locus to marker locus along the chromosome, can potentially provide us with the information we need to pinpoint the location of the disease gene.

KEYCONCEPT QUESTION

9.1 A population geneticist once quipped that "linkage disequilibrium can be neither." He meant that linkage disequilibrium can exist without physical linkage, and there can be linkage disequilibrium between loci in an equilibrium population. Explain.

Selective Consequences of Genetic Linkage

Predicting evolutionary change is much easier when we can model evolution one locus at a time, as we did in Chapter 7. But this is not always possible, because selection on an allele at one locus can drive changes in allele frequencies at another locus. In general, when do we have to worry about this problem? *Any time that the two loci are in linkage disequilibrium*. Thus, to predict allele frequency change at locus A, we also have to understand what is happening at other loci (B, C, D, and so forth) that are in linkage disequilibrium with A.

In this subsection, we will explore some of the evolutionary consequences of selection on loci in linkage disequilibrium. We have already discussed how to think about and quantify linkage disequilibrium in populations, and we have seen that the rate at which linkage disequilibrium breaks down depends on physical linkage. It is easiest to think about the effects of linkage disequilibrium when the entire genome is physically linked, so we will begin with such an example.

Periodic Selection

Linkage disequilibrium tends to be particularly strong in bacteria, which are haploid and typically have only a single chromosome. In most bacterial species, every locus in the genome is tightly linked with every other locus. This is because bacteria

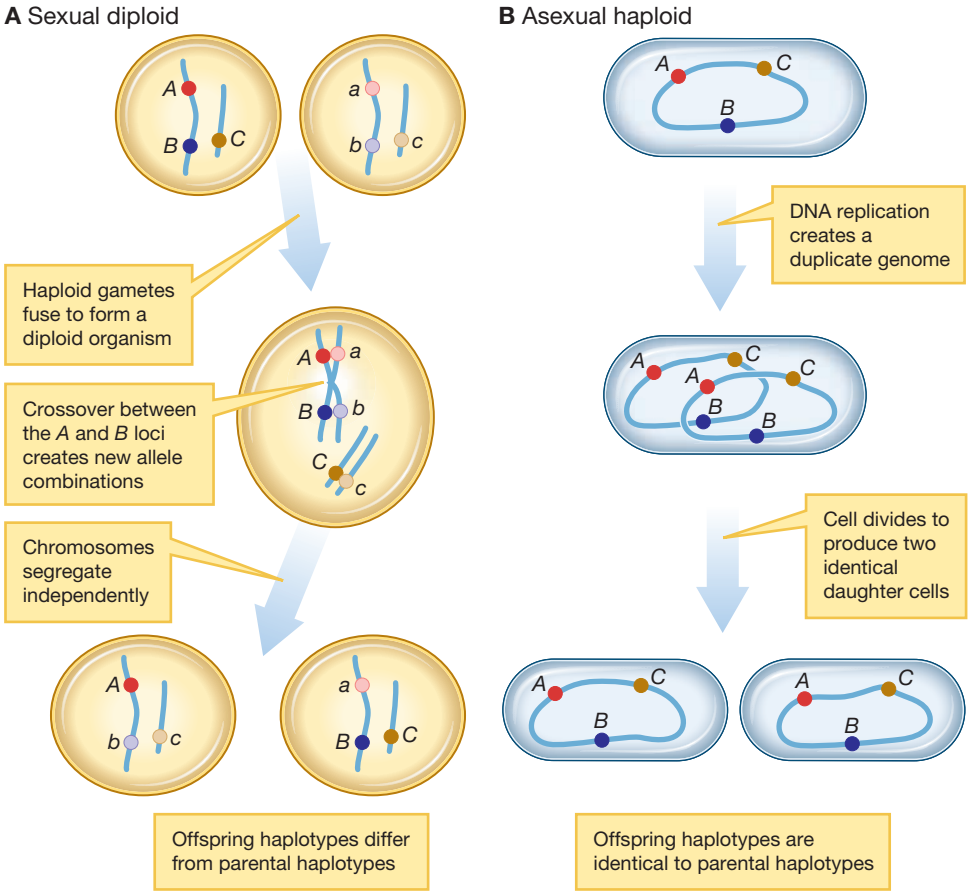


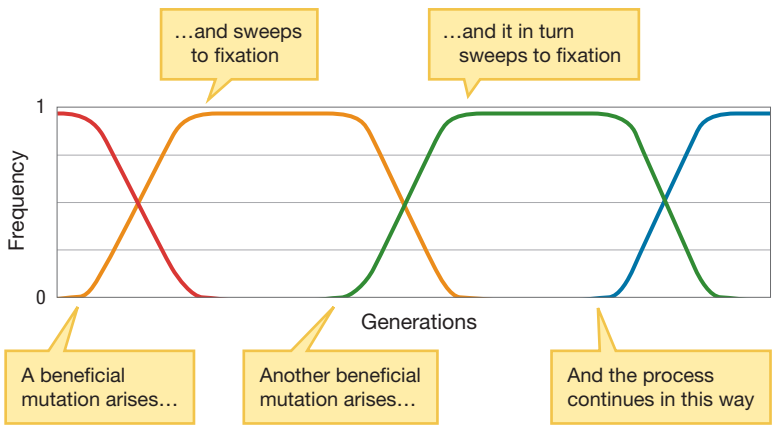
FIGURE 9.17 Physical linkage is particularly important in haploid prokaryotes. **(A)** Most pairs of loci in eukaryotes are not tightly linked because they can be separated by segregation if they occur on different chromosomes and by recombination if they occur on the same chromosome. **(B)** By contrast, linkage is much tighter in many bacterial species. Most bacteria have only a single chromosome, and there is no process of recombination between homologous chromosomes analogous to that in diploid eukaryotes.

use neither of the key processes that break up linkage disequilibrium in diploid eukaryotes: Bacteria don't have recombination between homologous chromosomes, and they don't have independent segregation of multiple chromosomes (Figure 9.17). For this reason, the bacterial chromosome is sometimes described as one single, albeit very large, locus.

When an allele goes to fixation as a result of strong natural selection, alleles at nearby loci are carried along to high frequency as well, because there is limited opportunity for recombination to occur between these loci and the selected locus. This process is known as a **selective sweep**. In eukaryotic species and other organisms with frequent recombination, only the selected allele and its near neighbors may rise to high frequency in a sweep. But in many bacterial species, linkage is tight across the entire genome and a selective sweep can carry an entire haplotype to fixation. When this process occurs repeatedly in succession, we refer to this phenomenon as **periodic selection** (Figure 9.18).

Periodic selection contributes to the phenomenon we described at the beginning of this chapter: the long-term persistence of bacterial resistance to antibiotics that have been withdrawn from general use. The process is illustrated in Figure 9.19. In an environment where antibiotics are used frequently, such as a

FIGURE 9.18 Periodic selection in a bacterial population. Periodic selection occurs when beneficial mutations arise infrequently in the population. Each time a new beneficial mutation does occur, it sweeps to fixation. In the absence of recombination, it carries to fixation the particular haplotype on which it arose.



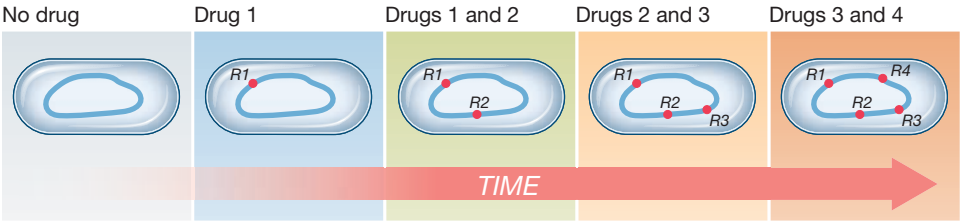


FIGURE 9.19 Periodic selection and the persistence of antibiotic resistance. We begin with a drug-sensitive strain. In response to the use of drug 1, a resistance gene to this drug (*R1*) is favored. When drug 2 comes into use, resistance to drug 2, labeled *R2*, arises and is favored in the drug 1–resistant strain. When drug 1 is phased out and replaced with drug 3, resistance (*R3*) to drug 3 evolves—on the common local strain, which is already resistant to drugs 1 and 2. When drug 2 is phased out and replaced with drug 4, resistance (*R4*) to drug 4 evolves—again on the common local strain, now already resistant to drugs 1, 2, and 3. As a result, we observe a multidrug-resistant strain that is resistant even to drug 1, which has not been used for a long period of time.

hospital or nursing home, resistance is strongly selected and often arises either by mutation or by acquisition of genes on small DNA molecules called *plasmids* or genetic elements called *transposons* (both of which we will examine in Chapter 10). When a new antibiotic is introduced into this environment, selection favors resistance to the new antibiotic. Alleles that confer resistance to the new drug will tend to arise by mutation or be incorporated by gene transfer onto a genetic background that includes resistance genes for previously encountered antibiotics. Even if the use of an older antibiotic is curtailed, selection on the new antibiotics continues. If alleles for resistance to the new antibiotics are linked to alleles for resistance to the discontinued drugs, the old resistance alleles will be maintained (because of physical linkage) by selection for the new resistance alleles.

In a 2001 study, Virve Enne and her colleagues wanted to test whether this process could explain the phenomenon with which we began this chapter: the persistence of sulfonamide resistance after sulfonamide use was discontinued in Britain (Enne et al. 2001). They reasoned that if this process were responsible, there should be a statistical association between resistance to sulfonamides and resistance to more recently used antibiotics. Indeed, they found that sulfonamide-resistant strains were significantly more likely to be resistant to a number of other antibiotics compared to sulfonamide-sensitive strains. From this, they concluded that, in addition to the compensatory mutations that we discussed in this chapter's introduction, physical linkage to other resistance genes had likely contributed to the long-term maintenance of sulfonamide resistance even after sulfonamide use had ceased.

Another consequence of periodic selection is that genetic diversity is greatly reduced in a population. Before heterogeneity has a chance to build up by mutation, it is wiped out in a selective sweep. This process happens over and over again, and, when combined with frequent population bottlenecks, it is responsible for many bacterial populations having relatively small effective population sizes despite being composed of huge numbers of individuals (Levin and Bergstrom 2000).

Genetic Hitchhiking

Periodic selection is a special case of a more general phenomenon, in which selection for an allele at one locus causes an increase in the frequency of certain alleles at other physically linked loci. Periodic selection is possible in bacterial species because their entire genome is physically linked. The more general process, observed in

species without such tight physical linkage across the genome, is known as **genetic hitchhiking.** A hitchhiking allele "rides along" with a nearby beneficial allele to which it is linked, and thus it increases in frequency even though it may be neutral or even deleterious itself (**Figure 9.20**) (Kojima and Schaffer 1967; Maynard Smith and Haigh 1974).

In the case of periodic selection in a bacterial population, homologous recombination is usually limited or nonexistent, and alleles that start together stay together. In recombining populations, however, the process of recombination will eventually break down the association between the favored allele and those around it. Associations with loci far from the selected allele break down quickly, but associations with nearby loci break down slowly. As a result, certain alleles at loci near a selected locus—namely, those in the "genetic background" on which the favored allele arose—will increase in frequency in the population through the process of genetic hitchhiking. In the process, other alleles at those nearby loci can be lost. As a result, genetic diversity at loci near the selected locus will be reduced relative to what we would expect in a neutral model.

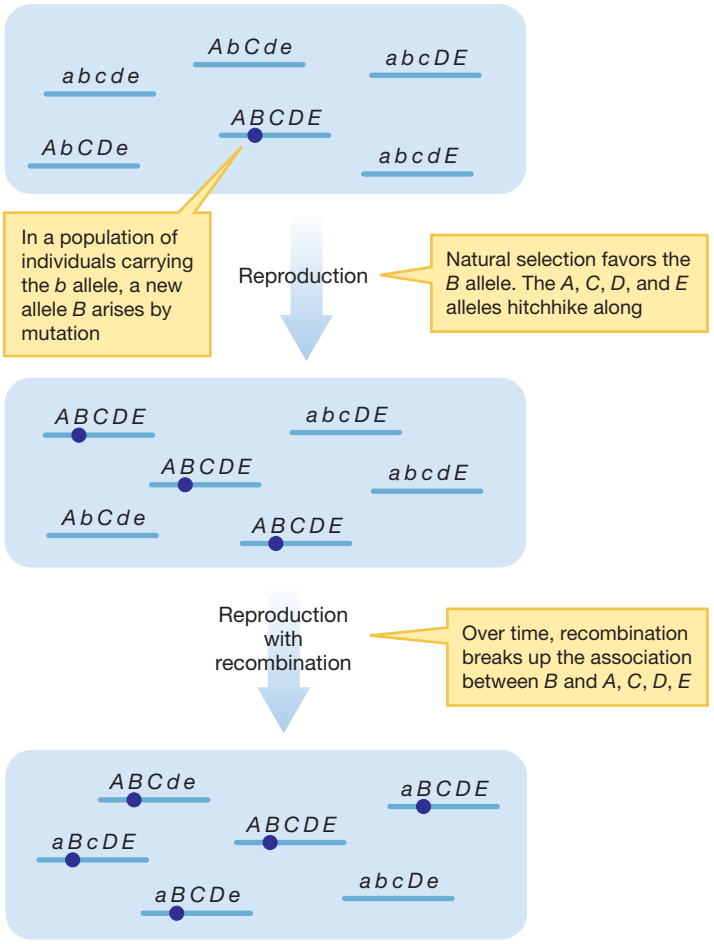


FIGURE 9.20 Genetic hitchhiking. A beneficial allele, *B*, arises on a genetic background with the *A*, *C*, *D*, and *E* alleles; that is, on a chromosome that has the *A*, *C*, *D*, and *E* alleles. These alleles hitchhike along with the *B* allele, increasing in frequency. Eventually, recombination breaks up the association between the *B* allele and the *A*, *C*, *D*, and *E* alleles. Adapted from Understanding Evolution (2008).

Pleuni Pennings and colleagues wanted to test whether genetic hitchhiking plays an important role in the evolution of human immunodeficiency virus (HIV). They used a valuable data set collected more than a decade earlier: an evolutionary history of HIV populations within single infected patients. Each patient was treated with a drug that inhibits the reverse transcriptase that the virus needs to replicate. At a number of stages in the process, the reverse transcriptase and protease genes in each patient were sequenced (Bacheler et al. 2000). The study revealed consistent patterns of evolutionary change, with similar mutations recurring in these genes that confer drug resistance.

Pennings and her colleagues realized that their data could tell us a great deal about hitchhiking in HIV (Pennings et al. 2014). Because the genetics of drug resistance in HIV are relatively well understood, the team knew which mutations were strongly favored by natural selection and thus could distinguish hitchhiking from direct selection. And by working with 30 different patients, they were able to observe multiple replicates of this process.

The researchers looked at the HIV populations of 30 patients. Each population began the study without resistance mutations but fixed at least one resistance mutation subsequently. If genetic hitchhiking occurred when resistance mutations were fixed, we would expect a decrease in nucleotide diversity at other sites to occur alongside the fixation event. This is exactly what the researchers found. At the time of fixation, per-site heterozygosity dropped to below half of its initial value (**Figure 9.21**).

We can see precisely how fixation reduces genetic diversity if we zoom in and examine sequence variants within the HIV population in a single patient.

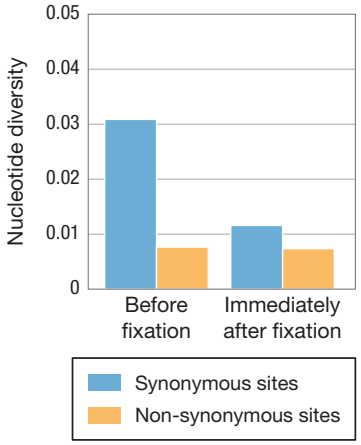


FIGURE 9.21 A decrease in genetic diversity associated with fixation of a beneficial mutation. HIV populations in 30 individuals underwent a loss of genetic diversity (measured as average pairwise genetic distance between virions from the same individual patient) when a beneficial mutation was fixed in a population. Diversity decreased at both synonymous sites (blue) and nonsynonymous sites (orange). Adapted from Pennings et al. (2014).

Figure 9.22 shows a set of sequences obtained from one patient on day 0, prior to fixation, and on day 84, immediately after a resistance mutation is fixed. While the population on day 0 is composed of many different haplotypes, the population on day 84 comprises a number of slight variations on a single haplotype. Presumably, the resistance mutation arose on this haplotype and the nucleotides at nearby sites hitchhiked to fixation, with a few minor changes due to additional mutations that occurred during the fixation process. Viewed in light of linkage disequilibrium, an initial mutation (from A to T at the third position of codon 103) resulted in linkage disequilibrium between that favorable mutation and the genetic background on which it arose. Direct selection favoring that one change indirectly favored variants at other sites in linkage disequilibrium with it.

Just as beneficial mutations cause a reduction in genetic diversity due to hitchhiking alleles around them, deleterious mutations can also cause a reduction in genetic diversity. When deleterious alleles arise in a population, they tend to be eliminated by selection. Much as beneficial mutations carry nearby alleles to fixation via hitchhiking, deleterious mutations carry nearby alleles to *extinction* via the process known as **background selection**. As a result of this process, genetic variation is reduced relative to what we would expect in a neutral model.

Hitchhiking Reveals Recent Positive Selection

We have seen that when an allele increases rapidly in frequency in a population as a result of natural selection, it carries along with it the alleles at nearby locations on the chromosome. Genetic hitchhiking thus leaves its mark on the genome. Population geneticists have developed numerous statistical tests that take advantage of this phenomenon to screen for loci that have recently undergone positive selection (that is, selection for a beneficial mutation).

Here we describe one such test, which Benjamin Voight and his colleagues developed to screen the human genome for loci that currently are or recently have been under strong positive selection — even before they are fixed in the population (Sabeti et al. 2002; Voight et al. 2006).

Suppose we have a polymorphic locus D with 70% D alleles and 30% d alleles. How can we tell which allele (if any) has been under positive selection; that is, which allele has been favored by natural selection? If we know only the frequencies of the D and d alleles, there is no way to tell. We don't know whether the D allele is at high frequency because it is neutral and drifting, because it is favored and on its way to fixation, or because it is disfavored but the d allele arose only recently. But, if we can look at the other nearby loci, we may be able to resolve this puzzle. The key insight is that, as a consequence of hitchhiking, strongly selected alleles will be surrounded by relatively long blocks of a single haplotype. Using this fact, Voight and collegues figured out not only how to locate loci under positive selection, but also to determine which allele at such a locus is the favored one.

Suppose that the D allele is ancestral, and that the d allele has recently increased in frequency as a result of strong natural selection. On haplotypes containing the D allele, recombination will have had plenty of time to mix up the alleles at surrounding loci. By contrast, because the d allele arose recently and reached its current frequency as a result of strong selection, recombination will not have had time to mix up the alleles at nearby loci. At the loci nearest to the D locus, we will tend to see the same alleles that were present on the haplotype where the d mutation first arose. **Figure 9.23** illustrates this idea.

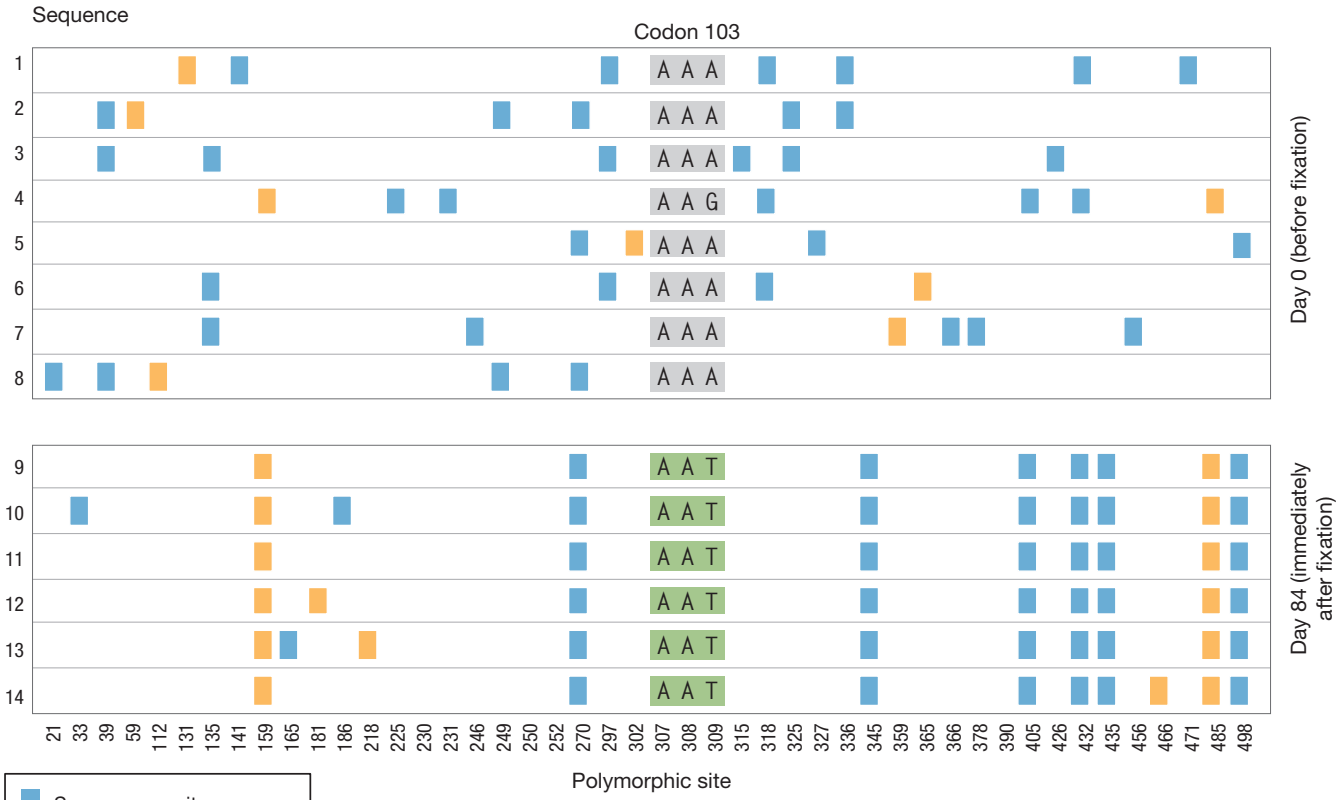


FIGURE 9.22 A selective sweep at a resistance locus drives fixation at other sites by genetic hitchhiking.
Each row in this figure corresponds to a single viral sequence from the HIV population in a single patient. Colored blocks indicate synonymous (blue) and nonsynonymous (orange) polymorphic sites. On day 0, all sequences were antiviral-sensitive, as indicated by the gray codon 103. Sometime before day 84, a mutation from A to T at the third position of codon 103 arose, conferring antiviral resistance. By day 84, all sequences were resistant, as indicated by the green codon 103. As the resistance mutation at codon 103 swept to fixation, sequence variants at other sites hitchhiked along. Adapted from Pennings et al. (2014).

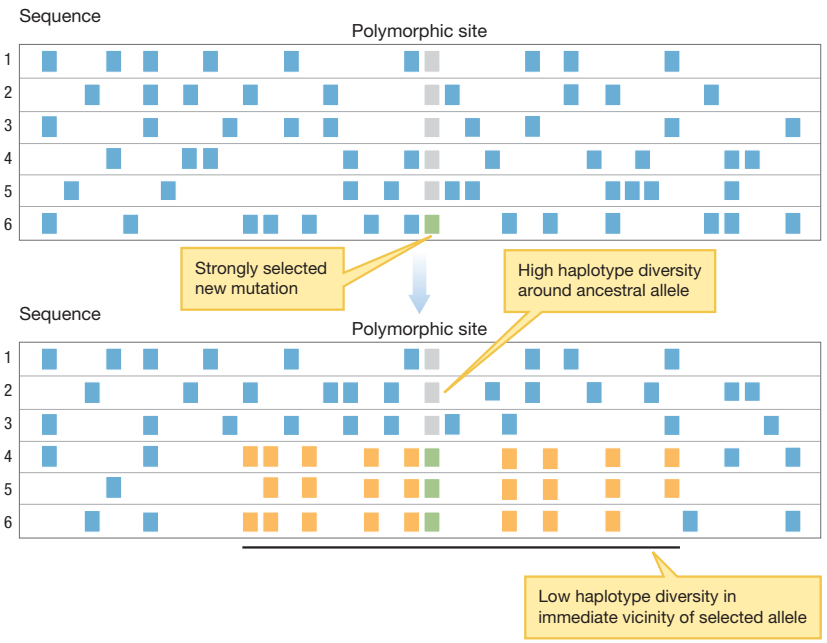


FIGURE 9.23 Voight's test for selection. As in Figure 9.22, each row corresponds to an individual sequence. Colored blocks indicate substitutions relative to the wild type. Top: A new strongly favored mutation (green) arises at a previously monomorphic locus. Bottom: After generations of positive selection, the new mutation has become common in the population. Haplotypes that include the original allele at this locus are highly variable at other loci, just as they were in the original population. Haplotypes that include the new allele are much less variable in the vicinity of this mutation (orange region). Further away from the mutation, recombination has separated the new allele from the background on which it arose.

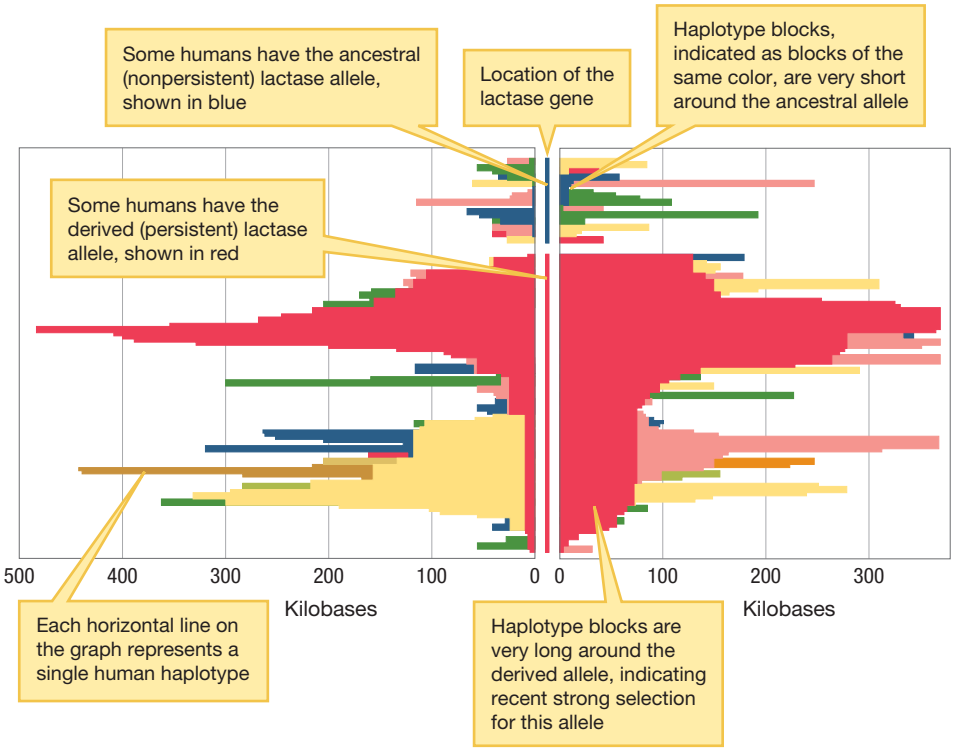


FIGURE 9.24 Recent strong selection in the human genome. Voight and his colleagues used haplotype variation to find alleles that have been under recent strong selection. Here, we see the haplotypes around the *Rs1561277* single nucleotide polymorphism. Lactase persistence has been under strong positive selection in cattle-growing populations, as we see from the strong conservation of haplotype structure around the selected lactase allele (red) relative to the haplotype structure around the other ancestral allele (blue). Adapted from J. K. Pritchard (personal communication).

Using genetic data from 209 individuals of African, Asian, and European descent, Voight and his colleagues applied this approach to explore how evolutionary processes have shaped the human genome. They identified a number of polymorphic loci for which one allele was surrounded by long haplotype blocks (indicating recent selection), while the other was not (indicating that it was the ancestral form). **Figure 9.24** shows a plot of the haplotypes around the lactase gene. This gene is responsible for the loss or persistence of lactose tolerance into adulthood. In populations that raise dairy cattle, lactase persistence confers a substantial fitness advantage (we return to this example in Chapter 18). At this locus the red allele, which confers lactase persistence, exhibits long haplotype blocks, whereas the blue variant has quite short ones. This indicates that, as expected, lactase persistence has been the subject of recent strong positive selection.

Hitchhiking Causes Genetic Draft

In the Pennings study on the evolution of HIV, genetic hitchhiking had several notable consequences. A number of loci that were initially polymorphic became fixed over time, (1) in a manner that would not have been predictable *a priori*, (2) without selection acting on them directly, and (3) with the consequence of reducing the genetic diversity of the population. This looks a great deal like genetic drift—but what is happening here is distinct from the process we studied in Chapter 8. Here, alleles are not becoming fixed or lost as a consequence of sampling effects, but rather because of the happenstance of being linked to a new beneficial mutation and the hitchhiking that ensues. To emphasize the analogy between widespread hitchhiking in the genome and the process of genetic drift, population geneticist John Gillespie dubbed this source of evolutionary randomness **genetic draft** (Gillespie 2000a,b; Gillespie 2010). The name is fitting. Genetic *drift* causes a neutral allele to fluctuate back and forth in frequency as if drifting on a lake, but genetic *draft* causes a neutral allele to follow effortlessly when linked to an allele under selection, like a bicyclist drafting behind a fellow racer.

Genetic drift and genetic draft are similar in that both processes reduce variation in populations and lead to stochastic fixation of alleles, but the precise behaviors of these two processes are somewhat different. Recall that genetic drift operates much more strongly in small populations. Not so genetic draft: The strength of genetic draft is approximately independent of population size (Gillespie 2001). This means

that under genetic draft, the amount of neutral variation in a population should be roughly independent of its size. Because genetic drift can be relatively weak in populations of even modest size, Gillespie argues that much of the random fluctuation in the frequency of common alleles, and much of the reduction in genetic diversity that we observe in natural populations, may be due to genetic draft rather than drift (Gillespie 2001; Leffler et al. 2012). Furthermore, the consequences of population size on fixation rates of selected alleles are distinctly different under drift and draft. Under drift, beneficial alleles become more likely to reach fixation and deleterious alleles become less likely to fix as population size increases. Under draft, the pattern is reversed: Mildly beneficial alleles become less likely to fix and deleterious alleles become more likely to fix with population size (Gillespie 2001).

To sum up thus far, we have ample evidence for two important general points about the evolutionary consequences of selection and physical linkage: (1) Alleles can increase in frequency due to selection either because they directly code for beneficial traits or because they are physically linked to beneficial alleles at other loci. (2) Natural selection, be it positive or negative, tends to cause a decrease in genetic variation at loci near the selected allele.

Clonal Interference

When we discussed periodic selection, we assumed that beneficial mutations arise one at a time, and that in the time between these events, the previous beneficial mutations have a chance to go to fixation. What happens if beneficial mutations arise more frequently, so that multiple beneficial mutations at different loci are segregating in the population at any given time? If recombination is nonexistent or limited, not all of the beneficial alleles can go to fixation at the same time. Moreover, the beneficial allele that goes to fixation does so more slowly, because it has to outcompete not only the lower-fitness wild type, but also the other beneficial mutations. In this way, the beneficial alleles "interfere" with one another, and the consequence is an overall reduction in the rate at which beneficial alleles are fixed (**Figure 9.25**). This slowing down of selection is known as **clonal interference** (Gerrish and Lenski 1998), and it has been observed in microbes including viruses (Miralles et al. 1999;

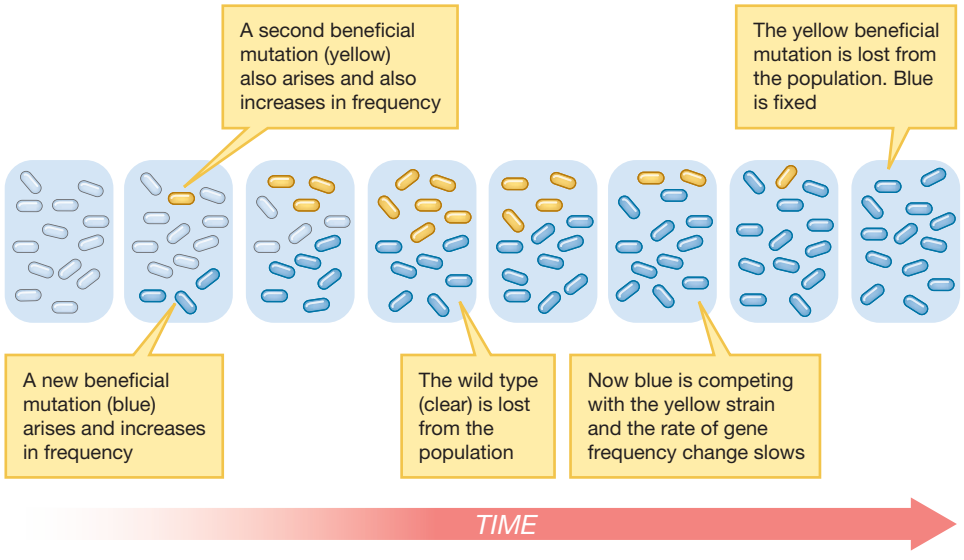


FIGURE 9.25 Clonal interference in a bacterial population. Clonal interference occurs when beneficial mutations arise frequently within a population. In a population with limited recombination, when two beneficial alleles arise concurrently, selection for one beneficial allele (blue) can interfere with the increase in frequency of the other beneficial allele (yellow).

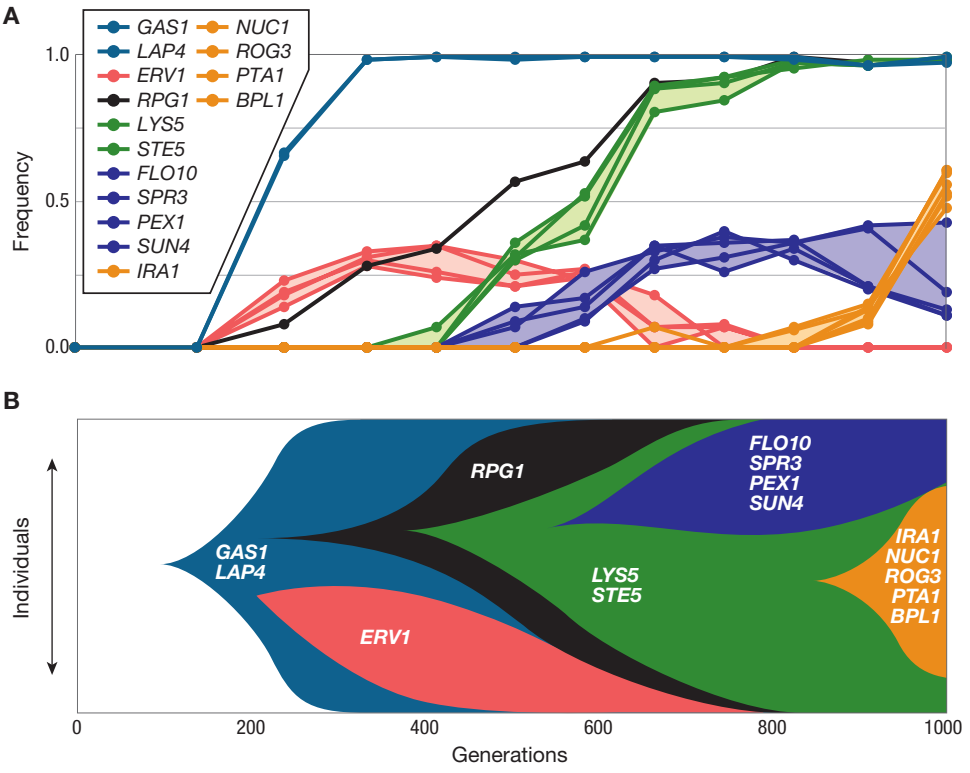


FIGURE 9.26 Hitchhiking and clonal interference in an experimental population of asexual yeast. This figure illustrates a single replicate population from the Lang et al. experiment. **(A)** A plot of allele frequencies over time shows groups of mutations, each represented by a single color, moving together as a cohort because of genetic hitchhiking. One cohort, shown here in pink, carries at least one beneficial mutation but is eventually lost because of clonal interference. **(B)** A schematic diagram of the same experiment illustrates the growth or decline of each cohort with time and reveals that the selective sweeps in this population are nested, with each new sweep beginning before the previous one has been completed. Each cohort is labeled with the mutations it carries. Adapted from Lang et al. (2013).

Strelkowa and Lässig 2012), bacteria (de Visser et al. 1999; Maddamsetti et al. 2015), and yeast (Kao and Sherlock 2008; Lang et al. 2013).

In experimental microbial populations, we can watch the process of clonal interference as it happens. Gregory Lang, Michael Desai, and their colleagues took advantage of recent advances in population-scale whole-genome sequencing to track the fates of new mutations over time in 40 replicate populations of an asexual strain of the yeast *Saccharomyces cerevisiae*. This study, which ran for 1000 yeast generations, provided the team with a detailed view of how genetic hitchhiking and clonal interference interact with selection to determine the fates of new mutations.

Lang and his team observed a fair degree of parallel evolution across the 40 replicate populations (Lang et al. 2013). Mutations in the same specific genes reached appreciable frequency in multiple populations. For example, many of the yeast lines acquired nonsense or frameshift mutations that inactivated components of the unused mating system. The researchers could infer that these frequently observed mutations were the ones most likely to confer strong selective benefits. By tracking these particular mutations, they could then follow the fate of selectively favored alleles.

Lang and colleagues found intricate interactions among the various linkage-associated processes that we have discussed. **Figure 9.26** shows the fate of nearly two dozen mutations that arose during the course of the experiment. Three important results are highlighted.

First, Figure 9.26A shows that groups of mutations, indicated by a common color, move together as a cohort. This is indicative of hitchhiking. Once such a clone acquires one or more beneficial mutations, it then increases in frequency due to positive selection. It is important to recognize that both neutral and beneficial mutations can spread through a hitchhiking mechanism. Neutral mutations simply go along for the ride, whereas we could say that beneficial mutations contribute gas money—they further increase the fitness of an already advantageous mutation with which they are associated.

Second, some cohorts—pink and purple in Figure 9.26—rapidly increase in frequency at first, but subsequently decline and may be lost from the population. This is a consequence of clonal interference. These genotypes, though favored relative to the ancestor, are eventually outcompeted by even more successful variants. Figure 9.26B provides a schematic representation of this process. We see cohorts

arise, grow, and then either decline to loss via clonal interference or form new cohorts by providing the genetic background on which new mutations arise and sweep toward fixation.

Third, selective sweeps can be nested. In Figure 9.26B, we see a cohort carrying mutations in *GAS1* and *LAP4* (blue) start to sweep through the population. Before it reaches fixation, two lineages arise on this genetic background: one adding a mutation in *RPG1* (black) and the other adding a mutation in *ERV1* (pink). Only one of these two lineages can ultimately succeed and one must go extinct. Although the *ERV1* lineage reached higher frequency initially, it ultimately loses out when a cohort with mutations in *LYS5* and *STE5* (green) arises in the *RPG1* lineage and sweeps to fixation. The process then continues. A cohort of four mutations arises in the background of the *LYS5*/*STE5* lineage, and increases in frequency until declining due to clonal interference by a cohort of five mutations (orange).

Clonal interference operates most strongly in asexually reproducing organisms like those in the Lang et al. study. This is because, in the absence of any mechanism of recombination, any two beneficial mutations that arise in different lineages will stay forever separate. As recombination becomes more frequent, beneficial mutations that arose in separate lineages can be assimilated into a genome. Released from the constraints of clonal interference, evolutionary change is accelerated. This is the core idea underlying the Fisher–Muller hypothesis for the evolution of sex, which we will discuss in Chapter 16 (see particularly Figure 16.15; the upper panel of that figure illustrates clonal interference as in Figure 9.26, while the lower panel shows how recombination accelerates the evolutionary process). But even in recombining populations, alleles at nearby loci can interfere with one another until their initial associations are broken down by recombination. This analog to clonal selection in a sexual population is known as the *Hill–Robertson effect* (Hill and Robertson 1966; Felsenstein 1974; Orr 2000).

Mathematical models predict and empirical studies confirm that the impact of clonal interference depends on the availability of new beneficial mutations and the level of recombination (Neher 2013). When beneficial mutations are sufficiently rare, most will be fixed before the next beneficial mutation comes along. The rate of evolution should then be proportional to the population size *N*: doubling the size of a population will double the rate at which new mutations arise in the population and will approximately double the rate of adaptive evolution (**Figure 9.27**, red line).

But when multiple beneficial mutations are segregating in the population at the same time, they will interfere with one another. In an asexual population, this interference is absolute in the sense that only one of the distinct mutations can be fixed at a time. (Multiple mutations can fix at once if one arises on a chromosome already carrying another, of course.) Thus, increases in population size have little effect on the rate of evolution beyond a certain point. In a sexual population, recombination allows multiple mutations on different genetic backgrounds to be combined into the same genome; these mutations can go to fixation concurrently. As a result, the rate of evolution grows faster with increasing population size in sexual populations than it does in asexual populations (Figure 9.27).

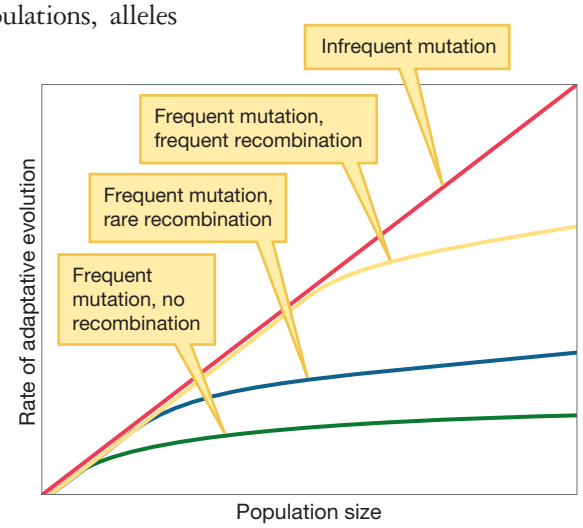


FIGURE 9.27 The impact of clonal selection depends on population size and mode of reproduction. When beneficial mutations are so infrequent that each one fixes before the next arises, the rate of adaptive evolution is proportional to population size (red line). When beneficial mutations arise frequently, clonal selection operates: In an asexual population, the rate of adaptive evolution does not substantially increase with population size (green line). In recombining populations with frequent beneficial mutation, adaptive evolution proceeds faster in larger populations (blue and yellow lines), albeit still not as fast as when beneficial mutations are infrequent. Adapted from Neher (2013).

Sources of Evolutionary Contingency

In his popular book *Wonderful Life*, Stephen Jay Gould speculated on what would happen if one could "replay life's tape" (Gould 1989; Beatty 2006). In other words, if we could rewind the history of life back to some point in the deep past, and then let it continue anew from that point, what would happen? Would we or anything remotely like us be here now? Would life have taken an entirely different direction? Could life have even died out entirely?

The key issue here is the role of contingency—chance—in the evolutionary process. Which aspects of this planet's biology are inevitable consequences of the evolutionary process, and which are mere happenstance? And if contingency is important, by what mechanisms does it come into play?

External environmental events are obvious sources of contingency in the evolutionary process. On the grandest scale, cataclysmic global events—asteroid impacts and mass volcanic eruptions—have been responsible for several of the mass extinctions we will explore in Chapter 15. At the opposite end of the spectrum, countless local populations have been extinguished and their genetic diversity lost when a fire scorched a forest glen or a river overflowed its banks and flooded a meadow. Such events need not be abiotic; for example, the chance introduction of a new predator can have dramatic consequences on the evolution of a prey species.

But even under stable environmental conditions, chance events within a population shape its evolutionary trajectory, and trait values or allele frequencies do not change in a deterministic fashion from one generation to the next. To describe this process properly, evolutionary biologists need to explain the mechanistic basis of evolutionary contingency on a population genetic scale. Traditionally, the process of genetic drift has received much of their attention: Biologists often decompose evolutionary change into an interaction between drift and selection, where the former provides the chance and the latter the necessity. However, recent studies of selection on linked loci—the work we have presented on the importance of hitchhiking, background selection, genetic draft, and clonal interference—are contributing to a shifting view of the mechanistic basis of contingency in the evolutionary process. In the place of a drift-centered view, a multifaceted view of chance is emerging.

One significant aspect of evolutionary contingency is simply the issue of which mutations arise. Almost all possible *single* mutations will arise in a modestly sized bacterial population because the genome size is on the order of 5×10^6 base pairs and thus far smaller than the population size, which is of the order 10^{10} or 10^{11} cells. Even though new beneficial mutations are often lost early-on because of drift, single mutants are readily regenerated. The situation is very different in modestly sized vertebrate populations, where the genome size is of the order 10^9 base pairs and thus much larger than the population size. In such populations, the happenstance of which single mutations arise can be important. Furthermore, when multiple mutations are required to generate selective advantage, the chance origin of mutational *combinations* matters as well. Even a large bacterial population will not generate all possible combinations of three or four novel mutations.

Mutational order can also affect evolutionary outcomes (Mani and Clark 1990). If there were no epistatic interactions between loci, mutational order would not matter much, because any given mutation would have the same effect regardless of

which other substitutions had preceded it. But when epistatic interactions occur, any early substitutions may influence the selective consequences of later mutations.

In most populations, drift plays its largest role when determining the fate of newly arisen alleles. Recall from Chapter 8 Haldane's approximation for the probability that a new beneficial mutation is fixed: If s is the selective advantage, the probability of fixation is only about $2s$. The happenstance of sampling is critical in determining what happens to a rare allele, irrespective of population size. Drift can also drive dramatic fluctuations in the frequencies of common alleles in small populations and in large populations going through short-term population bottlenecks. But in large populations of stable size, drift may not have a major effect on the frequencies of common alleles. The law of large numbers ensures that sampling effects will be minimal in these populations.

Thus, we need some other explanation of random changes in allele frequencies in large populations (Masel 2012). Here, genetic draft is particularly important. In the case of draft, the chance event that determines the fate of a given allele is the happenstance of what genetic background it arises upon. Notice that for draft, this contingency occurs at a single point in time: the origination of a new mutant. The mutation arises on either a beneficial background or a deleterious one, and its fate (should it become reasonably common) is largely determined by this initial chance event. This stands in contrast to the case of drift, where randomness is an ongoing phenomenon as allele frequencies change stochastically from generation to generation because of chance sampling effects. Thus, the frequency of a drafting allele will tend to change in the same direction from one generation to the next, whereas the frequency of a drifting allele will fluctuate back and forth over time (Gillespie 2010).

9.3 Adaptive Landscapes

Thus far, we've seen how statistical associations can build up between genes at two or more loci, and how we can mathematically model the changes of haplotype frequencies in populations. But the mathematics gets complicated quickly. It would be useful to have a conceptual way—even if it is only metaphorical—to think about evolution at multiple loci. The population geneticist Sewall Wright developed the **adaptive landscape** (or fitness landscape) metaphor for this purpose.

Wright was trained as a *physiological geneticist*—he studied the way that genes determine phenotype. From his doctoral research on heredity in guinea pigs and other species, Wright recognized that the relationship between genes and phenotype is seldom a simple and straightforward one (Provine 1986). Rather, interactions *among* genes are extremely important, generating a *genotype-to-phenotype map* that is complicated for many reasons that we have already discussed. To recap, these include:

- *Pleiotropy*. A single gene can have effects on multiple aspects of phenotype.
- *Epistasis*. A given phenotypic trait is often determined by complex interactions among multiple genes.
- *Norms of reaction*. A single genotype produces different phenotypes in different environments.

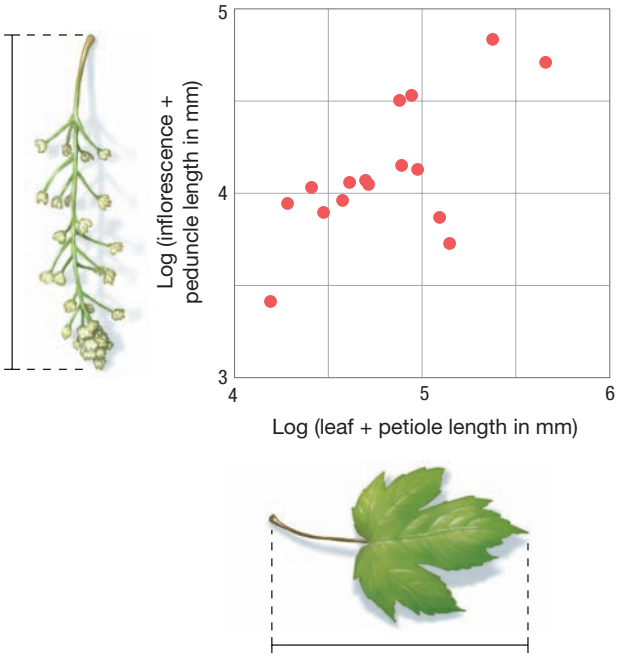


FIGURE 9.28 A two-dimensional phenotype space. The *x* axis indicates the length of a leaf plus its petiole (leaf stem). The *y* axis indicates the length of an inflorescence (flower cluster) plus its peduncle (flower stem). Each point represents the average phenotype for a different species of maple (*Acer* spp.). Adapted from Ackerly and Donoghue (1998).

- *Dominance*. One allele may cover up the effects of another allele at the same locus.
- *Multiple pathways*. A common phenotype may have a different genetic basis in different individuals.

In short, natural selection acts on the phenotype, the next generation inherits only the genotype, and the relation between genotype and phenotype is complex. In Wright's mind, this picture created considerable difficulties for simpler models in which natural selection brought about change through a series of mutations, each with small additive effects. Wright developed an extensive mathematical theory to deal with these challenges. To make his theory accessible to those without a mathematical background, Wright developed the adaptive landscape metaphor (Wright 1932; Provine 1986).

Phenotype Space

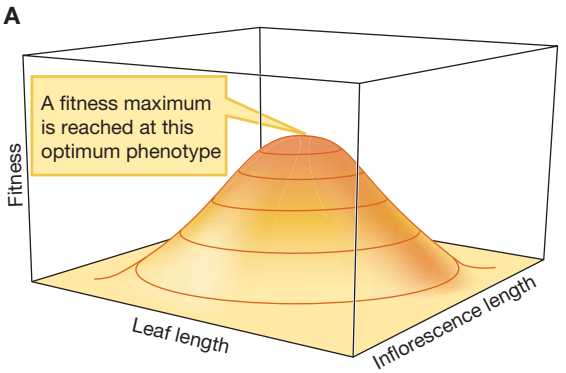
The idea behind the adaptive landscape approach is that we can think about different phenotypic or genotypic combinations as points on a map. This may be a map of **phenotype space**, in which the *x* and *y* axes represent the values of phenotypic traits, such as the size of a tree's leaves and the size of its flowers, as pictured in **Figure 9.28**. Within this phenotype space, each point corresponds to a pair of trait values. Thus, each individual in a population can be assigned a point in the phenotype space. Similarly, the average phenotype in a population can also be assigned a single point.

Note that Figure 9.28 specifically represents a *two-dimensional* phenotype space. In practice, we can consider three, four, or even more traits. A phenotype space that took all of these traits into account would necessarily have a high number of dimensions, and while it would be more realistic, it would also be very difficult to visualize. Fortunately, we can use even low-dimensional phenotype spaces to think about how evolutionary change occurs.

Adaptive Landscapes in Phenotype Space

Now suppose that we want to draw an adaptive landscape. The *x* and *y* axes of our map contain our phenotype space: the aspects of phenotype under consideration (for example, leaf length and inflorescence length). We can then introduce the *z* axis (elevation) to plot the corresponding fitness of an organism with that phenotype combination. This transforms our flat map of phenotype space into a landscape of "hills," where the highest points represent **fitness peaks**—combinations of traits associated with the greatest fitness values.

Figure 9.29A shows a *single-peaked* landscape: A single optimal phenotype lies at the peak of the "hill," and any movement that takes a population closer to the (*x*, *y*) coordinates of the peak necessarily moves the population to a higher fitness as well. **Figure 9.29B** shows a *multipeaked* landscape. Here, instead of a single hill, we have a range of multiple fitness peaks with **fitness valleys**—regions of lower fitness—between them.



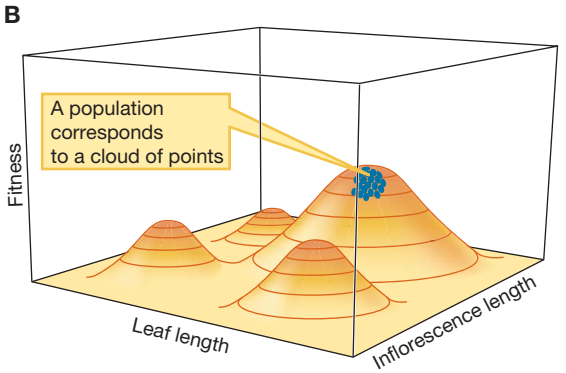


FIGURE 9.29 Fitness peaks and valleys in phenotype space. In these illustrations, hypothetical fitnesses are associated with the values of two phenotypic traits, leaf length and inflorescence (flower cluster) length. **(A)** The landscape is single peaked, with a unique local fitness optimum that is also a global optimum. **(B)** The landscape is multipeaked, with several local fitness optima; that is, several hilltops on the landscape. A population corresponds to a cloud of points on an adaptive landscape, as illustrated.

In the adaptive landscape metaphor, each point in phenotype space corresponds to a different phenotype. Thus, a population of individuals with different phenotypes corresponds to a set or "cloud" of points, as illustrated in Figure 9.29B.

By looking at an adaptive landscape, we can get a qualitative sense about how phenotypic evolution might proceed. In a large population with sufficient genetic variation for the traits in question, we would expect the population to move uphill on the adaptive landscape. Thus, selection will favor phenotypic values that result in increased fitness; that is, evolution might follow a hill-climbing trajectory on an adaptive landscape. If each genetic change has a small phenotypic effect, we would expect natural selection to follow a path that moves directly uphill until a local fitness maximum is attained, rather than crossing whatever fitness valleys are necessary to reach the global fitness maximum. This illustrates the shortsightedness of natural selection that we discussed in Chapter 3. Natural selection cannot plan ahead and aim for the highest peaks; rather, it simply sorts on the existing variation, causing the population to move across the adaptive landscape like a myopic mountain climber who takes small, incremental steps without being able to see a final goal (the red curve in **Figure 9.30**).

As we saw in Chapter 8, genetic drift joins selection as an important source of evolutionary change in smaller populations, and drift can even drive selectively disadvantageous changes in phenotype. This corresponds to downhill movement on the adaptive landscape. Drift—and processes that facilitate drift such as bottlenecks and founder effects—can help a population move across a fitness valley so that it can subsequently climb an adaptive peak on the other side (the blue curve in Figure 9.30).

Let's examine a set of studies by evolutionary biologist Edmond Brodie II that map a simple adaptive landscape in phenotype space. Brodie studied the fitness consequences of antipredator behavior and coloration pattern in garter snakes (*Thamnophis ordinoides*) (Brodie 1992). Garter snakes vary significantly in coloration, ranging from nearly unpatterned to mottled to dramatically striped (**Figure 9.31**). These snakes also differ in the escape behaviors that they use when threatened by a predator. Some individuals flee in a direct course, while others make a few or many "reversals"—evasive changes in direction that may confuse a pursuing predator.

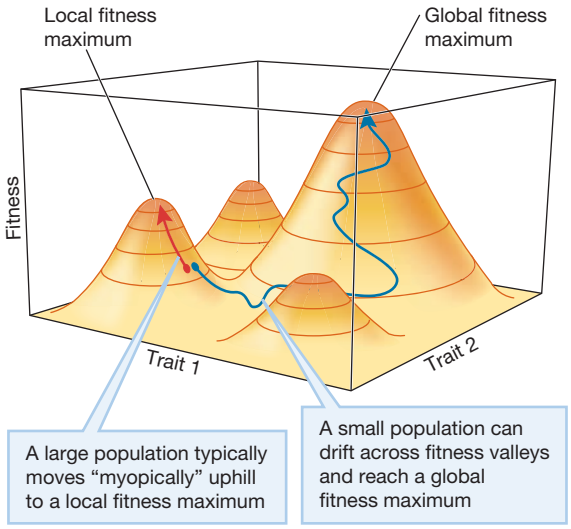


FIGURE 9.30 Movement on an adaptive landscape. In a large population (red curve), a population with sufficient genetic variation will climb myopically uphill to the nearest local fitness maximum. In a small population (blue curve), drift plays a significant role, and the population can drift down, into, and across fitness valleys.

FIGURE 9.31 The northwestern garter snake (*Thamnophis ordinoides*). This species exhibits dramatic variation in coloration (shown here) and behavior.

Brodie measured both coloration and reversal frequency in 646 newborn garter snakes, marked each individual, and released the lot of them into the wild. By recording the frequency with which the marked snakes were later recaptured over the course of a 3-year experiment, Brodie was able to estimate the fitness consequences of particular *combinations* of color pattern and reversal behavior. He observed strong nonadditive interactions between coloration and behavior: Spotted snakes that reverse and striped snakes that do not reverse have high fitnesses, while striped snakes that reverse and spotted snakes that do not reverse have low fitnesses. **Figure 9.32** shows an adaptive landscape inferred from these fitness estimates. This landscape is in phenotype space: The x and y axes reflect the phenotypes of reversal rate and degree of striping, respectively.

When evolution proceeds by a series of small, gradual changes, it will cause a population to move gradually across an adaptive landscape in small steps, as illustrated in **Figure 9.33A**. When mutations of large effect are available and selected, evolution may instead proceed as if "teleporting" from place to place on the adaptive landscape (**Figure 9.33B**). We saw an example of such a leap in the mouse coat color example discussed earlier and in Chapter 3; there, a single mutation in the *Mc1R* gene can cause a dramatic shift in coat color. Such mutations of large effect are more common than Darwin anticipated, and they play an important role in the evolutionary process.

Adaptive Landscapes in Genotype Space

When mutations have large phenotypic effects, movement on the adaptive landscape is not smooth and gradual. Worse still, the distance between two points on the landscape may be a poor indicator of how many genetic changes are needed to shift from one to the other. To get around these problems, population geneticists often conceptualize adaptive landscapes as occupying **genotype space** rather than phenotype space. This is the way that Wright initially presented his adaptive landscape metaphor. The idea here is that genotypes that are mutational

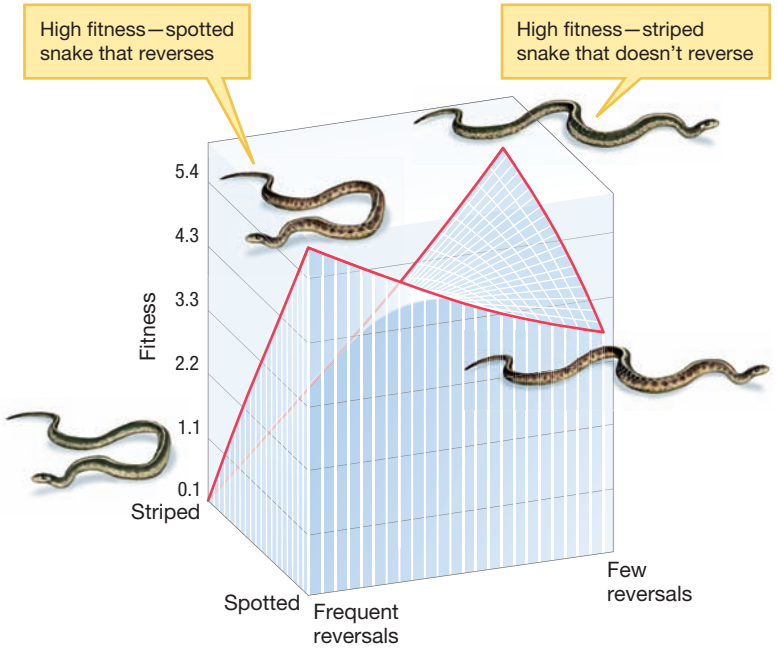


FIGURE 9.32 An adaptive landscape in phenotype space. The adaptive landscape for the garter snake *T. ordinoides* in the phenotype space defined by body coloration and reversal behavior during escape. Two bivariate phenotypes have high fitness; the fitnesses of the other two combinations are low. Adapted from Brodie (1992).

neighbors—namely, those separated by a single mutation—appear close together in the genotype space, whereas those that are separated by many mutations appear far apart. When adaptive landscapes are represented in this way, nearby points are genetically very similar, even if their phenotypes vary dramatically. By the same logic, distant points are very different genetically even if they happen to correspond to very similar phenotypes.

Because mutations are discrete rather than continuous changes, the true genotype space is actually a network of genotypes rather than a continuous space. In the paper in which Wright first proposed the adaptive landscape metaphor, he illustrated the genotype network concept as shown in **Figure 9.34** (Wright 1932).

Wright took these networks of loci and redrew them as adaptive landscapes in genotype space. **Figure 9.35** re-creates Wright's original sketch of such a landscape from his 1932 paper. In this figure, similar genotypes are close together, so individual mutations should correspond to small movements in this space, and evolution might be expected to trace out a nearly continuous path within this space as a sequence of mutations each become fixed in turn. But, as Wright was well aware from his studies of heredity, single mutations can cause large phenotypic changes, and they can thus have dramatic fitness consequences. As a result, the adaptive landscape in genotype space is likely to be *rugged.* Rather than being a smooth, gradual, and single-peaked surface, the landscape might consist of many sharp, jagged peaks and ridges. In Wright's view, this metaphorical space might look more like the saw-toothed limestone Tsingy of Madagascar than the smooth volcanic slopes of Mt. Fuji (**Figure 9.36**).

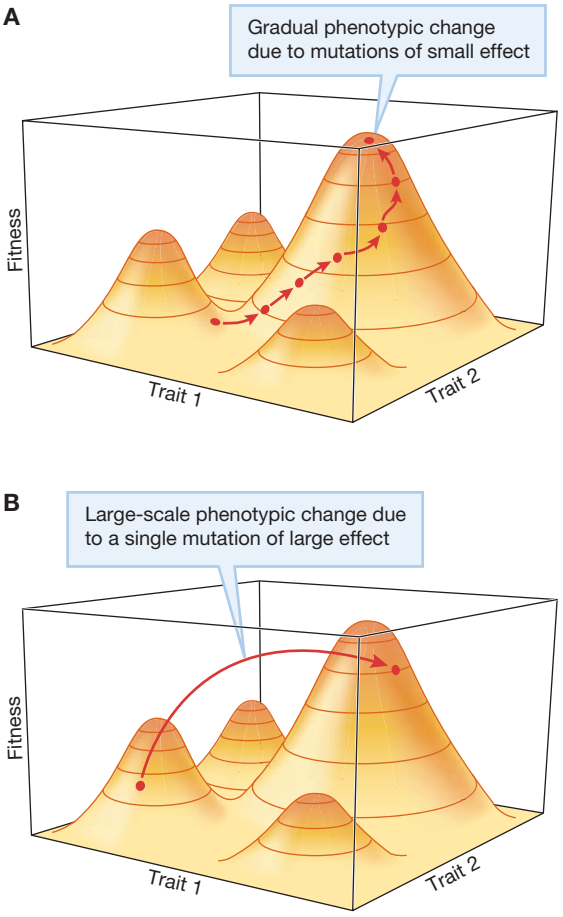


FIGURE 9.33 Evolutionary change can be represented as movement across the adaptive landscape. **(A)** When evolution proceeds gradually by a sequence of mutations of small effect, a series of substitutions causes the population to move gradually across the landscape. **(B)** When evolution occurs by mutations of large phenotypic effect, a single substitution can cause the population to "leap" from one part of the landscape to another.

KEYCONCEPT QUESTION

9.2 Do you think that increasing the amount of epistasis would increase or decrease the ruggedness of an adaptive landscape in genotype space?

Returning to the compensatory mutation story from the introduction to this chapter, we can see the value of thinking in terms of genotypic networks. We have already observed that compensatory mutations reduce the fitness cost of antibiotic

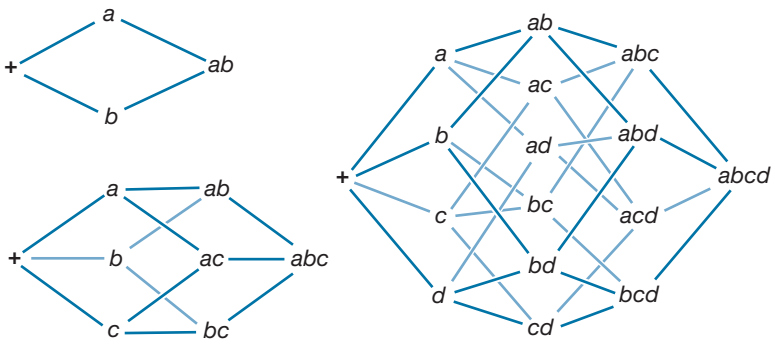


FIGURE 9.34 Genotypic networks. In a mutation network, the edges shown here as blue lines connect haplotypes that differ by only a single mutation. This figure illustrates mutation networks for two, three, and four loci. We have retained Wright's somewhat archaic notation: The wild type is indicated by "+", whereas the lowercase letters indicate alternative alleles at the *a*, *b*, *c*, and *d* loci. From Wright (1932).

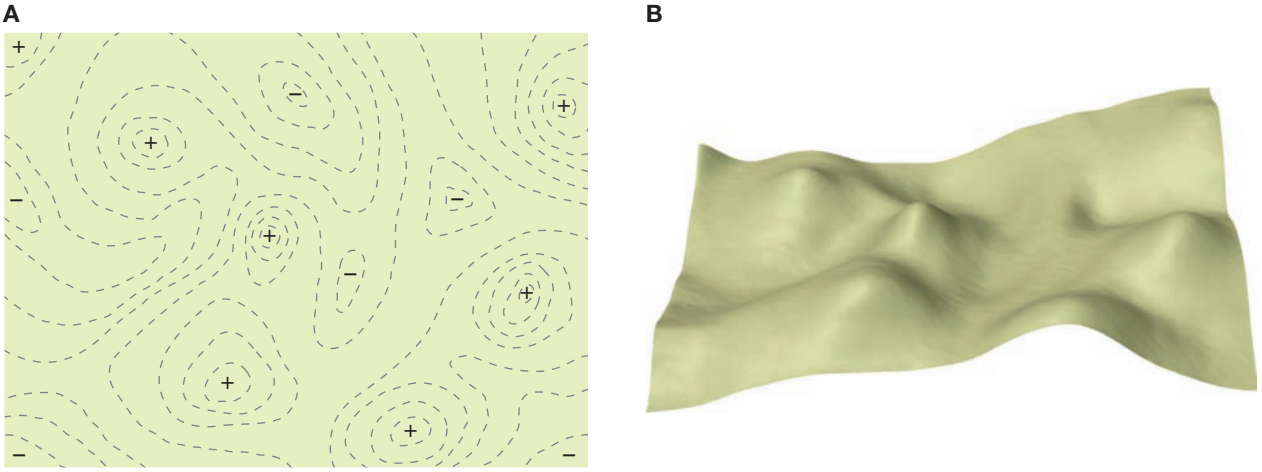


FIGURE 9.35 Wright's original sketch of an adaptive landscape. **(A)** Here the landscape is drawn as a topographic map. From Wright (1932). **(B)** A three-dimensional version of the same landscape.

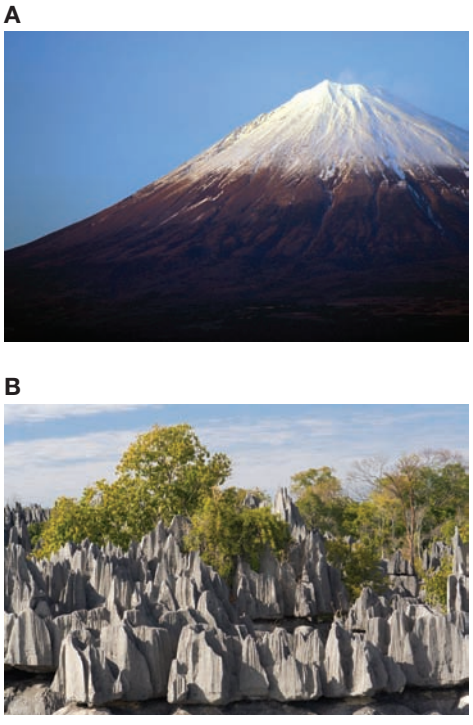


FIGURE 9.36 Smooth and rugged physical landscapes. R. A. Fisher envisioned selection moving on a smooth and single-peaked adaptive landscape analogous to the physical landscape of Mt. Fuji **(A)**, whereas Sewall Wright imagined that selection operated on a rugged adaptive landscape analogous to the Tsingy of Madagascar **(B)**.

resistance and thus make it hard to reverse the evolution of such resistance. The story is even more complex than this, however. Once resistance has evolved and been compensated, neither the loss of resistance nor the loss of compensation is immediately beneficial, even when no antibiotics are being used.

The genotypic network concept can help us develop a deeper understanding of how compensatory resistance works. Suppose that the *R* locus controls resistance, that the *C* locus is the compensatory locus, and that the wild type *rc* is neither resistant nor compensated. When antibiotics are present (**Figure 9.37A**), the resistance allele *R* confers a large selective advantage, and the compensatory allele *C* further increases fitness. Resistance evolution proceeds along the trajectory indicated by the red arrows in the figure. When antibiotics are not present in the environment (**Figure 9.37B**), the genotype with the highest fitness is the *rc* wild type. But once the resistant, compensated *RC* genotype has been fixed, there is no direct path to return to the *rc* wild type by a series of beneficial mutations. This is because either reversion, $R \rightarrow r$ or $C \rightarrow c$, imposes a fitness cost if it occurs first. Loss of the compensatory mutation in a resistant individual obviously causes a fitness decrease. But loss of the resistance mutation in a compensated individual also causes a fitness decrease. As shown in Figure 9.37B, the compensatory allele *C* provides a fitness advantage in the presence of the resistance allele *R* but imposes a fitness cost when paired with the wild-type allele *r*. This type of fitness interaction is common for compensatory mutations (Andersson and Hughes 2010).

Antibiotic resistance evolution has been described as an evolutionary lobster trap: One can get in easily, but once in, it's not easy to get out (Bergstrom and Feldgarden 2008; Tanaka and Valckenborgh 2011). In the presence of the antibiotic, natural selection can readily drive the population from *rc* to *RC* by means of two sequential substitutions, each of which increases fitness. But in the absence of the antibiotic, there is no comparable sequence of fitness-increasing mutations that leads from *RC* to *rc*. Returning to the sensitive uncompensated state requires that the population cross a fitness valley, and this can take a long time.

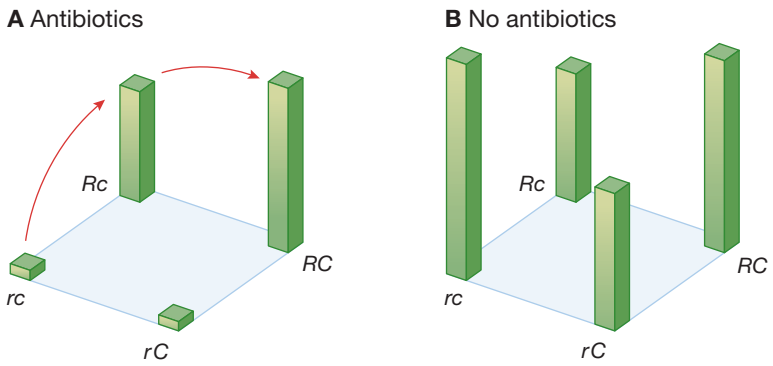


FIGURE 9.37 Adaptive landscapes in the presence and absence of antibiotics. Here we show the adaptive landscapes on a genotypic network in the presence **(A)** and absence **(B)** of antibiotics. The blue edges between *rc*, *Rc*, *rC*, and *RC* represent a genotypic network; these edges link genotypes that differ by only a single mutation. This figure is modeled on streptomycin resistance in *E. coli*, as studied by Schrag, Perrot, and Levin. In that system, a single base pair substitution in a ribosomal protein confers resistance, and a second mutation at a separate locus compensates for much of the fitness cost induced by the resistance mutation. Adapted from Schrag et al. (1997).

9.4 Quantitative Genetics

In Section 9.1, we saw how a nearly continuous range of phenotypes could arise via Mendelian inheritance when multiple genes influence the phenotype. In this section, we will revisit continuously varying traits and, in doing so, we will explore the field of **quantitative genetics**.

Consider a continuously varying trait such as the fruit size for a tomato plant (Frary et al. 2000) (**Figure 9.38**). Going from plant to plant, we see a continuous range of fruit sizes. But why is this so? The general question, applicable to almost any varying trait, is "Why does one individual differ from another?"

In the preceding chapters, we have seen that a number of different factors contribute to a phenotype. First, *genes* obviously influence phenotype. As we saw in Section 9.1, when gene effects combine additively, multifactorial inheritance can generate nearly continuous variation. Patterns of inheritance and variation can become even more complicated as a result of gene interactions or epistasis.

FIGURE 9.38 Continuously varying traits. Variation in fruit size across various tomato (*Lycopersicon*) species.

Second, the *environment* influences a phenotype. Tomato plants may generally tend to grow larger in sunnier environments, for example, or they may grow poorly if rainfall falls short of some critical threshold.

Finally, we expect some differences in a phenotype, even for genetically identical individuals raised under the same environmental conditions. Random chance events during the process of development can give rise to considerable phenotypic differences. This source of variation is known as *developmental noise*, and it contributes significantly to phenotypic variation in some populations (Babbitt 2008).

Given that continuously varying traits are shaped by these numerous influences, often with complex interactions among them, how can we make predictions about how continuous traits will change as a result of natural selection? The field of quantitative genetics provides a way of doing this and supplies additional tools for understanding the evolution and genetics of complex continuous traits.

The Phenotypic Value of Continuous Traits

The first step in constructing a theory of quantitative genetics is to develop a basic model of how phenotypes of the individuals in a population are determined (Christiansen 2008). *For a given individual*, we define P as the phenotypic value of the continuous trait that we are studying. In the case of tomato fruit size, P might be quantified as weight in grams. We then decompose P into two parts: the part due to the genotype (G), and the remainder, which we ascribe to environmental influences (E):

$$P = G + E \tag{9.7}$$

Here, the genotypic value (G) is defined as the expected phenotypic value of individuals of that particular genotype. Any deviation between P and G is attributed to environmental effects or developmental noise and is quantified as the environmental deviation (E). The average or expected value of E is zero, because the environmental deviation is equally likely to be positive or negative.

The key to the quantitative genetics approach is that it enables us to track not only the phenotypes of the individuals in the population but also the *variation* that is present in the population, and whether or not this variation has a genetic basis. This provides us with a way to make predictions about how natural selection—which requires genetic variation to proceed—will drive evolutionary change in the observed phenotypes over time.

We will measure variation by using a quantity known as the variance, which is a statistical measure of the variation in a sample. Different members of a population typically have different trait values, and the variance tells us *how different* from one another these trait values are. Let $x_1, x_2, x_3, \ldots, x_{n-1}, x_n$ be a set of observations (for example, the heights of the students in a class, measured in meters). The mean of these observations is

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

The sample variance of the observations is given by the expression

$$\mathrm{Var}[x] = \frac{1}{n-1}\sum_{i=1}^{n} (x_i - \bar{x})^2$$

The larger the variance, the more that individuals differ from one another and from the mean. **Figure 9.39A** illustrates samples from two populations with the same mean but different variances. **Figure 9.39B** illustrates samples from two populations with different means but the same variance.

Breaking up the phenotype P into genetic and environmental influences—G and E—is helpful not so much in that we have a model of what determines the phenotype of one particular individual. Rather, the point is that this decomposition also allows us to model the contributions to the *variance* in phenotypes observed in the population and to distinguish between the heritable and nonheritable factors involved. This means that we need to derive a mathematical equation for the phenotypic variance, denoted V_P. To do so, we use the basic fact from statistics that the variance of a sum of independent variables is equal to the sum of the variances. Because $P = G + E$, it follows that

$$V_P = V_G + V_E \tag{9.8}$$

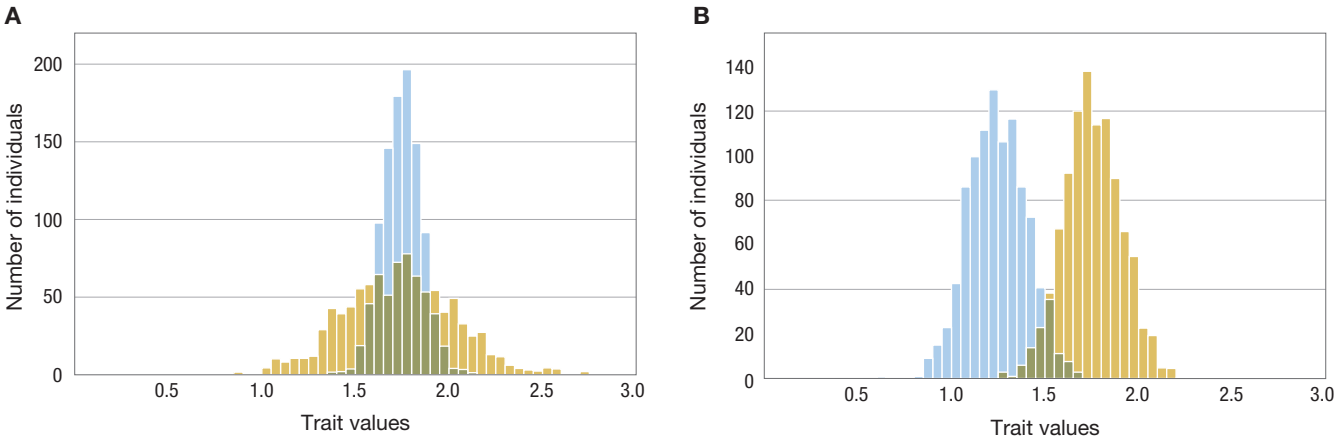


FIGURE 9.39 Population samples. Each histogram shows a sample of 1000 individuals from a population. **(A)** Both the blue and tan populations have the same mean, but the variance of the tan population is 3 times higher than that of the blue population. **(B)** Both the blue and tan populations have the same variance, but the mean of the tan population is higher than the mean of the blue population.

where V_G is the variance of the genotypic value G, and V_E is the variance of the environmental deviation E. (Here we have assumed that the genotypic value and the environmental deviation are uncorrelated.)

The contribution to phenotypic variance that derives from genotypic variance is, in principle, transmitted genetically from parents to offspring. The contribution that derives from environmental variance is not. We define **broad-sense heritability (H^2)** as the fraction of the variance that is potentially due to genetic causes:

$$H^2 = \frac{V_G}{V_G + V_E} \tag{9.9}$$

Broad-sense heritability quantifies the total fraction of the variation of a trait in a population that can be attributed to genetic causes. But it is not a particularly useful predictor of evolutionary change because, as we will see, selection is not able to operate effectively on all genetic variation. Therefore, evolutionary biologists more commonly work with a different quantity known as narrow-sense heritability, which is the fraction of the total variance due to additive genetic variation.

Before going into narrow-sense heritability, how can we estimate broad-sense heritability? To do so, we will need to consider how biologists can measure the terms in Equation 9.9. All we need to know are the relative magnitudes of the phenotypic variance due to genetic and environmental contributions. These we can find by comparing the amount of variation among genetically identical (or nearly identical) individuals with the amount of variation among unrelated individuals. For many model organisms, we can easily obtain or construct large numbers of nearly genetically identical individuals in the form of *inbred lines*. An inbred line is produced by multiple generations of repeated inbreeding (for example, between siblings) until the remaining genetic variation in the line is minimal.

Estimating the components of variation is then straightforward. Within an inbred line, there is negligible genetic variation. Therefore, all phenotype variation within an inbred line is due to environmental variation. Thus, we can estimate V_E as the average phenotypic variance among individuals *within* a single inbred line. We can then estimate the total phenotypic variance V_P by the phenotypic variance among individuals taken from different inbred lines. Subtracting V_E from both sides of Equation 9.8, we see that the genotypic variance V_G is the difference between these two quantities: $V_G = V_P - V_E$.

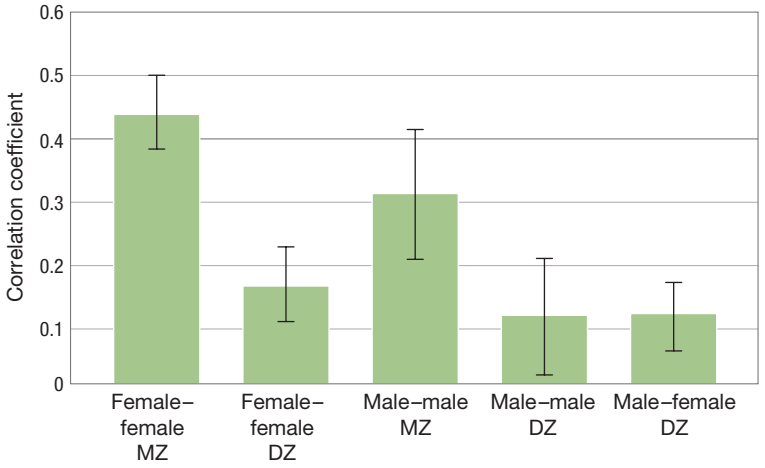


FIGURE 9.40 A study of the genetics of susceptibility to depression. Researchers compared monozygotic (MZ) twins to dizygotic (DZ) twins in order to estimate the influence of genetics on depression. The figure illustrates correlations by twin type (MZ or DZ) and sex. The error bars indicate the 95% confidence intervals for each of the correlation coefficients. These data reveal a higher correlation in depression among monozygotic twin pairs than among dizygotic twin pairs. This finding implies a genetic component to the susceptibility to depression. Adapted from Kendler et al. (2006).

When studying human genetics, it is obviously neither feasible nor morally acceptable to create inbred lines. In studies of humans, researchers can instead study pairs of monozygotic ("identical") twins as a way of measuring variation among genetically identical individuals. But there is a problem: Twins typically experience very similar environmental conditions as well. To control for this, researchers typically use one of two approaches. One approach is to study monozygotic twins who were adopted at an early age and reared in separate families. In this case, the genetics are the same, but the environments are different. Such twin pairs can be hard to find, so alternatively researchers can compare monozygotic twins reared together with dizygotic (fraternal) twins reared together. Monozygotic and dizygotic twin pairs alike experience similar environmental conditions, but the genetics of the two kinds of twin pairs differ. Monozygotic (MZ) twins should be essentially genetically identical, whereas dizygotic (DZ) twins should not be more closely related than are ordinary pairs of full siblings. **Figure 9.40** illustrates the use of monozygotic–dizygotic twin comparisons to study the genetic basis of depression.

Decomposing Genotypic Effects

The genotype is composed of many different genes. If all gene effects were to combine additively, an individual's genotypic value G could be represented as a simple sum of gene effects. But, of course, gene effects generally do not combine in so simple a fashion. Rather, we have to consider both the interactions between two alleles at the same locus, which we call **dominance effects**, and interactions between alleles at different loci, which we have already discussed in Section 9.1 as epistasis. **Box 9.3** provides an example of each.

To account for dominance and epistasis, we can break down our equations for P and V_P. We can think of an individual's genotypic value G as the sum of three contributing components: an additive component A, a dominance component D, and an epistasis or interaction component I. Here, A is the sum of the expected individual effects of each allele, D is the sum of the effects of dominance interactions between allele pairs at each locus, and I is the sum of the effects of epistatic interactions across loci (Barton et al. 2007). We can then write

$$P = G + E = A + D + I + E \tag{9.10}$$

If we assume that all components are independent of each other, we can write the variance of this sum as a sum of the variances, much as we derived Equation 9.8:

$$V_P = V_A + V_D + V_I + V_E \tag{9.11}$$

where V_A is the variance of the additive component A, V_D is the variance of the dominance component D, V_I is the variance of the interaction component I, and V_E is the variance of the environmental component E.

BOX 9.3 Additive, Dominance, and Epistatic Effects

To see the distinction between additive gene effects, dominance effects, and epistasis or interaction effects, let's walk through an example. Suppose that two loci, *A* and *B*, influence the height of a tomato plant, with the *A* and *B* alleles increasing height relative to the *a* and *b* alleles.

The following table lists average plant height, in meters, for several different genotypes.

Genotype	*aabb*	*Aabb*	*aaBb*	*AaBb*	*AABB*
Additive effects	1	1.1	1.3	1.4	1.8
Dominance effects	1	1.2	1.6	1.8	1.8
Epistatic effects	1	1.1	1.3	1.8	1.8

In the simplest case of additive effects, each *A* allele increases plant height by 0.1 meter, and each *B* allele increases plant height by 0.3 meter. The effect of each allele is independent of which other alleles are present.

In our example of dominance effects, one copy of the *A* or *B* allele is sufficient to have the full effect of increasing plant height by 0.2 or 0.6 meter, respectively. Having two *A* alleles instead of one or two *B* alleles instead of one adds nothing further to the plant height.

In our example of epistatic effects, the effect of having an *A* allele depends on whether or not the plant also has a *B* allele. In the absence of the *B* allele, the *A* allele increases plant height by 0.1 meter; in the absence of the *A* allele, the *B* allele increases plant height by 0.3 meter. But when both the *A* allele and the *B* allele are present, they together increase plant height not by the additive amount $0.1 + 0.3 = 0.4$ meter, but rather by a total of 0.8 meter. (In this particular example, *A* and *B* also have dominance effects; a single copy of each is sufficient for the full effect.)

The power of breaking down the variances in this way is that we can now write down a very simple and very general expression for how a phenotypic trait changes over time in a population in response to natural selection. The way we do this is considering which components of variation can be selected on directly by natural selection.

While all of the genetic contributors to a phenotype contribute to the genetic variance, the dominance component and interaction component are highly context dependent; that is, their effects depend strongly on the genetic background in which they occur. The additive component, by contrast, is independent of context. Irrespective of genetic background, the effects of this component are the same—and, as a result, the additive contributions are more accessible to natural selection. In other words, it is primarily the additive genetic variation on which natural selection operates.

In Equation 9.9, we defined broad-sense heritability H^2 to be the fraction of the variance due to any form of genetic variation: $H^2 = V_G/(V_G + V_E)$. But natural selection cannot easily act on all of this variation. To study the response of phenotype to selection, we need to look at the fraction of the total variation that is due to additive genetic variation alone. We call this fraction the **narrow-sense heritability (h^2)**. Mathematically, we define narrow-sense heritability as

$$h^2 = \frac{V_A}{V_A + V_D + V_I + V_E} \tag{9.12}$$

(If the broad or narrow sense is not specified, the term *heritability* typically refers to the narrow sense.)

Narrow-sense heritability, as a measure of what fraction of the variation is accessible to natural selection, plays a very important role in predicting how phenotypes change over time as a result of natural selection. Before seeing how this works, let's first consider a basic interpretation of narrow-sense heritability as a population-level measure of resemblance between parents and offspring:

Narrow-sense heritability reflects the degree to which offspring resemble their parents in a population, assuming that relatives do not have more similar environments than nonrelatives. Specifically, narrow-sense heritability can be estimated as the slope of a linear regression between the average phenotype of the two parents and the phenotype of the offspring. (In doing so, we are implicitly assuming that the environments experienced by parents and by their offspring are uncorrelated.) A subtle point: Because narrow-sense heritability is calculated as the *slope* of this regression, it is not always the case that the closer the resemblance between parents and offspring, the higher the heritability. As an extreme example, if all parents are identical and all offspring are identical to them, the heritability is undefined because there is no variability in the population.

Peter Berthold and Francisco Pulido used this expression for narrow-sense heritability to estimate the heritability of migratory behavior in a European species of blackcap warblers, *Sylvia atricapilla* (**Figure 9.41A**) (Berthold and Pulido 1994; Pulido et al. 2001). These researchers were interested in understanding whether migratory behavior—known to be under genetic control—could change by natural selection in response to climatic shifts such as global warming. If natural selection is going to shift migratory behavior, they reasoned, there must be additive genetic variation in the population for this behavior. For the blackcap warbler, this was at least plausible, as behavioral variation in migration in this species is well known—while many birds overwinter in the Mediterranean, some migrate as far as sub-Saharan Africa.

To estimate the heritability of migratory behavior, Berthold and Pulido designed a *parent–offspring regression study* (**Figure 9.41B**). They collected 186 newborn birds from wild nests, hand-reared these birds, and then measured their propensity for migratory behavior using a well-established assay: nocturnal restlessness in their cages during the autumn migratory period. The researchers then bred these captive birds (the parental generation) assortatively by nocturnal restlessness to produce a

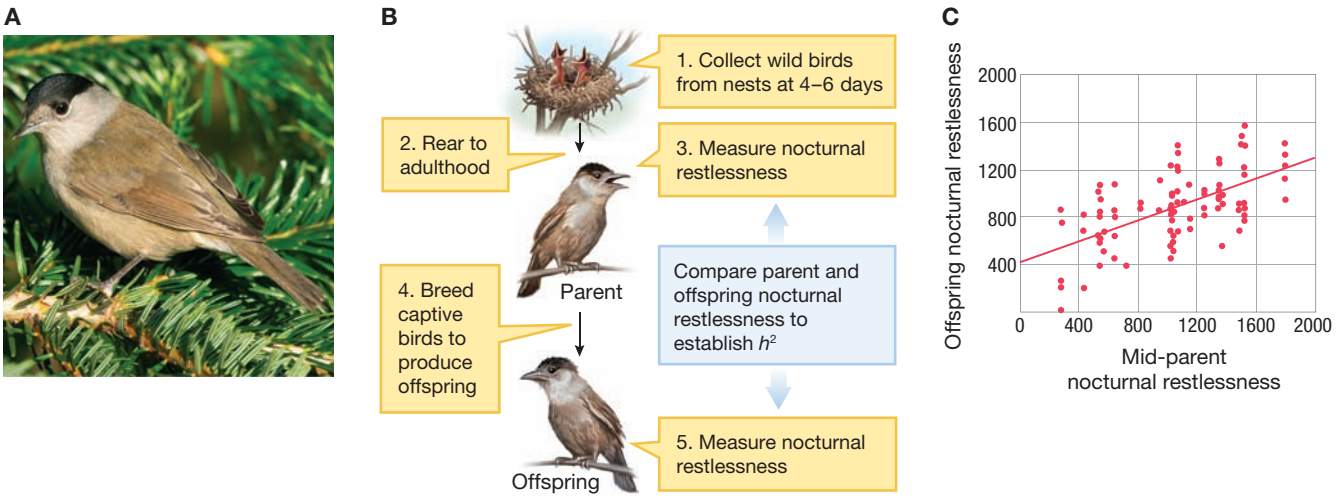


FIGURE 9.41 Heritability of migratory timing. **(A)** Berthold and Pulido estimated the narrow-sense heritability of migratory behavior in the blackcap warbler (*Sylvia atricapilla*). **(B)** To do so, they ran an experiment in which they captured and reared wild birds to adulthood and measured their nocturnal restlessness, then bred the birds to produce offspring and measured the offspring's nocturnal restlessness. Berthold and Pulido estimated the heritability of migratory behavior by looking at the relationship of offspring migratory activity as measured by nocturnal restlessness to mid-parent (average value for both parents) migratory activity as measured by nocturnal restlessness. **(C)** The axes on the graph indicate the number of half-hour periods of nocturnal restlessness in the offspring and the parents. Panel C adapted from Berthold and Pulido (1994).

set of offspring derived from parents with known migratory behavior. They reared these offspring to adulthood and measured their migratory behavior in the same way. To estimate the heritability of this behavioral trait, the researchers plotted the nocturnal restlessness of the offspring birds as a function of the average nocturnal restlessness of their parents (**Figure 9.41C**). The heritability estimate is simply the *regression coefficient*—that is, the slope of the best-fit line—between the offspring and the parental average.

Using the parent–offspring regression technique, Berthold and Pulido estimated the narrow-sense heritability to be 0.453 ± 0.080. Because this value is significantly higher than zero, Berthold and Pulido were able to conclude that migratory behavior in the blackcap warbler is heritable, and indeed strongly so. These findings suggest that migratory patterns could change rapidly by natural selection, even over the course of a few generations. This is consistent with recent observations that the migratory patterns of this species have already begun to shift, perhaps in response to warmer winter temperatures in Europe.

KEYCONCEPT QUESTION

9.3 Berthold and Pulido hand-raised both the first and second generations of blackcap warblers. Why was it important for the estimate of heritability that they not allow the parents to raise the second generation of birds themselves?

The Selection Differential and the Response to Selection

When studying the evolution of a quantitative character, we need a way of measuring the strength of selection on the trait. The simplest approach is to use the concept of the **selection differential (*S*)**. The selection differential S is defined as the difference between the mean trait value of the individuals who successfully contribute to the next generation and the mean trait value of all individuals in the population. We also want a way to measure the consequences of selection. Here, we can measure what is called the **selection response (*R*)**. The selection response R is defined as the difference between the mean trait value of the offspring population and the mean trait value of the parental population (**Figure 9.42**).

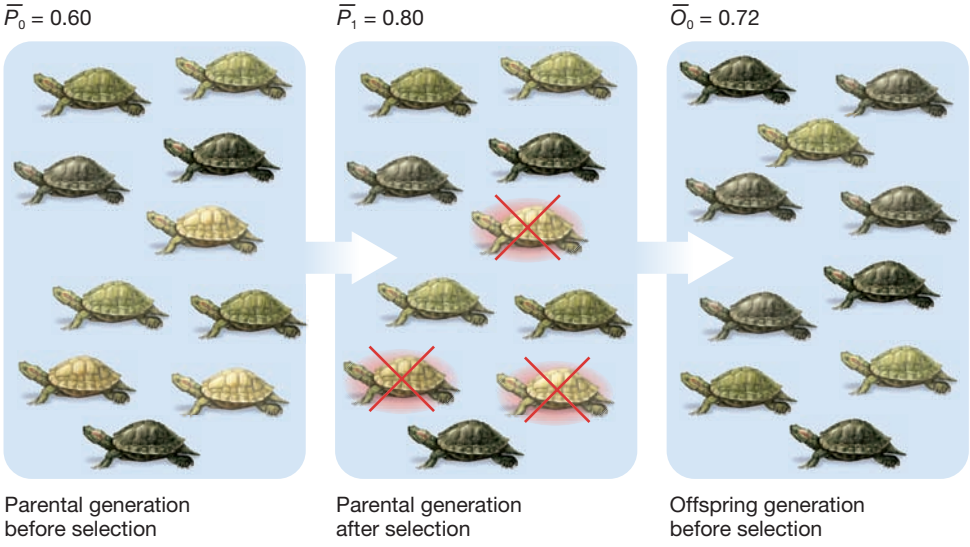


FIGURE 9.42 Calculating the selection differential and selection response. Here, a population of turtles differs in a quantitative trait, shell color, which can take on any value from light (0.0) to dark (1.0). In this example, selection favors darker-colored turtles, and most of the light-colored individuals in the parental generation die before reproducing. We calculate the selection differential S as the difference between the mean trait value of individuals in the parental generation who survive to reproduce and that of all individuals in the parental generation, whether they survive or not. In this example, $S = \overline{P}_1 - \overline{P}_0 = 0.20$. We calculate the selection response R as the difference between the mean trait value of individuals in the offspring generation prior to selection and the mean trait value of individuals in the parental generation prior to selection. In this example, $R = \overline{O}_0 - \overline{P}_0 = 0.12$.

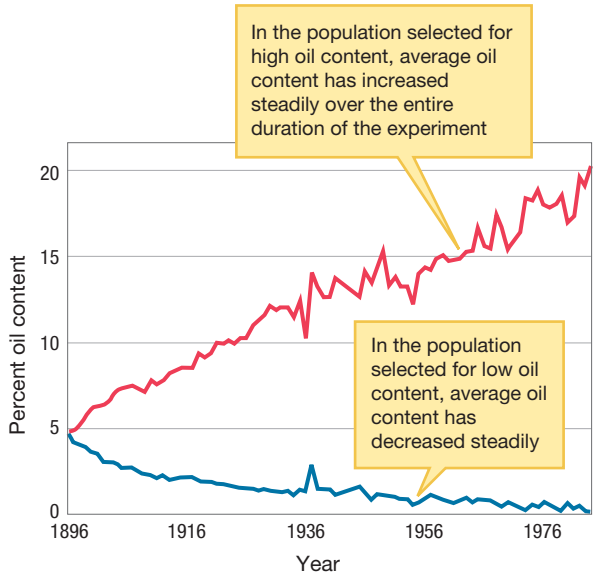


FIGURE 9.43 Long-term phenotypic response in the Illinois Long-Term Selection Experiment on Corn. Truncation in this experiment was relatively severe: Only the top 20% of the high oil content line was used to seed the next generation of the high oil content line, and only the bottom 20% of the low oil content line was used to seed the next generation of the low oil content line. The response of each line, over the subsequent century, is shown. Adapted from IDEALS (2011).

We are now in a position to write an expression for the mean trait value—that is, the average phenotype—of a continuously valued trait that changes over time as a consequence of natural selection. This expression is called the **breeder's equation**, which relates the narrow-sense heritability, the strength of selection measured as S, and the consequences of selection measured as R:

$$R = h^2 S \tag{9.13}$$

This simple equation predicts evolutionary change for quantitative traits. For example, suppose we select on fruit size in tomatoes, and the narrow-sense heritability of this trait is $h^2 = 0.5$. If the fruits from plants that we allow to reproduce are, on average, 2 grams heavier than the population mean, we can use the breeder's equation $R = h^2 S$ to predict the fruit size in the offspring population. In our case, $h^2 = 0.5$ and $S = 2$ grams, so $R = (0.5)\ (2\ \text{grams}) = 1$ gram. We would therefore expect the offspring generation to have a mean fruit size that is 1 gram heavier than that of the (preselection) parental generation.

KEYCONCEPT QUESTION

9.4 Based on the definition of narrow-sense heritability, what is the range of possible values that h^2 can take? Could the application of the breeder's equation ever lead one to *estimate* a narrow-sense heritability outside of this range? Why or why not?

Quantitative Genetic Analysis of an Artificial Selection Study

To see how quantitative genetics can be applied to understand the process and consequences of selection on a quantitative trait, we will look at the longest-running selection experiment in crop plants, the Illinois Long-Term Selection Experiment on Corn (*Zea mays*) (Moose et al. 2004). This study, parts of which are still running today, was initiated in 1896 by C. G. Hopkins and has operated continually since that time, except for a 3-year interruption during World War II.

The Illinois study is a long-running truncation selection experiment, so named because it involves truncating, or limiting, a population in terms of which individuals breed and which do not. The aim of this study was to better understand the genetic basis of kernel oil. To do so, the investigators initially set up two different breeding lines from the same starting stock. One line was selected for high oil content, and one was selected for low oil content. The truncation selection regime was relatively severe: Only the top 20% of the high oil content line was used to seed its next generation, and only the bottom 20% of the low oil content line was used to seed its next generation. The response of each line, over the subsequent century, is shown in **Figure 9.43**.

The degree to which phenotypes continued to shift under artificial selection is remarkable. In Section 9.1, we discussed the concept of latent variation and showed how selection could generate phenotypes beyond the range of those observed in

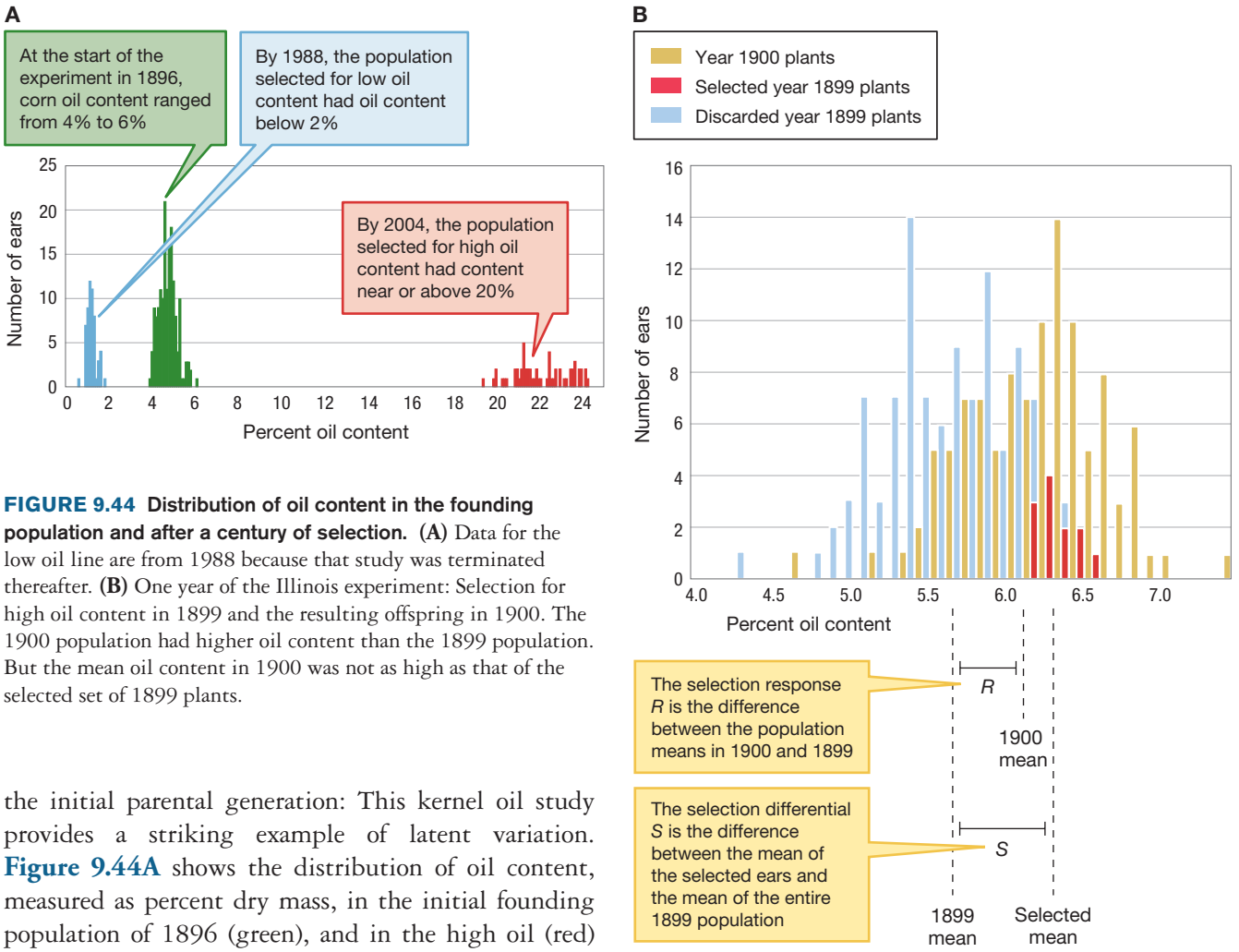


FIGURE 9.44 Distribution of oil content in the founding population and after a century of selection. **(A)** Data for the low oil line are from 1988 because that study was terminated thereafter. **(B)** One year of the Illinois experiment: Selection for high oil content in 1899 and the resulting offspring in 1900. The 1900 population had higher oil content than the 1899 population. But the mean oil content in 1900 was not as high as that of the selected set of 1899 plants.

the initial parental generation: This kernel oil study provides a striking example of latent variation. **Figure 9.44A** shows the distribution of oil content, measured as percent dry mass, in the initial founding population of 1896 (green), and in the high oil (red) and low oil (blue) lines nearly 100 years later. Selection has driven the production of phenotypes far beyond even the most extreme forms present in the founding population. In addition, novel mutations for oil content likely arose and were fixed over the course of the experiment.

In an artificial selection experiment such as this one, the experimenters can directly measure quantitative traits for each individual. Moreover, they know exactly which individuals reproduce and which do not (in **Box 9.4**, we discuss how we can also locate the loci involved when studying quantitative traits). Therefore, we can compute the selection differential S and the selection response R from the results of the experiment (**Figure 9.44B**). Using these values, we can then estimate narrow-sense heritability. For example, in 1899, the mean oil content was 5.65% dry mass, but the mean of the selected plants was 6.30% dry mass. This gives a selection differential of $S = 6.30\% - 5.65\% = 0.65\%$ dry mass. The mean of all plants in 1900 was 6.10% dry mass. This gives a selection response of $R = 6.10\% - 5.65\% = 0.45\%$ dry mass. From the selection response and the selection differential, we can compute the narrow-sense heritability h^2 directly using the breeder's equation (Equation 9.13):

$$h^2 = \frac{R}{S} = \frac{0.45}{0.65} = 0.69$$

BOX 9.4 Mapping Quantitative Trait Loci

It can be difficult to identify the precise loci that are responsible for quantitative traits (so-called **quantitative trait loci**, or **QTLs**), but **QTL mapping** is a powerful way of finding at least the general region of the genome in which quantitative trait loci reside. The idea is that we can use *marker loci* that are easily assayed, but causally unrelated to the trait in question, in order to identify the approximate locations of the unknown alleles that affect the trait of interest. **Figure 9.45** illustrates the basic concept behind the QTL mapping procedure.

Step 1. We typically begin the process by selecting two parental strains that (1) differ considerably in their values of the quantitative trait and (2) differ at a set of marker alleles. Parental strain 1 has a lower distribution of trait values than does strain 2; strain 1 is homozygous for the *A*, *B*, and *C* alleles, while strain 2 is homozygous for the *a*, *b*, and *c* alleles.

Step 2. The next step is to cross these two strains to produce a set of F_1 progeny. If the parents are homozygous at the marker loci, these F_1 progeny will be heterozygous at each marker locus, and typically they will manifest intermediate values of the quantitative trait.

Step 3. The F_1 individuals are then mated to produce an F_2 generation. For the F_2 individuals, we measure (1) the genotypes at the marker loci and (2) the value of the quantitative trait. From this information, we can infer which marker loci are most closely associated with QTLs for the trait in question. The F_2 generation in Figure 9.45 illustrates the basic logic behind this inference. In each frame, the quantitative trait values are plotted with the genotypes sorted according to one of the marker loci. At left in the bottom panel of Figure 9.45, we see a large difference in the quantitative trait values associated with the *AA*, *Aa*, and *aa* genotypes. This does not mean that the *A* marker locus is itself influencing the quantitative trait value, but it does imply that this locus is linked to an important quantitative trait locus. In particular, there appears to be a large positive QTL associated with the *a* allele. At center in the bottom panel of the figure, we see essentially no difference in the quantitative trait values associated with the different genotypes at the *B* locus. Apparently, there are no important QTLs near this *B* marker locus. At right in the bottom panel of the figure, we see a modest association between the value of the quantitative trait and the genotype at the *C* locus, again suggesting the presence of a quantitative trait locus in the vicinity of the *C* marker locus.

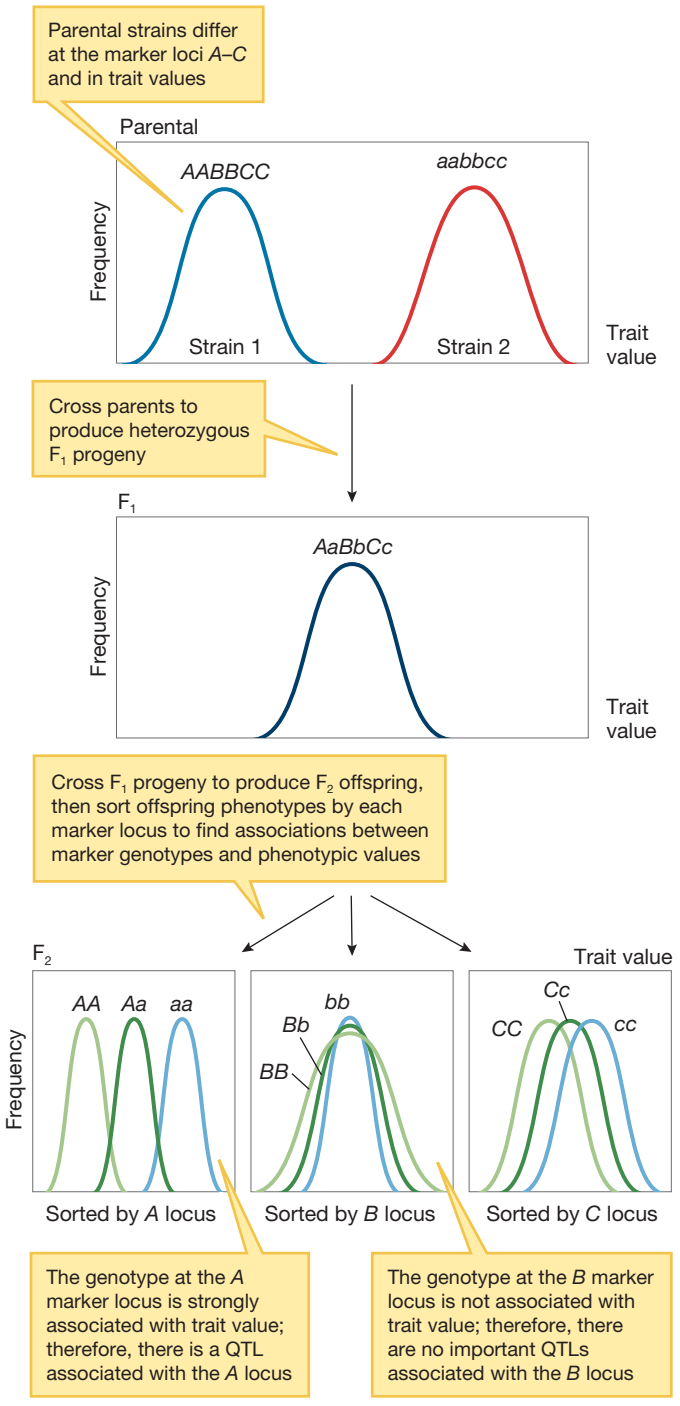


FIGURE 9.45 A schematic diagram of the principle behind the QTL mapping process. Note that in the F_2 generation, the heterozygotes will be twice as common as either homozygote, so the frequency distributions in the figure have been scaled to make each distribution directly comparable.

Heritabilities estimated from the selection differential and the selection response are sometimes referred to as **realized heritabilities**. In any given year, our sample sizes of breeding individuals are relatively small, and thus the heritability estimates are subject to considerable stochastic variation—that is, random fluctuation—from year to year. To deal with this problem, we might want to look at heritabilities over longer periods of time. Strictly speaking, the breeder's equation only holds for a single generation, but we can estimate the heritability over a modest number of generations (say, 10) as the sum of the selection responses in each year divided by the sum of the selection differentials in each year:

$$h^2 \approx \frac{\sum_{i=1}^{n} R_i}{\sum_{i=1}^{n} S_i} \tag{9.14}$$

Figure 9.46 shows the estimated heritability for oil content in the high oil content line of the Illinois experiment. Here, we have used a 10-year window; that is, each heritability estimate is based on the sum of 10 years' selection responses and the sum of 10 years' selection differentials. Heritabilities are initially quite high—approximately 0.4. Over time, heritability declines as some of the genetic variation for oil content is exhausted. Nonetheless, heritability remains above zero even after 100 years of continued directional selection. This suggests that if the experiment is continued, the oil content will continue to increase in response to continued selection.

The fact that the heritability of oil content changes over time highlights an important concept regarding heritability: *Heritability is a statistical property of a population, not a general fact about the genetic basis of a phenotypic trait* (Barton et al. 2007). It is meaningless to say something like "the heritability of seed weight is 0.4" without specifying a population and the associated environmental conditions. This is because one population may have considerable additive genetic variance for seed weight while another has little or none. One population may experience dramatic environmental variation in seed weight (leading to reduced heritability), while another experiences highly uniform environmental conditions, and thus minimal environmental variation. For the same reason, a heritability estimate obtained from one population does not tell us about the heritability of the same trait in another population unless we have other reasons to believe that environmental and genetic variance in the two populations are similar. Finally, it is important to realize that heritability estimates are *within-population measures*, not between-population measures; that is, heritability tells us about the sources of differences *within* a population, but not about the sources of differences between populations. Just because we observe a high heritability for differences within one population, we cannot conclude that differences between this population and another population are also due to genetic factors.

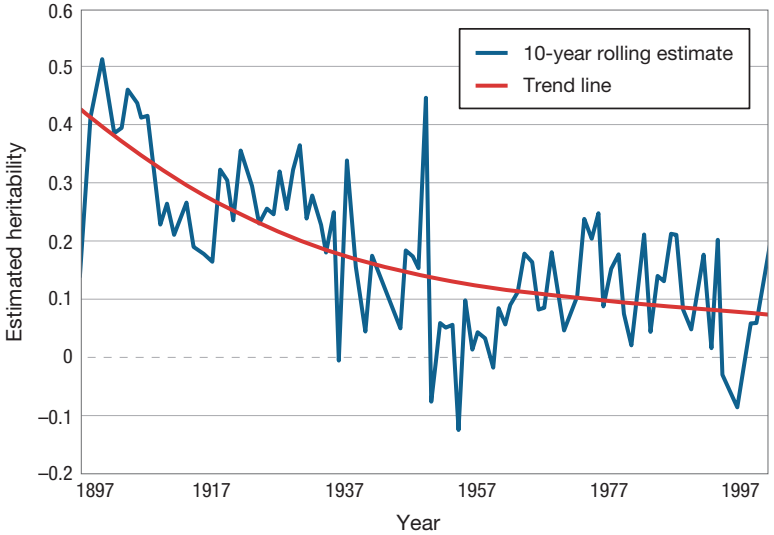


FIGURE 9.46 Estimated heritability of oil content. This graph shows the estimated heritability of oil content in the high oil content line from the Illinois experiment, based on a 10-year window. The red trend line is a cubic best-fit to the data. Heritability declines substantially over the course of the experiment, but it remains nonzero even after 100 years of continued selection.

Quantitative Genetic Analysis of Natural Selection in the Wild

Quantitative genetic tools are also useful for studying natural selection in the wild. By way of illustration, we return to an example from the start of Chapter 3: the drought-induced shift in flowering time of the annual plant *Brassica rapa* in southern California (Franks et al. 2007). In Chapter 3, we described one of the basic qualitative findings of that study: A *Brassica rapa* population sampled from a relatively wet habitat indeed evolved a more rapid flowering time over the period 1997–2004, presumably as a response to the drought of 2000–2004.

Researchers can go beyond simple qualitative assessments of this sort, however, as did Steve Franks and his colleagues. They wanted to determine whether the magnitude of the observed changes in flowering time was consistent with the operation of natural selection, given what could be determined about the genetic variation in the population and the strength of selection on this particular trait. Questions such as this one are not of purely academic interest. Our planet is currently going through a period of rapid climate change, and the ability of plant species to adapt to these ongoing changes in temperature and precipitation will depend on sufficient genetic variation for traits such as reproductive timing.

To address this question, the researchers used a quantitative genetics framework. As we have seen, the breeder's equation allows us to predict the magnitude of evolutionary change in a trait, given (1) the narrow-sense heritability of that trait and (2) the selection differential associated with the trait. Franks and his colleagues were able to measure both of these quantities in a straightforward fashion.

To determine the heritability of flowering time in the initial 1997 population, the researchers raised parent individuals in the greenhouse and recorded their flowering times. Using artificial pollination, they crossed known pairs of parents to produce F_1 offspring. They raised these F_1 offspring from seed in the same greenhouse, and they measured their flowering times. As we have already seen, narrow-sense heritability can be estimated directly from such data: It is the regression coefficient between the offspring flowering time and the average parental flowering time. Franks and his colleagues found that the heritability of flowering time was high in this population: $h^2 = 0.46$.

To determine the selection differential, the researchers first recorded the flowering time of each plant, and subsequently, once the seeds had set, they counted the number of seeds produced. From these data, they could estimate the selection differential. They found that in 2003, the selection differential in this population was −7.67 days; that is, plants that reproduced successfully had flowered, on average, 7.67 days earlier than those that failed to reproduce.

The breeder's equation predicts that in one generation, the change in mean flowering time should be $R = h^2S = (0.46) \times (-7.67 \text{ days}) = -3.53$ days. But in their study, Franks and his colleagues were not comparing flowering times of 2003 plants to flowering times of 2004 plants. They were comparing the flowering times of 1997 plants to 2004 plants; that is, they measured the changes over seven generations. If we assume that the selection response was the same in each year, the model then predicts that the total change in flowering time over the 7-year period should have been $-3.53 \text{ days} \times 7 = -24.7$ days. The plants should have flowered 24.7 days earlier.

In practice, average flowering time shifted by only 8.5 days in this population—still a large amount, but not as large as predicted. What can we make of this? One

conclusion we can draw is that given the heritability of flowering time, selection was more than strong enough to shift flowering times by the 8.5 days observed. The researchers not only observed rapid change in flowering time in response to a multiyear drought, but also were able to show that a response of this magnitude is easily consistent with the operation of natural selection. But why might the observed selection response have been smaller than predicted? Franks and his colleagues suggest that a number of factors may have contributed. Selection for early flowering may have been stronger—and thus the selection differential greater—in the 2003 population that they measured than in the other years between 1997 and 2004. This seems likely, given that the drought began only in 2000. Moreover, there may not have been seven full generations of selection between 1997 and 2004. If some fraction of the seeds remained dormant for one or more years before germinating, this would mean fewer generations of selection. Finally, the heritability estimate $h^2 = 0.46$ was based on studies in the greenhouse, not in the wild. Because environmental variance may be reduced under homogeneous greenhouse conditions, this heritability value may be an overestimate of heritability in the wild.

In this chapter, we have seen how multiple loci interact with one another in the evolutionary process. In the next chapter, we will further expand our view to look at evolution on a genome-wide scale.

SUMMARY

1. Interactions between alleles at different genetic loci play an important role in the evolutionary process.
2. When traits are polygenic—that is, influenced by alleles at multiple loci—Mendelian inheritance can give rise to a near-continuous range of variation.
3. To create population genetic models of evolutionary change at multiple loci, we need to track haplotype frequencies rather than merely tracking allele frequencies.
4. When there are statistical associations between alleles at different loci, we say there is linkage disequilibrium in a population. The magnitude of these associations is quantified by the coefficient of linkage disequilibrium D.
5. Linkage disequilibrium can be created by evolutionary processes, including mutation, selection, migration, and drift. It is broken down over time by genetic recombination.
6. Selection at one locus can influence allele frequency changes at other loci in linkage disequilibrium. In bacteria, we see this manifested as periodic selection when beneficial mutations are scarce and as clonal interference when beneficial mutations are frequent.
7. In the process of genetic hitchhiking, alleles ride to fixation on the coattails of beneficial mutations at nearby loci. In background selection, alleles are dragged to extinction by deleterious mutations at nearby loci.
8. Recent natural selection creates extended haplotype blocks in which linkage disequilibrium has not yet been removed by recombination. These extended haplotype blocks can help us identify not only loci under selection, but also which alleles have been favored at these loci.
9. Genetic draft caused by widespread hitchhiking parallels genetic drift as a source of evolutionary contingency. Both processes drive changes in allele frequency at a locus even without direct selection on that locus, and both reduce genetic variation in a population. But unlike genetic drift, the strength of genetic draft is roughly independent of population size.
10. The adaptive landscape metaphor provides a way to think about how phenotypes or genotypes change over evolutionary time as a consequence of natural selection.
11. Quantitative genetic approaches allow us to model and predict how continuous or quantitative characters change as a result of natural selection.
12. Narrow-sense heritability measures the fraction of phenotypic variation in a population due to the additive genetic variation on which selection can efficiently operate. If we know the narrow-sense heritability of a trait in a population, we can use the breeder's equation to predict how that trait will change in response to natural or artificial selection.

KEY TERMS

adaptive landscape (p. 339)
additive genetic effects (p. 313)
background selection (p. 332)
breeder's equation (p. 352)
broad-sense heritability (H^2) (p. 347)
clonal interference (p. 335)
coefficient of linkage disequilibrium (D) (p. 320)
coupling (p. 321
dominance effects (p. 348)
epistasis (p. 314)
fitness peaks (p. 340)
fitness valleys (p. 340)
genetic draft (p. 334)
genetic hitchhiking (p. 331)
genotype space (p. 342)
haplotype (p. 316)
latent variation (p. 314)
linkage disequilibrium (p. 319)
narrow-sense heritability (h^2) (p. 349)
periodic selection (p. 329)
phenotype space (p. 340)
physical linkage (p. 317)
polygenic traits (p. 312)
QTL mapping (p. 354)
quantitative genetics (p. 345)
quantitative trait loci (QTLs) (p. 354)
realized heritabilities (p. 355)
repulsion (p. 321)
selection differential (S) (p. 351)
selection response (R) (p. 351)
selective sweep (p. 329)

REVIEW QUESTIONS

1. Mendel's examples of inheritance involved transmission of discrete characters such as wrinkled or smooth seed shape, but most phenotypic traits vary continuously, as we easily see with height. How did early population geneticists reconcile these observations?
2. Which provides more information: haplotype frequencies or allele frequencies?
3. For two loci R and S with alternative alleles R/r and S/s, what are the coupling haplotypes? What are the repulsion haplotypes?
4. List five evolutionary processes that can generate linkage disequilibrium and one process that breaks it down.
5. Under what conditions will selection at a locus A drive allele frequency changes at another locus B?
6. Genetic drift and genetic draft are both important sources of evolutionary contingency. Why do you think genetic draft was not discussed with genetic drift in Chapter 8?
7. What does a point in genotype space represent? What does it mean if two points are far apart in genotype space?
8. In a quantitative genetics approach, why do we track the amount of variation in the population as well as the mean phenotype?
9. Which is more useful for predicting evolutionary change: broad-sense heritability or narrow-sense heritability?
10. Suppose you find that the narrow-sense heritability of tail length is 0.5 in a Washington State population of bald eagles. What can you conclude about the narrow-sense heritability of tail length in a separate Alaskan population of the same species?

KEY CONCEPT APPLICATION QUESTIONS

11. In a randomly mating population of fence lizards, haplotype frequencies at the A and B loci are as follows: 60% AB, 10% aB, 10% Ab, 20% ab.
    a. Calculate the coefficient of linkage disequilibrium in this population.
    b. From your answer, can you infer whether the A and B loci are on the same chromosome?
    c. If the population had the same allele frequencies but no linkage disequilibrium, what would be the frequency of the AB haplotype?
12. In a very large randomly mating population of mice, haplotype frequencies for the AB, Ab, aB, and ab genotypes are 0.1, 0.4, 0.3, and 0.2, respectively. Compute the coefficient of linkage disequilibrium D in this population. If Hardy–Weinberg assumptions are met and the recombination rate between these two loci is $R = 0.2$ per generation, what will the coefficient of linkage disequilibrium be one generation later? Three generations later?
13. To estimate the broad-sense heritability of chirping rate in crickets, researchers create a set of inbred lines. They

find that, at 68°F, the variance in chirping rate within inbred lines is 2 (chirps/minute)^2, and the variance between individuals taken from different inbred lines is 10 (chirps/minute)^2. Estimate the broad-sense heritability of chirping rate from these data.

14. a. Design an experiment in which you are able to estimate the narrow-sense heritability of flower size in an annual plant, without selecting on flower size or any other trait.

    b. Design an experiment in which you can estimate the narrow-sense heritability of bristle number in the fruit fly *Drosophila melanogaster*, in which you do not need to know which offspring come from which parent.

15. Which has a higher value, the broad-sense heritability or the narrow-sense heritability?

16. A professor collects a small sample of *Drosophila melanogaster* from her field site, an apple orchard in central Michigan. She brings them back to the lab and starts a colony from these initial stocks. After nurturing the colony under carefully controlled conditions in the lab for many generations, she then measures the narrow-sense heritability of bristle number and finds a value of $h^2 = 0.3$. Thinking about the definition of narrow-sense heritability, her student Jacques predicts that the narrow-sense heritability of bristle number will be lower at the field site. Present an argument that Jacques could make to support his prediction.

17. Anna, another student in the lab from question 17, disagrees with Jacques. Thinking back to her reading on genetic drift, she predicts that the narrow-sense heritability of bristle number will be higher at the field site. Provide an argument that Anna could make to support her prediction.

SUGGESTED READINGS

Andersson, D. I., and D. Hughes. 2010. Antibiotic resistance and its cost: Is it possible to reverse resistance? *Nature Reviews Microbiology* 8: 260–271. This review looks at the reasons why antibiotic resistance does not readily disappear after antibiotic use is halted. In doing so, it provides a number of good examples of how evolution operates on multiple interacting loci.

Berthold, P., and F. Pulido. 1994. Heritability of migration activity in a natural bird population. *Proceedings of the Royal Society B: Biological Sciences* 257: 311–315. This paper presents the study of the heritability of migration behavior that we described in this chapter.

Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America* 104: 1278–1282. This paper describes the clever study on *Brassica rapa* flowering time that we introduced in Chapter 3 and treated in further detail here.

Lang, G. I., D. P. Rice, M. J. Hickman, E. Sodergren, G. M. Weinstock, D. Botstein, and M. M. Desai. 2013. Pervasive genetic hitchhiking and clonal interference in forty evolving yeast populations. *Nature* 500: 571–574. We used this impressive experimental study to illustrate the concept of clonal selection.

10

Genome Evolution

◀ The colors of the Grand Prismatic Spring in Yellowstone National Park are formed by cyanobacteria and other heat-tolerant bacterial species that live in the scalding water. The hot spring takes its name from the order of its colors: from blue through to red like the light scattered from a prism.

Long before biologists had cause even to dream of whole-genome sequencing, researchers were already asking questions about the evolution of genomes and making comparisons of genomic data. Perhaps most notably, they were comparing the absolute sizes of genomes from species across the tree of life. Beginning in the 1950s, researchers compared genome size by measuring the amount of DNA per cell—originally called the C-value—across numerous species (Mirsky and Ris 1951). The results of such comparisons were surprising and decidedly counterintuitive. Researchers found that genome sizes vary by more than 100,000-fold across living organisms and by more than 10,000-fold even among the eukaryotes (**Figure 10.1**).

Yet, more perplexingly, as new genetic techniques were developed to estimate the number of genes in a genome, researchers discovered that differences in genome size do not correlate in any straightforward way with the number of protein-coding genes that an organism has, nor with its phenotypic complexity. This observation, known as the **C-value paradox**, or C-value enigma, was profoundly puzzling. If an organism's genes are

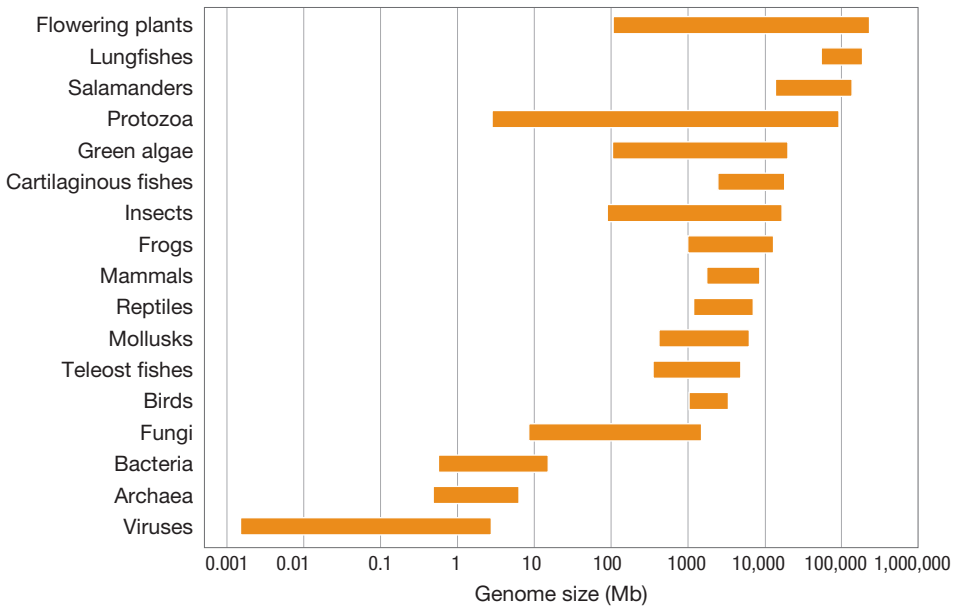


FIGURE 10.1 Genome size varies widely across the tree of life. Genome sizes are measured in millions of base pairs, called *megabases* (Mb). The data are displayed here on a logarithmic scale. Thus, the largest genomes (lungfishes, some flowering plants) are more than 100,000 times the size of the smallest cellular genomes (archaea) and nearly 10,000,000 times the size of the smallest viral genomes. Adapted from Gregory (2011).

encoded in its DNA, why is there scant correlation between the number of genes and the amount of DNA in a genome? Why should lungfish require 40 times as much genetic information as humans? Why would a single-celled amoeba have a genome that is 1000 times the size of the genome of a complex multicellular puffer fish?

We will address those questions and numerous others in this chapter. In Chapters 7–9, we explored the field of population genetics, which concentrates on evolutionary processes operating at a single locus or at sets of loci. With the advent of whole-genome sequencing, evolutionary biologists now have a rich set of tools for studying population genetics at the genome-wide scale and for exploring how entire genomes evolve over time. This is the field of **evolutionary genomics**.

A major theme of this chapter is that genome structure arises from a combination of selective and nonselective processes. Whereas many facets of genome organization may be explained by natural selection operating at the organismal level, others involve natural selection operating on "selfish" genetic elements within the genome, sometimes at a fitness detriment to the organism itself. Neutral or nearly neutral processes also play major roles in structuring genomes.

In this chapter, we present an overview of evolutionary genomics. We will

- Begin with a look at the development of whole-genome sequencing technology.
- Explore the evolution of genome size as a way to build up our intuition for thinking about how genomes evolve.
- Examine the genome structure and composition of viruses, prokaryotes, and eukaryotes and how they are fashioned by a combination of selective and nonselective processes.

Because the study of genome evolution is so young, open questions and unsolved problems still outnumber the resolved issues. As a result, this chapter is more descriptive than many of the others in this book. Rather than provide the last

word on genome evolution, however, our aim here is to provide an overview of the patterns found in the genomes of organisms from viruses to vertebrates and to discuss the various genome-scale processes that may be involved in generating these patterns. In many cases, the relative importance of these processes remains to be seen, and some processes may still be undiscovered. It is an exciting time to be working on evolutionary genomics.

10.1 Whole-Genome Sequencing

Genetic sequencing is unquestionably one of the most important technological achievements of the past half-century: It has had an enormous impact on nearly every area of the life sciences. In 1976, only a decade after Marshall Niremberg and many others deciphered the genetic code by mapping each RNA codon to the amino acid that it specifies, Walter Fiers and his colleagues reported the entire genome sequence for the **bacteriophage** MS2, an RNA virus that targets bacteria. This was the first whole-genome sequence for any microbe (Fiers et al. 1976). In 1977, Fred Sanger and his colleagues sequenced all 5386 base pairs of the genome of the bacteriophage ϕX174 (Sanger et al. 1978). This was the first entire DNA genome ever to be sequenced, a feat possible at that time only because of the exceptionally small size of the genome of this phage.

Even very small bacteria have genomes several orders of magnitude larger than the genomes of bacteriophages. Thus, it was another 18 years before researchers at Johns Hopkins University sequenced the entire genome of a bacterium, *Haemophilus influenzae.* In a 1995 paper, they reported this accomplishment, which was the first whole-genome sequence obtained for an independently living organism (**Figure 10.2**) (Fleischmann et al. 1995). A year later, the first genome sequence of a eukaryote was released: Researchers had sequenced the 12-million-base-pair (that is, 12-megabase [12-Mb]) genome of the yeast *Saccharomyces cerevisiae* (Goffeau et al. 1996). In 1998, the first genome of a multicellular organism, *Caenorhabditis elegans*, was published (*C. elegans* Sequencing Consortium 1998), and in 2001, the initial draft of the human genome was released (International Human Genome Sequencing Consortium 2001). The final draft was completed in 2003.

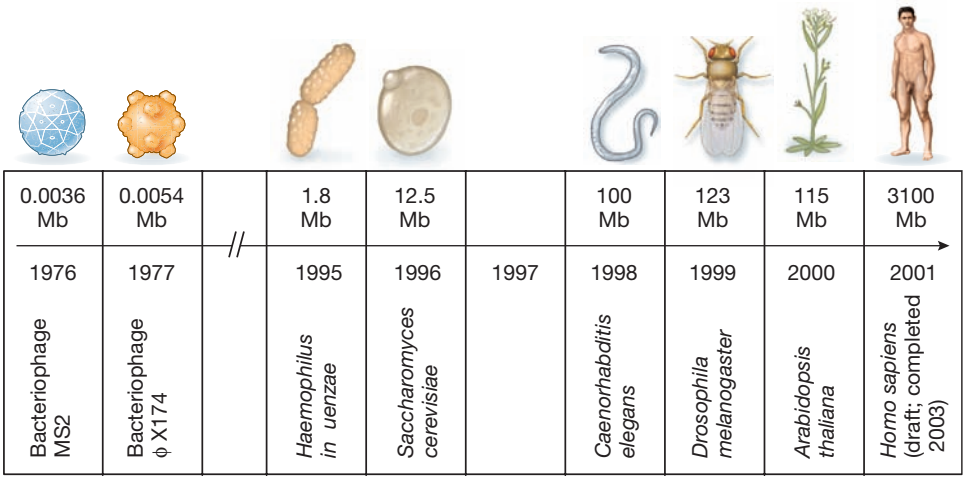


FIGURE 10.2 Some landmark genome sequencing projects. The numbers below each organism indicate the size of its genome in megabases (Mb).

Today, genome sequencing is commonplace in many areas of biological research. Costs have plummeted: Sequencing the first human genome cost roughly $3 billion; in 2008, sequencing of an additional human genome cost about $10 million; and by 2014, a human genome could be sequenced for about $5000 (Hayden 2014). Whole-genome sequences have been reported for more than 500 eukaryotic species and more than 17,000 prokaryotes (Azvolinsky 2014). Multiple genomes from a single species—such as the 1092 human genomes sequenced as part of an international collaboration called the 1000 Genomes Project—offer an unprecedented view of genomic variation (1000 Genomes Project Consortium 2012). Generating genomic sequence data is no longer the primary challenge. Now, the most pressing problems in genomics involve finding the best ways to organize and make use of the vast quantities of data we are obtaining.

10.2 Resolving the Paradoxes of Genome Size

In the introduction to this chapter, we posed the C-value paradox: Why is there such enormous variation in genome size, and why does organismal complexity not correlate well with genome size? The discovery of **noncoding DNA**—segments of genetic sequence that do not expressly specify a product such as a protein or ribosomal RNA—was a major step toward resolving this paradox. In organisms with small genomes—viruses, bacteria, prokaryotes, and even unicellular eukaryotes—the majority of the genome is made up of coding DNA. By contrast, organisms with large genomes, such as animals and land plants, have only a small fraction of the total genome devoted to coding sequence (**Figure 10.3**). The remainder is made up of noncoding DNA of various types. With this observation in hand, our previous question—why a lungfish would require 40 times as much genetic information as a human—becomes easier to resolve. The answer is that the lungfish does not require 40 times as much information; it just happens to have a genome that is 40 times bigger than the human genome, with most of that difference due to extra noncoding DNA.

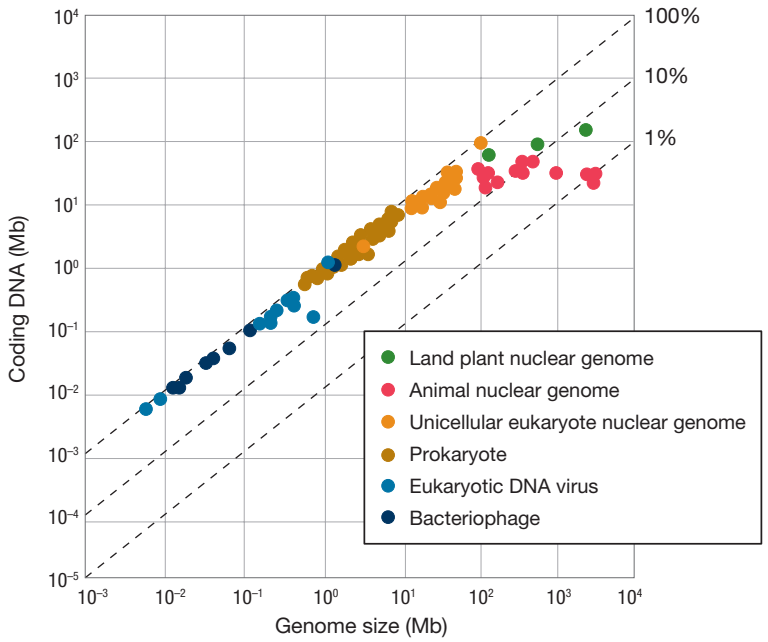


FIGURE 10.3 Coding DNA versus total genome size. Whereas smaller genomes consist largely of coding DNA, many larger genomes are made up mostly of noncoding DNA. Here, the number of coding base pairs is plotted against the total number of base pairs in the genome for organisms ranging from viruses to animals, with both axes on a logarithmic scale. The dashed lines indicate the fraction of the genome composed of coding DNA. In viruses, nearly 100% of the genome is coding sequence. In many prokaryotes and unicellular eukaryotes, the coding fraction drops below 50%. In land plants and in animals, the fraction drops further, to below 1% in some organisms. Adapted from Lynch (2007).

However, the mysteries of genome size are not entirely solved by the simple observation that genome size differences result largely from differences in the quantity of noncoding DNA. We would also like to explain the causes of the variation that we observe. Why do some species have vastly larger genomes than others, despite similar degrees of apparent phenotypic complexity?

A number of hypotheses have been proposed regarding possible mechanisms that determine genome size. One possibility is that changes in genome size are favored because of their structural effects on the size of the nuclear envelope, the volume of the cell, and other aspects of cell physiology (Cavalier-Smith 1978). One inescapable

consequence of large genome size is that larger genomes require larger cell nuclei, and thus larger cells. **Figure 10.4** shows the relationship between cell volume and genome size. Cell volume influences a number of aspects of phenotype that are relevant to fitness, including rate of cell division, metabolic efficiency, rates of protein and ion exchange, and, in many taxa, overall body size. This association between C-value and cell size may be one of the important drivers of selection on genome size (Gregory 2001)—but it is unclear that there has been sufficient individual-level selection on these factors to account for the enormous genome size differences.

A second hypothesis was proposed in 1980 in a pair of back-to-back papers published in the journal *Nature* (Doolittle and Sapienza 1980; Orgel and Crick 1980). This view holds that genome size is the result of a balance between two types of processes. On the one hand, proliferation of self-replicating genetic units such as **transposable elements** (or transposons)—small genetic elements capable either of catalyzing their own movement within the genome or of moving with the assistance of other transposable elements—may drive an increase in genome size over time. On the other hand, selection for replication speed, small cell size, and energetic efficiency may favor reductions in genome size. Different species face different ecological challenges—some may need cells that can divide very quickly, while others may not. Similarly, different species bring different evolutionary histories with them. For example, prokaryotic chromosomes have only a single *origin of replication* (ORI) from which DNA synthesis is initiated, so larger prokaryotic

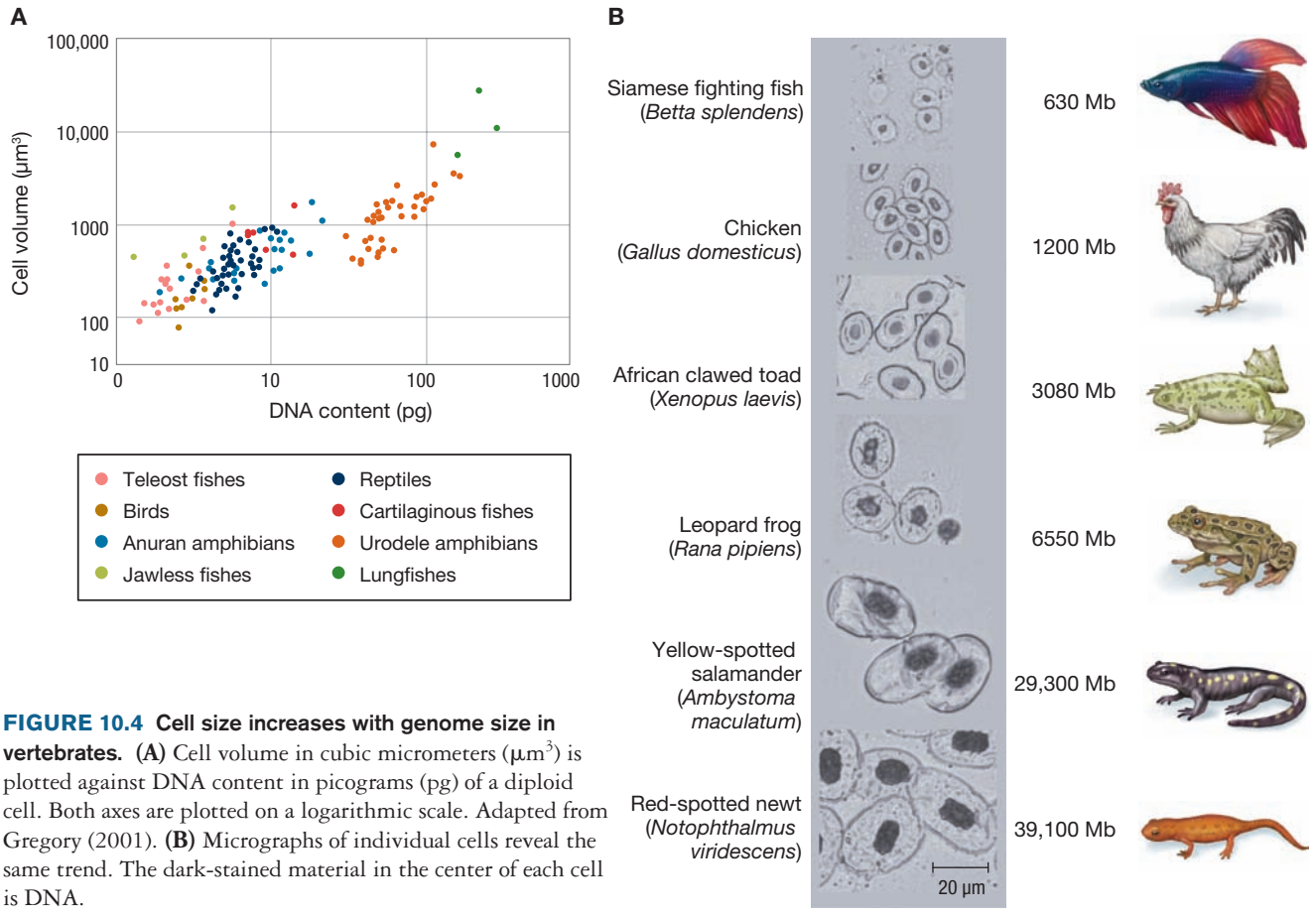


FIGURE 10.4 Cell size increases with genome size in vertebrates. **(A)** Cell volume in cubic micrometers (μm^3) is plotted against DNA content in picograms (pg) of a diploid cell. Both axes are plotted on a logarithmic scale. Adapted from Gregory (2001). **(B)** Micrographs of individual cells reveal the same trend. The dark-stained material in the center of each cell is DNA.

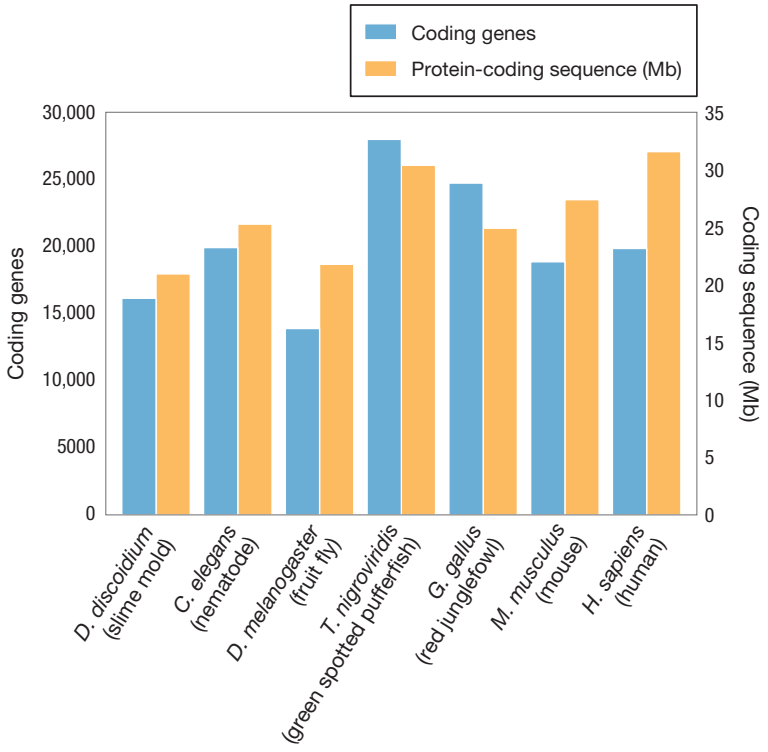


FIGURE 10.5 The G-value paradox. The number of protein-coding genes and the total amount of coding DNA do not scale in any obvious way with organismal complexity. Adapted from Taft et al. (2007).

genomes take proportionally longer to copy. This may be a critical consideration given that many prokaryotes are under strong selection for replication speed. In eukaryotes, some species such as those in the genus *Drosophila* have large numbers of active transposons in their genomes; others, such as humans, may have a history of transposon accumulation but relatively few active transposons in their current genome. As a result, processes driving genome expansion and genome reduction will balance one another in different ways in different species, leading to the broad variation in C-values that we observe across taxa.

Evolutionary biologist Michael Lynch has elaborated on this latter hypothesis. Recall from Chapter 8 that the strength of natural selection to eliminate deleterious mutations and to fix beneficial ones depends on the population size. As the population size grows larger, natural selection can operate effectively on smaller and smaller fitness differences. This may contribute to a general trend for prokaryotes to have smaller genome sizes than those of unicellular eukaryotes, which in turn tend to have smaller genome sizes than those of large multicellular eukaryotes. Larger organisms, because they tend to have smaller population sizes, will be less able to eliminate mildly deleterious variants that result from a minor increase in noncoding DNA. According to this view, the expanded genome sizes of eukaryotes result from nonadaptive processes at the organismal level. Nonetheless, the additional genetic material may be co-opted in any number of ways, allowing the subsequent evolution by natural selection of complex genome organization in eukaryotes (Lynch and Conery 2003; Lynch 2007).

The G-Value Paradox

Resolving the C-value paradox gives rise to a new puzzle, sometimes known as the **G-value paradox**. The G-value paradox states that the number of protein-coding genes—sometimes called the G-value—does not scale with organismal complexity. **Figure 10.5** illustrates this for seven fully sequenced model organisms. Surprisingly, slime molds have more protein-coding genes than do insects, and nematodes have approximately the same number of protein-coding genes as humans, despite a 300-fold difference in genome sizes and a large apparent difference in complexity.

How can this be? Why do more complex organisms not necessarily require more protein-coding genes? Although there is a great deal left to learn about this problem, one general principle appears to be emerging as an important component of the answer (Szathmary et al. 2001; Hahn and Wray 2002; Wray et al. 2003). In short, what matters more than the absolute number of genes is the complexity of the underlying gene regulatory network that generates the phenotype. Organisms

with similar numbers of genes can have very different gene regulatory network structures.

For example, **transcription factors** play a very important role in gene regulation. Transcription factors are proteins that bind to specific regions of DNA in order to regulate when, where, and to what degree specific genes are expressed. Despite similar numbers of protein-coding genes, the nematode, fruit fly, and human genomes have very different numbers of transcription factors: approximately 500, 700, and 2000, respectively (Szathmary et al. 2001; Tupler et al. 2001). Because transcription factors often act on one another, these differences can translate into even bigger differences in regulatory complexity. If transcription factors were to operate in pairs, then humans, who have 4 times as many such proteins as nematodes do, would have approximately 4^2, or 16, times as many possible combinations of transcription factor proteins. If transcription factors were to operate in trios, that ratio would be 4^3, or 64, times as many. Although the networks of interactions among transcription factors are obviously more complicated than simple pairwise or three-way interactions, the same general principles of scaling probably apply.

The noncoding regions of the genome are also becoming increasingly appreciated for the role they play in gene regulation. Rather than acting merely as useless repositories of "junk DNA," these noncoding regions are extensively transcribed, and their RNA products, particularly microRNAs (miRNAs), are heavily involved in regulating gene expression (Mattick et al. 2010; Berezikov 2011). Even supposedly "selfish" genetic elements such as the transposons that make up nearly half of the human genome have been co-opted to serve important regulatory functions (Feschotte 2008). Thus, organisms with larger noncoding genomic regions may have a larger number of regulatory elements that are involved in specifying complex patterns of development.

A third contributing factor is that one protein-coding gene does not necessarily correspond to one protein. Through the process of alternative splicing, a single gene with multiple exons can be spliced in a number of different ways to produce a variety of protein products. For example, humans have more alternatively spliced genes and more introns per gene (allowing a greater range of alternative splicing products) than do nematodes. In addition to alternative splicing, various forms of posttranscriptional modification—alterations made to newly transcribed RNA—can potentially increase the diversity of an organism's functional protein products (Hahn and Wray 2002).

Together, these observations lead us to at least a partial resolution of the G-value paradox. Complexity depends less on how many genes a species has than on how those genes are connected. The total number of coding genes in a genome matters less than the complexity of the regulatory network through which those coding genes interact.

10.3 Content and Structure of Viral Genomes

As we have just noted, genome sizes differ in part because the genomes of different taxonomic groups are made up of different types of genomic elements. In this and the two subsequent sections, we look at the components of viral, prokaryotic, and eukaryotic genomes in turn. We will look at what sorts of genetic elements are present,

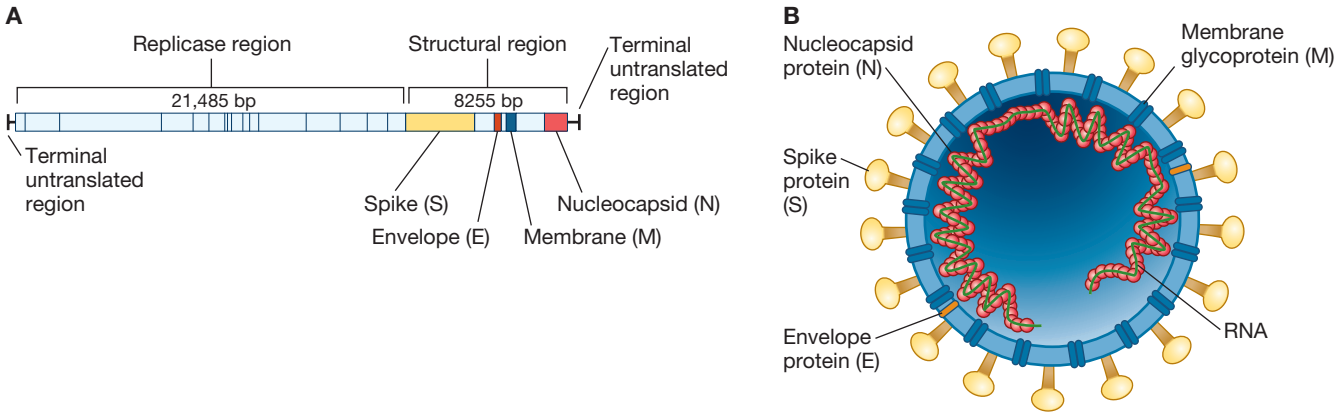


FIGURE 10.6 The SARS coronavirus. The SARS coronavirus, responsible for the 2002–2003 SARS epidemic, is a single-stranded positive-sense RNA virus with a genome composed of a single linear chromosome. **(A)** This diagram is a simplified map of the SARS genome showing the replicase region with the genes involved in genome replication and the structural region with the genes responsible for the structural proteins that the virus requires. **(B)** This virus diagram shows the structural components corresponding to the genes in the map. Adapted from Stadler et al. (2003).

and in what quantities, in genomes of different types. We will look at how these elements are arranged spatially within the genome. And we will look at the processes, selective or otherwise, that have shaped the content and organization of genomes. We begin in this section with the viruses. The primary characteristics of their genomes are that they are *diverse* and they are *compact*. We will treat these features in turn.

In comparison with cellular organisms, viruses have remarkably diverse genomic structures. Even the genetic material itself varies among viruses. Whereas many viruses have DNA-based genomes as do prokaryotes and eukaryotes, many others have RNA-based genomes. Some viruses, known as retroviruses, are capable of reverse transcription, in which DNA is synthesized from an RNA template. Many retroviruses, including the human immunodeficiency virus (HIV), use that capability to integrate their own genomes into the host's chromosome. Both DNA-based and RNA-based genomes may be either double- or single-stranded. Single-stranded RNA viruses may be either *positive-sense* viruses, in which case the genome is effectively the same as the viral mRNA, or *negative-sense* viruses, in which case the genome is complementary to the viral mRNA. Viral genomes may consist of a single linear chromosome, a set of linear chromosomes (in which case we refer to the genome as being *segmented*), or a circular chromosome. Most viruses are haploid in that the viral capsule carries a single copy of the viral genome, but some retroviruses including human immunodeficiency virus (HIV) are diploid in the sense that the capsule contains two copies of the single-stranded RNA genome. These diploid viruses can undergo recombination and thereby rapidly generate additional genetic diversity (Burke 1997). **Figures 10.6**, **10.7**, and **10.8** illustrate a number of different viral genome structures.

The remarkable diversity that viruses exhibit in something so fundamental as their genetic material suggests that they may be evolutionarily very ancient. This inference is supported by the fact that many viral genes lack *orthologs*—genes shared by common ancestry—in cellular life-forms. These observations have led some researchers to speculate that early in the history of life, a "virus world" may have preceded the origins of the cellular life-forms we see today, and that modern viruses may represent a continuation of certain lineages from that early virus world (Koonin et al. 2006; Koonin and Dolja 2012).

Viral genomes tend to be extremely compact (Carter and Saunders 2007). One reason is that many—although not all—viruses have RNA-based rather than DNA-based genomes. RNA is structurally more fragile than DNA, and it is also subject to higher mutation rates: These factors severely limit the maximum possible size of RNA-based genomes. The SARS coronavirus shown in Figure 10.6 is at the upper end of the RNA virus size range, with a 30-kilobase (kb) genome. As a result, RNA viruses typically encode only a few proteins, as illustrated in Figure 10.7.

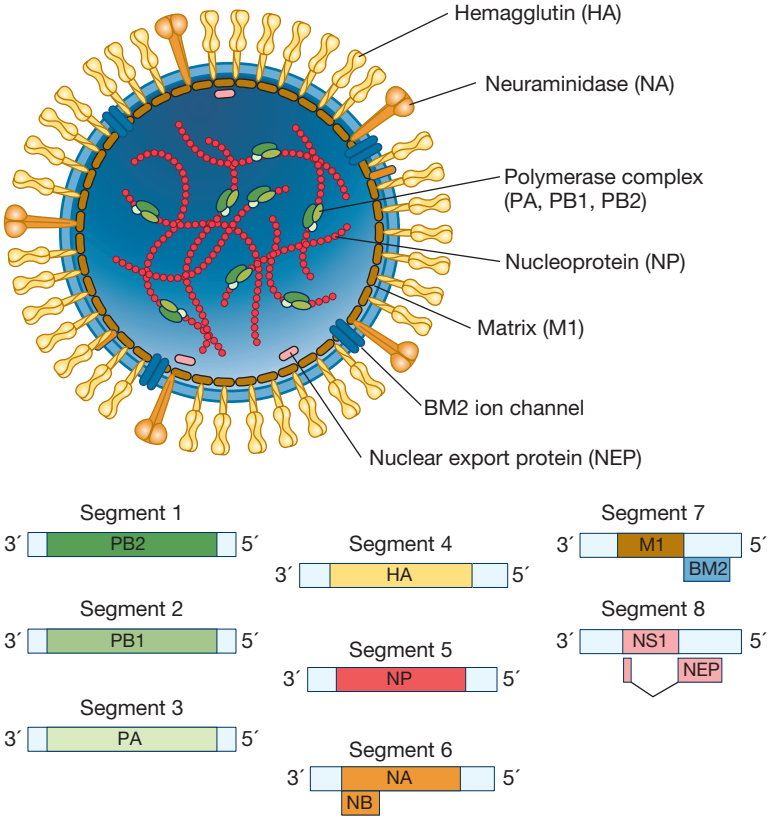


FIGURE 10.7 The influenza virus. The influenza B virus is a single-stranded negative-sense RNA virus of approximately 13,600 nucleotides in length. Its genome is segmented into 8 linear chromosomes encoding its 11 protein-coding genes. Some genes partially overlap other genes; these are indicated below the appropriate segment. Color coding indicates the correspondence between structural proteins in the virus and their respective protein-coding genes in the genome.

KEYCONCEPT QUESTION

10.1 Given the genome structures pictured in Figures 10.7 and 10.8, would you expect to see greater linkage disequilibrium between the HA and NA loci of the influenza virus or between the S and C loci of the hepatitis B virus? Explain.

DNA-based viruses can be much larger than RNA viruses, up to a megabase in length, but even this is relatively small in comparison with all eukaryotes and the vast majority of prokaryotes. One reason that even DNA viruses are relatively small is that most viruses undergo strong natural selection for rapid replication. The shorter the genome, the faster it can be copied. Another reason may involve natural selection on physical size. Viruses need to replicate many times within a host cell, so their capsules must be very small—and this in turn constrains the amount of genetic material that can be packaged within them (Cann 2005).

Because viral genomes are under such strong selection for reduced size, most of a viral genome consists of a protein-coding sequence, with terminal untranslated regions in the linear genomes. One of the most remarkable aspects of viral genomes

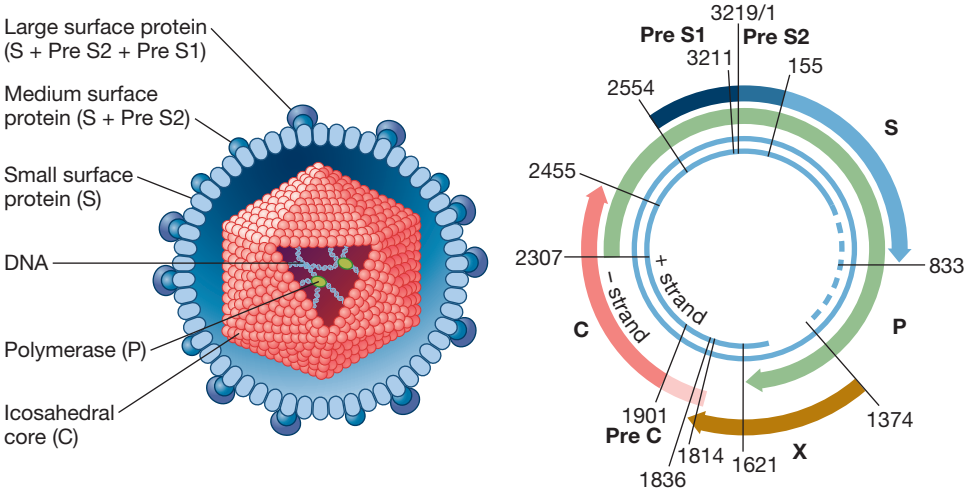


FIGURE 10.8 The hepatitis B virus. The hepatitis B virus (HBV) (left) has a very small, circular, double-stranded DNA genome of approximately 3.3 kb (right). The inner rings represent the DNA chromosome, with the numbers indicating the number of bases from the origin of replication. As shown by the dashed line, the positive strand is incomplete in the virus capsule but is completed by synthesis from the negative strand after infection. The colored arrows indicate the location and direction of each gene in the genome. Note the substantial overlap of different genes. Adapted from Park et al. (2006).

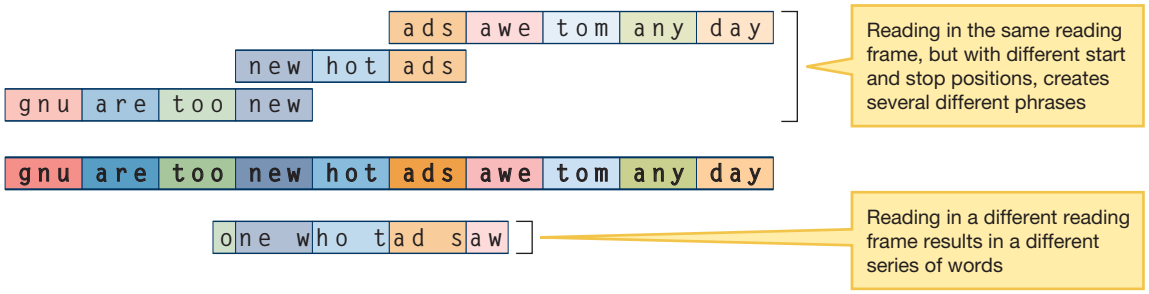


FIGURE 10.9 Two kinds of overlapping code. Here, a string of three-letter words represents a set of nucleotide triplets. From the basic string "gnu are too new hot ads awe tom any day," we can get multiple messages in two different ways. We can read in the same frame, but start and stop in different places. Alternatively, we can read in a different frame. Viruses employ both methods of coding for multiple proteins using a single region of the genome.

is the tremendous degree of compression achieved (Chirico et al. 2010). Multiple genes may be packed into a single region in two different ways: (1) in the same reading frame but only partially overlapping or (2) with different reading frames (**Figure 10.9**). The hepatitis B virus (HBV) in Figure 10.8 uses both methods. In HBV, the three different surface antigen proteins, Pre S1, Pre S2, and S, are all derived from a single gene (bases 2554–833) with different ATG start codons but a shared stop codon. In this case, all three proteins are produced by reading in the same reading frame; they just start in different places.

Even more remarkably, as seen in Figure 10.8, the entire S region overlaps with the longer polymerase gene P (positions 2307–1621), but their reading frames are offset by one base pair. If read in one frame, the polymerase is encoded; if read in the other, the surface antigens are produced! Because the reading frames are shifted by one nucleotide, the overlapping regions produce different sequences of amino acids. Similarly, two different core C proteins are produced from another gene with two start sites, and again the gene overlaps with a section of the polymerase gene (Zaaijer et al. 2007).

10.4 Content and Structure of Bacterial and Archaeal Genomes

Recall that there are three main branches to the tree of life: the domains Bacteria, Archaea, and Eukaryota. Extensive comparison of genetic sequences and biochemical pathways has revealed that the archaea are phylogenetically closer to the eukaryotes than to the bacteria. Despite this phylogenetic divide, archaeal and bacterial genomes have evolved to have qualitatively similar structures and organization (for a discussion of the differences, see Karlin et al. 2005). Thus, we will consider prokaryotic genomes—bacterial and archaeal—jointly in this section of the chapter.

One of the most important determinants of organization and content for prokaryotic genomes is the prevalence of **mobile genetic elements** such as plasmids, prophages, and transposons (Touchon et al. 2014). These elements move freely within and between genomes, lending fluidity to prokaryotic genome structure and shuttling important functional genes across species boundaries.

Most prokaryotic genomes are structured as a single circular chromosome, present in a single copy per cell. **Figure 10.10** illustrates this type of organization, using as an example the genome of the pathogenic *Escherichia coli* strain O157:H7. There are exceptions, however. Some bacteria have more than one circular chromosome. Others, such as species of *Borrelia* and *Streptomyces*, have a linear chromosome.

Although the genomes of bacteria and archaea tend to be larger than those of all but the very largest viruses, they remain relatively compact and indeed are smaller

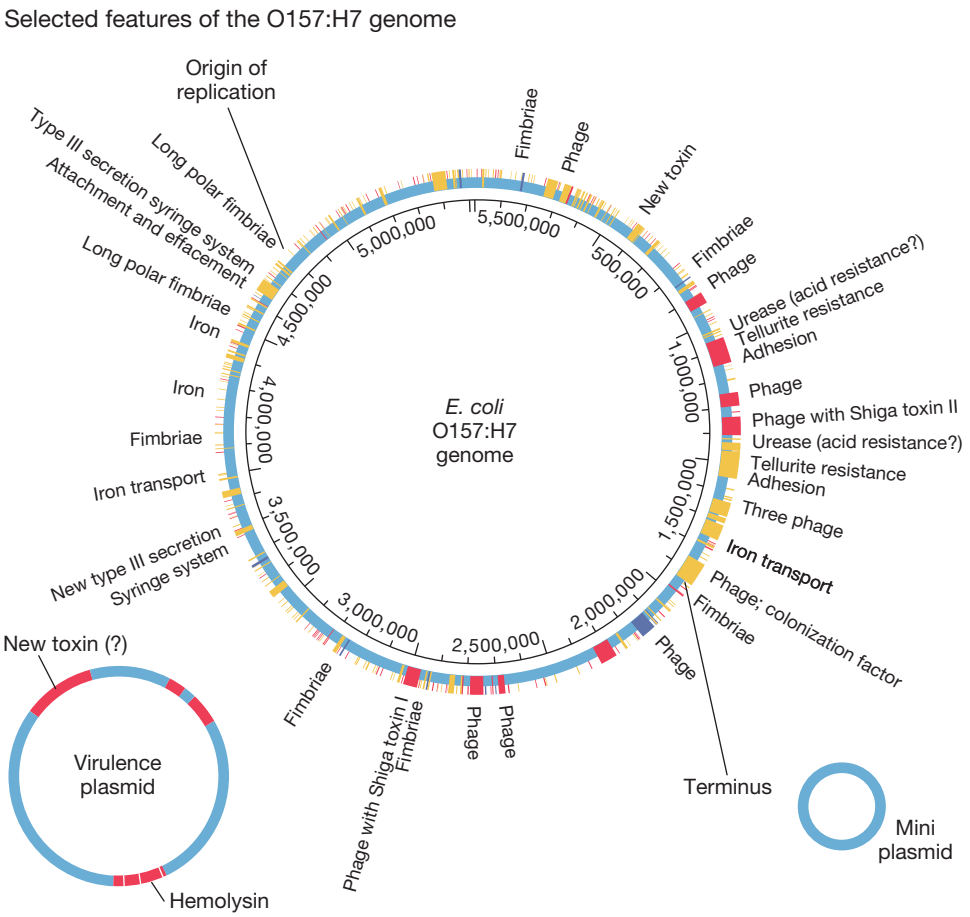


FIGURE 10.10 The *E. coli* O157:H7 genome. Like most bacteria, *E. coli* O157:H7 has a single circular chromosome. In addition, the strain carries two additional small DNA molecules: a virulence plasmid and a second miniplasmid. Here, the virulence genes are shown in red. Other genome islands—horizontally acquired genes—are shown in yellow. Adapted from Genome Center of Wisconsin (2011).

than those of all but the smallest eukaryotes. Typically, a prokaryotic genome consists of 85% to 95% protein-coding sequence. Intergenic regions—the stretches of DNA between genes—are minimal. In *E. coli*, for example, adjacent protein-coding genes are commonly separated by roughly 100 base pairs. Many of the prominent elements of eukaryotic genomes are absent or rare: Bacteria do not have the spliceosomal introns that are so prevalent in eukaryotes (see Section 10.5). The introns that are found in prokaryotic genomes tend to be located in ribosomal RNA (rRNA) or transfer RNA (tRNA) and are self-splicing. Prokaryotic genomes do have pseudogenes—recall that these are nonfunctional and typically untranslated sequences of DNA—although they tend to be less common in prokaryotes than in eukaryotes. Presumably, pseudogenes in prokaryotes are formed less often and are lost more rapidly than those in eukaryotes. Finally, although transposable elements are found in prokaryotes, they typically make up only a small fraction of the genome. In a few cases, however, that fraction can exceed 10% (Gregory and DeSalle 2005). Because prokaryotic genomes are mostly made up of coding DNA, there is a tight correlation between genome size and number of genes in these organisms, as shown in **Figure 10.11**.

Bacterial genomes often include DNA from bacteriophages, which are viruses that can insert their genomes into bacterial chromosomes. An inserted genome, known as a **prophage**, can remain latent for many generations before springing back into action and spurring the production of virus particles. Prophage DNA can make up 10% or more of the bacterial genome. Most of these prophages are no longer functional because of the accumulation of mutations that have eliminated

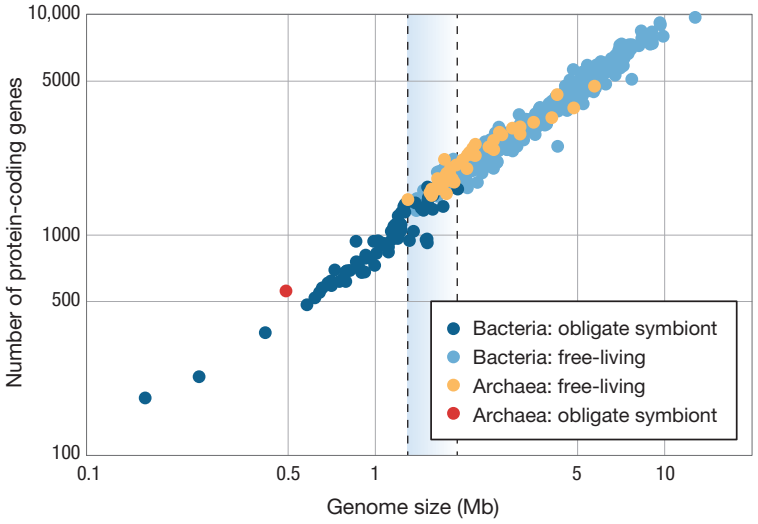


FIGURE 10.11 Prokaryotic genome size and the number of protein-coding genes. In bacteria and archaea, the number of protein-coding genes increases approximately linearly with genome size. Among bacteria, free-living species or facultative symbiont species (light blue) generally have larger genomes than obligate symbionts and parasites (dark blue). Free-living archaea (orange) tend to have genomes similar in size to the smaller free-living bacteria, though one obligate symbiont has a much smaller genome. Shading indicates the transition between symbiont and free-living species. Adapted from Podar et al. (2008).

their ability to replicate independently, but new prophages are readily incorporated into the genome. Over time, the breakdown and loss of old prophages and the introduction of new ones contributes to genomic differences not only between species, but even between strains of the same bacterial species. **Figure 10.12** shows how prophage DNA occurs in different places in three different strains of the human pathogen *Streptococcus pyogenes*.

Prophages often encode **virulence factors**, which are specialized genes that assist bacteria in exploiting eukaryotic hosts by aiding colonization, producing toxins, entering host cells, and evading immune responses (Wagner and Waldor 2002). For example, the Shiga toxins of the pathogen *E. coli* O157:H7 are encoded on prophages (O'Brien et al. 1984). When these prophages remain active, they can directly transmit Shiga toxins to new strains of bacteria. Evolutionarily, it remains unclear why virulence factors are so commonly encoded on prophage. One possibility is that the bacteriophages, which can often survive longer and in harsher environments than the bacteria they infect, serve to provide a stable long-term reservoir for the associated virulence genes (Muniesa et al. 1999).

In addition to a main chromosome, many prokaryotes carry one or more nonessential DNA molecules known as **plasmids** (**Figure 10.13A**). These accessory elements are usually circular in shape and may be present in sigle or multiple copies within the cell. Plasmids often code for genes that are usefull to their bacterial hosts in specific ecological circumstances, such as genes for resistance to antibiotics or heavy metals (**Figure 10.13B**). Plasmids vary considerably in size. Many code for only a few genes (or even none); others code for hundreds of genes, and some even exceed the size of smaller bacterial chromosomes (Smillie et al. 2010).

Horizontal Gene Transfer and Prokaryote Genomes

Horizontal gene transfer (HGT), also known as *lateral gene transfer*, is an important source of genetic variation for microbes. Both bacteriophages and plasmids are common vehicles of horizontal gene transfer, which involves the movement of genetic material from one organism to another by one of three processes: transduction, transformation, or conjugation (**Figure 10.14**).

FIGURE 10.12 Prophage DNA in three strains of *Streptococcus pyogenes*. Three different strains of *S. pyogenes* have prophages incorporated in different positions around the chromosome. Genetically similar prophages are shown in the same color, revealing that these three strains share many of the same prophages. However, these prophages have entered the genomes of each strain in separate insertion events at different genomic positions. Adapted from Canchaya et al. (2003).

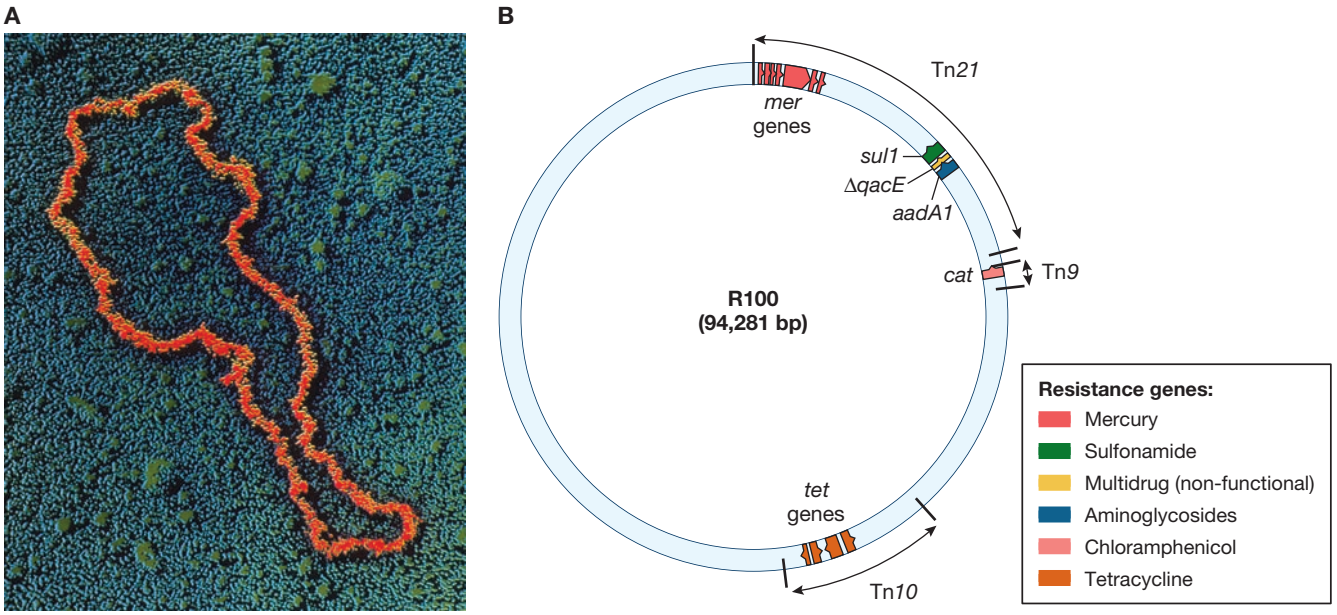


FIGURE 10.13 Bacterial plasmids. **(A)** An electron micrograph of a bacterial plasmid reveals that it forms a closed loop. **(B)** A genetic map of the antibiotic-resistance plasmid R100. Note that the genes conferring resistance are themselves located within transposons on the plasmid. These transposons are indicated as Tn*9*, Tn*10*, and Tn*21* on the diagram. Other regions of the plasmid genome predominantly include genes involved in plasmid replication and conjugation. Panel B from Nikaido (2009).

Transduction occurs when a phage packages bacterial DNA instead of its own within its capsule. When such a phage infects a new host, it injects the bacterial DNA into that host, where it can be incorporated into the genome by homologous or nonhomologous recombination.

In the process of **transformation**, a cell takes up double-stranded DNA—such as that released when other cells die—from the environment (Dubnau 1999). This DNA can subsequently be incorporated into the genome by recombination. Some species, including the human pathogens *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*, are naturally *competent*; that is, they have active mechanisms for acquiring DNA by transformation. Many naturally competent species also have mechanisms to make sure that they only take up DNA from members of the same species. Microbiologists have proposed several possible functions for competence, including (1) the acquisition of nucleotides as "food," (2) the use of acquired DNA in the process of DNA repair, and (3) the generation of variability. At present, the relative importance of each explanation remains unresolved.

In the process of **conjugation**, a plasmid is passed from a donor cell to a recipient cell. A donor bacterium creates hairlike *conjugative pili* that pull a recipient bacterium close, and then it opens up a *conjugative junction* between the two cells through which a copy of the plasmid is transferred. Both structures can be seen in **Figure 10.15**: The dark pili surround the cell on the left, and the conjugative junction joins the two cells. *Conjugative plasmids* encode all of the genes necessary for carrying out the conjugation process. Other *nonconjugative plasmids* do not encode this machinery and can only undergo horizontal gene transfer when they are facilitated by the presence of a conjugative plasmid in the same cell. Conjugation is not reciprocal: The donor passes a plasmid to the recipient but receives nothing in return.

KEYCONCEPT QUESTION

10.2 Which of the three processes—transduction, transformation, and conjugation—is least likely to have evolved by natural selection for its present purpose? That is, which of these processes is least likely to be an adaptation?

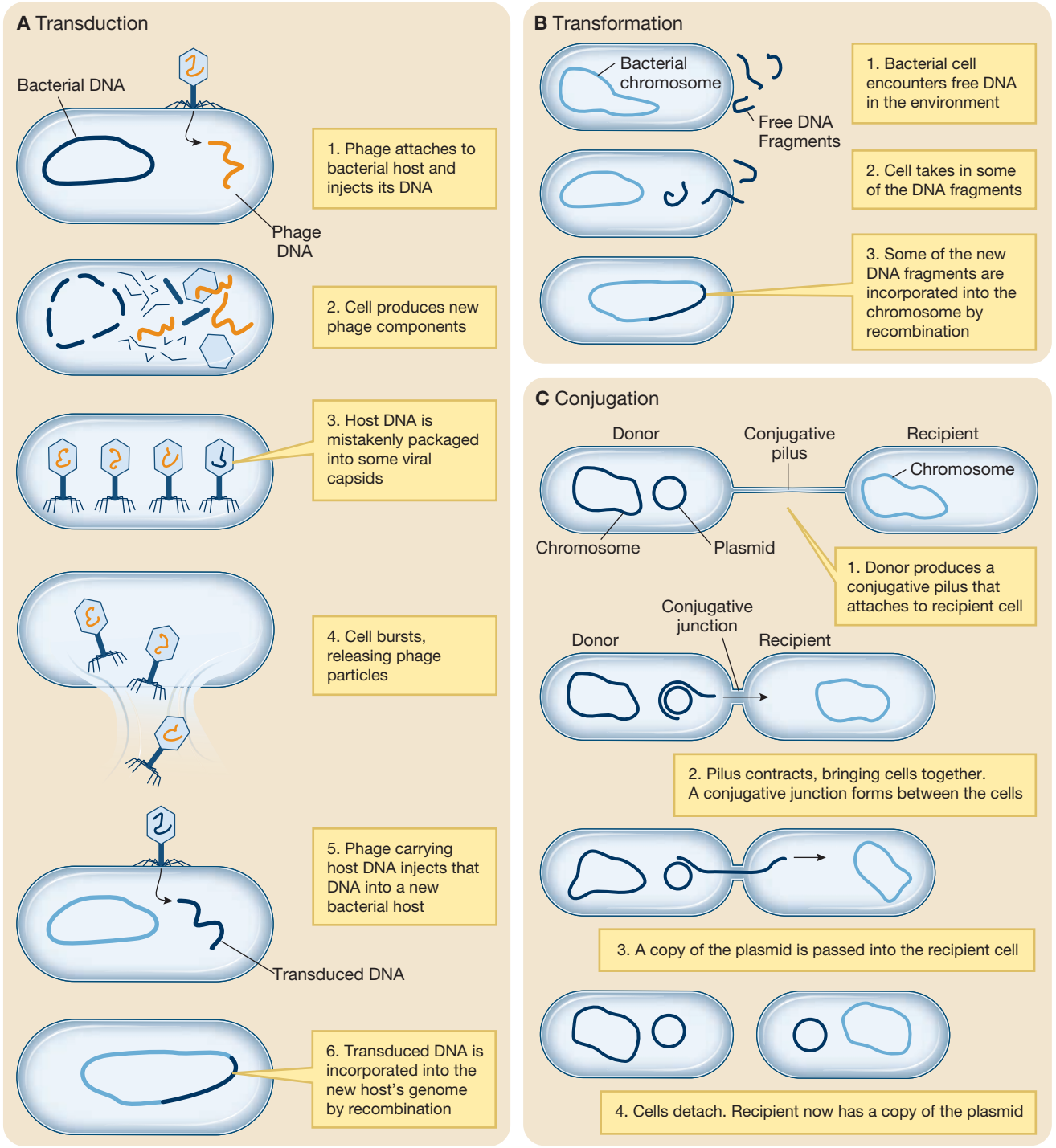


FIGURE 10.14 Three modes of horizontal gene transfer. Horizontal gene transfer occurs by the processes of **(A)** transduction, **(B)** transformation, and **(C)** conjugation.

Although horizontal gene transfer is sometimes referred to as "bacterial sex," the process is very different from sexual reproduction. First, although horizontal gene transfer is most common between individuals of the same or closely related species, bacteria are by no means limited by species boundaries when exchanging genes horizontally. In fact, genes have been transferred horizontally not only between different species but also between members of completely different domains of life, such as the transfer from prokaryotes to eukaryotes (Woese et al. 2000; Koonin et al. 2001; Thomas and Nielsen 2005). Second, genetic exchange in bacteria is

decoupled from the process of reproduction. In bacteria, genetic exchange occurs far less frequently than cell division.

A bacterium that acquires novel genetic material through horizontal gene transfer can incorporate these novel alleles into the chromosome by exchanging them with homologous alleles through the process of *homologous recombination*. The rate of such exchange varies by several orders of magnitude across bacterial species (Vos and Didelot 2009). The common model species *E. Coli* has a relatively low rate of homologous recombination, and the focus on this species perhaps delayed evolutionary biologists in appreciating the importance of recombination in bacteria.

Genetic exchange may be infrequent in bacteria relative to that in multicellular eukaryotes, but exchange need not be frequent to have a major impact on the evolutionary process (Didelot and Maiden 2010). Even a limited amount of genetic exchange can go a long way toward breaking up linkage disequilibrium. Some species, such as the causative agent of gonorrhea, *Neisseria gonorrhoeae*, have such frequent recombination that they show almost no linkage disequilibrium and appear, from a population genetic perspective, quite similar to sexual species (Feil et al. 2001). Others, including *E. coli*, exhibit extensive linkage disequilibrium—and thus exhibit periodic selection, clonal interference, and the other linkage-driven dynamics that we discussed in Chapter 9. In general, species with more rapid recombination, more mixing among subpopulations, and more stable population dynamics will exhibit less linkage disequilibrium (Maynard Smith et al. 1993).

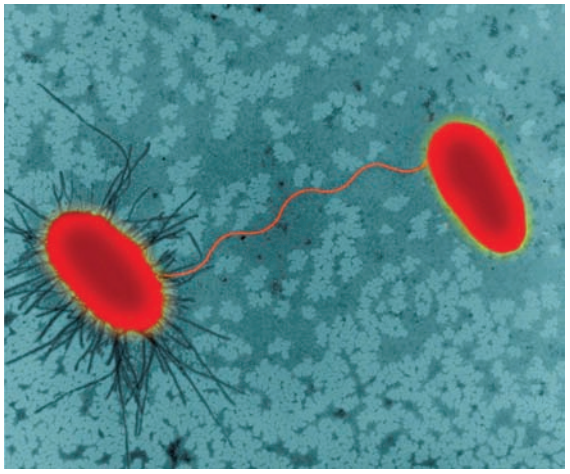


FIGURE 10.15 *E. coli* during conjugation. In the process of conjugation, a plasmid is passed from a donor cell to a recipient cell. Here, we see *E. coli* conjugation. A donor cell (left) creates hairlike pili that pull a recipient bacterium close, and then it opens up a conjugative junction between the two cells through which a copy of the plasmid is transferred.

Horizontal gene transfer among microbes can radically change the ecology of bacterial strains—and this can have dramatic health consequences for their human hosts. For example, *E. coli* K-12 is a harmless enteric strain that resides in the human gut. But the closely related strain of *E. coli* known as O157:H7 is a pathogen, and one that is potentially life threatening in humans. Often acquired by consuming undercooked beef, *E. coli* O157:H7 causes bloody diarrhea and, in some cases, hemolytic uremic syndrome, which leads to kidney failure. A comparison of the two strains suggests that many of the virulence genes (shown in red in Figure 10.10) that make *E. coli* O157:H7 a human pathogen were obtained via horizontal gene transfer (Perna et al. 2001).

Horizontal gene transfer is intriguing not only for its consequences—delivering important new genes and clusters of genes—but also as a selected trait in its own right. Why would natural selection favor the ability to engage in horizontal gene transfer? There are potential costs of "accepting" genetic material from other cells—particularly cells of a species that is only distantly related to the recipient cell. The genes of the donor species would have evolved in a different genetic background and would have been selected to work in a different cellular environment. Moreover, the donor species itself may have been exposed to very different selective conditions over evolutionary time. Such genetic material, even if beneficial to the donor species in its environment, may reduce the fitness of recipient cells that live in a different environment. Indeed, evidence suggests that, over time, natural selection has favored cells that more finely control the circumstances under which HGT occurs (Pal et al. 2005; Thomas and Nielsen 2005). Perhaps worst of all, the new genetic material could encode a prophage—a bacterial virus in latent form that could later be expressed and kill the cell.

Despite the costs and risks, HGT can also have beneficial consequences. Sometimes, genes obtained via HGT will increase the fitness of recipient cells and hence be favored by natural selection—which means that HGT is a contributor

to important evolutionary and developmental change (Ochman and Moran 2001; Yanai et al. 2002; Koonin 2003a). When organisms can receive new genes or groups of genes, new evolutionary pathways can emerge all at once. This process can lead to increasingly complex cellular life-forms that are better able to survive and reproduce in the environments in which they live. This process is especially true when the genes transferred are associated with one or more modular functions (Woese 2000, 2002), by which we mean some function that is not extensively integrated with other functions in a cell. Many fundamental cell functions are tightly integrated within the cell and are not likely to be replaced by horizontal gene transfer (Woese 2002). For example, genes associated with glycolysis—the process in which sugars are broken down and converted into energy—are tightly integrated with other genes and seldom appear to be taken up by cells via HGT (Pal et al. 2005). But other functions are easily decoupled from other cellular processes, and these are likely to undergo horizontal transfer. For example, *pathogenicity islands* are horizontally transferred stretches of DNA ranging in length from tens to hundreds of kilobases that encode suites of genes necessary to convert a bacterium from a commensal to a pathogenic lifestyle (Hacker and Kaper 2000).

It is difficult to overstate the importance of horizontal gene transfer in bacterial evolution. Recall that natural selection requires a supply of variation on which to act: This is what HGT supplies at a large scale for bacteria. If useful genetic variants arise anywhere in the bacterial world, the variants can be and often are transferred into other species by HGT. As a result, the supply of genetic variation available to a species such as the human gut microbe *Enterococcus faecalis* is not limited to the variation currently present in *E. faecalis*, but rather includes much of the variation in the entire bacterial domain. When humans developed the antibiotic vancomycin and thus imposed positive selection for vancomycin resistance on *E. faecalis*, the evolution of vancomycin resistance did not occur from scratch by de novo mutation. Instead, *E. faecalis* acquired genes for vancomycin resistance by horizontal gene transfer from soil microbes (D'Costa et al. 2006). Because these genes are found even in 30,000-year-old bacterial samples taken from permafrost, we know that the resistance alleles currently causing so many problems in clinical medicine have been around for far longer than we have been using antibiotics (D'Costa et al. 2011).

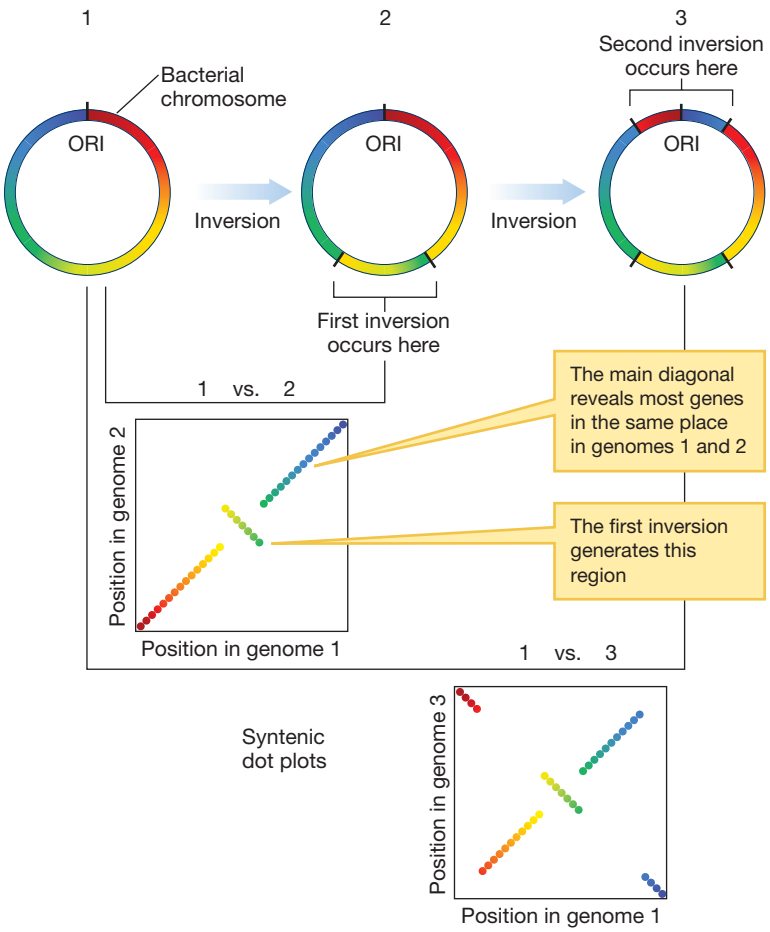


FIGURE 10.16 Syntenic dot plots. The arrangement of loci within bacterial genomes shifts rapidly over evolutionary time. Syntenic dot plots compare the gene order of two different strains or species, providing a picture of the genomic reorganization that has occurred. Here, we have two syntenic dot plots, one comparing genome 1 with genome 2, and one comparing genome 1 with genome 3. Each dot in the plot shows the relative position of a gene within the genome. ORI, origin of replication.

Gene Order in Prokaryotes

In large part due to rampant horizontal gene transfer, the arrangement of loci within bacterial genomes shifts rapidly on an evolutionary timescale. To get a picture of these changes, *syntenic dot plots* are useful tools for comparing the gene order of two different strains or species (**Figure 10.16**). They provide us with a picture

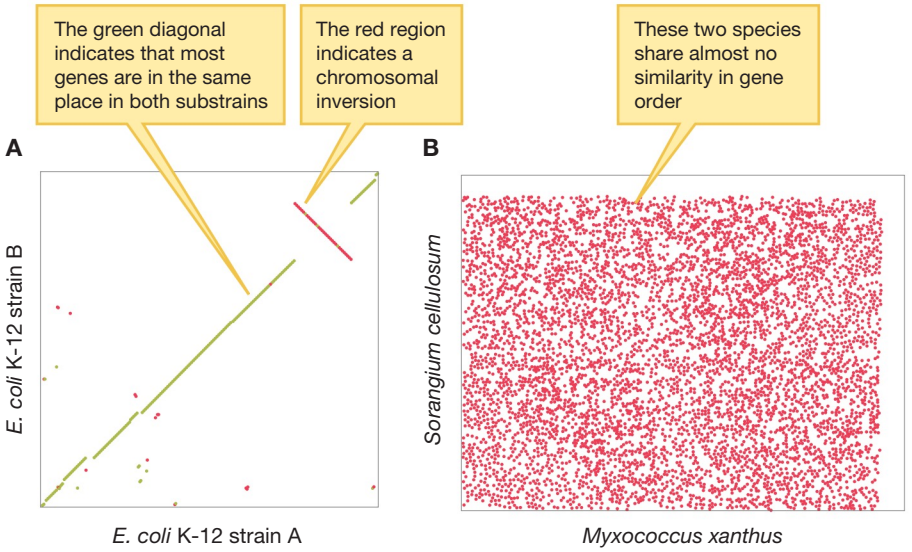


FIGURE 10.17 Comparing gene order across strains. A syntenic dot plot indicates the relative positions along the chromosome of homologous genes in two genomes. **(A)** Two closely related substrains of the K-12 strain of *E. coli*. The green diagonal shows that most genes are in the same place in both substrains; the red line with the opposite slope indicates a chromosomal inversion along one of the two lineages. Adapted from CoGePedia (2009a). **(B)** Even among closely related species pairs, similarity in gene order can be lost because of continual genomic reorganization. Here, we see a dot plot for two myxobacterium species: *Myxococcus xanthus* and *Sorangium cellulosum*. Essentially all similarity in gene order has disappeared. Adapted from Schneiker et al. (2007).

of the genomic reorganization that has occurred. In doing so, they allow us to deduce the translocations, inversions, deletions, and other genomic events that have occurred over evolutionary time since the divergence of the organisms in question. To create a syntenic dot plot comparing the genomes of two species, researchers choose one of the two organisms as a reference; this organism's genes are then represented from left to right along the x axis. The position of each homologous gene in the second organism is then plotted on the y axis. If no genetic rearrangement has occurred, the gene positions will form an unbroken band along the $45°$ ($x = y$) line. Other events have other characteristic patterns, as shown in Figure 10.16. Because most bacteria have circular chromosomes, gene position is typically plotted in the clockwise direction beginning at the origin of DNA replication.

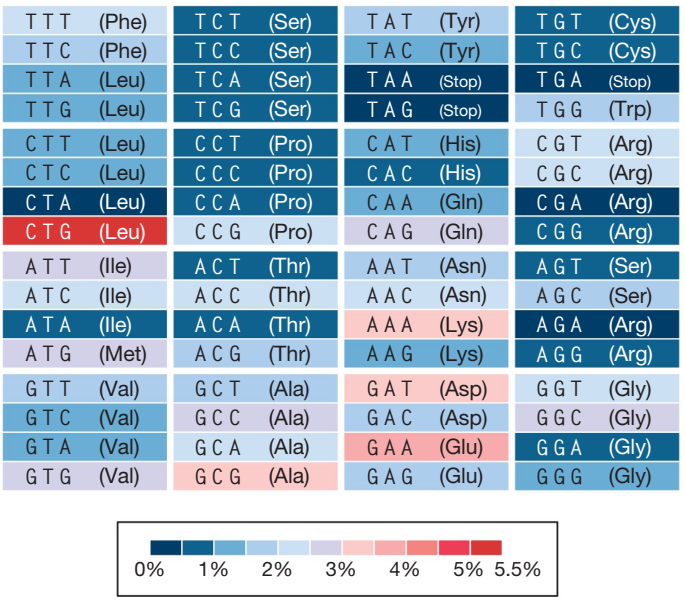
Syntenic dot plots for bacterial genomes reveal extremely rapid change in genome structure in prokaryotes. **Figure 10.17A** compares two closely related substrains of *E. coli* K-12. In the figure we see a single major inversion (red), but otherwise highly similar gene order (green). However, similarities in gene order can break down entirely in even closely related species (**Figure 10.17B**).

Codon Usage Bias

The genetic code is degenerate: As we saw in Chapter 6, most amino acids are coded by several different codon triplets. But in prokaryotes and eukaryotes alike, the various triplets encoding a given amino acid tend not to be equally common in a given organism's genome. Rather, when we look at the protein-coding regions within a genome, we observe **codon usage bias**, in which some codons occur more frequently than others that specify the same amino acid. Different species have different codon usage patterns: **Figure 10.18** shows codon usage in the *E. coli* O157:H7 genome.

Why do we see codon usage bias? Both nonselective and selective processes are likely involved. One nonselective process that contributes to this bias is mutation itself.

FIGURE 10.18 Codon usage in the *E. coli* genome. Synonymous codons are used at widely differing rates. For example, the CTG leucine codon is approximately 13 times as prevalent as the CTA leucine codon. Data are from the Kazura Codon Usage Database at http://www.kazusa.or.jp/codon/. The presentation is adapted for *E. coli* from Warnecke and Hurst (2011).



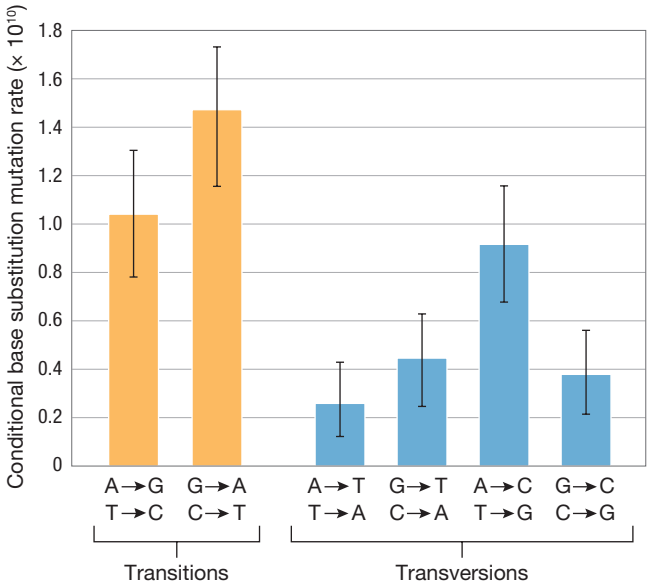


FIGURE 10.19 The mutation spectrum for *E. coli*. Heewook Lee and colleagues grew multiple lines of *E. coli* for 6000 generations, with frequent bottlenecks down to a single cell in order to minimize the consequences of selection. By sequencing the genomes of the progenitor and descendant populations in each line, they identified all mutations that occurred over the course of the experiment. From these data, they could estimate the *mutation spectrum*, the relative rate at which each base pair mutates to every other base pair. As expected, they found an excess of transitions over transversions. Error bars represent the standard error around the mean. Adapted from Lee et al. (2012).

Mutation rates from one base pair to another are not equal: As we noted in Chapter 6, transitions are significantly more common than transversions (**Figure 10.19**). As a result, even in the absence of selection, we would not expect all codons to be equally frequent. However, natural selection appears to play an important role as well: If a certain pattern of codon usage is advantageous, we would expect to see a bias toward it in genes that are more highly expressed. And, indeed, there is evidence in species including *E. coli* (dos Reis et al. 2003) and humans (Lavner and Kotlar 2005) that more highly expressed genes tend to have somewhat higher levels of codon usage bias.

But why would selection favor one codon over another synonymous codon, given that each specifies the same amino acid? There are a number of possible reasons, and evolutionary geneticists have amassed evidence for several of them. We consider two here.

One reason that a codon might be preferred over a synonymous alternative is that the frequencies of transfer RNAs (tRNAs) are not equal. A codon for which complementary tRNAs are common can be translated more quickly, with lower probability of error, than can a codon for which complementary tRNAs are rare. As a result, we might expect a match between the frequencies of tRNAs and the frequencies of codon usage. This is what Toshimichi Ikemura observed for bacteria and yeast in a now classic series of papers written in the early 1980s (Ikemura 1981a,b, 1982). The same pattern has now been established throughout the tree of life. Less clear is what aspect of this pattern is cause, and what is effect. Does natural selection set tRNA frequencies to match codon usage bias patterns that arise for other reasons or does selection favor codon usage bias patterns that track tRNA frequencies? The jury is still out on this.

Another possible explanation for codon usage bias is that codon usage choices influence the accuracy of replication and translation. Most notably, mononucleotide (single base pair) repeats of five or more bases, such as AAAAA, are particularly prone to replication slippage; that is, the DNA polymerase may slip forward or backward during replication. This results in frameshift mutations. Mononucleotide repeats also reduce the fidelity of transcription and translation. Thus, it is plausible that selection would favor either an increase in the frequency of mononucleotide repeats if a higher mutation rate is advantageous or a decrease in the frequency of such repeats if a lower mutation rate is advantageous.

To assess that hypothesis, Martin Ackermann and Lin Chao looked at the prevalence of mononucleotide repeats in the genomes of the bacterium *E. coli*, the yeast *S. cerevisiae*, and the nematode *C. elegans* (Ackermann and Chao 2006). They reasoned that if selection favors an increased mutation rate, they should see more mononucleotide repeats than expected at random (holding the amino acid sequence constant), whereas if selection favors a decreased mutation rate, they should see fewer mononucleotide repeats than expected at random. **Figure 10.20** shows their results for the entire protein-coding regions of the *E. coli*, *S. cerevisiae*, and *C. elegans* genomes. They found that short repeats of four to five base pairs were just as common as one would expect if the codon for each amino acid had been chosen at random. But long repeats of more than five base pairs were scarce

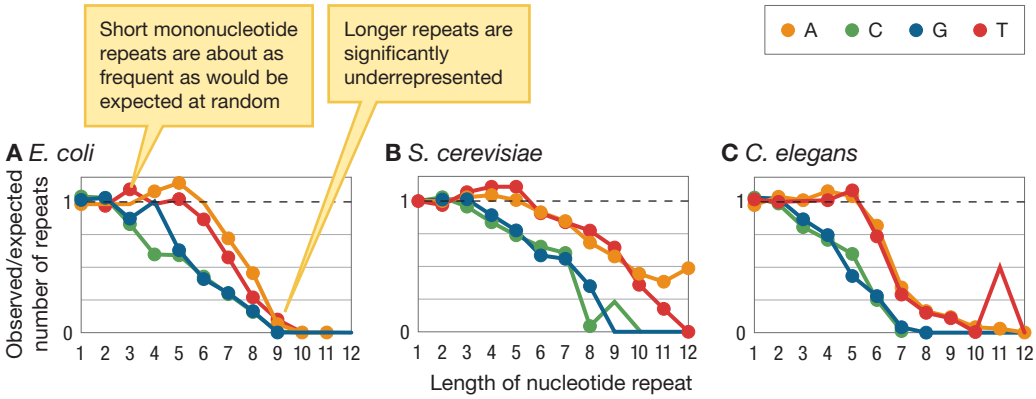


FIGURE 10.20 Long mononucleotide repeats are rare. Ackermann and Chao found that while short (four to five base pairs) mononucleotide repeats are about as common as would be expected at random, longer repeats are significantly underrepresented. This finding provides evidence of selection against inaccuracy in replication, transcription, or translation. The lengths of nucleotide repeats are shown in **(A)** bacteria (*E. coli*), **(B)** yeast (*S. cerevisiae*), and **(C)** nematode worms (*C. elegans*). Adapted from Ackermann and Chao (2006).

in the genome, indicating selection against long repeats due to selection against increased mutation rate, selection against transcriptional inaccuracy, or selection against translational inaccuracy.

To distinguish between selection against mutation or *transcriptional* inaccuracy and selection against *translational* inaccuracy, Ackermann and Chao devised an ingenious test. As we will discuss in detail in Section 10.5, eukaryotic genes often include untranslated regions known as introns that are spliced out of the transcribed mRNA before translation. Ackermann and Chao reasoned that repeats *that span introns* (**Figure 10.21**) have an effect on the process of translation, but because they are formed only after transcription occurs, they have no effect on replication or transcription. Such repeats are therefore ideal for distinguishing selection on replication and transcription from selection on translation. Ackermann and Chao found that mononucleotide repeats within an exon are much rarer than expected. By contrast, mononucleotide repeats that span introns are not uncommon in the genome. This indicates that translational accuracy has less of an impact on fitness than does the accuracy of replication or transcription.

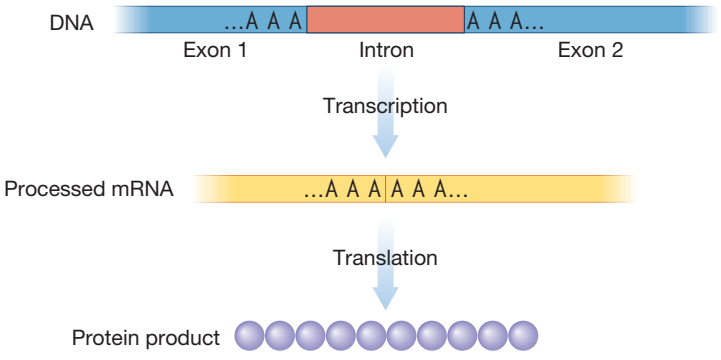


FIGURE 10.21 Mononucleotide repeat spanning an intron. Ackermann and Chao looked at the frequency of mononucleotide repeats spanning introns. The extended repeat structure of these intron-spanning repeats does not appear until the intron has been excised after transcription. Thus, these repeats do not reduce the accuracy of replication or transcription; their main effects are on the accuracy of translation.

GC Content

Because of base pairing, the fraction of A nucleotides in a genome will always be the same as the fraction of T nucleotides in that genome. Similarly, the fraction of G nucleotides will always be the same as the fraction of C nucleotides. But the fraction of G and C nucleotides need not be the same as the fraction of A and T nucleotides. Indeed, organisms vary widely in their **GC content**; that is, the fraction of G and C nucleotides. Some organisms, such as the prokaryotic soil microbe *Streptomyces coelicolor*, are GC-rich; others, such as the eukaryotic malaria parasite *Plasmodium falciparum*, are extremely GC-poor. **Figure 10.22** depicts GC content values for a number of fully sequenced genomes. As illustrated, GC content varies widely both in prokaryotes and in eukaryotes, so in this subsection we will consider eukaryotes as well.

As with codon usage bias, there are both nonselective and selective explanations for differences in GC content. One important nonselective consideration is a bias in mutation rates among transitions. As we saw in Figure 10.19, transitions from G to A and C to T are more common than transitions from A to G and T to C. As a result, mutation tends to drive genomes toward decreased GC content.

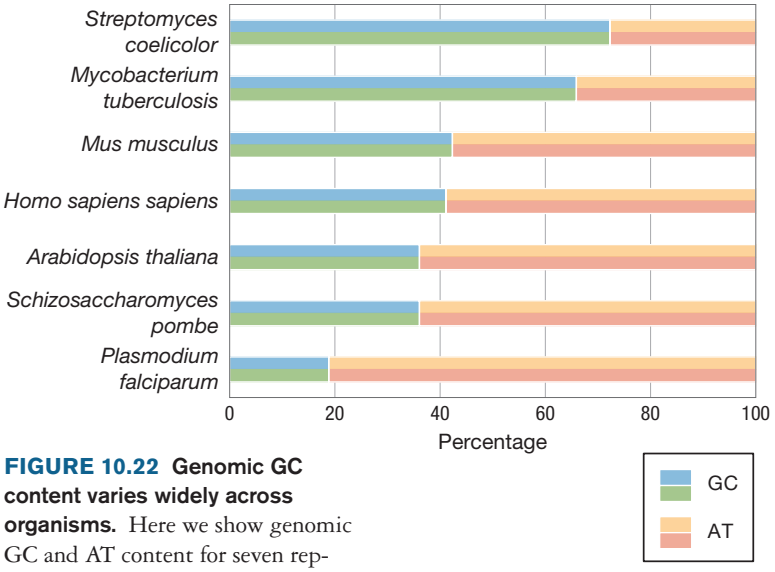


FIGURE 10.22 Genomic GC content varies widely across organisms. Here we show genomic GC and AT content for seven representative species. Adapted from Borodina et al. (2005), Cole et al. (1998), Gardner et al. (2002), Ruvinsky and Marshall Graves (2005), and Wood et al. (2002).

GC content in eukaryotes does not appear to be set by mutation rates alone, however. There are two lines of evidence for this. First, there is an overall excess of mutations from G to A and C to T. In a model at mutational equilibrium, we would expect to see the same number of mutations from G to A and C to T as from A to G and T to C. Second, when we look at GC composition in eukaryotes, it is not as low as we would expect given the excess rate of mutation from GC to AT relative to that from AT to GC. From both of these observations, we can infer that something other than mutation must be elevating GC content (Lynch 2010b).

One possible nonselective mechanism influencing GC content is *gene conversion,* a common process associated with homologous recombination (**Figure 10.23**). At the junction

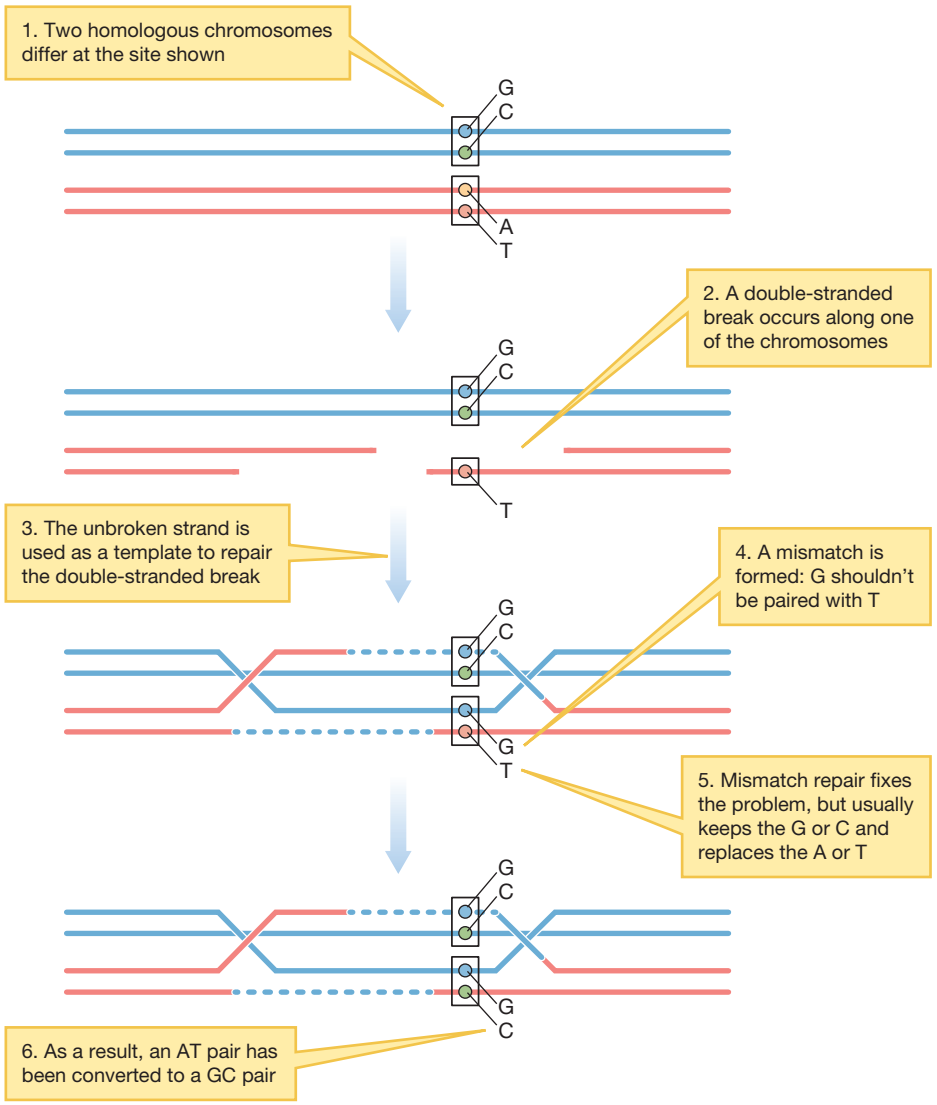


FIGURE 10.23 Gene conversion. A double-stranded break occurs in the red DNA molecule. A homologous stretch of DNA from the blue molecule is used as a template for repairing the double-stranded break, but the template differs at some sites. Mismatch repair fixes the discrepancies but usually removes an A or T and retains a G or C. As a result, AT pairs can be converted to GC pairs as shown. Adapted from Webster and Hurst (2012).

where a crossover occurs, a single strand of DNA from one chromosome can be paired with a single strand from another homologous chromosome. If the sequences in this paired region are not identical, sometimes pairing mismatches may arise. For example, a G or C on one strand may be mispaired with an A or T on the other. This triggers a corrective process known as mismatch repair, in which one of the mismatched strands is eliminated and the correct pairing is resynthesized using the other strand as a template. Due to the biochemistry of the repair mechanism, a strand that has a G or C is more likely to be used as the template than is a strand that has an A or T. This process tends to increase the frequency of G and C nucleotides at the expense of A and T nucleotides, thereby compensating for GC loss due to mutation (Galtier et al. 2001).

The interactions of mutation and gene conversion are not by themselves sufficient to explain the GC content levels observed across the tree of life, however. In the absence of other obvious nonselective processes influencing GC content, evolutionary biologists have hypothesized that natural selection also plays a role. A number of explanations have been proposed, including selection for codon usage bias and selection on thermal stability of DNA or, more likely, selection of functional RNAs. The relative importance of each remains unknown.

Returning our focus to prokaryotes now, GC content and codon usage bias provide powerful markers with which we can reconstruct the evolutionary history of genomes. The basic idea is that each species has its own characteristic GC content and codon usage bias: Genes acquired by horizontal gene transfer may not conform to those patterns, and thus they may stand out within the genome. As an example, Jeffrey Lawrence and Howard Ochman wanted to determine what fraction of the genome of the *E. coli* K-12 strain was acquired by horizontal gene transfer and when those transfer events occurred in the evolutionary history of this strain (Lawrence and Ochman 1998). To answer those questions, Lawrence and Ochman scanned the genome sequence of *E. coli* K-12 for regions where the frequencies of base pairs or of codon usage differed significantly from those that were characteristic of the genome as a whole. This scan led them to infer that at least 17% of the genes in the genome of *E. coli* K-12 have been acquired by horizontal gene transfer over the past 100 million years.

Lawrence and Ochman were also able to estimate when these various gene transfer events occurred by using a clever technique (Lawrence and Ochman 1998): When a gene is first acquired by horizontal transfer, it will have a GC content and codon usage pattern characteristic of the species from which it was received. But over evolutionary time, processes of mutation, gene conversion, and natural selection will act to drive GC content and codon usage toward patterns characteristic of the recipient species. If we knew the source of each horizontally acquired gene, we could simply see how much the GC content had changed, and we could use this information to estimate the time since acquisition. But the sources of the acquired genes are rarely known. Fortunately, there is another way to proceed. The first, second, and third positions of each codon have different probabilities of generating a synonymous versus a nonsynonymous change. We can observe in the codon table (Figure 6.7) that mutations at the first position in a codon are almost always nonsynonymous changes, whereas mutations at the third position are often synonymous changes. As a result, each codon position changes at a different rate toward the characteristic GC content and codon usage patterns of the recipient. This provides the information necessary to infer the time since acquisition by

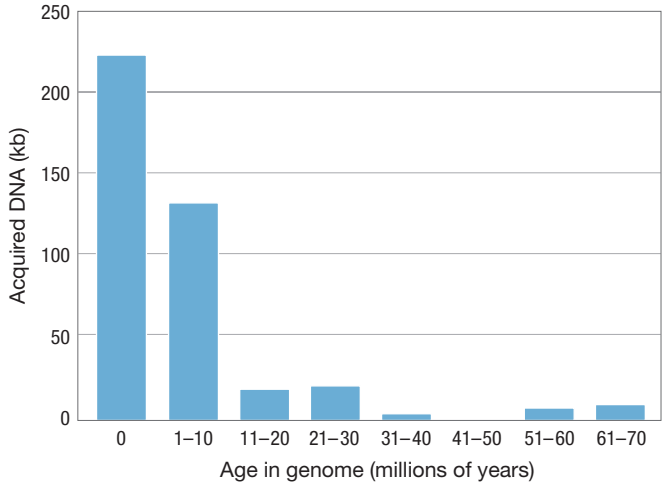


FIGURE 10.24 Age of horizontally acquired genes in the *E. coli* K-12 genome. Overall, at least 755 genes—more than one-sixth of the *E. coli* K-12 genome—have been acquired by HGT. Of these, the majority have been transferred quite recently. Adapted from Lawrence and Ochman (1998).

horizontal gene transfer. **Figure 10.24** shows Lawrence and Ochman's estimates of times since transfer for the horizontally acquired genes in the *E. coli* K-12 genome.

Studies of the genome teach us a great deal about the processes of divergence and speciation in bacteria. Of the genes that are present in either *E. coli* or its sister species *Salmonella enterica*, but not in both, the vast majority has been acquired by horizontal gene transfer subsequent to the divergence of the two species. As Lawrence and Ochman note, this suggests that speciation and diversification in bacteria proceed very differently than in eukaryotes. If *E. coli* and *S. enterica* are representative of bacteria more broadly, it appears that the ecological specializations responsible for evolutionary divergence are more often a result of wholesale acquisition of novel genes than a result of gradual accumulation of mutational differences.

GC Skew and Leading/Lagging Strand Gene Position in Prokaryotes

Prokaryote genomes typically have a single origin of replication. DNA replication is initiated at this AT-rich noncoding region, and it proceeds bidirectionally around the chromosome until a single replication terminus is reached at the other side. In prokaryotes, important genes tend to be located on the leading strand; that is, the strand that is synthesized continuously in the direction of the moving replication fork, rather than on the complementary lagging strand (Ellwood and Nomura 1982; Rocha and Danchin 2003) (**Figure 10.25**). This may function to reduce head-on collisions between the DNA polymerase involved in replication and the RNA polymerase involved in transcription; the processes of transcription and of DNA replication often occur concurrently in prokaryotes (Nomura and Morgan 1977; Merrikh et al. 2012). **Figure 10.26** illustrates an unusually strong excess of genes on the leading strand in the bacterium *Clostridium perfringens*.

Pairing constraints ensure that G = C and A = T in the genome at large, but on an individual leading or lagging strand, no such constraint is necessary. In principle, G might occur more often on the leading strand, while C might occur more often on the lagging strand. Because of mutational differences between the leading and lagging strands, this turns out to be exactly what we observe (a similar pattern holds for T on the leading strand and for A on the lagging strand). The difference is often measured as **GC skew**, the ratio $(G - C)/(G + C)$ in a sliding window moving along one strand of the chromosome. If G and C occur with equal frequency on each strand, GC skew will be zero. However, many prokaryotes exhibit substantial GC skew (McLean et al. 1998). In some of these, GC skew can be extremely dramatic, as illustrated in Figure 10.26. While the precise mechanisms responsible remain unknown, GC skew is most likely a consequence of different patterns of mutation and selection on the leading and lagging strands (Frank and Lobry 1999; Eppinger et al. 2004; Charneski et al. 2011).

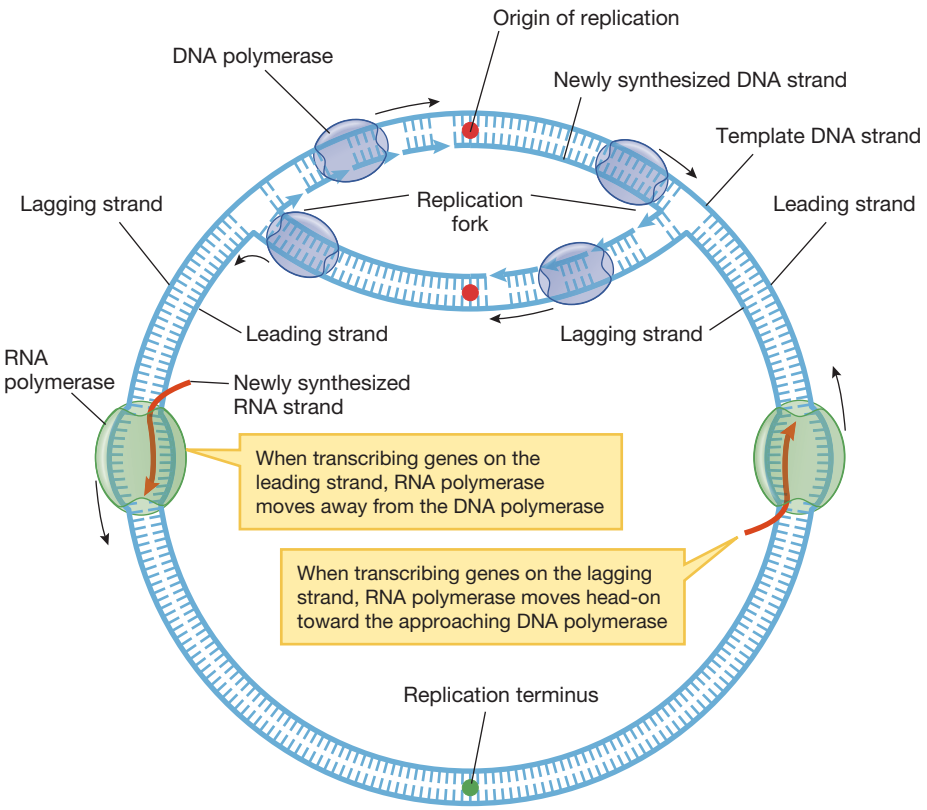


FIGURE 10.25 Genes on the leading strand minimize collisions between the RNA polymerase and the DNA polymerase. DNA replication in a bacterium proceeds bidirectionally from the origin of replication, with the replication forks moving outward along each side of the circular chromosome. When transcribing a gene on the leading strand of the chromosome, the RNA polymerase moves away from the replication fork and the approaching DNA polymer, reducing the likelihood of collisions between the polymerases. When transcribing a gene along the lagging strand of the chromosome, the RNA polymerase moves toward the replication fork and the approaching DNA polymerase, increasing the chance of a head-on collision. Adapted from Chen and Zhang (2013).

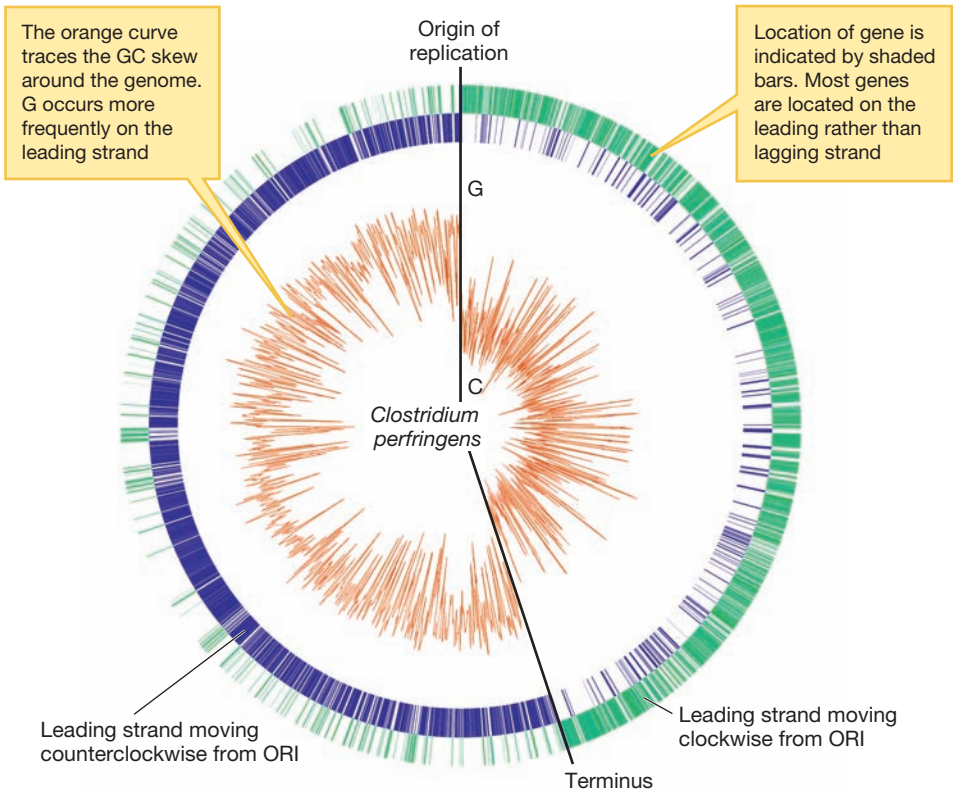


FIGURE 10.26 Extreme bias in gene location and extreme GC skew in *Clostridium perfringens*. On the outer two rings, genes are indicated by colored regions. Genes are predominantly located on the leading strand (purple moving counterclockwise from ORI; green moving clockwise from ORI) rather than on the lagging strand. On the inner orange ring, *C. perfringens* exhibits dramatic GC skew, with an excess of G over C on the leading strand.

10.5 Content and Structure of Eukaryotic Nuclear Genomes

Eukaryotic genomes differ dramatically from prokaryotic genomes both in structure and in content. First, a typical eukaryote can be said to have multiple genomes. The primary genome is the **nuclear genome**, which comprises a set of chromosomes contained in the nucleus. In addition, certain eukaryotic organelles—including mitochondria and chloroplasts—have their own separate genomes, relics of the ancient endosymbiosis events in which the formerly free-living life-forms were incorporated into the eukaryotic cell. We will defer our treatment of endosymbiosis and organellar genomes until Chapter 12; here, we will consider the nuclear genome only.

Most eukaryotes have nuclear genomes that are made up of multiple linear chromosomes. Unlike in prokaryotic genomes, only a relatively small fraction of the total DNA sequence in eukaryotic genomes codes for proteins. Another small fraction of the genome codes for functional RNAs such as tRNAs, rRNAs, and microRNAs. The rest of the genome is composed of noncoding regions, including transposons, introns, and structural elements such as centromeres and telomeres (Hellmann and Nielson 2008). While the details are not yet well understood, even these regions may be transcribed, and the corresponding RNAs may be involved in gene regulation (Mattick et al. 2010; Berezikov 2011). **Figure 10.27** illustrates the proportions of these elements in the composition of the human genome. In this section, we will look at each of these components and consider the evolutionary processes by which they came to be.

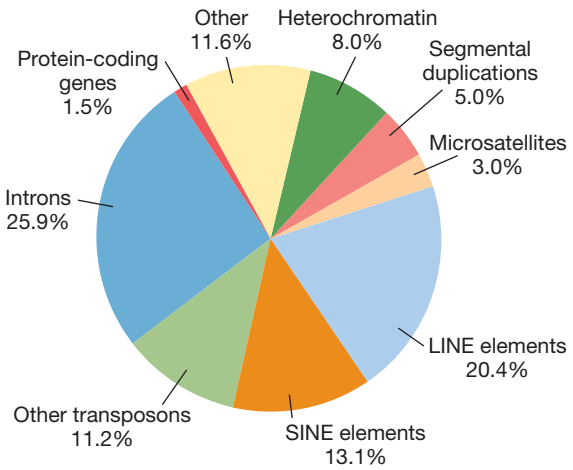


FIGURE 10.27 Composition of the human genome. In most eukaryotic genomes, only a relatively small fraction of the total genome is composed of protein-coding sequence, while a little more than a quarter of the genome is made up of introns. Transposable elements make up almost half of the genome: these include LINE elements and SINE elements. Other components of the genome include heterochromatin, segmental duplications produced by gene duplication events, and short nucleotide repeats known as microsatellites. Adapted from Gregory (2005) based on data from the International Human Genome Sequencing Consortium (2001).

Transposable Elements

Transposable elements, or transposons, represent a major fraction of the genomes of many multicellular eukaryotes: They make up approximately half of the human genome (see Figure 10.27). Most unicellular eukaryotic genomes also feature transposable elements, although at substantially lower frequencies (typically 1% to 5% of the genome). Transposons are distinguished by their ability to move within and between genomes. They do so in a variety of ways. *Conservative transposons* simply excise the original DNA element and reinsert it at another site. In this way, the transposon jumps to a new location, but the old copy is lost. *Nonconservative transposons* leave the original copy intact and create a new copy elsewhere. DNA transposons use a DNA intermediate, whereas retrotransposons copy the original element first to RNA and then back to DNA via a reverse transcriptase.

A number of classes of transposons are present in the human genome. The most common transposons in the human genome, by total sequence length, are retrotransposons known as **LINE-1 elements** (or *L1 elements*), where LINE is

an abbreviation for long interspersed element. The human genome includes more than 500,000 of these elements: Each is about 6000 base pairs in length, and together they make up roughly one-fifth of the human genome. L1 elements are called **autonomous transposons** because they encode the enzymes necessary to catalyze their own movement within the genome. But because of breakdowns that result from new mutations, the vast majority of these elements in the human genome have decayed and are no longer capable of transpositional activity. It is estimated that in the human genome, only 100 or so L1 elements retain the ability to transpose (Cordaux and Batzer 2009). Still, this 100 is a sufficiently large number to make L1 transposition events responsible for occasional instances of genetic disease in humans (Callinan and Batzer 2006).

SINE elements (or *SINEs*—short interspersed elements) represent another common class of transposable elements in the human genome. SINEs are **nonautonomous transposons** because they lack the capacity for independent replication. Like nonconjugative plasmids that rely on conjugative plasmids to move among bacterial cells, nonautonomous transposons rely on the machinery provided by autonomous transposons to move around the genome. In humans, SINEs rely on the protein products encoded by active L1 elements for their ability to move. A class of SINEs known as *Alu elements* outnumbers L1 elements by a substantial margin—there are more than a million *Alu* elements in the human genome. But *Alu* elements are much smaller in size than L1 elements—approximately 300 base pairs in length—and thus they represent a somewhat smaller total fraction of the genome (Cordaux and Batzer 2009). As with L1 elements, most *Alu* copies in the human genome are not currently active because the *Alu* promoter region is not by itself sufficient to initiate transcription. If it is to be active, an *Alu* copy has to be inserted by chance adjacent to the right types of flanking sequences (Batzer and Deininger 2002).

Alu elements appear to have arisen and proliferated at an extraordinary rate early in the evolution and radiation of the primate clade (**Figure 10.28**). During this initial phase, new *Alu* copies were substituted into the genome at a rate of one per generation. The process of expansion has continued throughout primate evolution, with ongoing amplification of various *Alu* families along different branches of the primate phylogeny. But in the lineage leading to humans, the rate of insertion has dropped approximately 200-fold, such that the rate of new insertions is now substantially reduced relative to that of 55 million years ago (Batzer and Deininger 2002).

Transposons are classic examples of **selfish genetic elements**. Selfish genetic elements are stretches of DNA that do not normally perform a useful function at the whole-organism level, but instead act to ensure their own survival and even replication within the genome. Transposons do this by copying themselves within genomes, which allows them to increase in frequency in at least three ways. First, consider a transposon in a haploid asexual organism. The transposon has no immediate way to move beyond the lineage in which it arises, but its ability to copy itself within the genome can reduce the chance that it is lost from its lineage. A single copy of any genetic element is always at risk of being

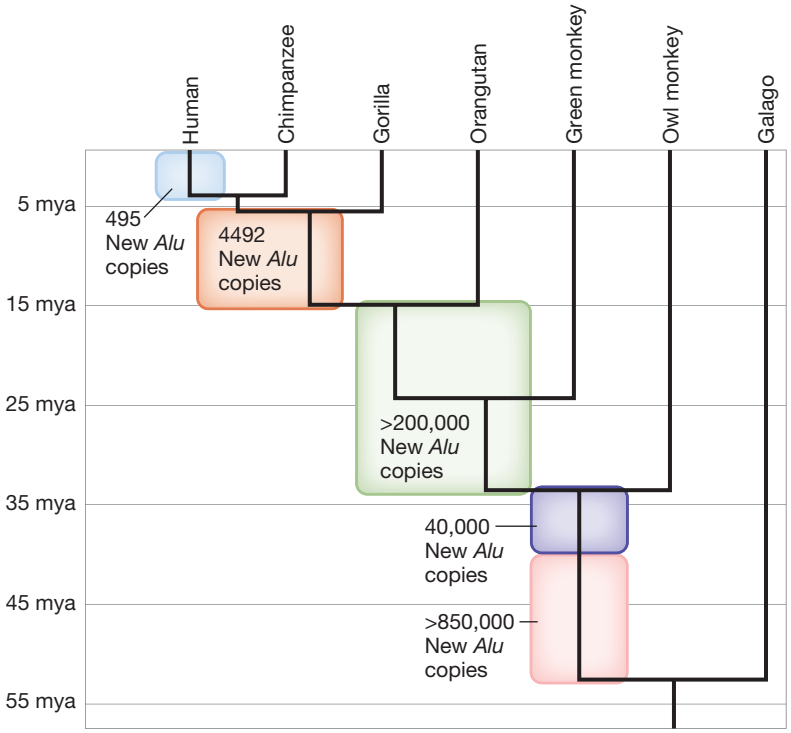


FIGURE 10.28 Expansion of the *Alu* elements along the lineage leading to humans. Around 55 million years ago, the initial expansion of the *Alu* copy number was extremely rapid, with new insertional substitutions occurring at a rate of one per generation. The rate of insertion has subsequently slowed considerably. Nevertheless, hundreds of *Alu* repeats have been incorporated into the human genome subsequent to the split between the human and chimpanzee lineages. *Alu* expansions have occurred along the lineages leading to the other primate species as well, but are not indicated in this figure. Adapted from Batzer and Deininger (2002).

lost, either by a segmental deletion or by mutational decay. But if that element can make multiple copies of itself within the genome, it is able to "hedge its bets" across those multiple copies. If one copy is lost by some mutational process, others will still remain and allow the transposon to persist within the genome (**Figure 10.29A**).

A reduced rate of loss is not the only benefit that transposition confers on transposons in asexual haploids. Many bacterial species have plasmids, which provide a further advantage to transposons. In such species, transposons also spread to new lineages, shuttled from one to the next on plasmids or other accessory genetic elements (**Figure 10.29B**).

In sexual diploid species, transposons can copy themselves onto new chromosomes. This increases their chances of being passed on to offspring of the next generation. If a transposon in the germ line jumps from one chromosome to a homologous chromosome that lacks that transposon, all subsequent meiotic products will include a copy of the transposon, and thus the transposon can spread through the genomes in the population (**Figure 10.29C**). This additional benefit of transposition may be one of the reasons that transposons are particularly numerous in sexual species.

In each of these explanations, the transposon does not benefit the organism in which it resides; that is, it does not confer any selectively advantageous trait on that organism. Rather, it benefits only itself, acting "selfishly" to minimize its own rate of loss from the genomes in which it resides and/or to maximize its own rate of spread into other genomes in the population. In this way, transposons are much like parasites. They are not capable of independent replication, but instead they rely on the replicative machinery of their "hosts"—in this case, the genomes of the organisms in which they reside. They persist over evolutionary time not because of any benefit that they confer to their hosts, but rather because the genes that they do encode operate to facilitate their own reproduction and spread into other genomes, possibly at the host's expense.

Indeed, retrotransposons are thought to have evolved directly from retroviruses. The LTR (long terminal repeat) retrotransposons are extremely similar in structure to retroviruses, and they appear to be essentially retroviruses that have lost the genetic machinery necessary to package themselves as independent replicating units. As a result, they are no longer capable of horizontal gene transmission from host to host, and instead they rely exclusively on vertical gene transmission from parent to offspring (Lynch 2007).

KEYCONCEPT QUESTION

10.3 If LINE-1 transposons are viewed as selfish genetic elements, why might we view *Alu* elements as hyperselfish genetic elements?

Transposition events can have a number of consequences. If a transposon inserts into the middle of a protein-coding gene, it will disrupt that gene and cause the loss of that protein. Even if it does not insert into the protein-coding region itself, it might interfere with the gene's promoter and alter expression of the gene. Transposons also play an important role in generating changes in gene

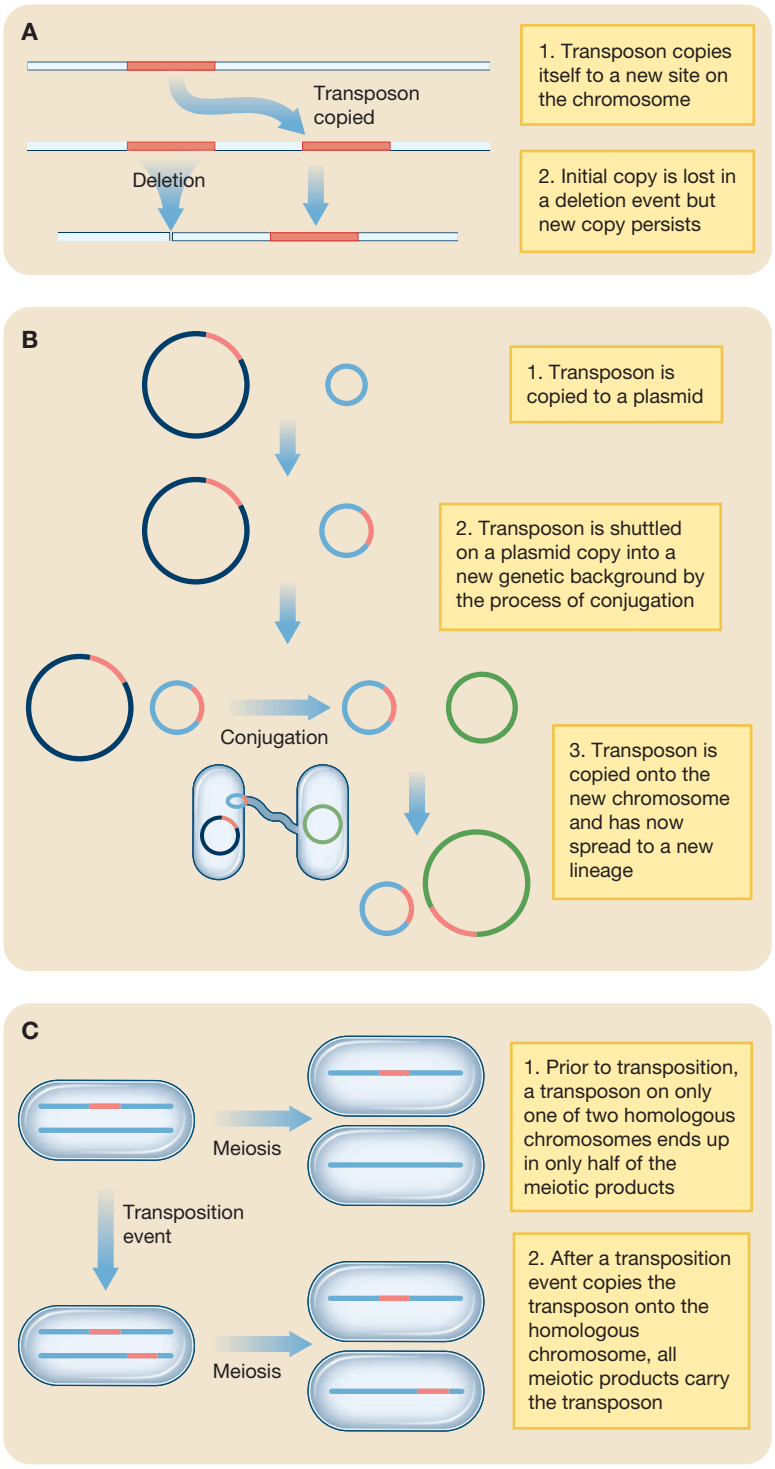


FIGURE 10.29 Three processes that favor transposition. Transposition confers a selective advantage at the level of the transposable element, but not at the level of the whole organism, in each of these cases. **(A)** Transposition creates additional transposon copies within the genome as a hedge against deletion events. **(B)** Transposition onto an accessory genetic element such as a plasmid facilitates the movement of a transposable element into a new genome. **(C)** In a sexual diploid species, transposition copies an element from one chromosome to its homologue, and thus it ensures that the transposon will be present in all meiotic products.

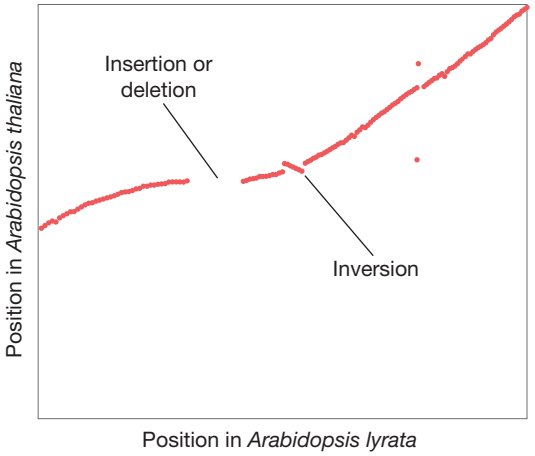


FIGURE 10.30 Changes in chromosome structure. A syntenic dot plot for two closely related plant species, *Arabidopsis thaliana* and *Arabidopsis lyrata*, which diverged roughly 5 million years ago. At left, we see that a set of continuous loci in *A. lyrata* is absent in *A. thaliana*; this indicates an insertion on the lineage leading to *A. lyrata* or a deletion on the lineage leading to *A. thaliana*. Toward the center of the figure, we see a short segment with the opposite slope; this corresponds to an inversion event. Adapted from CoGePedia (2009b).

order and chromosome structure, driving the sorts of inversions, translocations, deletions, and rearrangements that we see illustrated in **Figures 10.30 and 10.31** (Curcio and Derbyshire 2003).

Transposition events tend to have deleterous consequences for the host genome. Transposons can insert into the middle of other genes or can delete segments from the middle of other genes. They can create double-stranded breaks that generate mutation. Because of their high copy number throughout the genome, transposons also can set up an array of locations at which recombination errors can arise. As a consequence, *ectopic recombination* can occur when a transposon in one location is accidentally aligned with an identical transposon in another location and crossover occurs within the two misaligned transposons. Finally, a transposon may accidently copy some of the adjacent DNA as well, moving it to a new location in the genome when the transposon is inserted.

Because transposition events are typically deleterious, organisms have evolved a number of mechanisms that suppress the activity of transposons. A mechanism of *posttranscriptional gene silencing* known as *RNA interference* appears to reduce transposition activity by eliminating transposon messenger RNA. The same pathway may also be involved in *pretranscriptional silencing*, with the RNA products from the RNA interference pathway serving as guides to prevent the transcription of transposon DNA. Several other systems have been proposed as additional mechanisms to limit transposon activity (Lynch 2007).

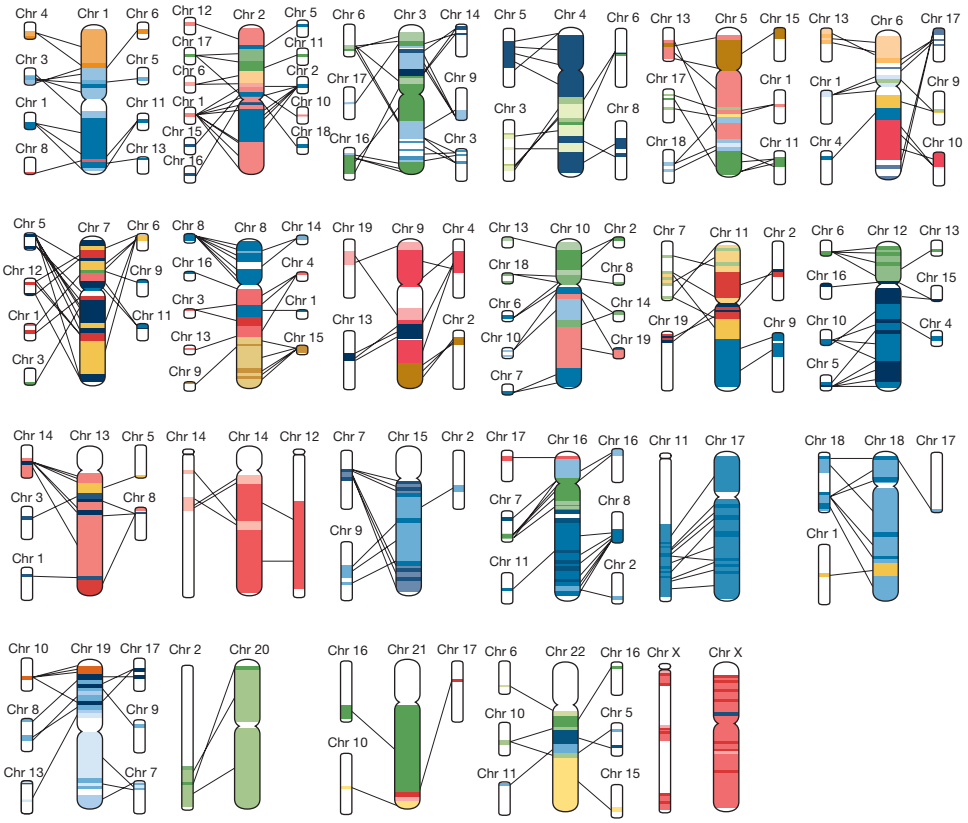


FIGURE 10.31 Changes in genome structure. This diagram shows the relationship between the genome structures of humans and mice. Each human chromosome is shown at the center, flanked by the corresponding mouse chromosome or chromosomes at each side. Overall, we see that genome structure has been shuffled considerably, with segments moving within or among chromosomes subsequent to the divergence of the lineages leading to mice and to humans approximately 80 million years ago. Nevertheless, we see that within segments, the basic arrangement and order of genes is conserved, and that in some cases—notably the X chromosome—rearrangement has been minimal. From Lewis et al. (2002).

Occasionally, however, the mutations caused by transposition will turn out to have beneficial effects. As such, transposition can potentially have advantages as well. As sources of mutation and particularly of genomic rearrangement, transposons almost certainly accelerate adaptive evolution of the host organism by generating additional variation—even though it is unlikely that the selective advantage from doing so can explain their widespread evolutionary success. As selfish genetic elements, transposons likely persist in huge numbers despite the costs they impose on their hosts, not because of the benefits they confer.

Origins of Replication, Centromeres, and Telomeres

Whereas prokaryotic chromosomes typically have only a single origin of replication, eukaryotic chromosomes have multiple origins of replication. There is good reason for this: Because eukaryotic genomes are so much larger than prokaryotic genomes, and because eukaryotic DNA synthesis is considerably slower, replication would take a prohibitively long time if eukaryotic chromosomes had only a single origin of replication.

Again in contrast to prokaryotes, eukaryotic chromosomes contain centromeres; that is, regions of DNA that form the attachment points for the kinetochore proteins to which the spindle binds in order to pull apart the chromosomes during cell division. Centromeres appear to be marked for this purpose not by specific DNA sequences, but rather by the presence of a particular type of DNA packaging protein, the centromeric histone CenH3. The centromeres are typically, although not always, a discrete region somewhere in the middle of a chromosome, and they are usually composed of satellite repeats extending for hundreds of kilobases, interspersed with frequent insertions of transposons.

Centromeres present a fascinating puzzle in genome evolution. While their function is critical to successful replication and their presence is a highly conserved trait, the actual sequences of the centromeres are evolving rapidly (Henikoff et al. 2001). In fact, the DNA sequence of the centromeric regions is among the most rapidly evolving of any region of the chromosome. At the same time, the CenH3 histones and other proteins involved in structuring the centromere are also rapidly evolving, in marked contrast to other noncentromeric histones, which are highly constrained evolutionarily; that is, the noncentromeric histones have not changed much over time (Malik and Henikoff 2001).

To explain these surprising patterns, Steve Henikoff and his colleagues proposed the **centromere drive** hypothesis (Henikoff et al. 2001; Malik and Henikoff 2001, 2002). When a female produces gametes by meiosis, only one of the four meiotic products forms a viable egg (oocyte); the other three form *polar bodies* that are discarded (**Figure 10.32**). As a result, selection at the level of the chromosome will favor any mutation to the centromere that increases its chance of segregating to the oocyte instead of to the polar bodies; for example, a change that allows the centromere to recruit more microtubules. Thus, the centromere might increase its number of repeat sections, providing a larger target area to which microtubules could bind. A chromosome with such a centromere would end up in a disproportionate number of oocytes and would rapidly sweep through the population because of its advantage during the process of meiotic segregation. (We will treat this phenomenon, known as meiotic drive, in detail in Chapter 17.)

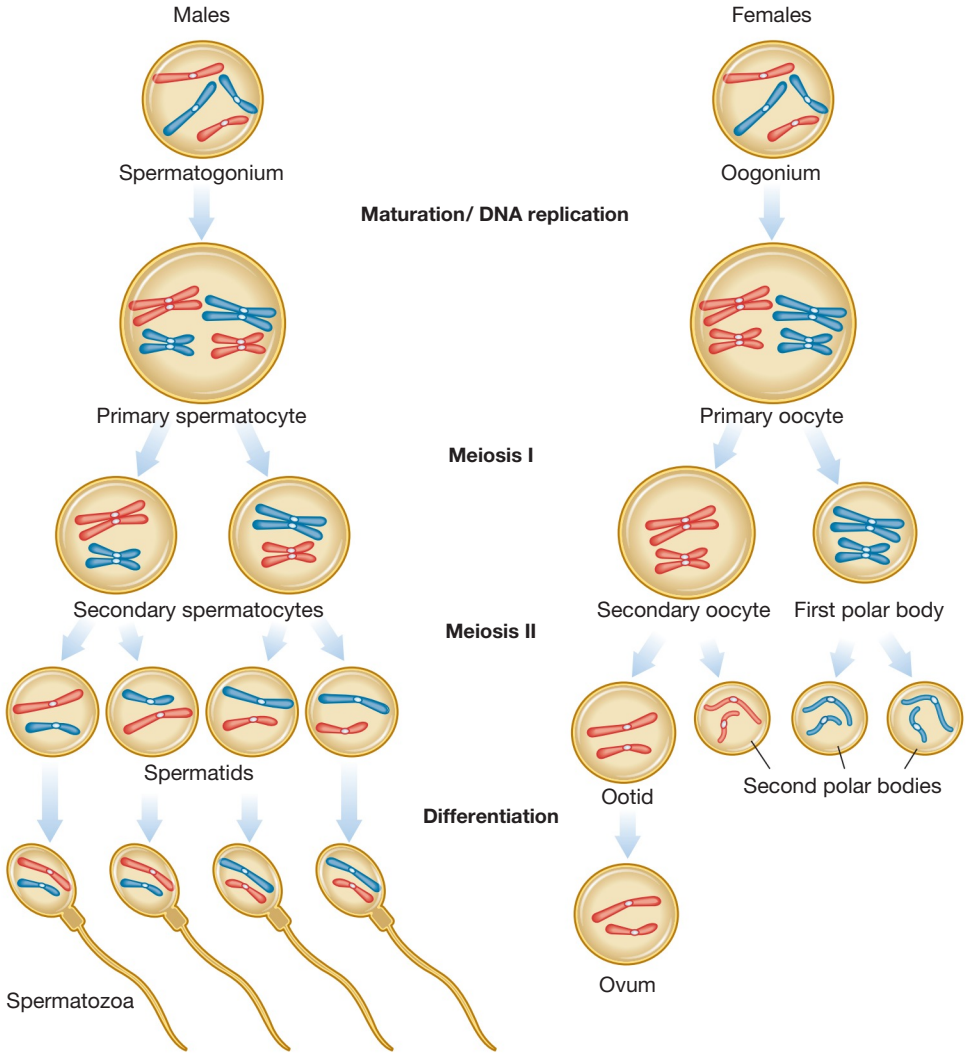


FIGURE 10.32 Meiosis in females and males. The process of meiosis in females produces one egg cell and three nonviable polar bodies; meiosis in males produces four sperm.

Yet, a centromere that increases its chance of segregating to the oocyte instead of to the polar bodies might cause meiotic problems such as nondisjunction; that is, the failure of homologous chromosomes to separate during meiosis I. In that case, natural selection would favor modifications at the protein level that counter the effects of the deleterious centromeric mutations. Such modifications are particularly likely to occur in the CenH3 histone, as illustrated in **Figure 10.33**. If this process played out repeatedly along different lineages, it would generate the observed patterns of genomic variation; that is, rapid evolutionary divergence between species both in the centromeric sequence and in the sequence of CenH3. Henikoff and his colleagues speculate that, by rapidly generating genetic differences in the meiotic machinery of closely related populations, this process could even contribute to reproductive isolation and eventual speciation.

Compared to prokaryote genomes, another major difference in the genome structure of eukaryotes is that they have telomeres. Telomeres, the extended regions of short repeats at the ends of eukaryotic chromosomes, are thought to be a solution to a problem that arises from having linear, instead of circular, chromosomes. Recall that DNA polymerase can operate only in the 3′ to 5′ direction along the template

strand. At the 5′ end of the template strand, this is not problematic: The DNA polymerase can simply begin at an origin of replication and continue until it runs off the end of the strand, with the 5′ end successfully replicated. But there is no way to replicate the far 3′ end of a linear chromosome. Along that strand, replication proceeds by ligating (joining together) short fragments known as *Okazaki fragments*; at some point, there is no longer sufficient room to add another such fragment, and the 3′ end will remain unreplicated (**Figure 10.34A**). As a result, the ends of the chromosome would shorten by approximately 100 base pairs with each replication (as indeed they do during ordinary mitotic cell division of somatic cells).

The solution to this problem is that eukaryotic chromosomes end with telomeres, which can be replaced by the action of a protein–RNA complex known as telomerase. Telomerase extends the 3′ end of a chromosome, adding a specific repeat sequence, such as TTAGGG in vertebrates (**Figure 10.34B**). This compensates for the loss of base pairs due to incomplete replication.

Much of the DNA in centromeres and telomeres is tightly packed in what is known as *heterochromatin*. Because it is so densely packed, it is largely inaccessible for transcription; therefore, gene expression from these regions is limited. Recombination is also greatly reduced in these regions.

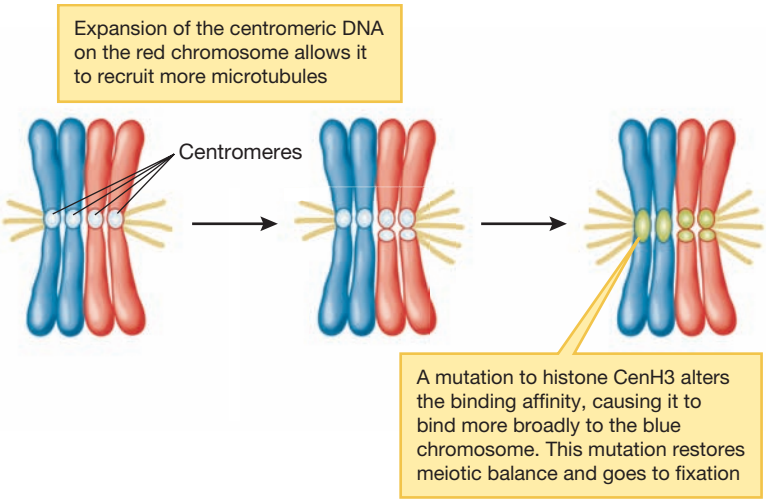


FIGURE 10.33 The centromere drive model. Prior to mutation, centromere strength is balanced. After mutation (here, an expansion of the centromeric DNA on the red chromosome), the mutant form recruits more microtubules. This mutant increases in frequency, but the mutation causes other problems in meiosis. Modifications to the CenH3 histone are selectively favored because they resolve the problem. Adapted from Henikoff et al. (2001).

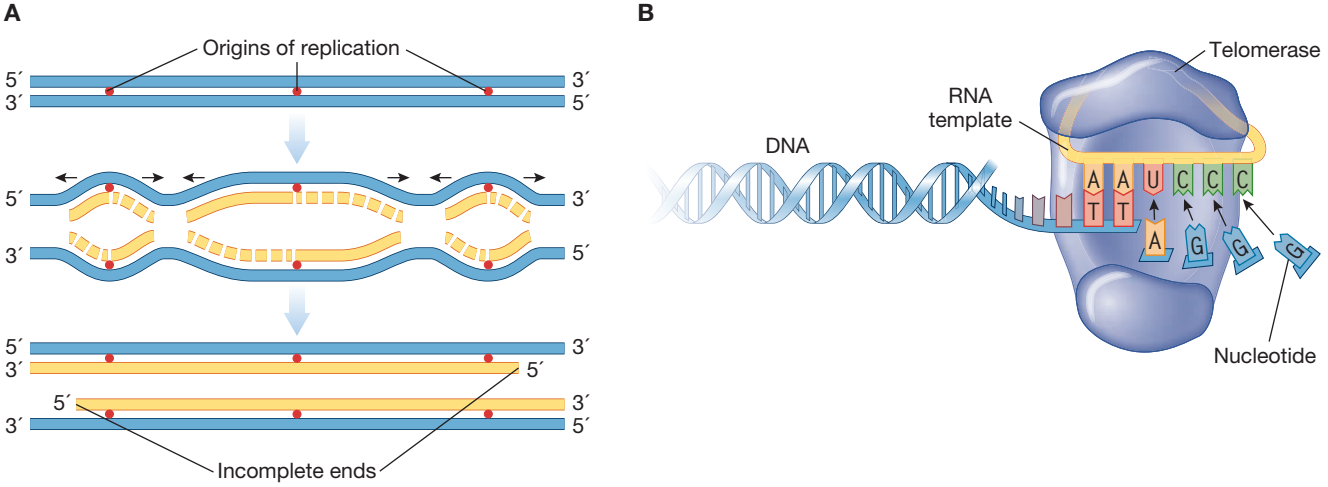


FIGURE 10.34 Telomerase compensates for incomplete replication at the 3′ ends of a linear chromosome. **(A)** Replication proceeds in the 5′ direction along the template strand by a single uninterrupted run of the DNA polymerase. In the 3′ direction along the template strand, replication occurs by ligating together a set of short Okazaki fragments. The fragments are unable to cover the terminus, and thus, for a linear chromosome, replication is incomplete at the 3′ ends of the template strand. **(B)** Telomerase, composed of a protein-based enzyme with an RNA template, adds a repeat sequence (such as TTAGGG in vertebrates) to the 3′ end of the chromosome. By extending the chromosome, telomerase compensates for the inevitable loss that occurs due to incomplete replication and prevents the eventual loss of coding sequence. Adapted from Kimball (2011).

KEYCONCEPT QUESTION

10.4 Describe three processes that are nonadaptive at the organismal level but that play an important role in the evolution of the structure and content of eukaryotic genomes.

Introns

Most protein-coding genes in prokaryotes comprise a single contiguous run of nucleotide bases, but this is not the case for most eukaryotic genes. Recall that in eukaryotes, protein-coding genes are typically composed of exon regions that code for protein products interspersed with intron regions that are spliced out before translation. If they are not translated, why are introns there at all? Recent work reveals that introns contain many microRNAs involved in gene regulation (Berezikov 2011). But this may not explain the origins of introns.

According to the *exon theory of genes*, the organization of eukaryotic genes into intron and exon regions is evolutionarily ancient, and many current genes arose by rearrangement of exons into new combinations. The idea is that individual exons often code for modular units of a protein, such as functional protein domains. When homologous recombination occurs within the introns between the exon-encoded domains, different allelic forms of each domain can form new combinations. When nonhomologous recombination occurs at locations within introns, the result is a new protein made up of a combination of functional domains—each coded by an exon (Gilbert 1987).

By increasing the length of protein-coding genes, introns increase the probability that recombination events can occur within individual genes. Moreover, they have a strong effect on *where* these events can occur. In the absence of introns, unequal recombination within the gene is likely to disrupt functional protein domains. But in the presence of introns, unequal recombination is now likely to occur between the exons, creating new combinations of protein domains without disrupting the structure of the individual domains themselves. Creating new proteins out of well-established modular subunits may be a particularly effective way to create new proteins that fold effectively and perform new biochemical functions. Furthermore, unequal crossing-over often causes frameshift mutations. When these occur in the middle of intron regions, they do not shift the reading frame of the processed mRNA, and thus they do not have the disruptive effect that they would if they had occurred in the middle of a coding region. For these reasons, intron structure may contribute to the combinatorial reuse of protein domains in genomes across the tree of life. This ability to recombine and reuse functional domains, rather than needing them to evolve from scratch, is thought to facilitate adaptive evolution (**Figure 10.35**).

Yet, introns may impose substantial fitness costs as well. First, introns increase the total size of the genome, thereby increasing metabolic costs and decreasing the maximal rate of cell replication. Mutations to the spliceosomal recognition sites can disrupt RNA processing, resulting in nonfunctional proteins. Introns also offer refuges for active transposons and other selfish genetic elements that can subsequently cause deleterious mutations.

There has been a major debate surrounding the evolutionary origins of introns (Rodriguez-Trelles et al. 2006). The *introns-early model* proposes that introns arose in ancestral prokaryotes. If so, they probably evolved to facilitate recombination

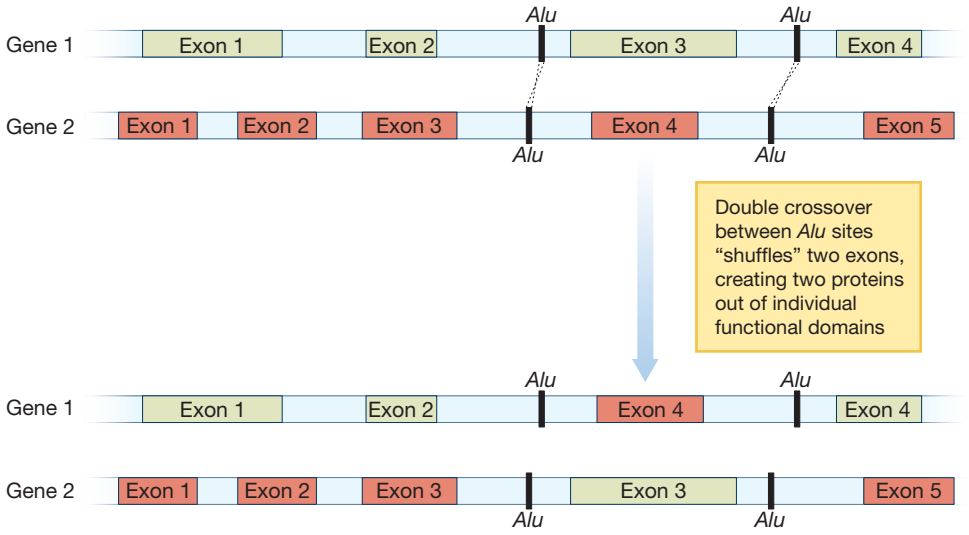


FIGURE 10.35 Exon shuffling. In the absence of introns, unequal recombination within the gene is likely to disrupt functional protein domains. In the presence of introns, the much greater length of the gene increases the probability of recombination within the gene. Moreover, with introns present and accumulating transposons, ectopic recombination is now likely to occur between the exons, creating new combinations of protein domains without disrupting the structure of the individual domains themselves. Adapted from Studentreader.com (2011).

between protein domains. One of the major challenges for the introns-early view is to explain the absence of spliceosomal introns in bacteria and archaea. Although those organisms do have introns known as *class I* and *class II* introns, these are simpler, self-splicing introns. Thus, the introns-early model requires that selection on genome size led to an elimination of spliceosomal introns and the subsequent loss of the spliceosome in these lineages.

In contrast, the *introns-late model* proposes that introns arose after the symbiotic event that gave rise to eukaryotes, possibly through the action of transposable elements (Cavalier-Smith 1978). According to the introns-late model, the current distribution of introns is due to their movement within the genome subsequent to that point rather than the result of phylogenetically conserved positions within the genome. With the additional evidence made possible by the genomics revolution, it is now clear that the common ancestor to modern eukaryotes had spliceosomal introns. But we still do not know precisely when these first evolved.

Recombination across the Genome

Homologous recombination plays an important role in structuring the genomic contents of most eukaryotic species. Recombination rates vary across species, with the general trend toward larger genomes having lower recombination rates per base pair (**Figure 10.36**). Rates also differ dramatically within the genome of any given species. To assess patterns of recombination, researchers use a number of different techniques. One of the most straightforward is *pedigree analysis*. By tracking how often two single-gene traits segregate together within a large pedigree, we can estimate the probability of recombination between those two genes. But the resolution of the method is low. We can obtain a much finer degree of resolution by *sperm-typing*, in which large numbers of sperm are genotyped. The sperm-typing method allows sample sizes that are vastly larger than those that can be obtained from pedigrees, but it can only provide estimates of recombination rates in males (Li et al. 1988). To obtain a recombination rate map of comparable resolution that is not male specific, but rather is averaged over the whole population, geneticists have developed a number of statistical tests that allow the use of population-wide

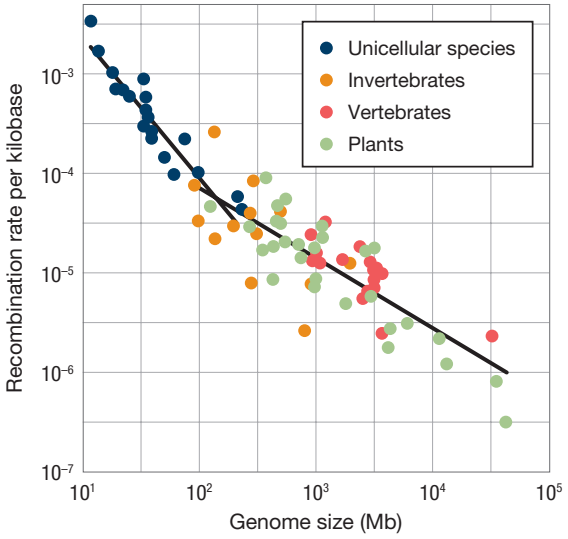


FIGURE 10.36 Recombination rate decreases with genome size. Recombination rates vary across species. Species with larger genomes tend to have lower recombination rates per base pair. Adapted from Lynch (2007).

patterns of linkage disequilibrium along the genome to estimate local recombination rates (Stumpf and McVean 2003).

By mapping recombination rates across the genome, researchers have found that in many organisms, recombination occurs largely at **recombination hotspots**—small regions of the genome that are particularly prone to serving as locations of crossover. Based on their analyses of linkage disequilibrium in human populations, Simon Myers and his colleagues estimated that 80% of recombination events in humans occur at sites located in only 10% to 20% of the genome (Myers et al. 2005). **Figure 10.37** shows a fine-scale recombination map for human chromosome 12. We see dramatic variation in recombination rates along the chromosome, with numerous hotspots at which recombination occurs at high rates and other regions where the local recombination rate approaches zero. Hotspots tend to occur near, but not within, coding genes.

The dramatic variation in recombination rate along each chromosome has important consequences for patterns of linkage disequilibrium in the human genome. The genome is broken up into a series of discrete **haplotype blocks**. Within the blocks, there is minimal genetic diversity, recombination is rare, and linkage disequilibrium is high (Daly et al. 2001). These blocks are bounded by recombination hotspots, so that linkage disequilibrium between even adjacent haplotype blocks is rapidly broken down over evolutionary time.

We do not yet have a detailed understanding of the factors that determine local recombination rate, but we do know that recombination hotspots appear to shift around the genome at an evolutionarily rapid pace. Highlighting this, evolutionary biologists have found that hotspots are not conserved between closely related species such as humans and chimpanzees (Ptak et al. 2005).

In this chapter, we have explored the rapidly growing field of evolutionary genomics, and we have looked at how genome-wide sequencing contributes to our understanding of the evolutionary process. This concludes Part II of the book. In the next chapter, we will turn to the origin and history of life.

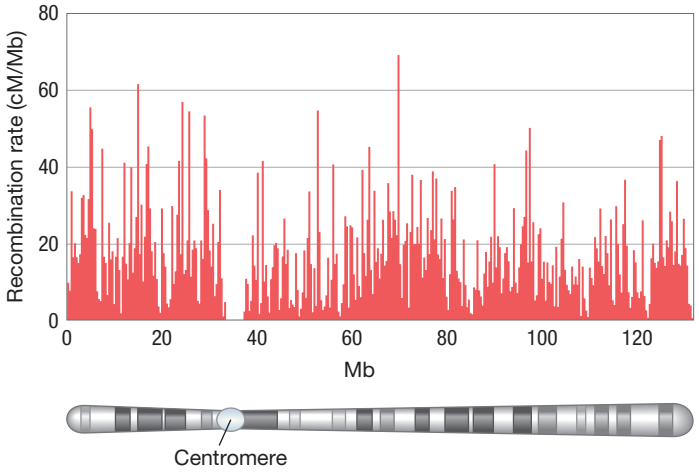


FIGURE 10.37 Recombination rate along human chromosome 12. The histogram shows the local recombination rate along human chromosome 12 in centimorgans per megabyte (cM/Mb). Note the frequent recombination hotspots represented by spikes in the recombination rate and the particularly low recombination rate around the centromere. Adapted from Myers et al. (2005).

SUMMARY

1. The development of techniques for sequencing large amounts of genetic data made whole-genome sequencing possible. With fully sequenced genomes for more than 500 eukaryotic species and more than 17,000 prokaryotic species as of 2015, we now have the data to study the content, structure, and organization of entire genomes and to consider how genomes themselves evolve.
2. Genome sizes vary dramatically across organisms. Viruses tend to have the smallest genomes, followed by prokaryotes, unicellular eukaryotes, and then multicellular eukaryotes.
3. Within multicellular eukaryotes, genome size does not correlate closely with organismal complexity. Much of the variation in eukaryotic genome size results from variation in the amount of noncoding DNA.
4. Viral genomes are extremely diverse. They may be made of DNA or RNA, may be single stranded or double stranded, and may consist of a single chromosome or a series of chromosomal segments. They are extremely compact, often achieving additional compression by means of overlapping coding regions. Some viral genomes, particularly those of RNA viruses, encode fewer than a dozen proteins.
5. Prokaryotic genomes are often organized as a single circular chromosome, supplemented by accessory genetic elements such as plasmids. They tend to be relatively compact, ranging in size from roughly 0.6 Mb to more than 10 Mb.
6. Bacteria engage in frequent horizontal gene transfer by the processes of transduction, transformation, and conjugation. Horizontal gene transfer is an important source of genetic variation in prokaryotic populations, and appreciable fractions of some bacterial genomes have been acquired by horizontal transfer.
7. In most organisms, the frequencies of GC versus AT base pairs and the frequencies of alternative synonymous codon triplets are not equal. GC content and codon usage bias can tell us about the evolutionary history of genes within genomes; for example, they allow us to identify regions of the genome that have been acquired by horizontal transfer.
8. Eukaryotic nuclear genomes vary tremendously in size, from just a few megabases in some unicellular organisms to more than 100,000 Mb in some large multicellular organisms.
9. Typically, only a small fraction of a eukaryotic genome is composed of protein-coding sequence. The remainder is made up of introns, transposons, and other genetic elements: Their distribution across the genome is the result of both selective and nonselective processes.
10. Transposons are selfish genetic elements that facilitate their own replication and movement within the genome of their eukaryotic "hosts." By moving and replicating within genomes, transposons increase their chance of being represented in the next generation. The action of transposons is an important driver of mutation, including changes in chromosome structure.
11. In addition to protein-coding regions and transposons, eukaryotic chromosomes include important structural regions such as centromeres and telomeres. Again, the structure of these components of the genome is fashioned by a combination of selective and nonselective evolutionary processes.

KEY TERMS

autonomous transposons (p. 385)
bacteriophage (p. 363)
centromere drive (p. 389)
codon usage bias (p. 377)
conjugation (p. 373)
C-value paradox (p. 361)
evolutionary genomics (p. 362)
GC content (p. 379)
GC skew (p. 382)
G-value paradox (p. 366)
haplotype blocks (p. 394)
horizontal gene transfer (HGT) (p. 372)
LINE-1 elements (p. 384)
mobile genetic elements (p. 370)
nonautonomous transposons (p. 385)
noncoding DNA (p. 364)
nuclear genome (p. 384)
plasmids (p. 372)
prophage (p. 371)
recombination hotspots (p. 394)
selfish genetic elements (p. 385)
SINE elements (p. 385)
transcription factors (p. 367)
transduction (p. 373)
transformation (p. 373)
transposable elements (p. 365)
virulence factors (p. 372)

REVIEW QUESTIONS

1. How did the discovery of noncoding DNA help resolve the C-value paradox?
2. How do differences in the number of transcription factors help resolve the G-value paradox?
3. Why do we say that the genetic material of viruses is much more diverse in form than that of cellular organisms?
4. Most eukaryotes have a fixed number of chromosomes. Explain how prokaryotes differ.
5. Briefly describe three modes of horizontal gene transfer.
6. Fill out the following table by briefly naming selective and nonselective processes that affect codon usage bias and GC content. You may wish to include multiple entries in some cells.

	Nonselective Process	Selective Process
Codon usage bias		
GC content		

7. Transposons are sometimes described as "selfish genetic elements." What do they do that is selfish, and at whose expense are they selfish?
8. Prokaryotes have a single origin of replication. Why do eukaryotes need multiple origins of replication?
9. Why do eukaryotic chromosomes need telomeres?
10. What are recombination hotspots?

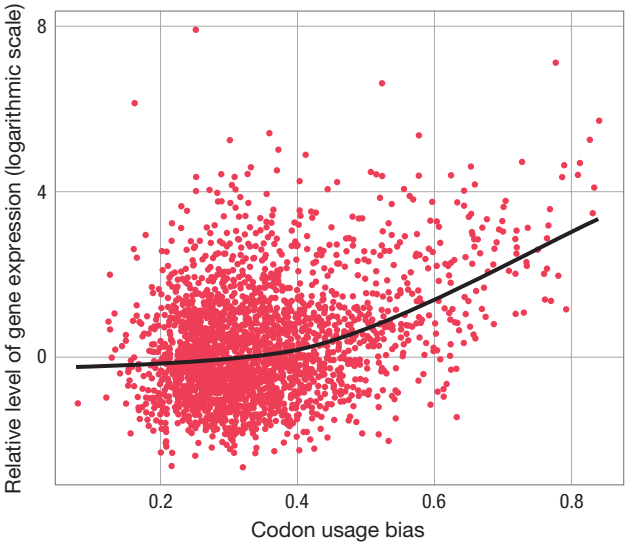
KEY CONCEPT APPLICATION QUESTIONS

11. Figure 10.9 illustrates a series of three-letter words that can be read in two different reading frames. Come up with your own example of this phenomenon.
12. In which organisms do you expect a transposon could more easily spread despite imposing a small fitness cost on its host: in an asexual haploid species or in a sexual diploid species? Explain.
13. Consider an ancestral species of bacterium A_1 that gives rise to two modern species S_1 and S_2. Along the lineage from ancestor A_1 to modern species S_1, gene order along the chromosome remains unchanged. Along a lineage from ancestor A_1 to modern species S_2, chromosome translocation occurs as follows:

ABCDEFGHIJKLMNOP → EFGHIJKLMNOPDCBA

Sketch a syntenic dot plot comparing this chromosome in species S_1 and S_2.

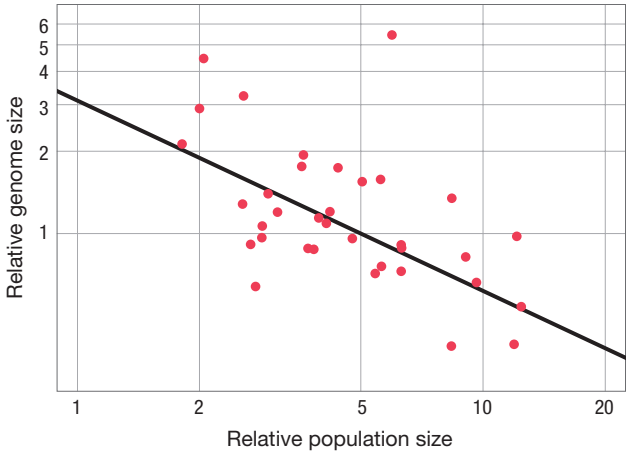
14. In a study of codon usage bias in genes across the *E. coli* genome, dos Rios and colleagues observed the following pattern.



Briefly summarize the trend shown in the graph, and propose a hypothesis to explain the trend.

15. Suppose that researchers created two phylogenetic trees for a clade of bacteria. One tree is based on the *rpoB* gene, a core functional gene that encodes part of the RNA polymerase enzyme. The other tree is based on the *TEM-1* gene, which confers antibiotic resistance by encoding an enzyme that breaks down antibiotics. How would you expect these two trees to differ?

16. Yi and Streelman (2005) compiled the following data about genome size and population size among ray-finned fish:



Briefly summarize the trend shown in the graph, and propose a hypothesis to explain this trend.

SUGGESTED READINGS

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Lawrence, J. G., and H. Ochman. 1998. Molecular archaeology of the *Escherichia coli* genome. *Proceedings of the National Academy of Sciences of the United States of America* 95: 9413–9417. A beautiful study in which genome sequence is used to reconstruct evolutionary history.

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PART III

The History of Life

Prior to the Permian–Triassic mass extinction 251 million years ago, crinoids were abundant in the Earth's oceans. Although the stalked crinoids here look somewhat like plants—hence, the name "sea lillies"—these creatures are echinoderm animals, related to starfish and sea urchins.

11

The Origin and Evolution of Early Life

◀ Rings spread out from water drops on the ocean's surface in Paradise Bay, Antarctica.

In the Agres gold mine of South Africa, Emmanuelle Javaux and her team of researchers found something that may turn out to be more precious than gold. Using sediment samples that they obtained by drilling five holes 600 meters below the surface of the gold mine, they discovered remarkable evidence of life that had existed billions of years ago: tiny fossils, called *microfossils*, that were approximately 3.2 billion years old. At the time of their discovery, these microfossils were 1.4 billion years older than the oldest microfossil samples known (Buick 2010; Javaux et al. 2010), although a set of recently discovered microfossils may be even more ancient—3.4 billion years old (Wacey et al. 2011). Using state-of-the-art microscopy, Javaux and her team found that 22 of their 55 samples contained fossils that were 30–300 micrometers (μm) in diameter (300 μm = 0.01 inch) (**Figure 11.1**). Because such fossils are so very small and sometimes resemble structures that are abiotic, a rigorous procedure is in place for classifying something as a microfossil. Javaux outlines this procedure: "Any purported ancient microfossil must pass essential

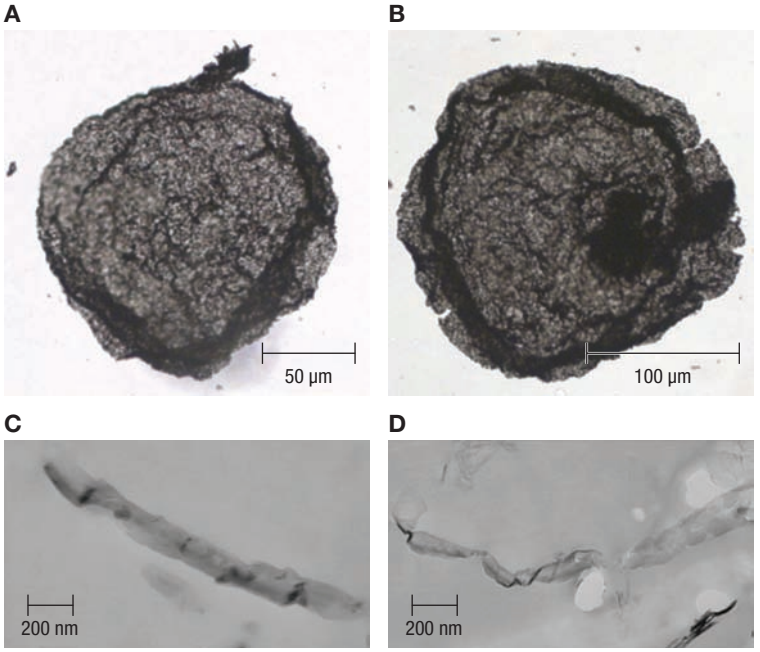


FIGURE 11.1 Microfossils of organisms from 3.2 billion years ago. (A, B) Examples of microfossils from the approximately 3.2-billion-year-old sample from South Africa. Scale: 100 micrometers (µm) = 0.004 inches. **(C, D)** Wall structure in the microfossils. Scale: 200 nanometers (nm) = 0.000008 inches.

tests before it can be considered evidence of early life. Evidence for contamination must first be discarded. Microbes can enter existing rock through borings or fluids in veins and pores or during sample preparation. . . . The microstructures must also be shown to be contemporaneous to the enclosing rock. Once these tests are successful, the biological origins of the microstructures must be demonstrated, considering all possible abiotic explanations for the observations" (Javaux 2011, pp. 663–664). Detailed analysis of the remains discovered by Javaux and colleagues suggests that what they uncovered were very likely fossilized creatures with organic "cell-like" walls around them and with other cell-like qualities, and that these organisms were part of populations of other such entities, probably in an environment on the edge of a marinelike coast. Although questions remain about exactly what kind of organisms were captured in these microfossils, Javaux's detailed analysis suggests that more than 3 billion years ago, microorganisms possessed something similar to cell walls.

Understanding that life on Earth likely existed more than 3 billion years ago is difficult for most people, as this is an almost incomprehensibly long time in the past (**Figure 11.2**). Until the late eighteenth century, most people thought that Earth was only a few thousand years old, and they could not imagine that it could actually be billions of years old. As more information was gathered from across the sciences, however, estimates of Earth's age began to increase from thousands to millions to billions of years, with current estimates being about 4.5 billion to 4.6 billion years. But these were largely estimates about the age of the planet, not estimates of how long life has been present on Earth. Once scientists began to understand how old the Earth really is, they began to wonder just how far back life on Earth could be traced, what processes could have produced such life in the first place, and how the early evolution of life could lead to the diversity of life we see today.

In this chapter, we will examine the following questions:

- What is life?
- What are some of the challenges for understanding how life originated?
- How did complex organic molecules first arise on Earth?
- What were the first living organisms like, and how did they originate?
- How did the modern organization of life based on DNA, RNA, and protein originate?
- How might early evolution and diversification of life have occurred?

Conceptually, evolutionary biology can readily adapt well-established ideas and theories to address the early evolution and diversification of life. Addressing the *origins* of life on Earth is also possible, but more difficult, for reasons we will

discuss shortly. But, before we consider the *origins* of life, we will step back and think about what we mean when we talk about life in the first place.

11.1 What Is Life?

What does it mean for something to be alive? While this may appear to be a straightforward question, the harder we try to pinpoint the defining features of life, the more difficult it turns out to be (Schrödinger 1944; Fox and Dose 1977; Crick 1981; Dyson 1985). The following thought experiment illustrates some of the difficulties of defining exactly what we mean by life.

Imagine that you are the lone person on a remote island and that you have never heard of or seen fire. You then observe a fire and watch what it does. Should you conclude that the fire is alive? The fire grows, and it appears to move. It engulfs living material in its path, and smoke and ash appear to be waste products produced by fire. The fire even seems to reproduce by splitting off new, smaller fires. Based on your everyday intuition that living things acquire nutrients to grow and reproduce, you might well conclude that the fire is alive. But, fire isn't alive; it just shares some of the characteristics of living things.

KEYCONCEPT QUESTION

11.1 Can you think of another example of something that at first may appear to be alive but isn't?

Rather than trying to construct a definition of life, perhaps the best we can do is to identify a set of properties that are typically, if not always, associated with living things. These properties include

- homeostasis: the ability to adjust the internal environment to maintain a stable equilibrium;
- structural organization: the ability to maintain distinct parts and the connections between them;
- metabolism: the control of chemical reactions;
- growth and reproduction;
- response to environmental conditions or stimuli.

In addition to these characteristics, there is a very important property shared by all the living things that we have observed on this planet: All life is subject to, and appears to have evolved by, the process of natural selection. This is a critical observation because it shapes our explanations of how life originated on Earth. The origin of life on Earth was more than just the origin of self-replicating entities: The origin of life that we are interested in as biologists is

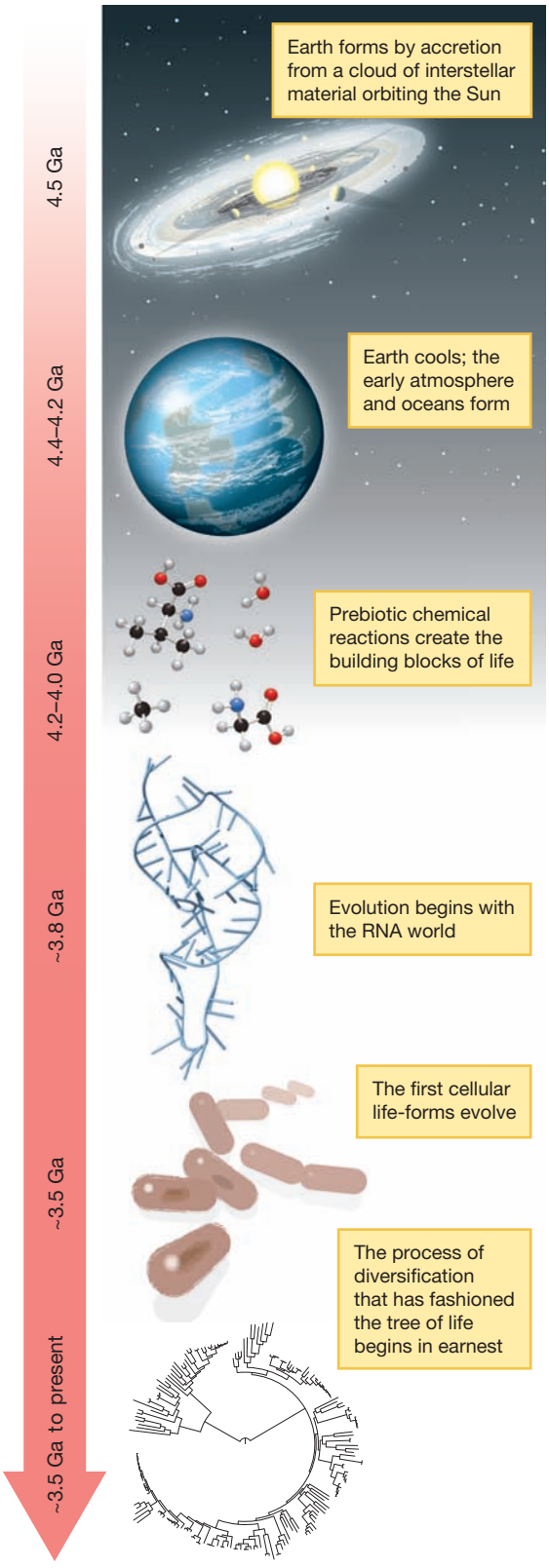


FIGURE 11.2 Early events in the history of life on Earth. Dates are in billions of years, or *gigaannum* (Ga), before the present time. Adapted from Joyce (2002).

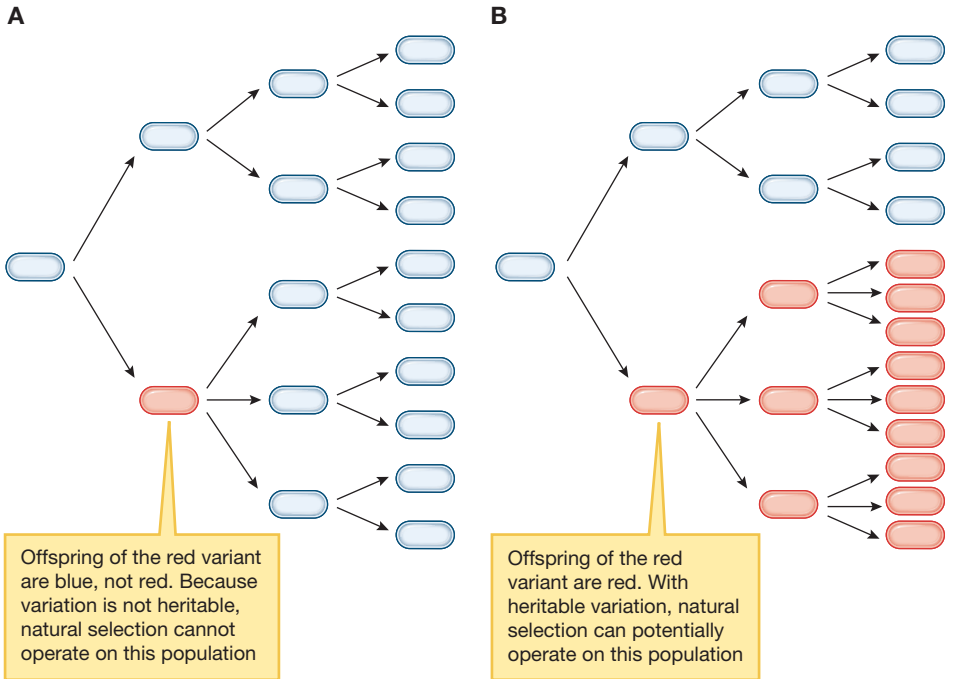


FIGURE 11.3 Self-replication and the origin of natural selection. **(A)** A lineage of organisms capable of self-replication but not capable of passing on any variations that might occur. **(B)** A lineage of organisms that reproduce with heritable variation. When variation is heritable, natural selection can drive phenotypic change provided that different types leave different numbers of offspring. The point is that the origin of life, as we observe it on Earth, involved not only the origin of self-replication but also the origin of heritable variation and thus of natural selection. Adapted from Maynard Smith and Szathmary (1999).

the origin of life that is subject to natural selection—we need replication, but we also need heritable variation in traits that cause fitness differences (see Chapter 3). To highlight the difference between a population of self-replicating entities and a population of self-replicating entities subject to natural selection, **Figure 11.3** illustrates a thought experiment devised by evolutionary biologists John Maynard Smith and Eörs Szathmary (Maynard Smith and Szathmary 1997, 1999) in which a self-reproducing organism that uses energy and materials from the environment to make copies of itself. Unless those copies inherit the properties of their parents, natural selection cannot act upon the population. Later in the chapter, we will consider how both self-replication and heritable variation tied to fitness differences could arise in a world in which RNA was the basis of life: what is known as the **RNA world**.

11.2 The Origin and Evolution of the Building Blocks of Life

Evolutionary biologists have a conceptual and theoretical foundation from which to work when it comes to understanding the evolution and diversity of early life. If variation, fitness differences, and heritability are present, evolution by natural selection will occur. Selection will weed out some life-forms and favor others, resulting in organisms that are well suited to their environments. This same process applied billions of years ago, just as it does today. Of course, it is not easy to understand how natural selection and other evolutionary processes acted on organisms that were present more than 3 billion years ago and to use that information to generate testable predictions, but we will examine numerous ways that evolutionary biologists do just this. Our point here is that evolutionary biologists can use well-established theories and techniques to understand both the process of natural selection and the phylogenetic relationships among early organisms.

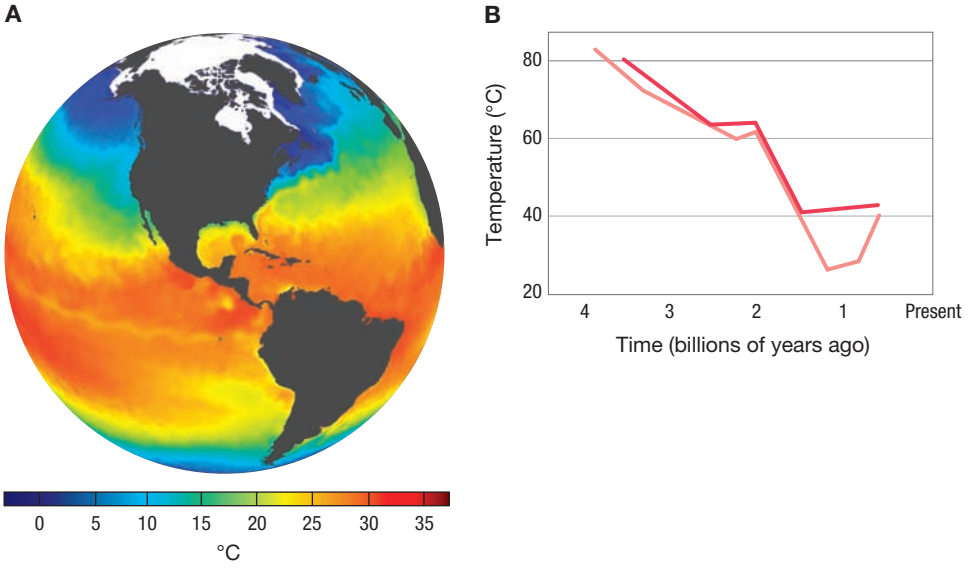


FIGURE 11.4 Ocean temperatures now and over the past 3.5 billion years. **(A)** A snapshot of Earth showing present-day ocean temperatures. **(B)** The average ocean temperature decreased dramatically from 3.5 billion to 0.5 billion years ago. The lines represent two different estimates of temperature based on maximum levels of ^{18}O in chert rocks. Panel B adapted from Gaucher et al. (2007).

Understanding the *origin* of life is another matter; in a sense, this topic lies somewhat beyond the purview of evolutionary biology. The theory of evolution, both as conceived by Darwin and Wallace and as developed subsequently by thousands of scientists over the past century and a half, neither offers nor aims to offer an explanation of how life arose on Earth. Rather, the theory of evolution explains how life diversified subsequent to its origin. Understanding the origin of life itself is an inherently interdisciplinary project, requiring evolutionary biologists to collaborate with chemists, geologists, atmospheric scientists, and researchers from other disciplines (Gould 1987; Rice et al. 2010). Chemists, particularly geochemists and biochemists, can help us to understand what the initial chemical building blocks of life might have been, and geologists and atmospheric scientists can shed light on the possible physical characteristics of the environment in which life originated. Work from these areas has provided information on the conditions on Earth 3.5 billion years ago, including the composition of the atmosphere, the geology and chemistry of Earth's surface, and Earth's temperature patterns (Knauth and Lowe 2003; Robert and Chaussidon 2006). For example, studies of the chemical signatures from the remains of ancient oceans suggest that ancient Earth was much hotter than today's planet. Ocean temperatures, for example, have cooled by 30°C from 3.5 billion to 0.5 billion years ago (**Figure 11.4**). Studies involving the phylogenetic reconstruction of the way that proteins reacted to temperature in organisms that lived billions of years ago lead to the same conclusion (Gaucher et al. 2007).

KEYCONCEPT QUESTION

11.2 How do data such as ocean temperatures from billions of years ago help evolutionary biologists understand the early evolution of life?

But there is a problem. When addressing questions about the *origin* of life, evolutionary biologists are deprived of one of their strongest tools: phylogenetic

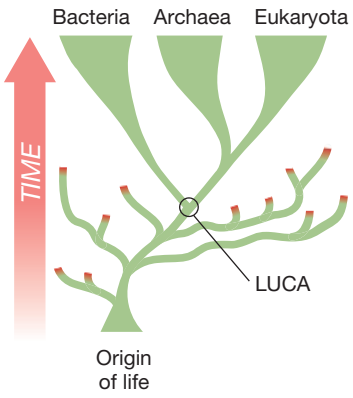


FIGURE 11.5 The last universal common ancestor (LUCA). LUCA is defined as the common ancestor of all currently extant life, but LUCA was not the first living thing. LUCA represents a *phylogenetic event horizon* beyond which phylogenetic analysis cannot directly inform our understanding of the history of life. Red tips on branches denote extinction.

reconstruction. To understand why, think about life at the base of the tree of life—what is often referred to as the **last universal common ancestor (LUCA)**. LUCA is not meant to be thought of as a single organism, but rather as a population of organisms. And LUCA itself was not the first life-form, nor was it the only life-form present at the time. LUCA was presumably just one of many life-forms on Earth at the time, but it is—by definition—the only one that left any descendant lineages that remain to this day. And because whatever else that was present left no descendants to the present day, we cannot reconstruct a phylogeny of its descendants, and so we cannot see back beyond LUCA. That means that if we use phylogenetic analysis based on extant (currently living) species, LUCA is the common ancestor to any group of living species that we might choose to analyze. As such, we might say that LUCA represents a **phylogenetic event horizon**: a point in the history of life beyond which phylogenetic analysis cannot possibly see. As a result, it is impossible to use the tools of phylogenetic analysis to infer what happened during the period of time prior to LUCA when life on Earth first originated (**Figure 11.5**).

Fortunately, there are other tools besides phylogenetic analysis that evolutionary biologists can use when studying the origin of life on Earth. For example, we can use population thinking and our understanding of evolutionary processes, including genetic drift and natural selection, to analyze questions relating to the origin and evolution of early life. As we will see, evolutionary biologists have used these tools, in collaboration with chemists, molecular biologists, geologists, and atmospheric scientists, to make progress in understanding the origin of life on Earth.

Broadly, our aim in understanding the origin of life is to understand how living organisms arose from the simple molecules present on the primordial Earth. To do this, we need to identify a set of plausible stages leading from simple chemical compounds such as water (H_2O), methane (CH_4), and ammonia (NH_3) to cellular organisms able to evolve by natural selection. **Figure 11.6** illustrates some of the transitions that we wish to understand. The result would be a self-replicating primitive cell—a **protocell**—such as the one shown in **Figure 11.7**.

To understand the origin of life, the first step is to understand how prebiotic chemistry could transform simple inorganic molecules into the complex building blocks of life: amino acids, nucleic acids, and lipid molecules. Darwin himself envisioned this process as taking place in a "warm little pond, with all sorts of ammonia and phosphoric salts, lights, heat, electricity, etc., present, so that a protein compound was chemically formed ready to undergo still more complex changes" (letter from Darwin to Hooker, February 1, 1871).

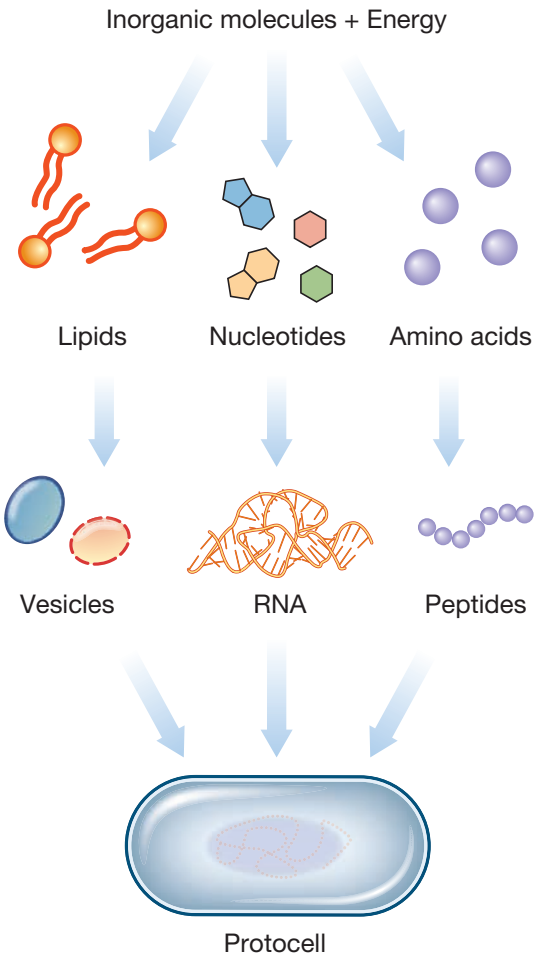


FIGURE 11.6 Early stages in the origin of life. To understand how life evolved, we need to know how small inorganic molecules gave rise to the organic compounds critical to life; how the organic compounds were assembled into structures such as vesicles, RNA, and peptides; and how these structures came together to form early protocells. Adapted from iBiology.org.

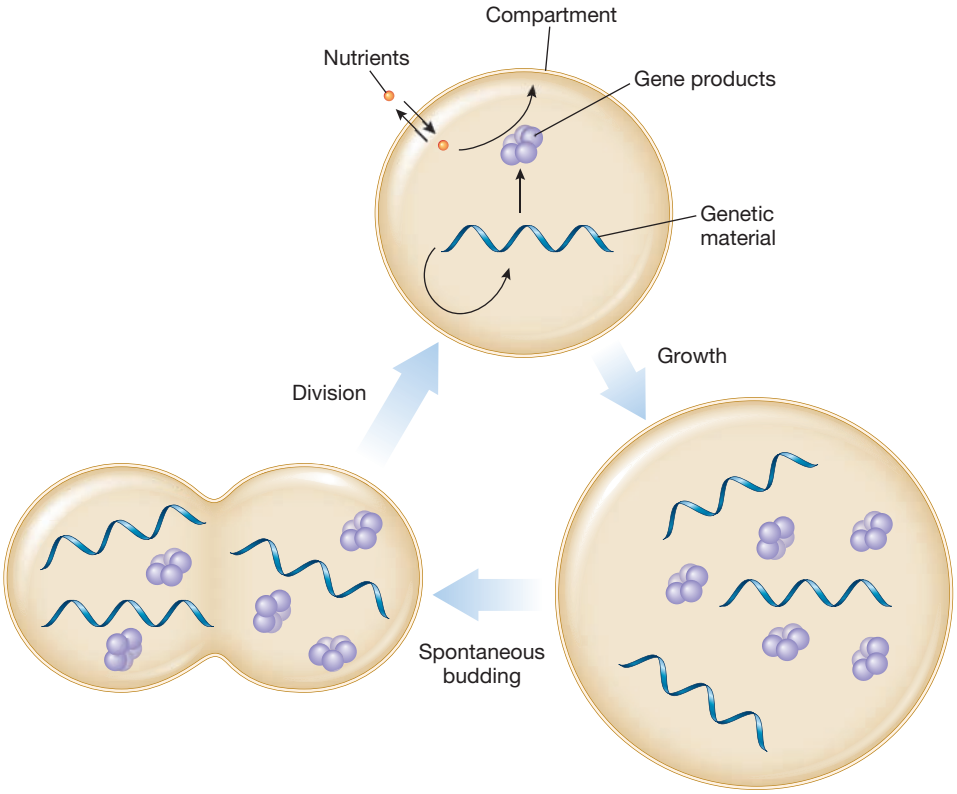


FIGURE 11.7 Elements of a self-replicating protocell. As shown here, a protocell can control the flow of nutrients across a boundary layer, copy its genetic material and produce gene products, and divide to form new daughter cells. Adapted from Blain and Szostak (2014).

In the 1920s, both Aleksandr Oparin and J. B. S. Haldane elaborated on Darwin's "warm little pond" idea and proposed the following hypothesis for the origin of life on Earth: In an atmosphere that lacked oxygen—as primitive Earth's atmosphere did—ultraviolet light and lightning might have served as sources of energy that converted atmospheric gases into a range of molecules that served as the basis for early life on Earth. Other energy sources involved in the early history of life on Earth might have included cosmic rays, volcanic eruptions both above ground and below the ocean surface at deep-sea vents, and Earth's own internal heat (Martin et al. 2008).

This **prebiotic soup**—meaning the pool of organic molecules suspended in water before life arose—would have grown richer in complexity over time. Oparin and Haldane hypothesized that in a process of **abiogenesis**—the chemical formation of life from nonliving material—the earliest life-forms may have emerged from such a prebiotic soup, and that they may even have used various parts of this soup as a source of energy and nutrients. In what follows, we consider the kinds of chemical reactions that allow simple molecules in a prebiotic environment to give rise to the critical elements of early cells.

KEYCONCEPT QUESTION

11.3 What is the difference between abiogenesis as discussed here and the process of spontaneous generation that we discussed in Chapter 2?

Small Molecules to Amino Acids, Lipids, and Nucleotides

A first experimental step in understanding the prebiotic origins of life came in the 1950s from Stanley Miller and Harold Urey, who tested the *plausibility* of the **prebiotic soup hypothesis** for the origin of life. Their approach was to simulate in the lab—on a very small scale—the conditions outlined by Oparin and Haldane to test whether some of the chemical precursors to life would emerge. To simulate lightning in the ancient atmosphere, they set an electric

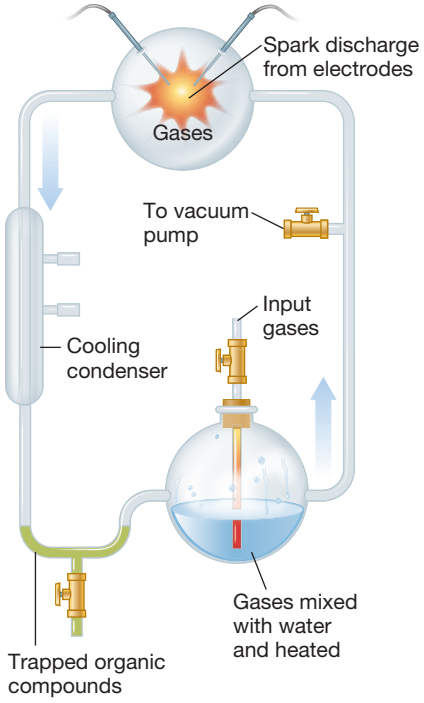


FIGURE 11.8 The Miller–Urey experiment. The experimental device used in the 1953 experiments by Stanley Miller and Harold Urey. Adapted from Lazcano (2006).

current between two electrodes. This current, in turn, interacted with a mixture of gases—for example methane (CH_4), hydrogen (H_2), and ammonia (NH_3)—that, based on evidence from chemistry and physics, was thought at the time to best represent the characteristics of the atmosphere of Earth about 4 billion years ago (Miller 1953) (**Figure 11.8**).

Depending on which combination of gases was used, numerous common amino acids, such as glycine, alanine, and valine, were produced in the experimental apparatus. From their simple experimental protocol, Miller and Urey produced some of the building blocks of life.

There's an interesting coda to this story. Miller carefully saved the samples from his experiments and nearly 50 years later passed them on to his former student Jeffrey Bada (**Figure 11.9**). Beginning in 2008, Bada and his colleagues reanalyzed these samples using sensitive modern equipment and found that Miller had actually synthesized more than 40 different amino acids, far more than existing technologies had enabled him to detect when he performed the experiments in the 1950s (Johnson et al. 2008; Bada 2013). But how did Bada's team know that these amino acids were products of the original experiments rather than bacterial contaminants that accumulated over the intervening half-century? In principle, amino acids can exist in one of two different chiral forms, or enantiomers, which demonstrate different "handedness": an L-enantiomer or a D-enantiomer. But biologically produced amino acids almost exclusively take L-enantiomeric form. If the amino acids in Miller's vials arose as contaminants from biological sources, the team would see only L-enantiomers. Instead, they saw an even or near-even mix of D and L forms, indicating that the amino acids had arisen from the original abiotic reactions in Miller's experiments (Parker et al. 2011).

FIGURE 11.9 Miller's old vials. Samples from Miller's origin-of-life experiments conducted in the 1950s were reanalyzed in 2008 by Jeffrey Bada and colleagues using modern technology.

In retrospect, the combination of gases that Miller and Urey used in their experiment is probably not an accurate representation of what was present in the ancient atmosphere. The atmosphere in the Miller–Urey experiment was a so-called *reducing* atmosphere, with hydrogen, methane, and ammonia but lacking carbon dioxide and nitrogen. On the basis of evidence accumulated since their experiment in the 1950s, we now have reason to believe that the ancient atmosphere was not strongly reducing, but instead relatively similar to the atmosphere today, albeit without the atmospheric oxygen (Trail et al. 2011). This, however, does not change the basic findings of the Miller–Urey experiment: Numerous studies have shown that organic molecules can be formed under a wide variety of atmospheric conditions supplied with a wide range of energy sources (Rode 1999).

In addition to the mechanisms we have just discussed, the pool of molecules in the prebiotic soup might have grown more diverse as a result of matter arriving from outer space. Extraterrestrial objects such as carbon-rich meteorites, comets, and interstellar ices were raining down on the planet during its early days. Such meteorites are now known to carry many of the necessary components of life: lipids, amino acids, and nucleobases

(Oro 1961; Kvenvolden et al. 1970; Cooper et al. 2001; Callahan et al. 2011) (**Figure 11.10**). They also carry amphiphilic molecules (molecules with both hydrophilic and hydrophobic ends) that can self-assemble into **vesicles**—small, fluid-filled spaces enclosed by a lipid membrane (Deamer et al. 1985). Similarly, interstellar ices are composed of gaseous mixtures that through simple chemical reactions can also self-assemble into vesicles when energy is provided in the form of ultraviolet radiation (Dworkin et al. 2001).

Complex molecules may also have arisen deep in the ocean. Then as now, hydrothermal vents on the ocean floor were leaking sulfide-rich compounds from giant "black smokers" rising as high as 20 meters above their bases. These sulfide-rich compounds interacted with iron-rich waters on the ocean floor (Martin and Russell 2003; Martin et al. 2008; Mielke et al. 2010). These reactions, which would have occurred under high pressure and temperature, would have led to the production of compounds that may have played a role in the early formation of life.

Hydrothermal fields of *alkaline vents* may be an even more hospitable environment for the formation of organic compounds and possibly for the origin of life (Martin et al. 2008; Lande and Martin 2012). The towering carbonate vents of these fields, such as the vents of the Lost City in the middle of the Atlantic Ocean (**Figure 11.11**), offer much more moderate temperatures (about 40°C to 90°C) than those of the black smokers. They rise up to 60 meters in height and persist for far longer than the black smokers—up to hundreds of thousands of years instead of mere decades. The porous construction of carbonate vents offers plentiful microstructure for complex chemical reactions, and their reducing chemistry is similar to that in the Miller–Urey experiment: prevalent methane and hydrogen but little carbon dioxide (Kelley et al. 2005).

FIGURE 11.10 The Murchison meteorite. A sample of the Murchison meteorite that fell on Murchison, Australia, on September 28, 1989, is shown here. This meteorite is particularly rich in molecular diversity, with tens of thousands of different organic compounds including at least 70 amino acids (Schmitt-Kopplin 2010)—substances that may have enriched the prebiotic soup that was present early in Earth's history.

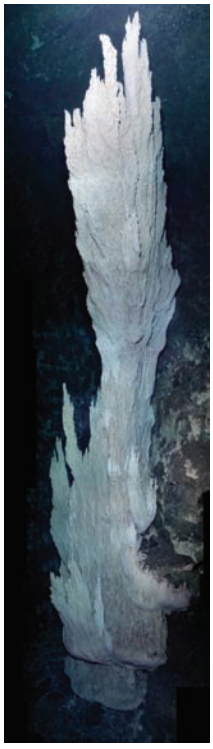


FIGURE 11.11 Alkaline vents. A towering carbonate chimney, about 10 meters in height, from an alkaline vent in the Lost City hydrothermal field provides conditions conducive to the origin of life.

Lipids to Vesicles, Nucleic Acids to RNA, and Amino Acids to Proteins

We would also like to understand how the complex organic molecules produced by the processes described earlier could assemble into yet more elaborate structures: lipid membranes, nucleotide chains, and proteins. One problem with the basic prebiotic soup explanation is that while floating free in sizable bodies of water, the chemical ingredients of life would be far too dilute to interact and form more complex molecules and structures. The substrates of life need to be contained or concentrated somehow. Once concentrated, lipid molecules in solution can spontaneously self-assemble into vesicles composed of a bilayer membrane; we explore this process and how these vesicles can reproduce in a subsequent section.

For the assembly of other complex structures, surface chemistry may play an important role. While many of the chemical reactions necessary to form complex organic molecules can take place in liquid solution, some of these reactions are greatly accelerated when they occur on solid surfaces. For example, both long RNA chains and complex amino acid structures can self-assemble on minerals (Ferris et al. 1996). The microscopic spaces between clay layers may concentrate the reagents,

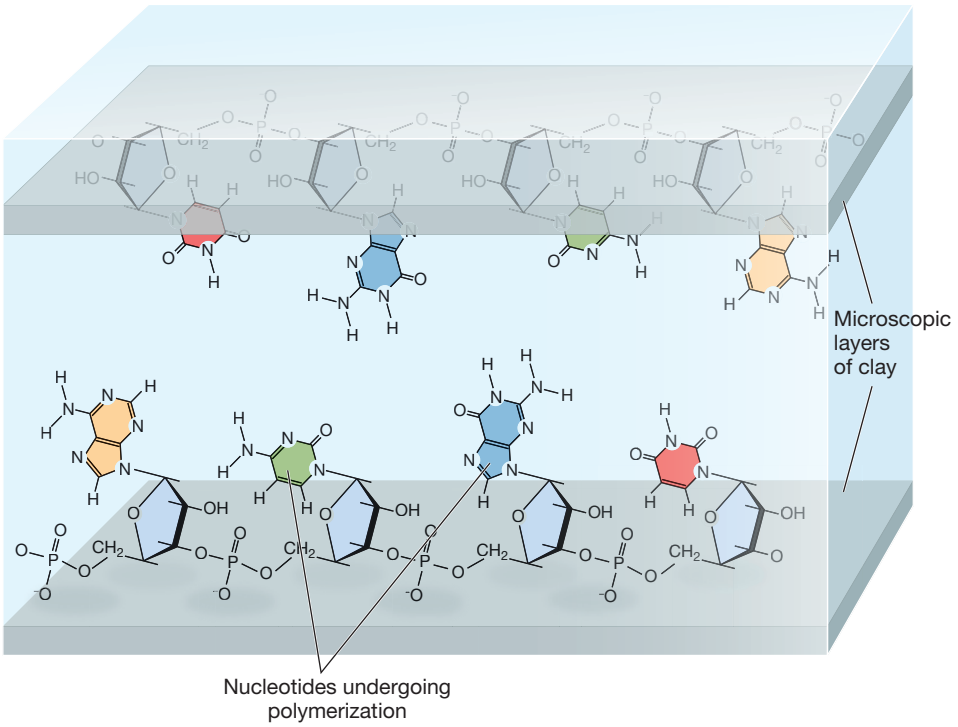


FIGURE 11.12 RNA assembly on mineral clay surfaces. On a solid surface such as clay, free nucleotides are much more likely to encounter one another in configurations favorable to polymerization. Adapted from Ricardo and Szostak (2009).

and binding to the clay surface may align molecules so that they readily polymerize (**Figure 11.12**). A similar process may happen when a solution freezes: Complex molecules such as nucleotides can be concentrated in the spaces between growing ice crystals, facilitating reactions involving these reagents (Attwater et al. 2010).

As with almost all groundbreaking experiments in science, the Miller–Urey experiment described earlier raised as many questions as it answered. For example, in this and other similar experiments, the critically important sugar ribose was found in very low supply, and a mechanism for joining the amino acids together to make proteins was completely absent. Some of the key parts of complex proteins could be produced in an environment that was meant to simulate that of early Earth, but how did those parts come to be joined together? This problem was addressed in 1977, when Sidney Fox mixed a number of different amino acids together at a high temperature (120°C) in an environment lacking water (Fox and Dose 1977).

When Fox mixed large amounts of the amino acids aspartic acid and glutamic acid, and subsequently placed the mixture into water, the amino acids present were strung together in a peptide-like structure. The bonds between the amino acids, however, were weak and unstable, and they were different in structure from the peptide bonds that join amino acids in most organisms. Subsequent work by Claudia Huber and her colleagues found that amino acids do link together via stable peptide bonds if a compound such as carbon monoxide (CO)—which is thought to have been present in early Earth's atmosphere—is used in the laboratory experiments (Huber and Wachtershauser 1997; Huber et al. 2003).

Many questions remain regarding the origin of life. The studies described above provide plausible mechanisms by which organic molecules came to populate the early Earth. But they do not explain how the first organisms capable of variation,

multiplication, *and* heritability came into existence. For that we must understand how these complex organic molecules self-assembled into the replicating vesicles that would evolve to become protocells.

11.3 The Evolution of Protocells

Cells—compartments enclosed by a membrane that separates an "inside" from the rest of the world—are so fundamental to life today that it is hard to imagine a world without them. But how did cells arise in the first place, and what were the earliest cells like? We have little, if any, phylogenetic evidence with which to answer this question. All organisms on the tree of life have a cellular structure, so presumably LUCA did as well. It would be nice if we could use phylogenetic inference to determine the structure of the first life-forms, but we run into the phylogenetic event horizon that we discussed earlier. Fortunately, we can use our understanding of physics, chemistry, and the process of natural selection to generate hypotheses about how and why the first cells may have arisen. In this section, we will explore hypotheses about how cell membranes arose and how natural selection might have favored the early evolution of single-celled life-forms from acellular precursors.

Lipid Membranes and Reproduction in Early Cells

The membranes that surround modern cells are complex structures. Cell membranes consist of a phospholipid bilayer embedded with numerous transmembrane proteins that ferry molecules from one side to the other and transduce signals across the membrane. Early membranes would have been assembled from simple organic molecules in the environment, and thus would have had a simpler structure than that of more modern membranes (Deamer and Dworkin 2005). For example, they may have been composed of single-chain fatty acid molecules such as oleic acid (**Figure 11.13A**). Under appropriate conditions, these molecules can spontaneously self-assemble into lipid bilayers and form enclosed vesicles (Hargreaves and Deamer 1978; Gebicki and Hicks 1996). In the presence of micelles—small spherical assemblages of fatty acid molecules with hydrophobic tails inside and hydrophilic heads outside—these vesicles can grow as they incorporate the micelles into their walls (**Figure 11.13B**).

Cells need to be able to import nutrients and export wastes. But without protein channels, it is not immediately clear how substrates would move in and out of lipid vesicles. To answer this question, Jack Szostak and colleagues explored the transport of molecules such as nucleotides across a fatty acid membrane (Mansy et al. 2008). They found that molecules

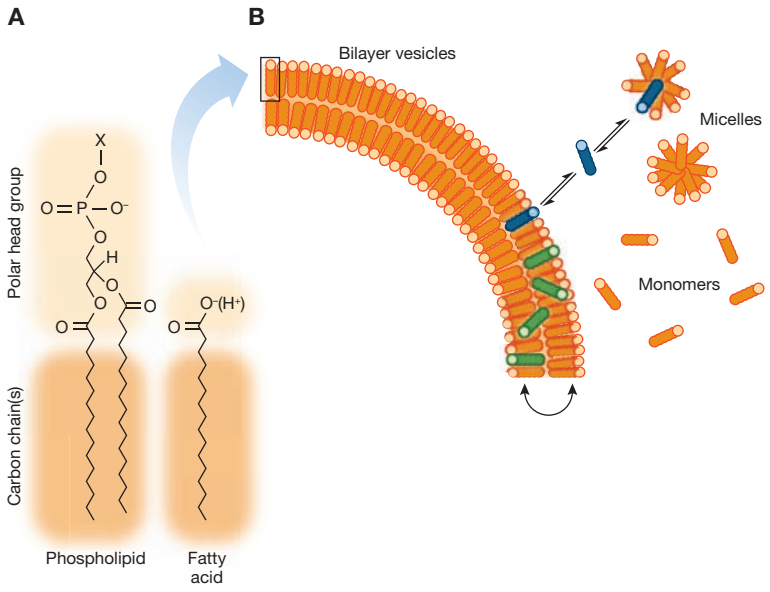


FIGURE 11.13 Fatty acid vesicles. **(A)** Simple fatty acids have a polar head and a single carbon chain, whereas the phospholipids that make up the membranes of modern cells have an additional phosphate group and two carbon chains. **(B)** Vesicles composed of simple fatty acids can grow by incorporating micelles and free fatty acid molecules (Chen and Szostack 2004). Individual single-chain fatty acid molecules exchange rapidly between layers of the membrane, external solution, and external micelles. Adapted from Budin and Szostak (2010).

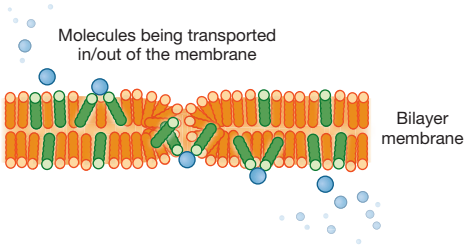


FIGURE 11.14 Flipping lipid molecules traffic solutes across a membrane. Simple single-chain fatty acids flip back and forth between the inner and outer layers of a bilayer membrane, trafficking other molecules from outside to inside or vice versa. Adapted from Mansy and Szostak (2009).

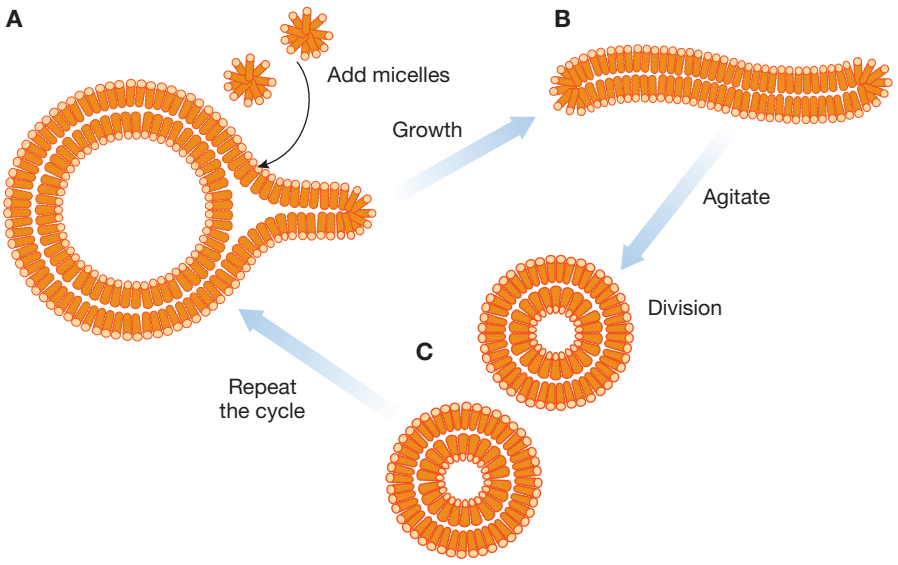
pass relatively easily across such membranes, trafficked by the lipid molecules that flip-flop from the inner to the outer membrane at a much higher rate than in phospholipid membranes (**Figure 11.14**).

Szostak and colleagues went on to study how simple vesicles of this sort could divide (Zhu and Szostak 2009). They looked at multilaminar vesicles—vesicles made up of multiple lipid bilayers—which can be formed by spontaneous reactions. They observed that as additional lipid molecules were absorbed into the outer membrane by integrating micelles, an extrusion would form on one side of the cell (**Figure 11.15A**). With further growth, the vesicle would take on the form of a thin, elongated strand (**Figure 11.15B**). Strands of this sort are fragile and can be broken up even by gentle motion, forming a set of new "daughter" vesicles (**Figure 11.15C**).

At this stage in the evolutionary history of cells, the cell membrane would have still been made up of single-chain lipid molecules rather than more complex phospholipids. But Szostak and Itay Budin have identified a process of natural selection that would lead from the former to the latter (Budin and Szostak 2011). Using a simple one-chain fatty acid, they produced lipid vesicles—but in some of the vesicles they added a small fraction of phospholipid molecules. To their surprise, they discovered that the presence of these few phospholipid molecules *slowed down* the rate at which fatty acid molecules moved out of the bilayer. Recall that fatty acid molecules rapidly move in and out of the membrane, so two adjacent vesicles will be constantly exchanging lipid material. As a result, vesicles with higher phospholipid content will tend to grow in size while vesicles with lower phospholipid content will shrink (**Figure 11.16**).

This process confers a selective advantage in terms of growth on cells that include phospholipids in their membrane. As a result, selection favors vesicles that contain the acyltransferase machinery to synthesize phospholipids. An evolutionary arms race would follow, as cells with greater and greater ability to synthesize phospholipids outcompete their neighbors. Eventually, membranes would be dominated by phospholipids—and selection would favor the evolution of transport structures to move metabolites across the membrane.

FIGURE 11.15 Growth and replication in multilaminar lipid vesicles. **(A)** As fatty acids are incorporated into the outer membrane of the vesicle, a membrane tail is extruded. **(B)** As the membrane grows, a long filament is formed. **(C)** This filament is easily broken into multiple daughter vesicles, which can then themselves grow and repeat the process. Adapted from Zhu and Szostak (2009).



Szostak's team started with extremely simple chemical ingredients to demonstrate how physical processes can lead to vesicle replication. An alternative approach is to start with a complex modern organism and then simplify it. Romaine Mercier and his team did exactly this. They took advantage of the fact that under certain conditions, some species of bacteria do not produce an outer cell wall (what is known as the peptidoglycan cell wall) (Mercier et al. 2013). When a cell wall is absent, the bacteria are called the *L-form* of the species. In such cases, the bacteria vary substantially

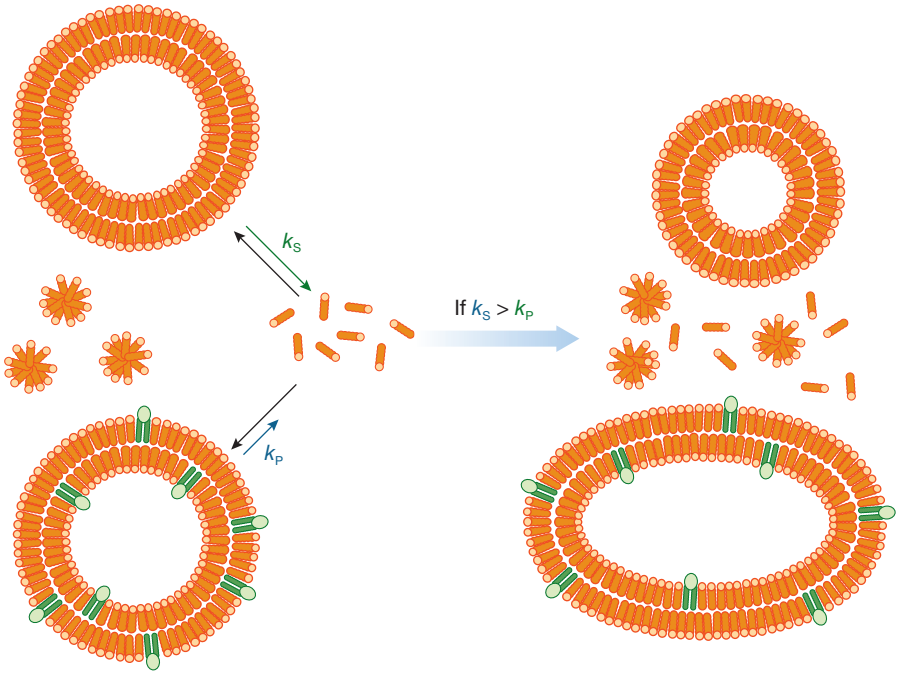


FIGURE 11.16 A selective process favoring membrane phospholipids. Fatty acid molecules move rapidly in and out of a simple fatty acid vesicle, dissociating from the membrane at a high rate (k_S). As phospholipids are incorporated into the vesicle membrane, the rate k_P at which fatty acid molecules dissociate from the membrane decreases, so that $k_S > k_P$. As a result, the simple vesicle shrinks in size, and the one with phospholipid grows. Adapted from iBiology.org.

in shape, and their form is maintained primarily by a fatty acid membrane layer. In this way, they mimic the presumed structure of early life. When Mercier and his team artificially increased the level of fatty acids in the membrane of the L-form of *Bacillus subtilis*, the buckling that occurred because cell surface area increased more quickly than cell volume led to cell division.

If the components of the original cell were divided between the daughter cells, this process would represent the early stage of cellular reproduction. Once cell reproduction—even an imperfect system of cell reproduction—exists, natural selection will favor any changes to the process of reproduction that lead to more rapid reproduction or to increased numbers of daughter cells surviving to reproduce in the next generation.

Hypercycles

To understand how selection may have driven the evolution of encapsulated cells from pools of replicating biotic molecules, it is helpful to look at an analogy from modern forms of life. When members of two species interact in ways that benefit both, we say that the individuals are engaged in a mutualistic interaction, or **mutualism**. Mutualistic relationships—in which each species provides something to the other—are win–win scenarios in that they increase the fitnesses of all parties involved. When biologists talk about mutualisms, they are usually referring to interactions between two or more *species*. But mutualistic relationships can also occur at the *molecular* level. If two or more molecular substrates each contribute in a positive way to the replication of the others, we would call this a **molecular mutualism**. Such molecular mutualisms may have been important among *replicators*—entities that can replicate themselves.

The **hypercycle model** proposed in 1977 by Manfred Eigen and Peter Schuster suggests the following possible molecular mutualism among replicators leading

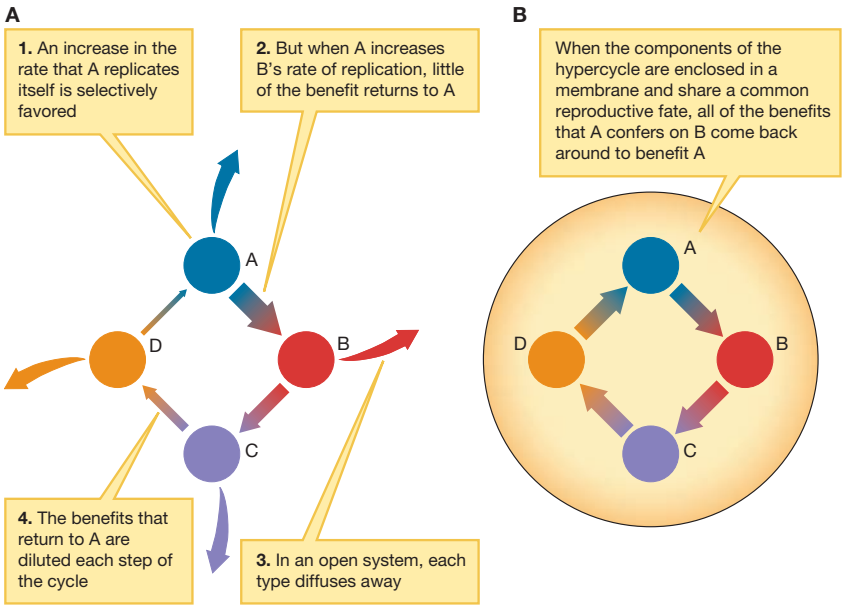


FIGURE 11.17 Hypercycles. **(A)** In a hypercycle, each of four types of replicators—indicated by the colored circles—self-replicates, and each also facilitates another's replication. These four types are shown in a free solution here. As indicated by the arrows, B replicates faster when more A is present, C replicates faster when more B is present, and so on. This chain of facilitation forms a closed loop: A → B → C → D → A. But each type of replicator diffuses away, so only a small fraction of the benefit that A confers on B will return to A through the cycle. Thus natural selection favors variants that increase their own rate of replication but does not favor variants that increase the rates of replication of other types. **(B)** If a hypercycle is enclosed in a membrane, the benefits that A confers on B return to A through the cycle. Natural selection now favors any reaction in which one type of replicator increases the rate at which another type replicates.

to early cellular life: Imagine four independent replicators labeled A, B, C, and D (**Figure 11.17A**). Suppose that these replicators are all found in the same environment and in close proximity to one another, and that they interact in a cycle; that is, a closed loop of the form A → B → C → D → A, where an arrow from A to B indicates that A facilitates the replication of B, and so on (Eigen and Schuster 1977).

In a hypercycle, a positive feedback loop exists, such that the rate of replication of any one replicator is a function of the concentration of the replicator that preceded it in the cycle—the more A present, the greater the replication rate of B; the more B present, the greater the replication rate of C; the more C present, the greater the replication rate of D; and, finally, to close the loop, the more D present, the greater the replication rate of A. This hypercycle is a type of molecular mutualism, in that replicators affect each other's reproduction in a positive manner. And this might become an even more prominent factor if two separate hypercycles were somehow linked to produce an even more complex new hypercycle.

To see why hypercycles may have been an important stage in the evolution of the cell, we need to think about how mutations in hypercycles generate *variation* and how *natural selection* may favor variations that lead to more complex life-forms, including cell-based life. Consider a mutation that causes replicator A, at some cost to itself, to increase the replication rate of replicator B. Let's call this mutation A′. Such a mutation might, for example, make A′—or the chemical products that A′ produces—more readily accessible to replicator B. In the hypercycle we have described so far, natural selection would not favor a mutant like A′. Why? Because

A, B, C, and D are all independent entities. The mutation we have posited, however, would be costly only to replicator A′, and, at the same time, it would have a direct positive effect on replicator B.

But other conditions might allow our mutation to increase in frequency. Very early replicators were capable of synthesizing fatty acids. What would happen if the four replicators in our hypercycle were all enclosed within some sort of surrounding membrane made from the fatty acids they produced or that they extracted from their environment? That is, what would happen if the four replicators were encased in a primitive protocell? Now when a mutant A′ increases the rate of B, this will feed back to help A′ for two reasons. First, the B replicators that benefit from A no longer diffuse away. They stay around, facilitating the replication of Cs, which facilitate Ds, which then accelerate replication of the A′s. Second, when replicators live together inside a membrane, we can speak of the entire membrane-enclosed ensemble of replicators as an organism, and so the four independent types of replicators in our original example have become a single organism with four constituent parts in a cellular hypercycle.

Anything that accelerates the replication of the organism as a whole will be selected. For example, if the rate of cell reproduction is a function of the total concentration of all replicators within it, then this type of mutualistic relationship might be favored. In other words, natural selection might favor mutualistic replicators enclosed in cells over those not encapsulated in cells (**Figure 11.17B**).

We say that natural selection *might* favor such cell life—whether selection *would* actually favor encapsulation of several cells by a membrane depends on all of the costs and benefits of our replicators encapsulated in a cell. Building the membrane requires resources that might otherwise be used for the growth of each replicator. Another significant cost of such encapsulation to A, B, C, and D is that resources must now be brought across a cell membrane, which was not necessary before the replicators were enclosed together. On the benefit side of our cost–benefit analysis, in addition to the selective benefits previously described, other benefits of encapsulation to A, B, C, and D include controlling the microenvironment inside the cell; creating chemical gradients across membranes to let in certain chemicals and keep out others; using the cell membrane as a defensive mechanism against predatory replicators; and partitioning various functions to operate more efficiently than if they were not encapsulated in a cell (Zenisek et al. 2007). If the total benefits outweigh the costs, then selection will favor cellular life. We know that at some point this happened, because all living organisms today are composed of cells.

While biologists distinguish between a variety of different types of cells, for our purposes we can define two basic cell forms: prokaryotic and eukaryotic. Eukaryotic cells have membrane-bound organelles and a distinct nucleus containing DNA. Prokaryotic cells are structurally simpler and evolved much earlier than eukaryotic cells: They typically lack membrane-bound organelles, and their DNA is not contained in a nucleus. The protocells described in this section most likely led to the first prokaryotic cells. We will return to the evolution of eukaryotic cells in the next chapter.

11.4 The RNA World

In modern cells, DNA and RNA encode the information used to make proteins, but the enzymatic activity of proteins is necessary to replicate DNA and transcribe it into RNA (**Figure 11.18A**). We need nucleic acids to make proteins, and we need

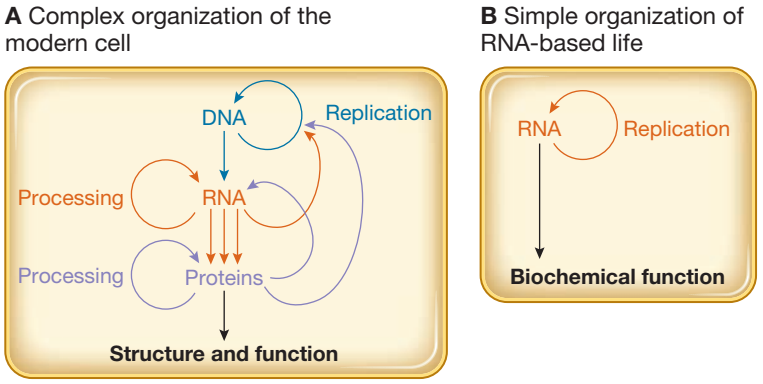


FIGURE 11.18 Organization of life now and in the earlier RNA world. **(A)** Modern cells produce and regulate the production of new DNA, RNA, and proteins through complex interactions of existing DNA, RNA, and proteins. DNA plays an information storage role, RNA an intermediary role, and protein a structural and enzymatic role. **(B)** Crick, Orgel, and Woese hypothesized that the first cellular life had a much simpler organization, with RNA playing all three roles. Adapted from ibiology.org.

proteins to make nucleic acids. Neither can be produced without the other. So which evolved first—the nucleic acids or the proteins? To answer this chicken-and-egg question, several leading scientists (Woese 1967; Crick 1968; Orgel 1968) in the 1960s proposed that very early in the history of life, RNA played both roles: information carrier *and* enzymatic molecule (**Figure 11.18B**).

This hypothesis received considerable support in the early 1980s, when research teams led by Thomas Cech and Sidney Altman independently discovered that enzymes need not be proteins. Rather, RNA itself can act as an enzyme (Kruger et al. 1982; Guerrier-Takada et al. 1983). Such RNA enzymes are called **ribozymes** (**Figure 11.19**). Although ribozymes are much less stable and much less efficient than protein enzymes, a number of different ribozymes have since been documented (Lilley 2003; Orgel 2004). For example, small virus-like particles known as viroids code for one class of ribozymes, which cause damage to commercial plants such as chrysanthemums (Yang et al. 1997; Daros et al. 2006; de la Pena and Garcia-Robles 2010; Kaddour et al. 2011).

Based on these discoveries, Walter Gilbert coined the phrase "RNA world" to capture the idea that early life—from about 4 billion to 3.5 billion years ago—may have been RNA-based rather than DNA-based (Gilbert 1986). Gerald Joyce describes understanding life in such a world as akin to an archaeological mystery:

> It is as if a primitive civilization had existed prior to the start of recorded history, leaving its mark in the foundation for a modern civilization that followed. Although there may never be [direct] evidence for an RNA-based organism, because the RNA world has likely been extinct for almost four billion years, molecular archaeologists have uncovered artifacts of the ancestral era. (Joyce 2002, p. 214)

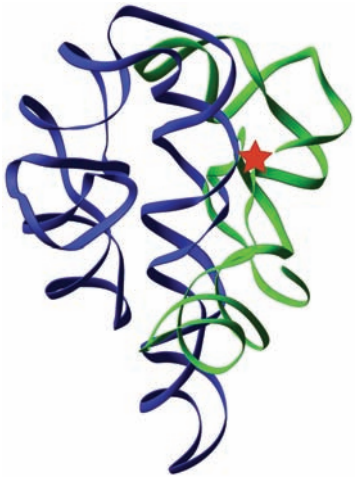


FIGURE 11.19 The chemical structure of the first ribozyme discovered. Colored ribbons show the path of what are called RNA "backbones," and the red star indicates the active site of the ribozyme. This particular ribozyme is a self-splicing intron: It catalyzes its own excision from a precursor RNA molecule.

Several lines of evidence support the hypothesis that life was originally RNA-based (Stozack 2011; Cech 2012). First, many present-day protein-based enzymes have *cofactors*—nonprotein components needed for enzymatic function—that are RNA nucleotides or based on such nucleotides. These may be relics of an RNA world in which catalytic and structural functions were performed by RNAs (White 1976; Maynard Smith and Szathmary 1999). Second, in modern organisms, the deoxyribonucleotides of DNA are constructed by first synthesizing a ribonucleotide intermediate (as used in RNA) and then removing a hydroxyl group through the action of a ribonucleotide reductase (**Figure 11.20**). Adding and later removing the hydroxyl group seems curious if DNA preceded RNA, but it makes perfect sense if DNA evolved as a replacement for an earlier system in which RNA was the informational molecule.

Third, the catalytic site of the ribosome (which is responsible for translation of RNA to protein) is formed entirely from RNA (**Figure 11.21**). In other words, the ribosome is effectively a ribozyme, strongly suggesting that it had a fully functional RNA-only precursor (Steitz and Moore 2003). The ribosome is not the only part of the gene expression pathway that is catalyzed by RNA. Researchers have recently discovered that the eukaryotic spliceosome, a molecular catalyst responsible for the critical process of gene splicing, also has an active site composed entirely of RNA (Fica et al. 2013). These core pathways seem to preserve the remnants of a long-lost RNA world.

Finally, laboratory work mimicking conditions on the early Earth suggests that ribose, phosphate, purines, and pyrimidines—all the essential parts of RNA—likely

existed in the prebiotic environment (Robertson and Miller 1995a). Evolutionary biologists hypothesize that these molecules may have emerged from conditions similar to those simulated in the prebiotic soup experiment or they may have arrived on meteorites, which often contain high amounts of carbon. In either case, if these compounds had bonded together, RNA-based life-forms might have resulted (Benner et al. 1989; Joyce 2002).

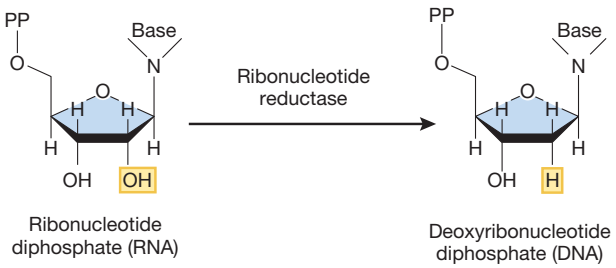


FIGURE 11.20 DNA synthesis passes through an RNA intermediate. In modern organisms, deoxyribonucleotides as used in DNA are synthesized from ribonucleotides as used in RNA by the action of a ribonucleotide reductase. PP indicates two phosphates.

KEYCONCEPT QUESTION

11.4 Was LUCA likely an RNA-based or a DNA-based organism? Why?

Experimental Evidence on the Origins of Natural Selection

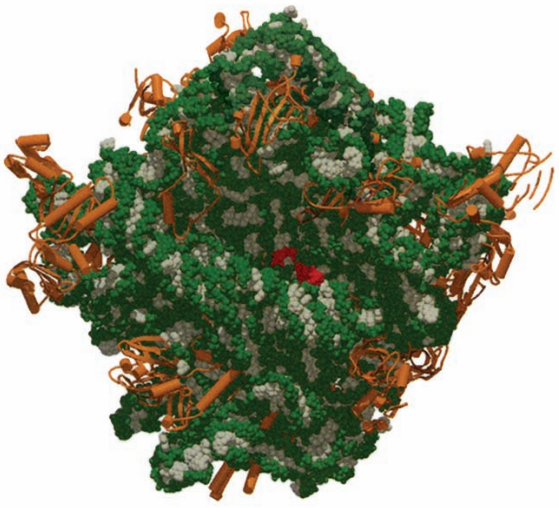
A critical step in understanding how basic chemical reactions could lead to RNA-based entities capable of variation, multiplication, and heritability came in a fascinating experiment by Sol Spiegelman and his colleagues (Mills et al. 1967; Spiegelman 1970). In this early experiment on the origins of natural selection, Spiegelman and his colleagues placed a "primer" strand of RNA, made up of about 4000 RNA nucleotides—adenine (A), guanine (G), cytosine (C), and uracil (U)—into a small test tube. To this mixture, they added more A, G, C, and U nucleotides and a replicase enzyme, which functions to make copies of the RNA molecule. The researchers heated and incubated the mixture, and then they transferred a small drop to a new test tube.

The new test tube contained the replicase enzyme and nucleotides A, G, C, and U, but it did *not* contain primer RNA. This new test tube was heated and incubated, followed by yet another transfer to a new test tube, and so on, for 75 serial transfers (**Figure 11.22**). Spiegelman and his colleagues found that RNA made copies of itself in these test tubes. The interesting thing was not that the RNA was copied—Spiegelman had added replicase enzyme to ensure that this would happen—but rather that natural selection took place on these copies, leading to a change in their characteristics. Let us examine how.

The process of RNA replication carried out by the replicase enzyme involved errors; these errors produced new mutant forms of RNA that differed both in their length and their nucleotide sequence. This generated variation in RNA types on which natural selection might act. Because the replicase enzyme copies whatever strands are present, these changes should be heritable as well.

We already have mentioned that two of the three ingredients for natural selection were present in the experiment: variation and heritability (which was built into the design of Spiegelman's experiment). The third ingredient for natural selection is differential survival or replication, and we would expect this to be present in Spiegelman's experiment as well. We know that shorter RNA sequences will replicate more quickly, and so, in general, selection should favor sequences shorter than the original 4000-nucleotide primer strand. But, if RNA is very short—fewer

FIGURE 11.21 The ribosome is a ribozyme. The active site of the large 30S subunit of the ribosome, shown in red, is composed entirely of RNA (green and white) rather than protein (brown). From the Thomas Steitz lab.



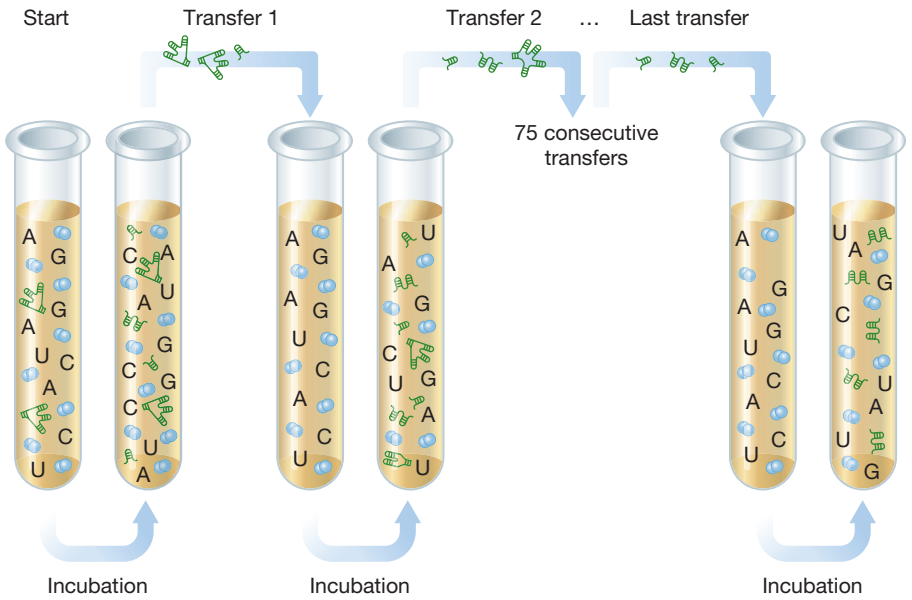


FIGURE 11.22 Spiegelman's experiment on the origins of life. A "primer" strand of RNA made up of about 4000 nucleotides, along with other nucleotides of A, G, C, and U, and a replicase enzyme were placed into a small test tube. The mixture was incubated, and then a small drop was transferred to a new test tube. The new test tube contained replicase enzyme and units of A, G, C, and U, but no additional primer RNA was added. Incubation then occurred, followed by transfer to a new test tube, and so on, for 75 serial transfers. RNA is shown in green; replicase enzyme in blue. At the end of the experiment, the RNA strand that remained was a little over 200 nucleotides long. Adapted from Maynard Smith and Szathmary (1997).

than 50–100 nucleotides or so—the error rate in replication is so very high that these strands can no longer be copied reliably by the replicase enzyme. As a result, we would expect strands shorter than 50–100 base pairs to be selected against.

At the end of the serial transfer experiment, if natural selection was operating in Spiegelman's test tubes, we would expect to see a strand that is less than 4000 nucleotides long and greater than 50–100 nucleotides in length. And that is what Spiegelman found—a strand that was a little over 200 nucleotides long. Apparently, selection for a moderately short strand was very strong, as the end-product RNA was much closer to the 50- to 100-nucleotide minimal length we discussed than to the 4000-nucleotide-long original primer. Shorter RNA sequences, which took a shorter time to replicate, were favored by selection.

Using Spiegelman's protocol, Manfred Sumper and his colleagues further examined natural selection and the early stages of RNA-based life (Kuppers and Sumper 1975; Sumper and Luce 1975; Eigen et al. 1981). They ran an experiment similar to the one described earlier; in addition, they added a chemical called acridine orange. Acridine orange is a dye used in fluorescence microscopy that binds to RNA and typically inhibits replication by the replicase enzyme. Researchers found that while replication was initially inhibited, within a few hours there arose RNA variants that could replicate effectively, despite the presence of acridine orange—and in fact these variants replicated faster with acridine orange present than without. Moreover, these "acridine orange–adapted" variants did not evolve in other experiments where acridine orange was not present. Taken together, these observations suggest strong natural selection for the ability to replicate in the particular chemical environment that the RNA molecules experienced.

KEYCONCEPT QUESTION

11.5 Compare the Spiegelman and Sumper experiments: Why was Sumper's acridine orange experiment a stronger test of the power of natural selection to act on RNA-based life?

In essence, the results from the studies by Spiegelman and by Sumper show how the genetic building blocks of life may have evolved. If small variations in RNA sequences exist, if the sequences can replicate themselves, and if there are fitness differences between these sequences, natural selection will lead to evolutionary change.

Scientists have hypothesized that in the RNA world, RNA molecules would have replicated by using ribozymes. But because the RNA world and its abiotic and biotic conditions have long vanished, evolutionary biologists have created laboratory conditions to examine whether RNA molecules are capable of using ribozymes for independent reproduction (Issac and Chmielewski 2002; Voytek and Joyce 2007).

In an ingenious experiment, Natasha Paul and Gerald Joyce demonstrated that RNA can catalyze reactions involved in its own assembly by using a ribozyme known as R3C (Paul and Joyce 2002). In their work, R3C ligated—that is, joined together—two RNA molecules (let's call them RNA A and RNA B) by forming a bond between them (**Figure 11.23**). Using genetic engineering techniques, Paul and Joyce redesigned R3C so that the product formed by R3C joining RNA A to RNA B was identical to the R3C itself. In ligating RNA A and RNA B, R3C operated both as a template (positioning A and B in relation to each other) and as an enzyme (catalyzing the chemical reaction that joined them). From simpler

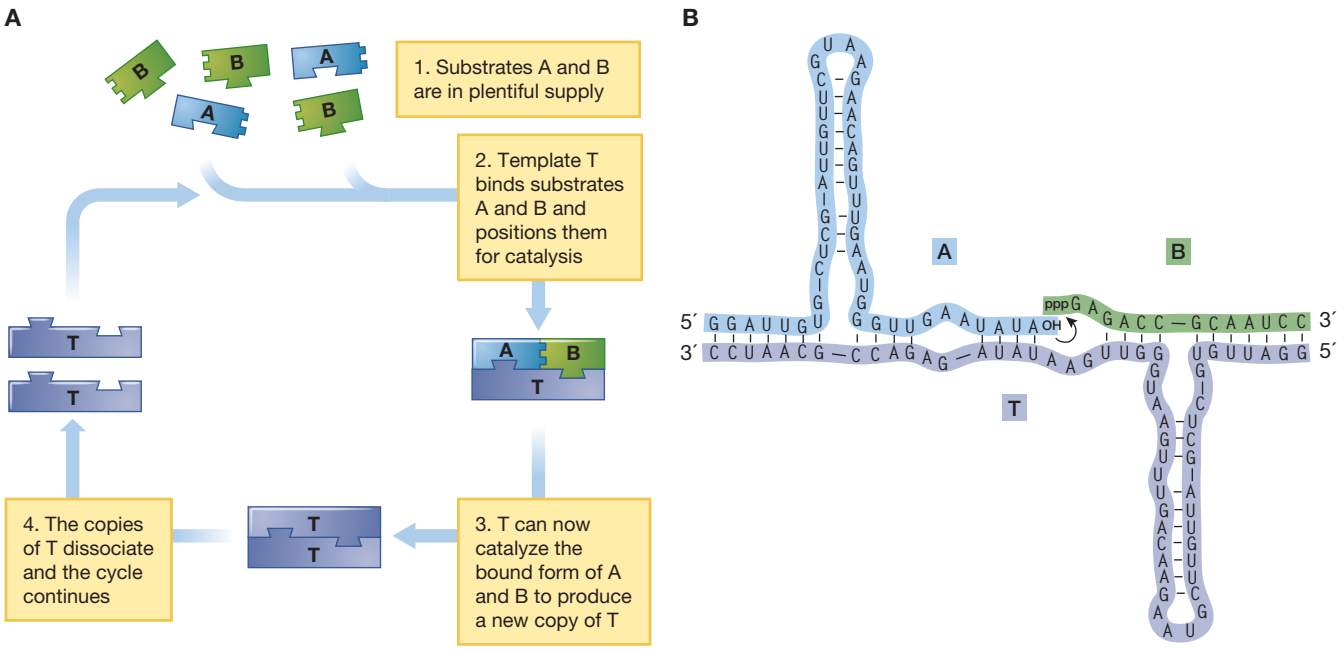


FIGURE 11.23 A self-replicating RNA system. **(A)** A schematic diagram of a simple self-replicating system in which a template molecule T positions the substrates A and B and catalyzes the chemical reaction that joins them together. **(B)** In Paul and Joyce's experiment, the substrates A and B and the template molecule T were constructed out of RNA as shown. Once they are bound together, A and B form a new copy of T. Adapted from Paul and Joyce (2002, 2004).

precursors RNA A and RNA B, R3C created a copy of itself; these copies could in turn create copies of *themselves*. But Paul and Joyce found that their experimental system could only undergo about two rounds of replication before further copying reactions failed, because at that point, the RNA A and RNA B were producing AB compounds instead of binding to the R3C ribozyme.

In a follow-up experiment, Lincoln and Joyce (2009) were able to design a similar system that could replicate indefinitely as long as substrate was present. Whereas the Paul and Joyce experiment demonstrated that RNA species can self-replicate, that system was not capable of evolution by natural selection because it lacked variation for natural selection to act on. To construct a system capable of evolution by natural selection, researchers needed to design a system featuring variation that would affect successful replication in the RNA molecules present. Moreover, these self-replicating molecules needed to have the property illustrated in Figure 11.3B: A variant template T′ needs to catalyze synthesis of other variant T′ molecules rather than copies of the original template T.

In 2009, Lincoln and Joyce found a way to do this. They constructed a system much like the one from the 2002 experiment, but with two different ribozyme templates and four different substrates. After establishing that the template ribozymes in this system could self-replicate, they conducted an experiment in which several template molecules were supplied with various substrate molecules. In this system, new variant forms of the templates arose via various mutations. This variation was heritable, and different templates replicated at different rates. The conditions for natural selection were now met in their system. Lincoln and Joyce found that those self-replicating ribozymes that had more efficient catalytic activity and the ability to grow quickly soon began to dominate their populations of self-replicating ribozymes (Lincoln and Joyce 2009).

11.5 Genetic Information and Genetic Exchange

Assuming early evolution occurred in an RNA world, how do evolutionary biologists explain the shift to a world in which DNA is the primary means by which genetic transmission occurs? To understand how this might have occurred, we need to answer two questions: (1) What sort of biochemical changes produced DNA in the RNA world, and (2) why would DNA be favored once it was present? Let us address the latter question first.

From RNA to DNA

In the RNA world, natural selection would have favored any transmission system that was more efficient than that of RNA and ribozymes (Paul et al. 2006). For a number of reasons, evolutionary biologists have hypothesized that DNA-based transmission may be just such a system. DNA is chemically more stable than RNA, primarily because DNA's deoxyribose sugar is less reactive than RNA's ribose sugar (**Figure 11.24**).

The double-stranded structure of DNA reduces the potential for outside molecules to interact with and disrupt the nitrogenous bases that encode sequence information. DNA replication systems also have "proofreading" capabilities that

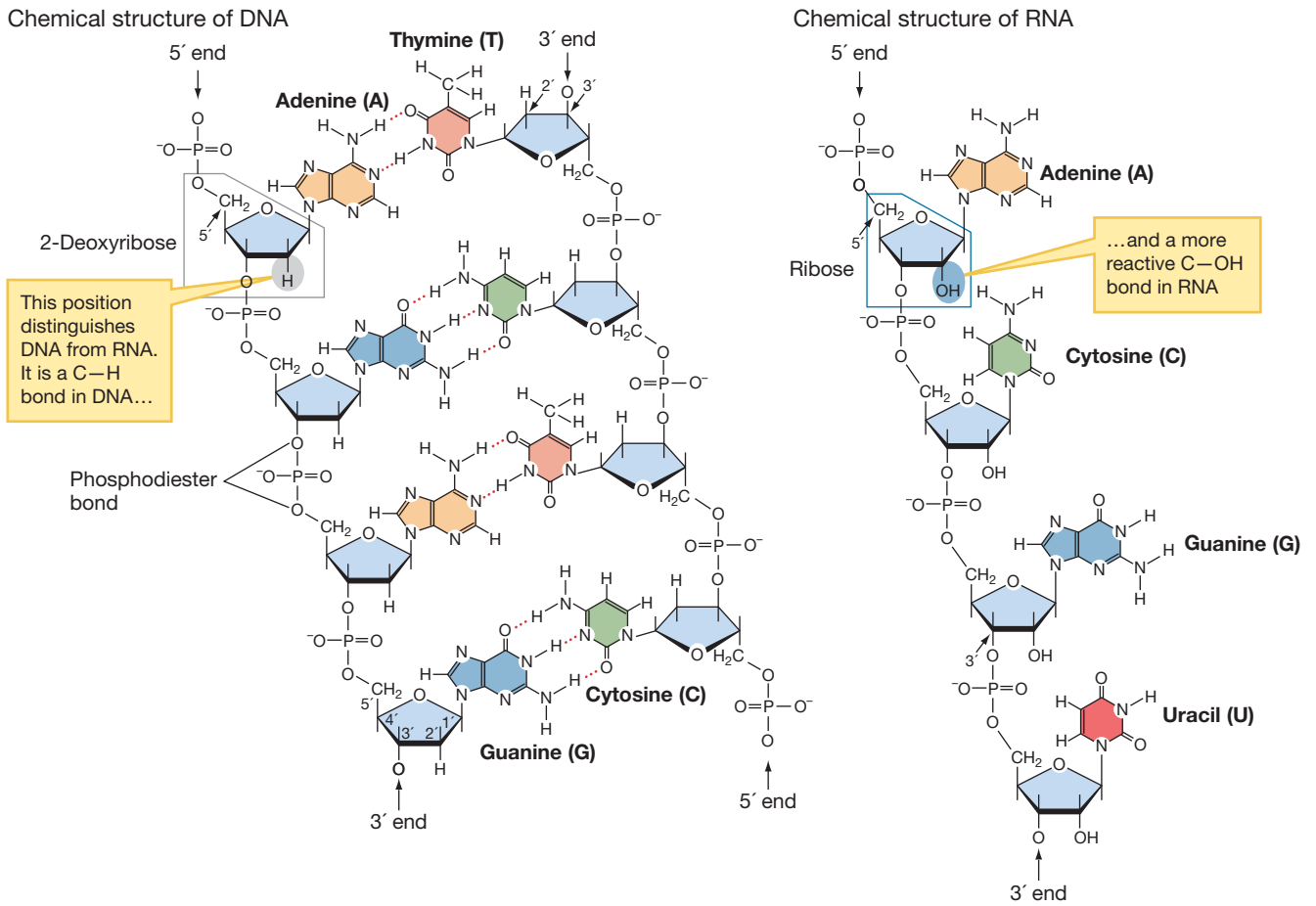


FIGURE 11.24 DNA is chemically more stable than RNA. The C–H bonds on each sugar of a DNA molecule are less reactive than the corresponding C–OH bonds of RNA. Moreover, the double-stranded structure of DNA protects the nucleic acid bases from chemical interactions with other molecules.

are not present in RNA replication. During DNA synthesis, an exonuclease checks each newly added base to make sure it is complementary to that on the parent strand (**Figure 11.25**). Finally, as a result of its double-stranded structure, DNA has repair mechanisms that are not available to RNA. For example, if only one strand of the double helix is damaged, cells can use the complementary strand as a repair template to correct errors.

The higher fidelity associated with DNA proofreading and repair is evolutionarily important because it dramatically lowers mutation rates. Lowered mutation rates allow for longer genes and thus more information to be stored in the genome. When mutation rates are as high as they are in the RNA genomes of RNA viruses, there is a relatively low upper limit—approximately 10,000 base pairs—to the size of a genome that can reliably create error-free or nearly error-free copies of itself (for more on virus evolution, see **Box 11.1**). By lowering mutation rates, DNA proofreading and repair allow DNA genomes to increase in size by many orders of magnitude, thus allowing organisms to store and transmit far more genetic information than would be possible with an RNA genome. Another reason that DNA-based transmission may have been favored by natural selection is because it allowed for specialization within cells—DNA could act as a genetic storage system, while RNA could be involved in other cell functions (for example, it could serve as a cell messenger system), and proteins could perform most enzymatic functions.

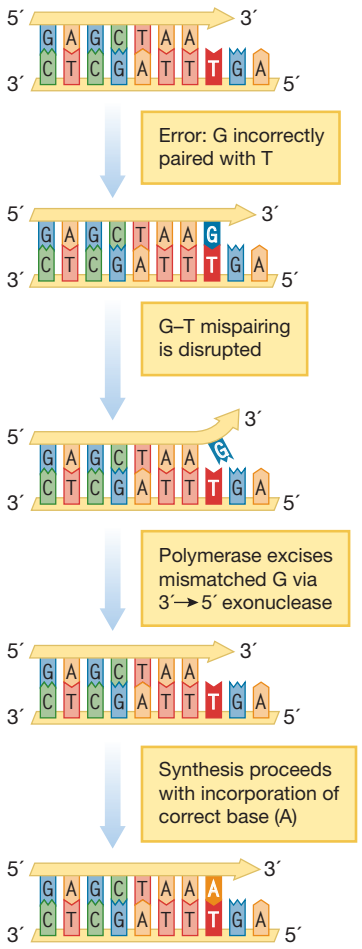


FIGURE 11.25 DNA proofreading. During DNA synthesis, an exonuclease associated with the DNA polymerase removes inappropriate pairings, allowing the polymerase another try to make the correct pairing. Adapted from Cooper and Hausman (2009).

Given the selective benefits that are associated with DNA-based over RNA-based transmission, evolutionary biologists are building mathematical models to decipher what sort of molecular genetic changes might have occurred to produce DNA in an RNA world. In addition, they are constructing experiments to simulate the conditions of an RNA world to find the molecular bridge from RNA to DNA (Robertson and Miller 1995b; Alberti 1997; Saladino et al. 2004).

One question about the RNA to DNA transition is whether DNA can also act as a catalyst. Some laboratory work has used experimental evolution to select for a DNA-like version of a ribozyme (Eisenstein 2006; Paul et al. 2006). Paul and her team began with the R3C ribozyme we discussed in the previous section. They reengineered this ribozyme into what would be its DNA equivalent. To see how this was done, look back at Figure 11.23, which shows the structure of the original R3C ribozyme. Paul and her team replaced each RNA base of uracil with the DNA equivalent, thymine. They took this new DNA-like structure and subjected it to natural selection: Each generation, the DNA-like structures that were best able to ligate the RNA in the medium were selected for the next round of replication. What they found was that after multiple rounds of selection, they had a DNA-like structure that was almost half as effective as the original R3C ribozyme at ligating RNA.

KEYCONCEPT QUESTION

11.6 If the DNA-like structure in Paul's experiment was only half as effective as the original ribozyme, how can this be evidence for the evolutionary move out of the RNA world?

To examine what sort of biochemical changes might have led to the production of DNA in the RNA world, Michael Robertson and Stanley Miller investigated whether formaldehyde (CH_2O)—which is thought to have been produced on the early Earth—played a role in producing DNA. To test this hypothesis, Robertson and Miller mixed formaldehyde with the RNA nucleotide uracil (Pinto et al. 1980; Robertson and Miller 1995b). From the resulting chemical reaction, they found that formaldehyde added something called 5-hydroxymethyluracil to uracil. This is important because 5-hydroxymethyluracil has structures that are similar to the side chains of most of the 20 essential amino acids in proteins, providing an indirect link between the RNA world and the proteins that are so critical in DNA-based genetic transmission. Much work remains to be done to understand fully the move from an RNA world to a world dominated by DNA and proteins, and the studies described in this subsection are examples of the type of research that is being conducted to address this problem.

Horizontal Gene Transfer

In Chapter 10, we looked at the role that horizontal gene transfer (HGT) has played in the evolution of contemporary prokaryotes and their genomes. Most likely, horizontal gene transfer was also very important in the early history of life.

There are large differences between the early cells we have been describing in this chapter and the single-celled life we see today. How can we explain the long-term accumulations of amazing adaptations we see in modern single-celled organisms? By now, we hope the most basic answer to that question is obvious: Natural selection would have favored cells that were better suited to survive

BOX 11.1 Where Did Viruses Come From?

The tree of life contains all known cellular life-forms, but it does not include viruses (Moreira and Lopez-Garcia 2009). We still do not know the exact phylogenetic relationship between viruses and cellular organisms. What can we say about where viruses came from? This remains an unresolved question, but there are three leading hypotheses (Forterre 2006; Koonin 2006; Domingo et al. 2008):

1. The *escaped genes hypothesis* posits that viruses have their origins as selfish genetic elements that replicate within a "host" genome. At some point in their evolutionary history, these rogue stretches of parasitic DNA or RNA somehow evolved or assimilated the necessary protein capsules and packaging mechanisms to allow themselves an independent existence outside of their cellular hosts.
2. The *reduction hypothesis* proposes that viruses have their origins in parasitic cellular organisms. Over evolutionary time, the genomes of these cellular parasites were greatly reduced as the parasites came to rely more and more on the functions of their hosts. Eventually, they abandoned cellular structure, metabolism, and independent replication entirely, taking on protein capsules for existence outside of their hosts.
3. The *relics of the RNA world hypothesis* suggests that viruses are remnants of the original RNA world. According to this hypothesis, they have existed alongside cellular life for its entire history, and as such represent a link back to the first precellular life that existed long before LUCA.

It is possible that more than one of these answers are correct. Viruses almost certainly do not have a single origin, but rather they arose multiple times in the history of life. Different origins could easily have occurred along different pathways. For example, RNA viruses may have arisen as escaped genes. The massive DNA-based mimiviruses (La Scola et al. 2003; Raoult et al. 2004) and pandoraviruses (Philippe et al. 2013) may have come from reduced organisms, possibly even from organisms belonging to previously unknown domains distinct from the current domains of Eukaryota, Archaea, and Bacteria (Pennisi 2013). Viroids (small, circular, noncoding RNAs that infect plants) may be relics from the RNA world.

and reproduce in their environment—in this case, the environment that existed billions of years ago. But we can say more than that if we return to the distinction between modular and nonmodular cellular functions that we discussed in Chapter 10. Along these lines, Carl Woese hypothesized that during *early* cell evolution, horizontal gene transfer was a powerful force leading, in general, to more and more complex cellular organisms (Woese 1998a,b). This is because the metabolic processes within cells would have been far from integrated during this period of time, and many cell functions would have been modular. As a result, horizontal gene transfer may have been the primary means for propagating copies of a gene (Kandler 1994a,b; Woese 1998b). Even if the frequency of horizontal gene transfer was quite low relative to ordinary vertical transmission (in which genes are transferred from parent to offspring), it could have had a very big impact on the diversity of genes present and the structure of the early phylogenetic tree. Indeed, if Woese is correct, then the very idea of a single population of "primordial life-forms" starts to unravel. Instead, early cell life would more closely have resembled a hodgepodge of different cell forms readily exchanging genetic information.

As cell structure and function became more complicated over time, they would also have become more integrated, less modular, and less likely to take up new genes by horizontal gene transfer—HGT would still occur, but its role in promoting adaptation would decrease.

The hypothesis that HGT was important in early evolution has ramifications for building the tree of life. The tree of life has been constructed based on patterns of common descent that presuppose the primary way that genes are transmitted is vertically. During the early evolution of life, *if* HGT was the predominant mode by which genes were transferred—and this hypothesis is still being actively

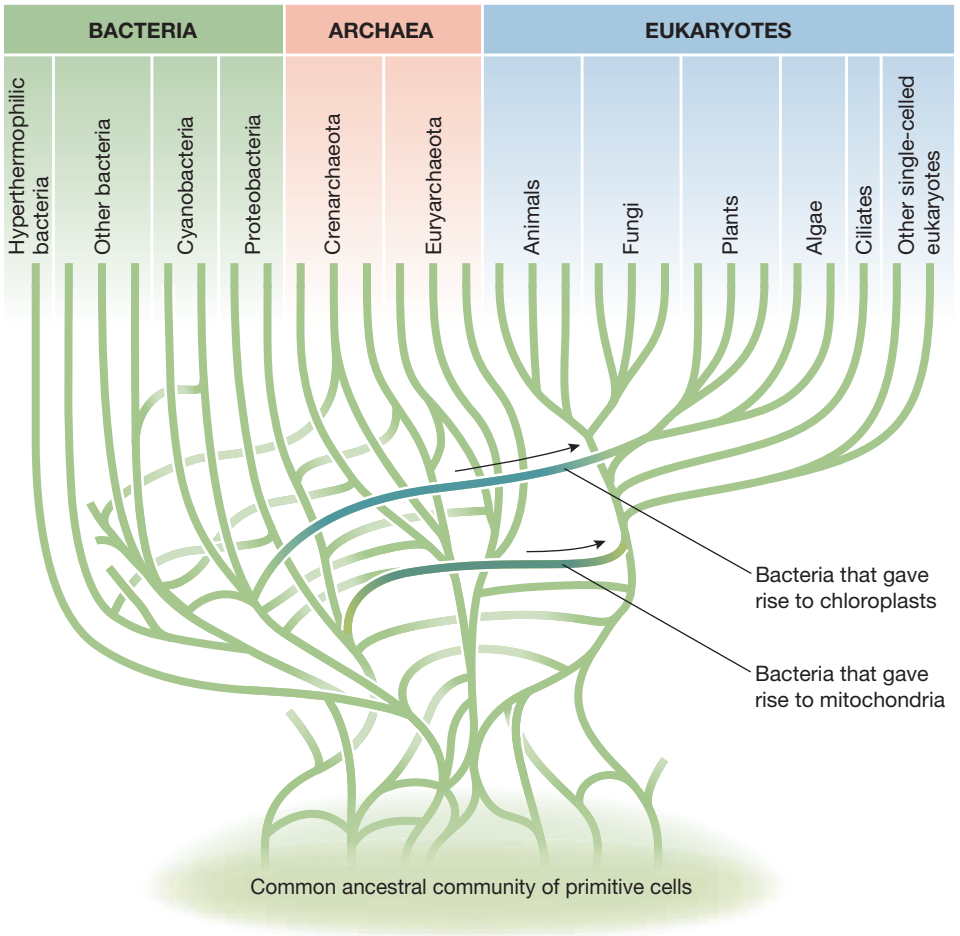


FIGURE 11.26 Horizontal gene transfer and the early evolution of life. During the early evolution of life, *if* HGT was the predominant mode by which genes were transferred, the tree of life might have had a base that reflected a pool of early life-forms that readily swapped gene components. Adapted from Doolittle (2000).

investigated—rather than being rooted on a single universal common ancestor, the tree of life might have had a base that reflected a pool of early life-forms that readily swapped gene components (**Figure 11.26**). Once this period of intense HGT was complete and vertical transmission became predominant, the three main branches of the tree of life—archaea, bacteria, and eukaryotes—would have emerged.

It is important not to misinterpret what the implications of intense HGT might have been early in the evolution of life. The hypothesis that the tree of life might have a base that reflects a pool of early life-forms that readily swapped gene components does *not* imply that all life-forms do not share a history of common descent. Instead, what we are saying is that it is *not* possible to delineate what ancestral species were present during the early evolution of single-celled creatures because HGT blurs the concept of a species.

11.6 Metabolic Networks, Minimal Gene Sets, and Cell Evolution

Genomic analysis provides us with another tool in our efforts to understand the early events in the evolution of life. Although we cannot make direct phylogenetic inferences about what organisms were like prior to the last universal common ancestor, comparative genomic data are nonetheless useful, as they can help us to generate, *on functional grounds*, hypotheses about what kinds of genes may have been present in pre-LUCA genomes. With this sort of genomic analysis, researchers can

try to calculate the minimal characteristics that a cell would need to operate as a living organism (Mavelli and Ruiz-Mirazo 2007).

How might we go about estimating what constitutes the most basic cellular functions and estimating how many genes are necessary to code for such functions? As we noted in Chapter 10, more than 50,000 prokaryotic genomes have been fully sequenced and annotated as of late 2015, with thousands more on the way (see the Genomes Online Database at www.genomesonline.org for the most up-to-date list of fully sequenced genomes). These whole-genome sequences collectively provide us with a comparative perspective on the cellular functions necessary to support life.

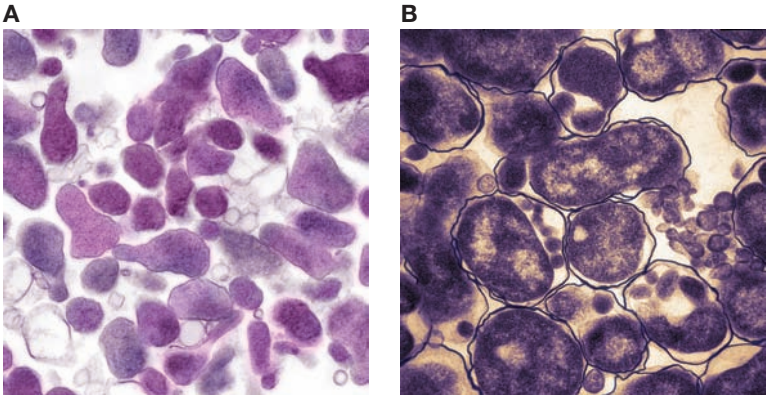


FIGURE 11.27 Microbes with very small genomes may shed light on early life. (A) *Mycoplasma genitalium.* **(B)** *Chlamydia trachomatis.*

To pinpoint the basic and essential cellular functions, researchers have focused on a number of bacterial species with unusually small genomes (**Figure 11.27**). For example, the bacterium *Mycoplasma genitalium* has one of the smallest genomes of any organism that can be grown in the laboratory. This microbe is a parasite of the human urogenital system, and phylogenetic analysis suggests that over evolutionary time, a large decrease in genome size in the genus *Mycoplasma* has occurred. Indeed, *M. genitalium* has only 482 protein-coding genes and 43 RNA-coding genes. Most of the sequenced bacterial species with the smallest genomes are, like *M. genitalium*, parasitic in lifestyle, though some species are symbiotic. For example, *Wigglesworthia glossinidia* and *Buchnera aphidicola* are both *endosymbiotic* species that live in specialized organs within insect hosts; *Rickettsia prowazekii* and *Chlamydia trachomatis* are obligate intracellular parasites (that is, they live only within eukaryotic cells).

Because of their associations with eukaryotic hosts, species such as *W. glossinidia, B. aphidicola, R. prowazekii*, and *C. trachomatis* face relatively stable environmental conditions, and as a result, they may have reduced metabolic requirements and hence reduced genome sizes. This makes them easy to analyze. Nonetheless, a note of caution is required as well. All of these species have small genomes as a consequence of reductive processes; that is, because of the loss of genes over evolutionary time. While they may share many features in common with simple early organisms, there may also be important differences between cells that have small genomes because of loss from a more complex state and those early life-forms that had small genomes formed from the ground up by adding genes. With this caveat in mind, we turn to experimental work on the minimal set of genes needed for cellular life.

Using what is called transposon mutagenesis—a technique that allows researchers to disrupt gene function systematically—evolutionary biologists have found that all 43 RNA-coding genes and at least 380 of the 482 protein-coding genes are essential in *M. genitalium*. If any of these genes are disrupted, *M. genitalium* is not capable of growth. These essential genes are involved in energy metabolism, regulatory functions, fatty acid and lipid metabolism, nucleotide synthesis, transcription, DNA metabolism, and protein binding.

Comparative analyses can tell us yet more about what sorts of genes appear to be essential for basic cellular life. When researchers compared the genome of *M. genitalium* with other microorganisms having small genomes—for example,

B. aphidicola, R. prowazekii, C. trachomatis, and other microbes such as *Escherichia coli* and *Staphylococcus aureus*—a number of interesting patterns emerged (Gil et al. 2004b). A comparison of these genomes showed that certain functions were found in *all* of these organisms, and that these functions were associated with about 206 different genes—referred to as the **minimal gene set**. This gene set included 16 genes associated with DNA metabolism, 106 genes linked to RNA metabolism, 15 genes associated with the processing and folding of proteins, 56 genes linked to energetic and intermediate metabolism, and 13 genes associated with other cellular processes.

What are we to make of this minimal set of genes and the functions associated with it? To begin with, there is nothing fundamental about the absolute number of genes listed. It is based on data from a small subset of species; no doubt, these numbers will change when data from additional genomes are added. Rather than the absolute number of genes, it is the basic functions that are most critical. From this list, we might hypothesize that DNA and RNA metabolism, the processing and folding of proteins, and energetic and intermediate metabolism are the central building blocks that natural selection favored during early cell evolution. One way to test this hypothesis is to see whether we can predict the distribution of such critical gene sets in one species based on what is found in other species. When the distributions of genes in the minimal gene sets of *Haemophilus influenzae* and *M. genitalium* were used to predict the distribution of genes in the minimal gene set for *Bacillus subtilis*, the fit was encouraging (Koonin 2003b) (**Figure 11.28**).

In the long run, we would really like to have comparative information about the genomes of many different creatures with small genomes so that we could search for patterns and make more general predictions. That is, if we could uncover functions that we see in all these microorganisms, then these might constitute the minimal set of functions necessary for cellular life (Koonin 2000; Gil et al. 2004b).

Let's consider another set of experiments on minimal gene sets by Csaba Pal and his colleagues (Pal et al. 2006). These researchers chose *E. coli* as their test species because a tremendous amount is known about *E. coli* cells; for example, when Pal and his colleagues began their work, it was already known that cellular metabolism in the K12 strain of *E. coli* involved approximately 904 genes and 931 unique biochemical reactions (Reed et al. 2003). The researchers ran the following experiment using this strain of *E. coli*: They randomly selected one of the 904 genes, and they deleted it from the genome of *E. coli.* They then assayed whether this deletion decreased the fitness of the cell by measuring its rate of biomass production and comparing this to the rate of biomass production of an *E. coli* cell with no deletions. If the deletion did not affect fitness in any measurable way, it was permanently removed from the genome of that cell. If the gene deletion did decrease fitness, it was restored to the cell. This procedure was repeated as many times as necessary to reach the state in which deleting any of the remaining genes would decrease fitness. At the end of this deletion process, the remaining metabolic

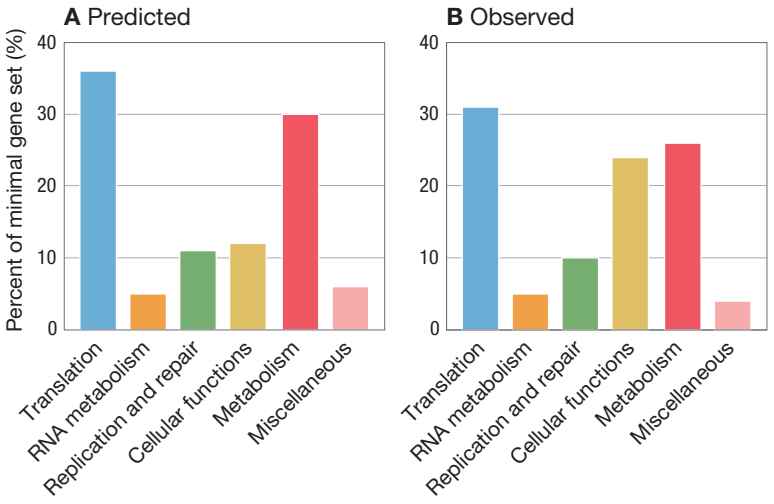


FIGURE 11.28 The distribution of genes in the minimal gene set of *Bacillus subtilis*. **(A)** The predicted distribution of genes in the minimal gene set of *B. subtilis* made through a comparison of the *Haemophilus influenzae* and *Mycoplasma genitalium minimal* gene sets. **(B)** The experimentally determined distribution of genes in the minimal gene set of *B. subtilis*. Note that these results very closely match the predicted distribution. Adapted from Koonin (2003b).

pathways were documented. This experiment was repeated 500 times to mimic 500 independent evolutionary scenarios.

Pal and his colleagues found that metabolic networks that remained in these 500 replicates were quite similar—about 77% of the metabolic pathways remaining were shared across all 500 experimental replicates. That is, of all the metabolic pathways that we know of in *E. coli*, these 77% seem to be essential to the organism—we might call this the "minimal set of metabolic pathways" in *E. coli* (K12). If an *E. coli* strain were to lose these metabolic pathways, natural selection would act against such a strain, and it would eventually be lost from the population. Pal and his colleagues used this information to make predictions about the sort of characteristics that cells might have possessed during the early stages of cell evolution. They hypothesized that if they were to examine the cellular metabolism of species that were closely related to *E. coli* but had genomes that were much smaller than that of *E. coli*, they would find that metabolic networks in these species would resemble the minimal set of metabolic pathways that they had found in their *E. coli* experiment. In other words, they attempted to test ideas about very simple cell life by working backward from a modern organism that has a very long evolutionary history.

The minimal set of metabolic pathways from the *E. coli* deletion experiment was compared to the metabolic pathways of two closely related species: *B. aphidicola* and *W. glossinidia.* Both *B. aphidicola* and *W. glossinidia* were chosen not only because they are evolutionarily close to *E. coli*, but also because they are endosymbiotic organisms, and so they often obtain all their resources from their hosts. What this means is that some of the genes and the metabolic pathways typically associated with resource acquisition and processing are not necessary in endosymbiotic organisms. Moreover, as we noted earlier, because endosymbionts cannot live outside their host, they typically experience only a narrow and controlled range of environmental conditions, further reducing the necessary set of genes for these organisms. As such, we predict that natural selection should have favored a reduction in genome size and the number of metabolic pathways in these sorts of species—and, indeed, the entire genome of *W. glossinidia* is 75% smaller than that found in *E. coli.*

Pal and his colleagues used published information on the genomes and metabolic networks in these two endosymbiotic species to examine whether their metabolic pathways were similar to the minimal metabolic pathways in *E. coli* (Gil et al. 2004a,b). They found support for their hypothesis, as those networks that were most commonly found at the end of their *E. coli* deletion experiments coincided with the metabolic networks that are present in *B. aphidicola* and *W. glossinidia.* Thus, by studying a reductive process of genome evolution, they were able to make predictions about which metabolic pathways would be present in *B. aphidicola* and *W. glossinidia*—two modern organisms that are presumably derived from reductive processes of genome evolution. This analysis also sheds light on what sorts of genes may be essential for basic cellular life, and hence on early evolution on Earth.

The approach we have described in this section is a powerful one, in that it allows researchers to integrate genomics, hypothesis testing, and experimental manipulations to address general questions about the evolution of early life.

In this chapter, we have outlined conceptual, theoretical, and empirical work on the origin and evolution of early life on Earth. Of course, much of what interests

biologists has happened since LUCA and the early evolution of cellular life. Indeed, many major evolutionary transitions have taken place since the evolution of the prokaryotic cell. We will explore these major transitions in the next chapter.

SUMMARY

1. Properties of living organisms include homeostasis, structural organization, metabolism, growth and reproduction, and the ability to respond to environmental conditions or stimuli. In addition, all life is subject to the process of evolution by natural selection. Thus, the origin of life encompasses more than just the origin of self-replicating entities: Heritable variation for natural selection to operate on was also a necessary component.
2. Understanding the origin of life requires interdisciplinary collaboration among biologists, chemists, geologists, atmospheric scientists, and researchers from many other fields.
3. At the base of the tree of life is the last universal common ancestor (LUCA). LUCA was not a single organism, but rather a population of organisms. LUCA was not the first life-form or the only life-form present at the base of the tree of life. But, by definition, it is the only one that left any descendant lineages that remain to this day.
4. When we use phylogenetic analysis, we cannot see back beyond LUCA, for LUCA is a common ancestor to any group of living species that we might choose to analyze. LUCA represents a phylogenetic event horizon: a point in the history of life beyond which phylogenetic analysis cannot possibly see.
5. In the 1920s, Oparin and Haldane proposed the prebiotic soup theory for the origin of life. Miller and Urey found that by simulating the conditions on the early Earth, they could produce some of the building blocks of life; namely, amino acids. Additional organic molecules may have come from space via asteroids, comets, and interstellar ices and from hydrothermal vents deep in the oceans.
6. Simple chemical processes in liquid or on the surface of mineral clays can assemble amino acids into proteins and nucleic acids into nucleic acid polymers such as RNA strands.
7. Lipid molecules can self-assemble into vesicles that grow, divide, and even facilitate selective processes. The hypercycle model provides one explanation for how selection could drive the origin of protocells.
8. Several lines of evidence support the hypothesis that early life was RNA based. The discovery of RNA enzymes called ribozymes led to the RNA world hypothesis, which supposes that in the first living organisms, nucleic acids played both informational and catalytic roles.
9. Evolutionary biologists have built mathematical models and conducted experiments to simulate the conditions of the RNA world, in part to find a bridge from the RNA world to a world in which life is dominated by DNA and protein.
10. The origin and early evolution of bacteria were accelerated by what is known as horizontal gene transfer (HGT). HGT of genes or gene clusters may be especially important with respect to modular cell functions—those not extensively integrated with other functions in a cell. Depending on the extent of HGT, early cell life might resemble a hodgepodge of different cell forms readily exchanging genetic information.
11. Using genome analysis and experimental manipulations, scientists are attempting to understand early cellular evolution by calculating the minimal characteristics that a cell would need to operate as a living organism.

KEY TERMS

abiogenesis (p. 407)
hypercycle model (p. 413)
last universal common ancestor (LUCA) (p. 406)
minimal gene set (p. 426)
molecular mutualism (p. 413)
mutualism (p. 413)
phylogenetic event horizon (p. 406)
prebiotic soup (p. 407)
prebiotic soup hypothesis (p. 407)
protocell (p. 406)
ribozymes (p. 416)
RNA world (p. 404)
vesicle (p. 409)

REVIEW QUESTIONS

1. Why are such rigid requirements necessary to identify something as a microfossil?
2. What is LUCA?
3. What is meant by a phylogenetic event horizon?
4. What is the prebiotic soup hypothesis?
5. What is the possible role of clay in the origin of life?
6. What is the RNA world hypothesis, and why are ribozymes central to this idea?
7. List three pieces of evidence supporting the RNA world hypothesis.
8. Why do evolutionary biologists think that DNA-based transmission systems are more efficient than RNA-based systems?
9. What are the three leading hypotheses for the evolution of viruses?
10. What is the core idea behind the hypercycle model for the evolution of cells?
11. What functions appear to be most critical for cells and are associated with genes in the minimal gene set?

KEY CONCEPT APPLICATION QUESTIONS

12. Besides ocean temperature, what other variables might shed light on the abiotic environment upon which natural selection acted during the early evolution of life?
13. How does work on the chemical composition of extraterrestrial objects such as asteroids and meteors inform the study of the origin of life?
14. What additional experiments might follow the "one-gene-at-a-time" gene deletion experiments that are now being used to study minimal gene sets?
15. Draw a phylogenetic tree that incorporates both a pool of early life-forms that readily swapped gene components and LUCA.
16. Why don't we see life originating again and again from scratch on Earth now?

SUGGESTED READINGS

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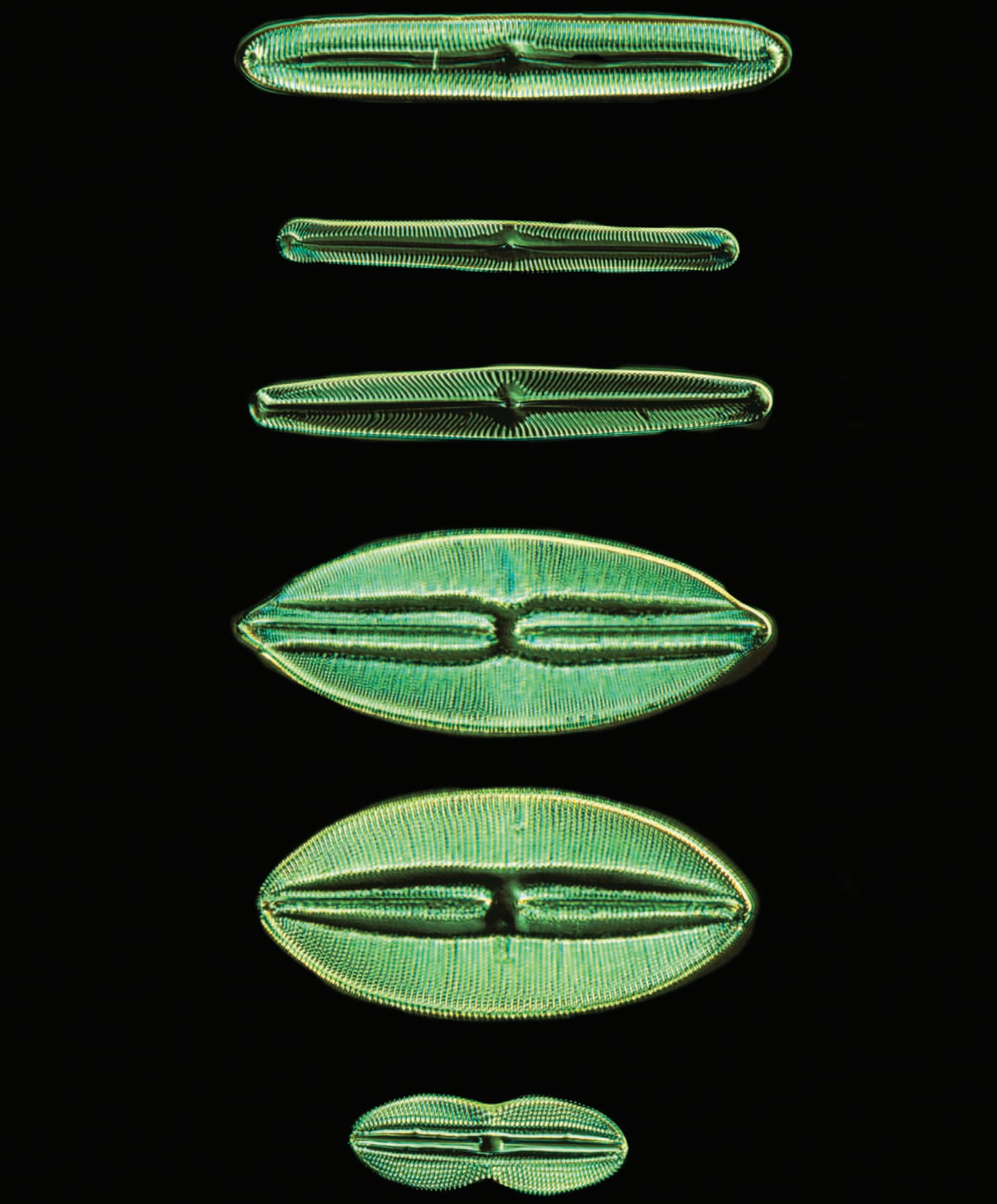

12

Major Transitions

◀ Unicellular planktonic algae known as diatoms display intricate geometric forms.

Cellular slime molds—also known as social amoebas—spend much of their lives as single-celled creatures. There is nothing unusual about that. But then they undergo a radical developmental shift, in which thousands of these free-living cells come together to form a multicellular group called a "slug" (Kessin 2001; Bonner 2009). This is unusual. Rarely do we see free-living, single-celled organisms relinquish their autonomy to become one of many cells in what amounts to a sort of primitive multicellular-like creature. Because of this feature of their development, slime molds, which first appeared about 1 billion years ago, are a model system for looking at what are called *major transitions* in evolution—fundamental organizational changes in the history of life. Slime molds provide some hints about one of these major transitions: from single-celled organisms to multicellular organisms.

The best studied of the slime molds is *Dictyostelium discoideum* (Raper 1935; Kessin 2001). In this species, the earliest developmental stage is a *single-celled* individual—often referred to as an amoeba—that feeds on bacteria living in the soil. A single small patch of soil may contain millions

of *D. discoideum* feeding independently of one another. Although individual cells can reproduce asexually, they usually do not. Instead, once an area of soil is depleted of available food supplies, between 8000 and 500,000 single-celled *D. discoideum* in that area come together and form a multicellular slug. The newly formed slug then migrates to an area closer to the surface of the soil, where reproduction occurs.

After the slug form of *D. discoideum* has moved to the surface of the soil, it breaks up into a collection of multicellular structures called fruiting bodies. Fruiting bodies are composed of two kinds of cells: those that form a stalk section—these cells anchor and secure the fruiting body in place, but they sacrifice the ability to reproduce—and other cells that produce the reproductive spores of the fruiting body. Spores are enclosed in a capsule at the top of the stalk. The spores are released and disperse when an invertebrate predator, such as a tiny roundworm, disturbs the fruiting body, or when the fruiting body sticks to the invertebrate, or when the soil is flooded. When spores mature they form individual amoebas, and the cycle begins again in a new generation (**Figure 12.1**). During the fruiting body stage, the once solitary amoebas are part of a pseudo-multicellular creature. The cells in the stalk of the fruiting body function as **somatic cells**, the cells that grow and maintain the body of a true multicellular organism. The spore cells act as **germ cells**, the cells of a multicellular organism that are specialized for reproduction. The transformation of the single-celled amoebas into multicellular slugs and fruiting bodies provides hints as to how one of the major transitions in evolutionary history may have occurred. We will explore this transition in greater depth later in this chapter.

In the previous chapter, we considered the origin and early evolution of life, and we described some of the very simple organisms that may have represented the first steps in the evolution of life on Earth. In this chapter, we will examine some of the major transitions that have occurred since those first steps.

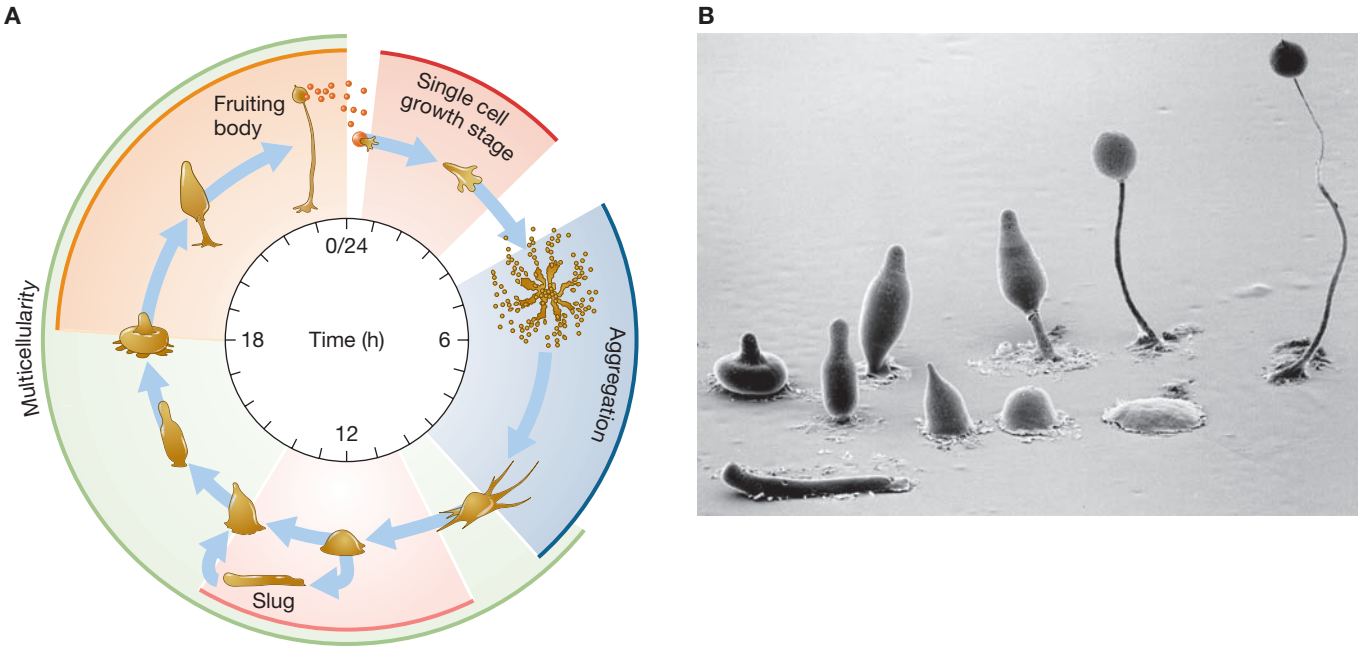

FIGURE 12.1 Stages of development in slime mold. (A) Developmental stages in *Dictyostelium discoideum*. Multicellular stages are in the green arc. Adapted from Fey et al. (2007). **(B)** Electron micrograph of the different developmental stages in *D. discoideum.*

To conceptualize the idea of a major transition, compare your own physiology to those of the earliest organisms: Compare your body to ensembles of autocatalytic molecules, to protocells, to primitive prokaryotes, to a slime mold. Your body is composed of approximately 10^{13} cells, organized into extensive and elaborate organs and tissues. Each cell contains within it the detailed intracellular organization that we observe in eukaryotes. Within the cell nucleus, we find more than just a random collection of genes: We find a highly structured genome, arrayed along 23 pairs of homologous chromosomes. In short, the structure of our bodies is vastly more *complex* than any early life-form. The same is true along any number of branches of the tree of life: It is an astonishingly long way from autocatalytic cycles and protocells to plants and animals, forests, coral reefs, and dolphin pods (**Figure 12.2**).

Yet there is nothing in the process of evolution by natural selection that should *necessarily* entail a buildup of complexity over time. Indeed, along some branches of the tree of life, we have seen very little increase in complexity for billions of years. Modern bacterial and archaeal lineages may be scarcely more complex than their ancestors that lived before the origin of multicellular life. Sometimes we even see complexity evolve, only to be lost again later.

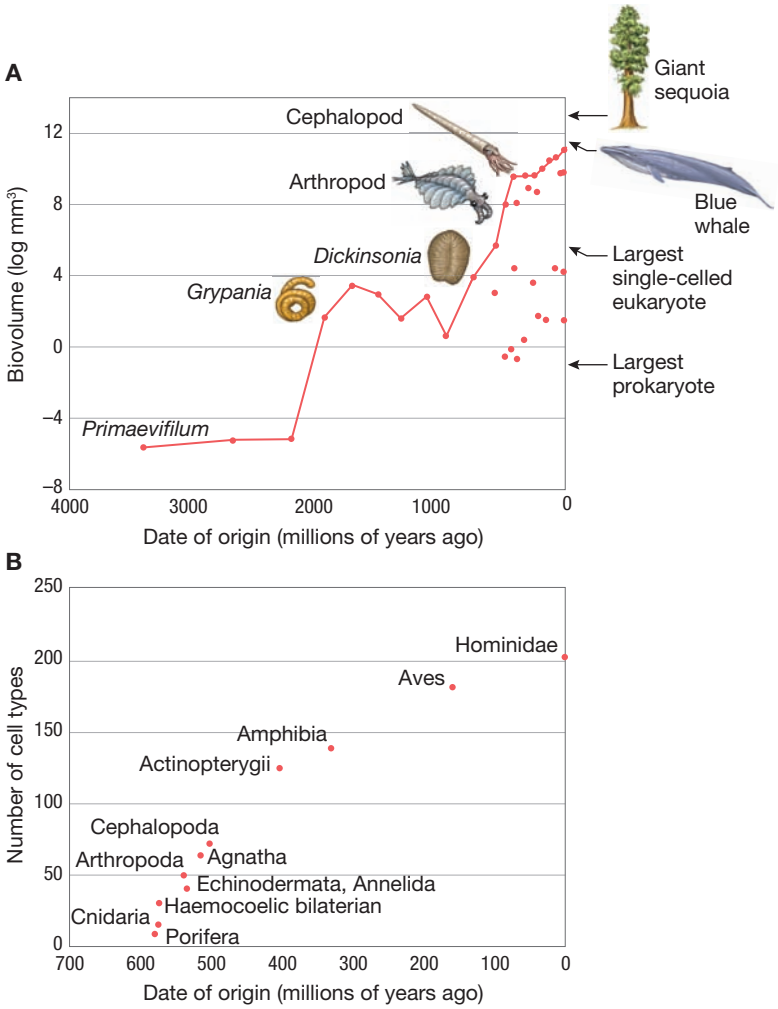

FIGURE 12.2 Some organisms have become larger and more complex over evolutionary time. **(A)** The body size, measured as total volume, of the largest living organisms has increased over evolutionary time. The largest living things today are 10^{18}—that is, a billion billion—times larger than the earliest life-forms. Adapted from Payne et al. (2009). **(B)** The complexity of multicellular organisms—here as measured by the number of cell types—has also increased. Adapted from Valentine et al. (1994).

KEYCONCEPT QUESTION

12.1 Figure 12.2B, with its linear hierarchy of complexity placing humans at the top, bears a disquieting similarity to the *scala naturae*, or great chain of being. One possible reason for this similarity is that humans really do have an exceptionally high number of cell types. Can you think of any alternative reasons why there might be more known cell types in humans than in the other species shown in the figure?

In this chapter, we will explore the following:

- What are considered to be the major transitions in evolution?
- What are explanations for some of the major transitions?
- Why was the evolution of the eukaryotic cell a major transition?
- How can the evolution of multicellularity be understood as a second example of a major transition?
- Why did the evolution of individuality in multicellular creatures constitute a major transition?
- How can the shift from solitary to group living be seen as a major transition?

12.1 Overview of Major Transitions

How did the complexity that we observe along some branches of the tree of life arise? What were the major events in the history of life that led to the elaborate forms that we see around us today? To answer such questions, we will focus here on what evolutionary biologists John Maynard Smith and Eörs Szathmary have called major transitions in evolution (Szathmary and Maynard Smith 1995; Maynard Smith and Szathmary 1997). Maynard Smith and Szathmary looked at some of the most critical events in the evolution of life on Earth—events that have changed the way that life is organized. These include

- The origin of self-replicating molecules capable of heredity.
- The transition from RNA as both catalyst and genetic material to a division of labor with proteins as catalysts and DNA as genetic material.
- The origin of the first cells.
- The emergence of eukaryotic cells.
- The evolution of sexual reproduction.
- The evolution of multicellular organisms from single-celled ancestors.
- The evolution of developmental complexity within multicellular organisms.
- The evolution of individuality, including the evolution of germ cells, a specialized line of cells that became gametes.
- The evolution of groups, including complex societies.
- The evolution of eusocial societies, like those seen in some species of bees, ants, and wasps, with a division of labor and sterile castes.

We have already treated the first three items in the previous chapter. We will treat the evolution of developmental complexity in Chapter 13. We will examine the evolution of sex in Chapter 16. Because the evolution of eusociality requires a background in relatedness and kin selection theory, we will postpone that topic until Chapter 17. In this chapter, we will look at the remaining evolutionary transitions: the emergence of eukaryotic cells, the evolution of multicellularity, the evolution of individuality, and the evolution of groups.

Some of the major transitions were most likely unique events in the history of life—the origin of the genetic code, for example, was a unique occurrence—while other transitions such as the evolution of multicellularity and the evolution of group living have evolved independently numerous times. Regardless of whether a major transition occurred just once or many times, Maynard Smith and Szathmary hypothesize that many of the major transitions in evolution share a common structure and lead to common consequences. Each major transition encompasses some of the following processes, and a few feature all of them.

1. *Individuals give up the ability to reproduce independently, and they join together to form a larger grouping that shares reproduction.* For example, early in the history of life, independently replicating molecules joined together within a lipid membrane to form protocells (as we discussed in Chapter 11). Later, independently and along numerous branches on the tree of life, unicellular

organisms joined together to form multicellular creatures. Repeatedly and along numerous branches of the tree of life, solitary individuals started living together in groups, sometimes even giving up the possibility of independent reproduction, as we see in many species of social insects. In all these cases, formerly autonomous individuals join together, and the result is a major transition in which these formerly autonomous units now have a shared reproductive fate.

2. *Once individuals aggregate into higher-level groupings, they can take advantage of economies of scale and efficiencies of specialization*. An **economy of scale** arises when a group can perform a task more efficiently than a single individual or when a group can do things that a lone individual cannot do at all. For example, groups of social insects such as ants and bees can acquire food in ways that individuals working alone cannot. Collectively, ants can capture far larger prey than could any single individual (**Figure 12.3**). They can even engage in a sort of agriculture, as we see with leaf-cutter ants and their fungal gardens (we explore this in more depth in Chapter 18).

   Efficiencies of specialization arise because once groups are collectively engaged in a task, they can benefit not only from larger numbers, but also from a division of labor, allowing different individuals to specialize in different tasks. We see this sort of task specialization in social insects, with some individuals acting as guards, others as foragers, and others as "nurses" that take care of developing eggs. Within a single multicellular body, different cells may specialize in generating movement, digesting food, processing information, or other tasks. Perhaps most critically, we see a division of labor between reproductive functions and growth and maintenance functions—the *germ–soma distinction*.

3. *Aggregation and specialization facilitate changes in information technologies. Organisms develop new and increasingly efficient ways to acquire, process, transmit, and store information.* For example, once simple cells form and protein replaces RNA as a catalytic molecule, the fundamental method of storing biological information and passing this information across generations can change. Single-stranded RNA with low replication fidelity is replaced as an informational molecule by double-stranded DNA with high replication fidelity (Chapter 11). The evolution of sex changes the way that genetic information is transmitted through populations (Chapter 16). Another example of a change in information technology is the acquisition of language in humans. Because we deal with the RNA to DNA transition and the evolution of sex in other chapters, in this chapter we will not concentrate on how organisms develop new and increasingly efficient ways to acquire, process, transmit, and store information.

FIGURE 12.3 Economies of scale in ants. *Formica hemorrhoidalis* ant workers attacking a caterpillar. This is a benefit of economies of scale, as a single worker could not capture such a prey item by itself.

KEYCONCEPT QUESTION

12.2 In Section 11.3 of Chapter 11, we discussed possible evolutionary explanations for the origin of protocells. Explain why this would be considered a major transition in evolution.

Explaining Major Transitions

Given the huge advantages that come with economies of scale, division of labor, and advances in handling information, we might think it obvious that natural selection would favor these major transitions. So why do they pose a puzzle to evolutionary biologists? As we learned in Chapter 3, if we want to invoke natural selection as an explanation, we need to explain each change by the "immediate selective advantage to individual replicators," rather than by turning to group-level benefits (Maynard Smith and Szathmary 1997, p. 8). This is tricky in the case of major transitions, because it means that we have to identify advantages at the individual level not only once the transition is complete, but also during the transition so that it can proceed.

Once the transition takes place and individual units group together to form a higher-level individual/organism, we also have to be able to explain why this higher-level individual continues to exist and doesn't break down (**Figure 12.4**). Why don't cooperation and coordination collapse in the face of individual incentives for selfish replication? For example, when cells first band together to form multicellular organisms, why don't individual cells cheat, exploiting the other cells of the organism? Alternatively, why do they not revert to reproducing themselves alone to avoid being exploited? The answer is probably that cheaters often do emerge, and many incipient instances of major transitions were likely stopped in their tracks as a result. But, putting those instances aside, why don't we see the collapse of higher-level organisms that make it past this initial hump?

Part of the answer is that policing mechanisms can evolve. Higher-level individuals that evolve ways to suppress cheating are favored in competition with other higher-level organisms. For example, consider Mendel's first law (the law of segregation). This law states that the process of segregation is "fair," in that on average, at a given locus, half the gametes produced by a heterozygote will contain one allele at that locus, and half the gametes will contain the other allele. In Chapter 17, we will examine alleles called segregation distorters that cheat the system so as to appear in more than half of the gametes, potentially at a cost to the organism. Selection may have favored mechanisms that enforce fair segregation by suppressing segregation distorters. As another example, worker bees may "cheat" and produce their own direct offspring, instead of helping to rear the queen's brood. This has driven the evolution of policing behavior, whereby the eggs of cheating workers are destroyed by their sisters (Chapter 17).

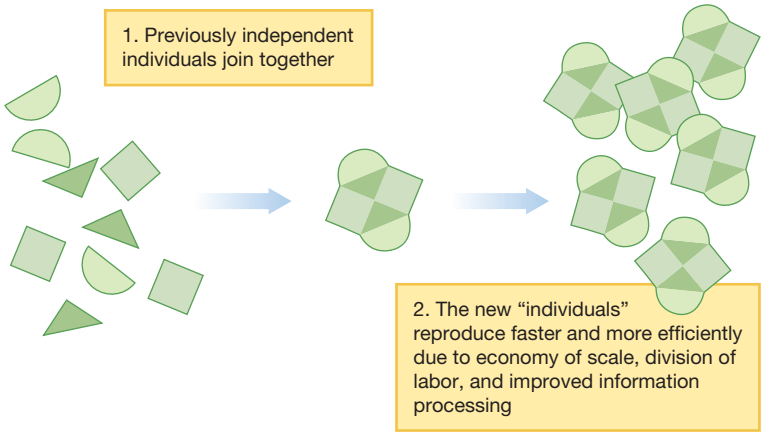

FIGURE 12.4 Steps in a major transition. Individual "replicators" (left) band together to form a new, high-level individual (center) that can then replicate more effectively (right). To provide an adequate evolutionary explanation for this transition, we need to be able to explain both how the process of banding together is beneficial to the individual replicators and why there is not an incentive for the components of the higher-level individual in the middle panel to cheat and revert to independent replication.

Another part of the explanation for why higher-level individuals don't break down due to selfish replicators is that over evolutionary time, the higher-level individuals get "locked in" by some detail of their biology and cannot easily revert to their previous states. Sexual reproduction is a classic example. Why are there no *parthenogenic* mammals that reproduce asexually via unfertilized eggs? That is, why are there no asexually reproducing mammals? Why doesn't sexual reproduction break down due to "cheaters" who reproduce parthenogenically, and hence pass down their entire genomes intact (Chapter 16)? The process of **genomic imprinting**—in which alleles are

differentially expressed according to whether they are inherited from the mother or from the father—seems to be one contributing factor (Chapter 17). Once genomic imprinting evolved in mammals, potentially parthenogenic females faced a new and major barrier: Any parthenogenically produced offspring would have a mother but not a father, and they would thus fail to express a number of important genes that are expressed only from the paternally derived copy (Szathmary and Maynard Smith 1995; Maynard Smith and Szathmary 1997).

While parthenogenesis is common among plants, there are no parthenogenic conifers. This is due not to imprinting, but instead to the way that organelles are inherited. In conifers, unlike in most other plants, the chloroplasts are transmitted through the pollen rather than through the seed. As a result, a parthenogenically produced conifer would lack chloroplasts. Of course, neither imprinting nor pollen-derived transmission of chloroplasts initially evolved as a safeguard to prevent sexual females from reverting to parthenogenesis, but once present, these traits serve this purpose in mammals and conifers.

While there may be later developments that inhibit reversion to the pretransition state, these are not adequate explanations for the *initial occurrence and stability of the transition itself*. To explain any major transition, we have to look at factors that would have been present at the time of the transition.

12.2 Major Transition: The Evolution of the Eukaryotic Cell

In the previous chapter, we discussed the evolution of prokaryotic cells—cells that lack complex membrane-bound organelles and whose DNA is not enclosed within a nucleus. Prokaryotic cells are ancient, having originated approximately 3 billion years ago (Schidlowski 2001). The other basic cell type is the eukaryotic cell, which has membrane-bound organelles—for example, chloroplasts or mitochondria—and a distinct nucleus containing the genomic DNA (**Figure 12.5**). Eukaryotic

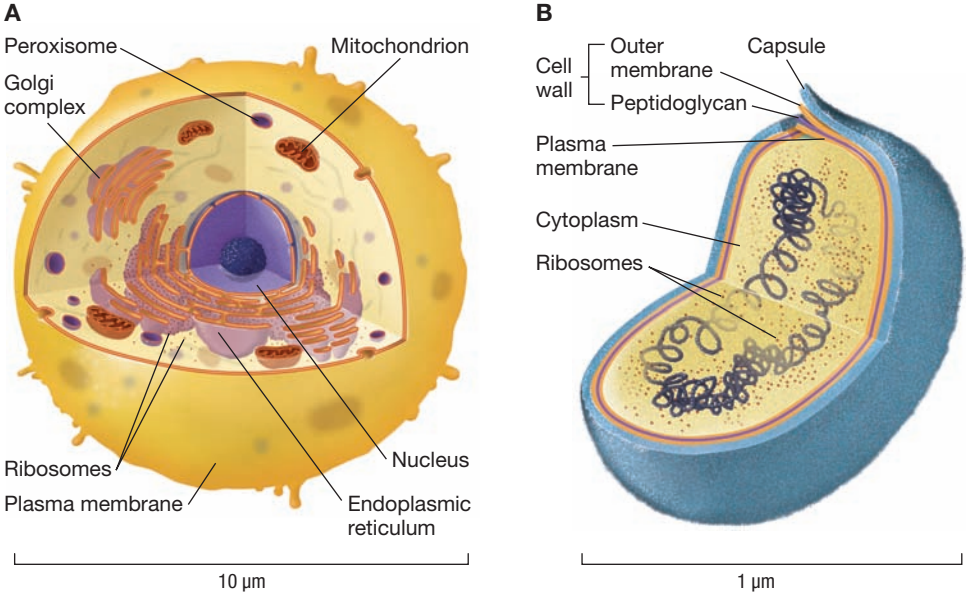

FIGURE 12.5 Eukaryotic and prokaryotic cells. **(A)** A eukaryotic cell has membrane-bound organelles and a distinct nucleus containing DNA. **(B)** Prokaryotic cells lack membrane-bound organelles, and their DNA is not contained in a nucleus. Note the different scales for the two cells.

cells evolved between 1 billion and 2 billion years after prokaryotes—fossil evidence leans toward the earlier estimate, while molecular genetic evidence favors the latter estimate (Berney and Pawlowski 2006; Knoll 2014). In many ways, eukaryotic cells are more complex than prokaryotic cells, as they have very complicated within-cell communication networks that coordinate interactions among organelles, cytoplasmic elements, and the nucleus and target the appropriate proteins and other resources to the appropriate substructures within the cell (Knoll 2006) (**Figure 12.6**). How did the major transition from prokaryotes to eukaryotes unfold? The answer is complex of course, and we are still in the process of building a complete picture of this major transition. Here, we highlight some of the leading theories proposed to explain such a transition.

Endosymbiosis and the Evolution of Eukaryotic Organelles

Because the presence of complex membrane-bound organelles is one of the critical traits that distinguish eukaryotic cells from prokaryotic cells, we need to understand where these structures came from if we are to understand the major transition associated with the evolution of the eukaryotes. In 1970, Lynn Margulis proposed the endosymbiotic theory to explain the origin and evolution of two eukaryotic organelles: the mitochondrion and the chloroplast (Margulis 1970).

Margulis hypothesized that mitochondria and chloroplasts did not evolve de novo as internal components of a eukaryotic ancestor, but rather through a long-term symbiotic, or mutually beneficial, relationship. She proposed that independent bacterial species capable of energy production and photosynthesis began to reside within cells of another species, resulting in **endosymbiosis**, or symbiosis within a cell. These endosymbionts provided their hosts with critical resources such as energy and food, and in return they were protected from various dangers in the environment by residing inside another organism. Over evolutionary time, Margulis hypothesized, this facultative symbiotic relationship became so strong that it developed into an obligate relationship: The endosymbionts, which evolved into organelles of the host cells, were no longer able to live on their own (**Figure 12.7**). This is a classic example of one of the distinguishing characteristics of a major transition: Formerly independent units merge, and the process creates a new unit with a shared reproductive fate. In addition, this major transition also involved individuals aggregating into higher-level groupings and taking advantage of efficiencies of specialization: Chloroplasts specialized in converting light to chemical energy that a cell could use, and mitochondria specialized in converting complex organic molecules into more immediately useful sources of chemical energy.

Margulis's endosymbiont hypothesis was supported by the fact that both mitochondria and chloroplasts have their own genomes—distinct from that found in the cell nucleus. These organellar genomes consist of single circular chromosomes resembling those found in bacteria. Furthermore, phylogenetic analyses based on molecular genetic data have shown that chloroplast RNA is more closely related to that of the cyanobacteria than to that of other eukaryotes. This suggests that chloroplasts were once free-living photosynthetic cyanobacteria before they formed a symbiotic relationship with an ancestral eukaryotic species (Giovannoni et al. 1988). In a similar vein, mitochondrial genes in eukaryotes more closely resemble the genes in α-proteobacteria than other genes in their eukaryotic hosts (Gray et al. 1999; Gray 2012).

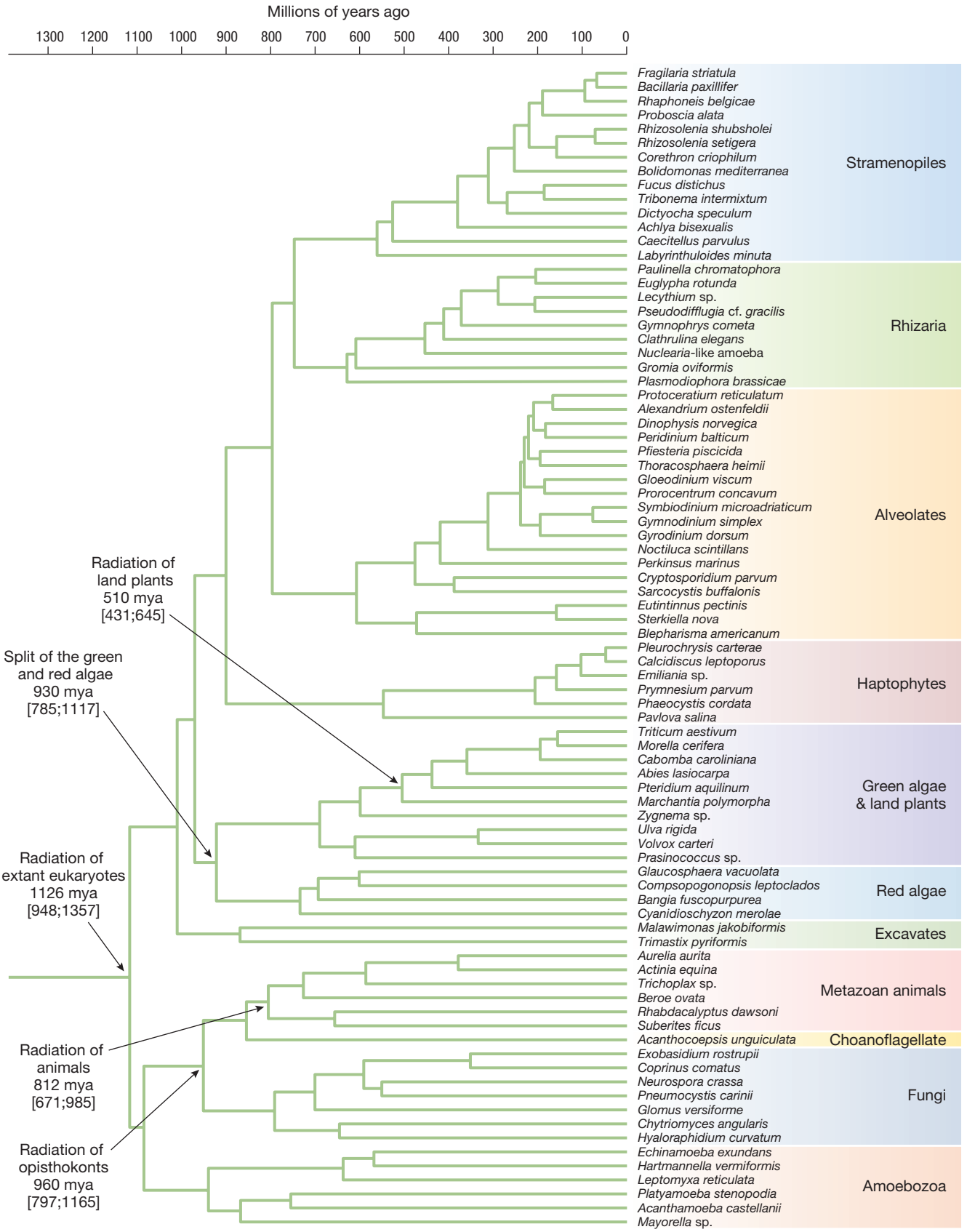

FIGURE 12.6 Major events in the evolution of eukaryotes. A chronogram of major events in eukaryotic evolution, based on 83 ribosomal RNA gene sequences and calibrated using the fossil record. Under each major event is the estimated time of the event (million years ago; mya) and the confidence interval around this estimate. Adapted from Berney and Pawlowski (2006).

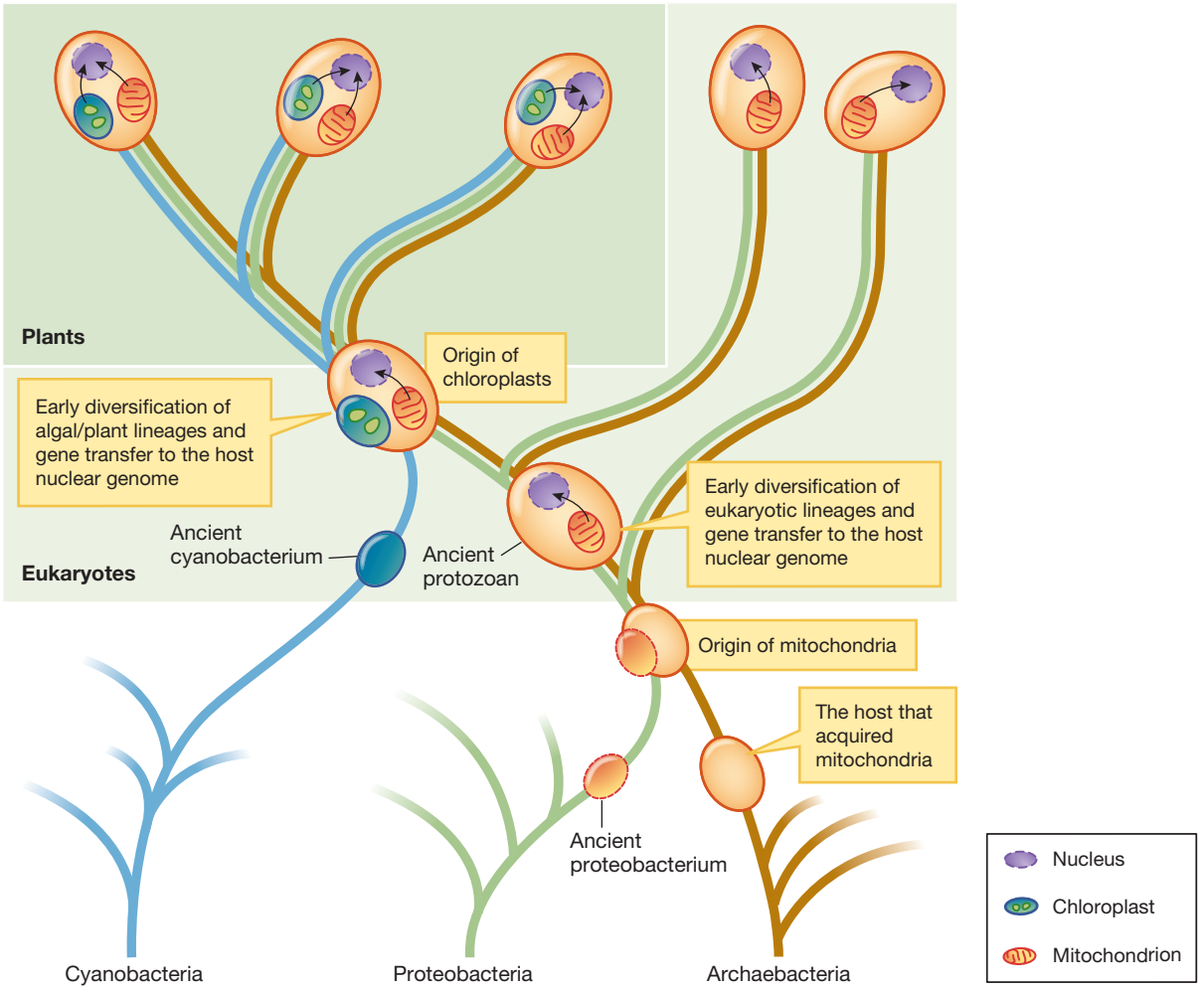

FIGURE 12.7 Endosymbiosis and the evolution of mitochondria and chloroplasts. This schematic diagram illustrates one possible endosymbiotic origin for mitochondria and chloroplasts in eukaryotes. Here, we assume an archaebacterium served as the original host. Arrows within the cells indicate gene transfer from organelle to nuclear genome. Adapted from Timmis et al. (2004).

Several early evolutionary studies on RNA, enzymes, and ribosomes showed a strong phylogenetic link between prokaryotic and eukaryotic cells. Some of this early work suggested that eukaryotes shared a common ancestor with species in the prokaryotic domain Archaea (Woese et al. 1990; Gribaldo and Brochier-Armanet 2006), while other studies suggested that eukaryotes traced their evolutionary roots to the other prokaryotic domain, Bacteria (Eubacteria) (Martin et al. 1996; Brown and Doolittle 1997; Feng et al. 1997; Gupta 1998). Subsequent work has shown that the situation is more complicated (Alvarez-Ponce et al. 2011, 2013). Phylogenetic analyses indicate that eukaryotic "informational" genes—genes associated with transcription and translation—are most closely related to archaeal genes, whereas "operational" genes associated with metabolic processes, cell membrane formation, and amino acid production are most closely related to bacterial genes (Thiergart et al. 2012). The number of bacterium-derived genes tends to be higher than the number of archaea-derived genes across eukaryotes. Moreover, while the number of archaea-derived genes

is similar across eukaryotic genomes, the number of bacterium-derived genes is considerably more variable—why this should be and its implications are not yet understood (Rivera et al. 1998; Cotton and McInerney 2010; Alvarez-Ponce et al. 2011, 2013).

Maria Rivera and James Lake tested the hypothesis that the eukaryotic nuclear genome may have emerged from a fusion between an ancient bacterium and an ancient archaeal cell. They compared genomic sequences from 10 prokaryotic and eukaryotic species (Gupta 1998; Margulis et al. 2000; Horiike et al. 2001; Hartman and Fedorev 2002; Rivera and Lake 2004). The researchers used the similarities and differences between molecular genetic sequences to construct a phylogeny using tree-building software that was specifically designed to handle the case in which the origin of one group was the result of the fusion of other groups in such a tree (McInerney and Wilkinson 2005; for more on this approach, including potential problems with such analyses, see Bapteste and Walsh 2005).

Rivera and Lake's analysis suggests that ancient eukaryotic cells emerged from the fusion of an archaeal cell (most likely from the phylum Eocyta) and a bacterium (Rivera and Lake 2004; McInerney et al. 2014). But, as is always the case with the phylogenies produced by evolutionary biologists, this phylogeny is a *working hypothesis*—a hypothesis that could be falsified or supported by future analyses that might, for example, include species that were not included in the Rivera and Lake study.

The fusion outlined by Rivera and Lake probably involved some sort of endosymbiosis, in which either the archaeal or bacterial cell type began residing within the other, most likely when one cell engulfed the other but did not metabolize it. It is unclear which cell type—archaeal or bacterial—was the original "host." There is some evidence, however, that this relationship began when a bacterial cell became integrated into an archaeal cell, and, through time, this relationship became a mutualistic one in which each provided benefits to the other (Timmis et al. 2004; Godde 2012). Nonetheless, more work remains to be done in this area before a better resolution to the "original host question" can be obtained (Esser and Martin 2007; Pisani et al. 2007).

The intricate and complex relationship between archaeal and bacterial genes in the origin of Eukaryota has spurred a debate about the phylogenetic relationships in the tree of life that we first discussed in Chapter 4. The massive amount of horizontal gene transfer that likely occurred early in the history of life combined with endosymbiotic events means that we cannot use a single gene tree to construct the tree of life. A widely accepted consensus version of this tree represents Archaea, Bacteria, and Eukaryota as three monophyletic domains. In this tree, Archaea and Eukaryota share a common ancestor that is not shared with Bacteria; that is, Archaea and Eukaryota are sister domains. Yet recent work using molecular sequences of previously unknown archaeal species and the development of improved methods in phylogenetic reconstruction have led to an alternative to this three-domain tree of life. This alternative, known as the *eocyte hypothesis*, proposes that Archaea is in fact paraphyletic, and that Eukaryota is a subclade nested within the paraphyletic archaeal domain. More specifically, Eukaryota is a sister group to the archaeal eocyte group, rather than a sister domain to all of Archaea as in the three-domain model. Both the three-domain hypothesis and the eocyte hypothesis account for the critical role of endosymbiosis in the origin of Eukaryota, but the eocyte hypothesis, if correct, would reduce the number of

domains of life to two: Archaea and Bacteria (**Figure 12.8**) (Gribaldo et al. 2010; Williams et al. 2013). Future work drawing upon the continually increasing number of archaeal genome sequences should help evolutionary biologists better distinguish between the classic three-domain tree of life and the two-domain tree of life suggested by the eocyte hypothesis.

Endosymbiosis and the Evolution of the Eukaryotic Nucleus

Endosymbiosis may also have played a role in the evolution of another of the defining characteristic of eukaryotes—the nucleus. Indeed, the same sort of endosymbiotic relationship that led to the origin of eukaryotes also may shed light on the origin and evolution of other structures found within the eukaryotic cell. Although evidence suggests that the cell nucleus may have evolved from archaeal ancestors and that the organelles may have evolved from bacterial ancestors, after the major transition to eukaryotic life-forms occurred, many genes initially found in these organelles migrated to the nuclear genome. Sometimes, the ancestral gene was then lost from the organelle; in other cases, the ancestral gene was maintained both in the organelle and in the nucleus (Brown and Doolittle 1997; Ribeiro and Golding 1998; Rivera et al. 1998; Horiike et al. 2001, 2002).

Early studies demonstrating the migration of genes between the eukaryotic organelles and nucleus were conducted on maize and yeast (Farrelly and Butow 1983; Jacobs et al. 1983). Subsequently, so many additional studies have found further evidence for organelle-to-nucleus migration that the term "promiscuous DNA" has been coined to describe such genes. Humans, for example, have somewhere between 296 and 612 insertions of mitochondrial DNA (mtDNA) into the nuclear genome (Mourier et al. 2001; Tourmen et al. 2002; Hazkani-Covo et al. 2003). In the plant *Arabidopsis,* a remarkable 18% of the genes in the nucleus appear to have migrated from their chloroplasts (Martin 2003; Burt and Trivers 2006; Rand et al. 2004).

This migration from organelle to nucleus can even be observed in real time, in essence allowing us to re-create part of one of the major transitions in evolution. In a remarkable genetic engineering experiment, researchers inserted a gene called *neoSTLS2* into the *chloroplast genome* of tobacco plants (*Nicotiana tabacum*). The *neoSTLS2* gene confers resistance to kanamycin, an antibiotic that also inhibits seedling growth, but it only provides that resistance to antibiotics when it is found as a *nuclear gene*. That is, the only way that the *neoSTLS2* gene in this experiment could protect against kanamycin is if it migrated from the chloroplast to the nucleus of the tobacco plant. What Chun Huang and his colleagues found was that no kanamycin

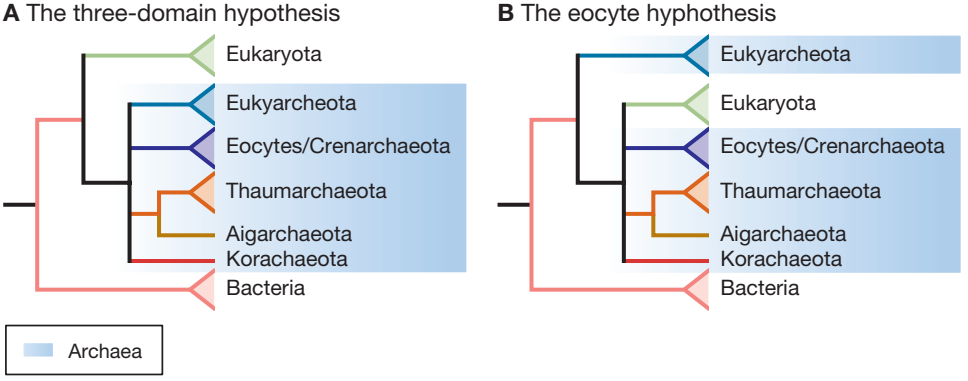

FIGURE 12.8 Three domains of life or two domains of life? **(A)** In the current consensus version of the tree of life, there are three domains: Archaea, Bacteria, and Eukaryota. Archaea, shaded in blue, is a monophyletic domain, and Eukaryota is its sister domain. **(B)** The eocyte hypothesis proposes that Archaea is paraphyletic, with Eukaryota as a subclade within the Archaea. Adapted from Williams et al. (2013).

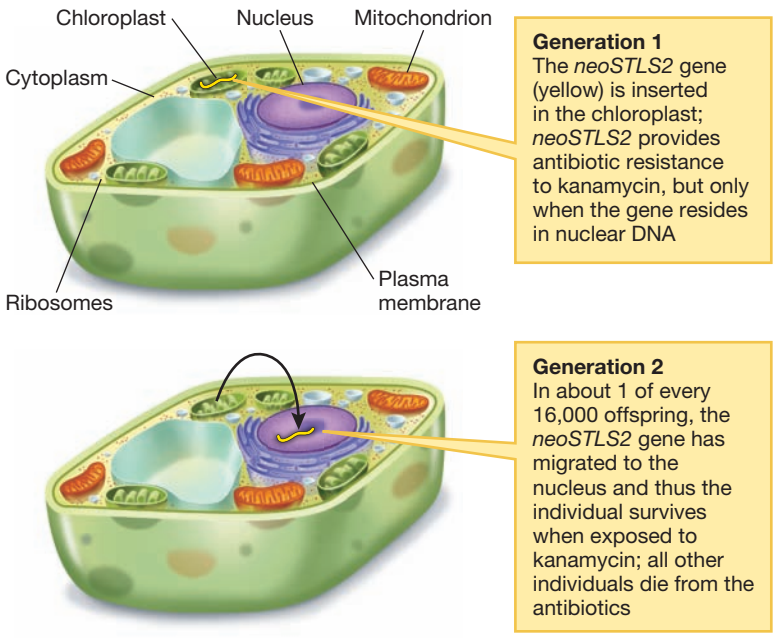

FIGURE 12.9 Gene migration from chloroplast to nucleus. The *neoSTLS2* gene is shown in yellow. This gene was initially inserted in the chloroplasts of tobacco cells. The *neoSTLS2* gene confers resistance to the antibiotic kanamycin, but only when it resides in nuclear DNA. Offspring that possess the *neoSTLS2* gene in the nucleus survived in the presence of the antibiotic kanamycin. All other offspring died when exposed to kanamycin. In about 1 of every 16,000 offspring, the *neoSTLS2* gene had migrated to the nucleus.

resistance was present in control lines. In the experimental line that had *neoSTLS2* inserted into its chloroplast genome, most plants were not resistant to kanamycin, but 16 of the 250,000 offspring they examined could survive in the presence of kanamycin. That is, in about 1 of every 16,000 offspring produced by the tobacco plant, there was evidence that a gene initially found only in the chloroplast had migrated to the nucleus *in a single generation* (Huang et al. 2003, 2004) (**Figure 12.9**).

The evolution of these sorts of endosymbiotic relationships can have important medical implications (Roos et al. 2002; Huang 2004; Ralph et al. 2004). We discuss this in more detail in **Box 12.1**.

BOX 12.1 Apicoplasts and the Medical Implications of Endosymbiosis

The apicoplast is an organelle found only in species in a phylum called Apicoplasta—a phylum that includes such eukaryotic pathogens as *Plasmodium falciparum*, one of the agents responsible for malaria. Using morphological evidence, molecular genetic tools, and phylogenetic analysis, researchers have reconstructed the history of the apicoplast.

The apicoplast probably arose through a secondary endosymbiosis event, as illustrated in **Figure 12.10**. First, an initial eukaryote, probably a red algae, arose by a primary endosymbiosis event in which one prokaryotic host engulfed a cyanobacterium. Once the ancestral cyanobacterial species became involved in an endosymbiotic relationship with its original host, its photosynthetic properties appear to have been lost (Funes et al. 2002, 2004; Waller et al. 2003). Subsequently, that species was itself engulfed in a secondary endosymbiosis event. The original cyanobacterium, now surrounded by four membranes as illustrated in Figure 12.10, became the apicoplast (Lim and McFadden 2010). The apicoplast plays a very important role in the cells of such organisms as *P. falciparum*, where it is involved in the production of at least 500 different gene products (**Figure 12.11**).

How can we use this knowledge of the endosymbiotic history of the apicoplast, together with information on its modern function, to improve the medical treatment of malaria? The answer revolves around what metabolic pathways in malaria should be targeted by antimalarial drugs. Think about it like this: Most metabolic pathways in *P. falciparum* are similar to pathways found in other eukaryotes, because *P. falciparum* is a eukaryote. When we target these pathways with our antimalarial drugs, we risk disrupting similar pathways in eukaryotic hosts of malaria—in particular, in humans. Sometimes such risks must

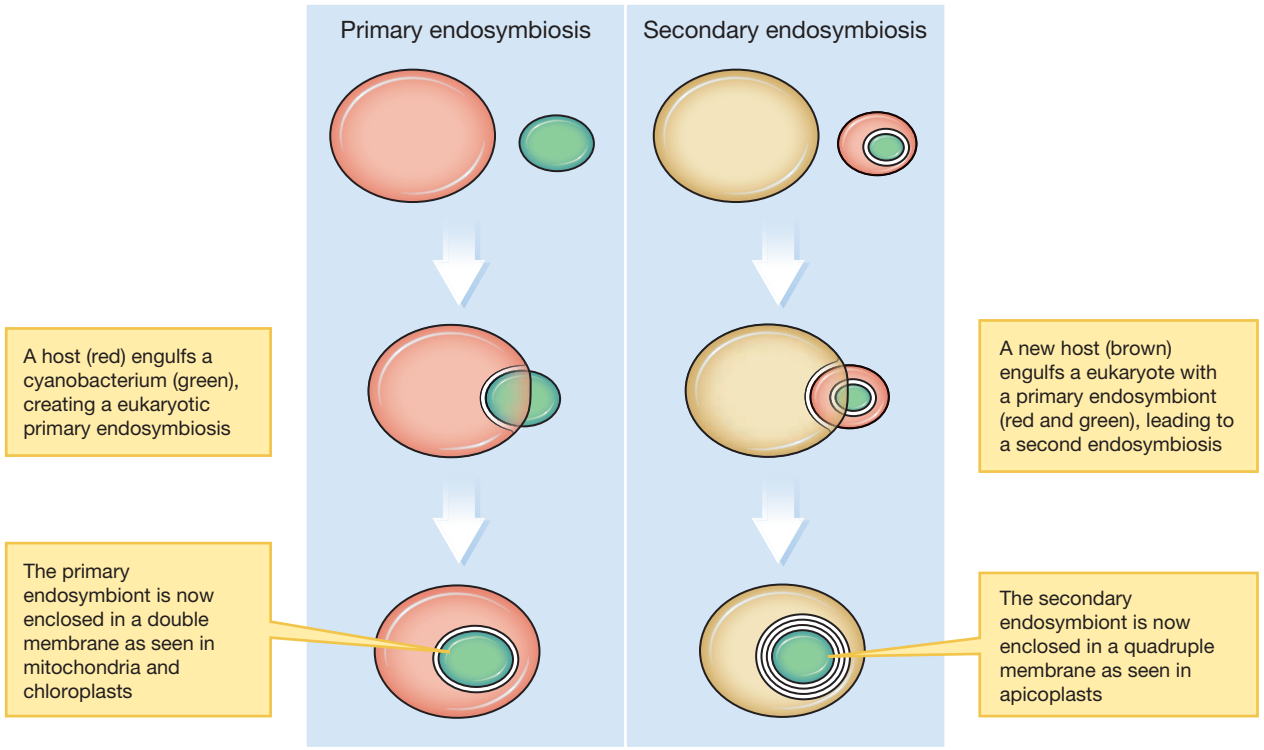

FIGURE 12.10 The process of secondary endosymbiosis. First, a primary endosymbiosis arises; subsequently, a secondary endosymbiosis occurs when the primary endosymbiont is itself engulfed by a new host. Adapted from Gschloessl et al. (2008).

be taken to combat deadly diseases. But because humans lack the apicoplast organelle and because we know the evolutionary history of the apicoplast, we have a safer route we can take for targeting metabolic pathways in *P. falciparum*: We can target the pathways associated with protein production by the apicoplast. Targeting these pathways, because they have prokaryotic evolutionary roots, reduces the chance of disrupting similar pathways in human hosts (Ralph et al. 2004). Ongoing work suggests that this may be a productive line of research in developing antimalarial drugs (Dahl and Rosenthal 2008).

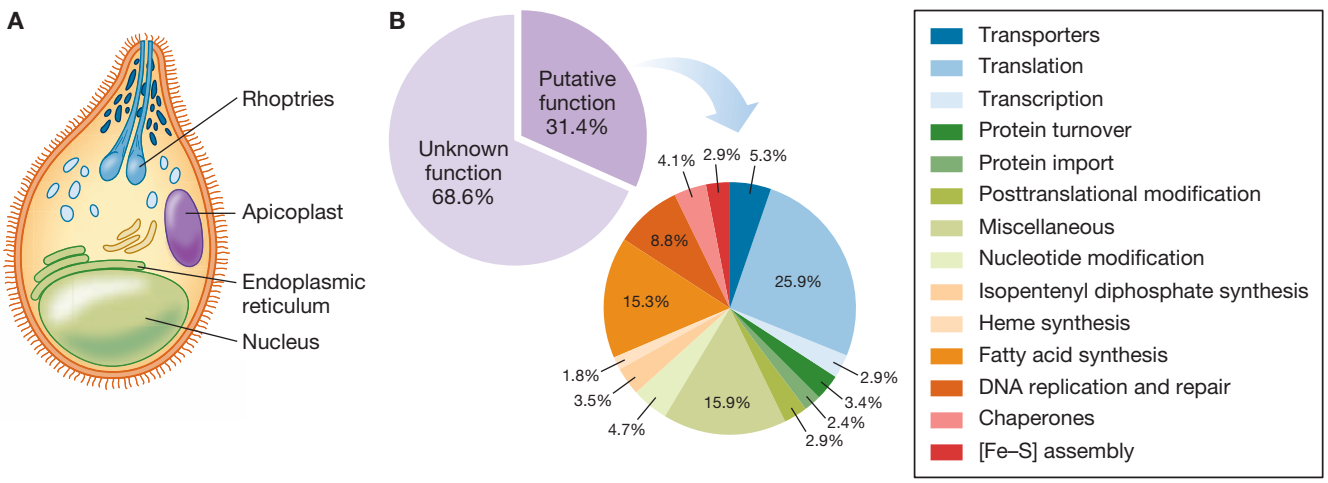

FIGURE 12.11 Apicoplasts and their functions. **(A)** The apicoplast found in a *Plasmodium* cell. **(B)** Functions of apicoplast genes in *Plasmodium*. Adapted from Ralph et al. (2004).

The major transition leading to eukaryotic cells and their complicated within-cell communication networks, then, centered on a series of endosymbiotic mergers, the first of which had either an archaeal or bacterial cell type residing within the other, which was followed by the endosymbioses associated with the nucleus and organelles within eukaryotes.

12.3 Major Transition: The Evolution of Multicellularity

Our focus in this chapter thus far has been on the evolution of single-celled organisms because these made up the earliest communities found on Earth. Now we will turn to the evolutionary transition from single-celled to multicelled organisms. Such a transition has occurred independently many times, in many taxa, over evolutionary history: This distribution of **multicellularity** across the tree of life is a dramatic example of convergent evolution (**Figure 12.12**) (Michod 1997, 2007; Bonner 2000; Grosberg and Strathmann 2007; Herron and Michod 2008).

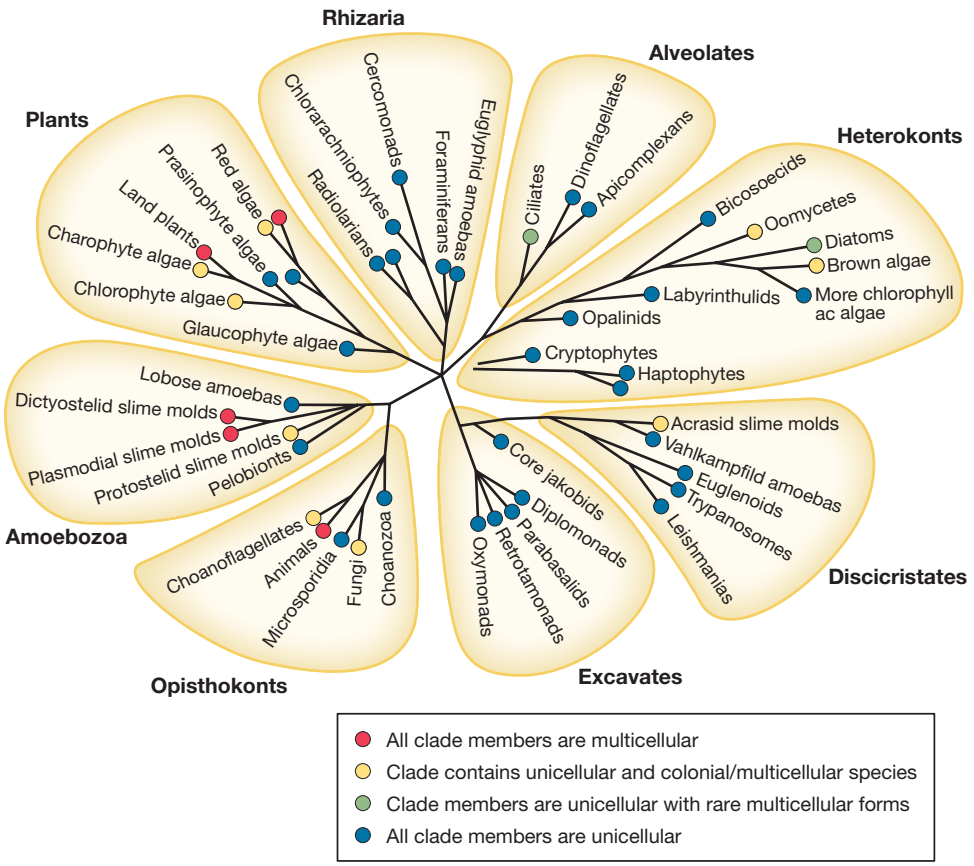

FIGURE 12.12 The phylogenetic distribution of multicellularity. Multicellularity has evolved numerous times within the eukaryotes. Adapted from Grosberg and Strathmann (2007).

KEYCONCEPT QUESTION

12.3 Across many of the independent transitions to multicellularity, there has been an increase in the number and complexity of gene families that are associated with cell adhesion and cell signaling pathways (Rokas 2008). Why might this be? Why is it not all that surprising that the evolution of multicellularity is often associated with an increase in gene families linked to cell–cell signaling?

Evolutionary biologists posit two routes leading to multicellularity: "staying together" and "coming together" (Grosberg and Strathmann 2007; Bourke 2011; Olson 2013; Tarnita et al. 2013). The basic idea behind the staying together route—also known as the clonal route—is that multicellularity arose when, for one reason or another, cells in an ancestral unicellular lineage remained together after cell replication. In contrast, the coming together route involves formerly free-living cells joining together during the early stages of the evolution of multicellularity (**Figure 12.13**).

The staying together route is thought to be much more common than the coming together route and is considered to explain most cases of multicellularity in plants and animals (Grosberg and Strathmann 1998; King 2004; Rokas 2008). In the staying together route, primitive multicellular creatures go through a single cell stage: a "unicellular bottleneck." A single cell divides, but parent cells produce offspring cells that fail to separate, and subsequent growth follows the same pattern. Cells that fail to separate after replication are close genetic relatives—indeed, they are clones of one another—and so there is little if any genetic difference between cells. Contrast this to the coming together route, in which formerly free-living cells unite: Such cells may or may not to be genetically similar to one another.

This difference in genetic similarity between cells in the staying together versus coming together routes may be important in explaining why the staying together route is thought to be more common. When cells in putative multicellular creatures are genetically similar, this has the effect of reducing genetic conflict among cells and cell lineages within an organism. When genetic similarity is lower, conflict should be greater.

In general, the more genetically similar cells are, the more closely their reproductive interests are aligned, and so the less genetic conflict is expected between cells. We delve into why this is so in Chapter 17, but for now, it suffices to say that when the reproductive interests of cells are aligned, as opposed to in conflict, the easier it is for natural selection to produce a well-integrated cluster of cells; that is, a better-functioning multicellular creature.

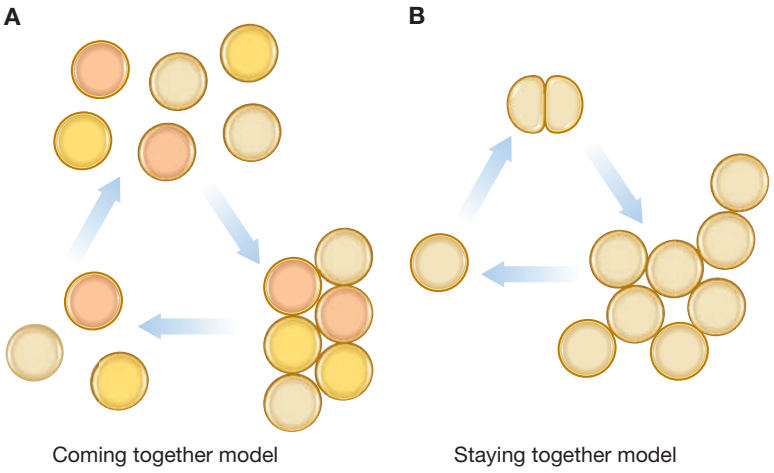

FIGURE 12.13 The coming together model versus the staying together model for the evolution of multicellularity. The coming together model **(A)** posits that independent cells came together during the early stages of the evolution of multicellularity, producing unicellular nonclonal propagules, while the staying together model **(B)** hypothesizes that cells of a single lineage remained together after cell replication, producing unicellular clonal propagules. Adapted from Olson (2013).

Staying Together: Yeast and Multicellularity

Using the experimental evolution approach we first outlined in Chapter 5, Will Ratcliff and his colleagues have investigated the staying together route to multicellularity in yeast (*Saccharomyces*

cerevisiae) (Ratcliff et al. 2012, 2013, 2014). Their aim was to experimentally manipulate selective conditions that might favor multicellularity in yeast, and then to examine what, if any, genetic changes occurred in unicellular yeast populations subject to such conditions.

Their experiment—which ran for 60 days or approximately 500 generations in yeast—began with 10 identical replicates, each containing cells from a unicellular strain of yeast. Each replicate occupied its own test tube, nourished by a broth containing all of the resources necessary for yeast growth. Replicate strains were maintained in an asexual mode of reproduction, and so any new variant that arose during the experiment was a de novo mutation, rather than one that came about as a result of recombination. Every 24 hours, Ratcliff and colleagues withdrew the 100 microliters of yeast at the bottom of the test tube. This 100-microliter sample of yeast was then transferred to a new test tube with new resources, and the process was repeated each day of the experiment.

The "settling selection" that Ratcliff and his colleagues imposed by using the yeast at the bottom of a test tube favors yeast cells that sink. Such settling selection should favor yeast cells that cluster together—a possible step toward multicellularity—because clusters of cells sink faster than solitary cells.

Initially, during the early days of the experiment, larger single yeast cells were favored in three of the 10 replicates. These cells had almost twice the amount of DNA of standard, smaller single yeast cells, and hence they sank to the bottom of the test tubes more quickly than lighter cells. But this was a transient stage. Somewhere between 7 and 60 days into the experiment, the researchers began to observe *snowflake clusters* (so named because of their resemblance to snowflakes) of yeast cells in every one of the 10 replicates (**Figure 12.14**). By the end of the experiment, these snowflake clusters had outcompeted single cells—heavyweight or lightweight solitary cells—in every replicate. When selection for settling was made more intense by selecting for cells at the bottom of the test tube at earlier and earlier stages of the experiment, larger snowflake clusters were more strongly favored. Moreover, selection began to favor not only larger clusters, but also clusters with a hydrodynamic, spherical shape that led to even faster settling.

To distinguish between the coming together and staying together routes to multicellularity, Ratcliff and his team then looked at how snowflake clusters were formed. Under certain conditions, independent yeast cells can stick together (flocculate) by producing adhesive glycoproteins and bumping into other independent cells—an example of the coming together route. This was not what was causing the snowflake clusters to form in the Ratcliff experiment. Instead, as in the staying together model, independent mutations in each replicate line produced cells that remained attached after cell division rather than completely separating as in normally asexually reproducing yeast (Figure 12.14). When snowflake cell clusters were split apart by Ratcliff and his team, the resulting cells eventually

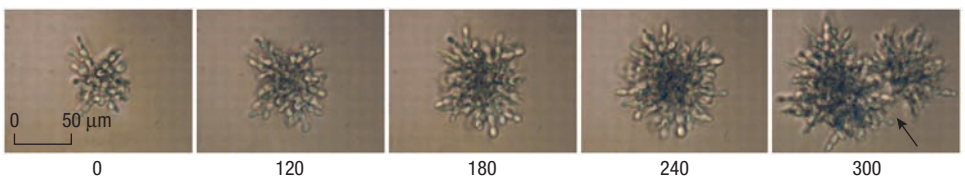

FIGURE 12.14 "Snowflake" multicellularity in yeast. Though at the start of the experiment all yeast were found in the form of solitary cells, the yeast in each replicate line of the experiment evolved the ability to cluster together into snowflake-like assemblages such as that shown. In the figure, we see a snowflake cluster growing and ultimately dividing (at the arrow in the far right photo) as it gets very large. From Ratcliff et al. (2012).

produced new clusters by the same mechanism, showing that this sort of snowflake cluster formation is heritable.

Ratcliff's team next examined how a snowflake cluster produced new snowflake clusters; in essence, looking at how reproduction had evolved in these multicellular strains of yeast. By the end of their 60-day experiment, what they found was that when a snowflake cluster grows large, a branch from the cluster breaks off and forms a new cluster. Apoptosis—cell death—seems to be the mechanism that leads to one branch breaking off of a "parent" cluster and forming a new cluster. They found high rates of apoptosis at the break point. Experimental work suggests that cell death at the break point was not caused by breaking off from a cluster, but rather the cause of breaking off: natural selection had favored increased rates of apoptosis at the break point in snowflake clusters.

The yeast experimental evolution studies demonstrate one way that we can examine the staying together route to multicellularity. Next, we return to the slime mold studies we introduced at the opening of this chapter and focus on that work as an example of the coming together route to multicellularity.

KEYCONCEPT QUESTION

12.4 What molecular genetic manipulations to snowflake cluster yeast, similar to the genetic manipulations performed in the *neoSTLS2* experiment in tobacco plants, can you imagine that may shed light on the transition to multicellularity?

Coming Together: Slime Molds and Multicellularity

We tend to think of multicellularity as an obligate condition—something that cannot be turned on or off. Worms, for example, don't break apart into single-celled creatures for a period of time and then form back into worms. But the coming together model for the evolution of multicellularity suggests that early on, cells may very well have joined together and disbanded. Work on the slime molds illustrates this point.

Recall from the opening of this chapter that when resources in the soil become depleted, individual slime mold cells form a multicellular slug. These multicellular slugs respond to environmental cues when moving about (Bonner 2000, 2003). But how does the slug—composed of cells that were completely independent before its formation—orient itself in its environment? In *D. discoideum*, the many cells that make up a slug coordinate their behavior by communicating with a chemical called cyclic adenosine monophosphate (cAMP). In the region of soil with the highest concentration of cells, cAMP is released, and this signals cells "downstream" of this point to orient toward the source of the cAMP (**Figure 12.15**). Once the individual cells arrive where cAMP is being emitted, they adhere to each other, surrounding the cells that have emitted the cAMP. The aggregating cells then produce proteins that cause them to stick to each other and form the slug. The slug is then able to orient itself and to move quickly and efficiently toward stimuli such as light and bacterial nutrients.

The cAMP signaling system does more than inform us about *how* a multicellular slug moves; it also helps us to understand the *benefits* of multicellularity in the life of a slime mold, and so provides insight into the evolution of the early stages of

multicellularity. The signaling system allows the slug to orient to ambient environmental cues such as light, temperature, and ammonia and oxygen gradients, which it uses to move up toward the surface of the soil, where reproduction will occur (Yamamoto 1977; Sternfeld and David 1981; Fisher 1997; Bonner et al. 1998; Kessin 2001). The slug can sense and respond to information in the form of environmental cues in a way that individual cells cannot. These new possibilities illustrate some of the economies of scale that play a key role in major transitions.

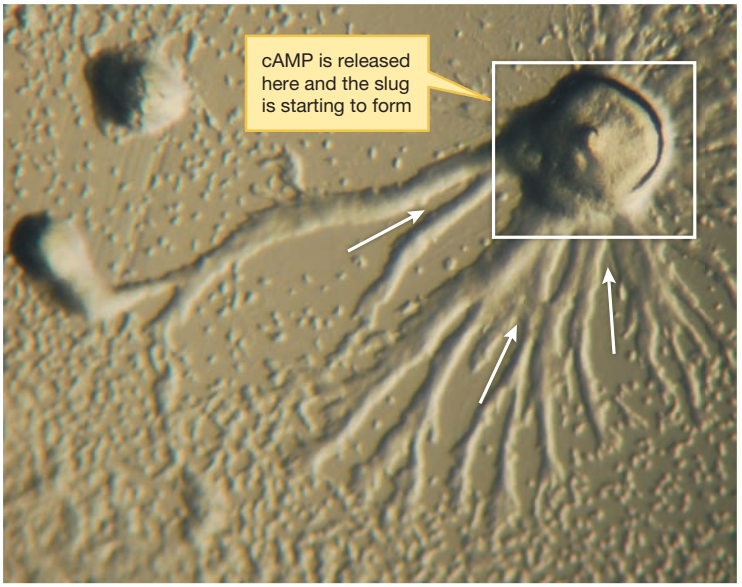

FIGURE 12.15 cAMP stimulates slug formation. Single-celled *D. discoideum* move (as indicated by the white arrows) toward an area where cAMP is being released (white square).

The benefits of multicellularity in slugs are not limited to orienting to stimuli and migrating to the surface for reproduction. The slug is also able to form a slime "sheath" around itself that helps protect it from nematode predators (Wang et al. 2001). This sheath is made up of cellulose and protein-rich substances, and it coats only the surface of the slug; it is completely absent in the single-celled amoeba stage. This confers an antipredator benefit above and beyond what slime mold cells would get by just moving together in groups (**Figure 12.16**). Production of a protective layer around the slug is also another example of an economy of scale, as the surface-to-area ratio makes such a slug sheath much less expensive to produce than many individual sheaths would be. This slime sheath thus provides additional benefits to a multicellular developmental stage in slime molds.

Joan Strassmann and her colleagues hypothesized that yet another economy of scale is important in this system: Slugs may be able to reach new food sources more quickly than individual slime mold cells (Kuzdzal-Fick et al. 2007; Gilbert et al. 2012a). To test this hypothesis, Strassmann's team had to construct an experiment that allowed them to separate the effects of (1) single-cell movement versus slug movement and (2) developmental stage per se. To understand why both of these were necessary, remember that in normal *D. discoideum*, the amoeba stage precedes the slug stage. So, if slugs were able to navigate faster than amoebas, it could be because they were multicellular (rather than single-celled amoebas) *or* it might be that in later developmental stages—whatever those stages might be—slime molds could move more quickly. To distinguish between these alternatives, Strassmann used a standard strain

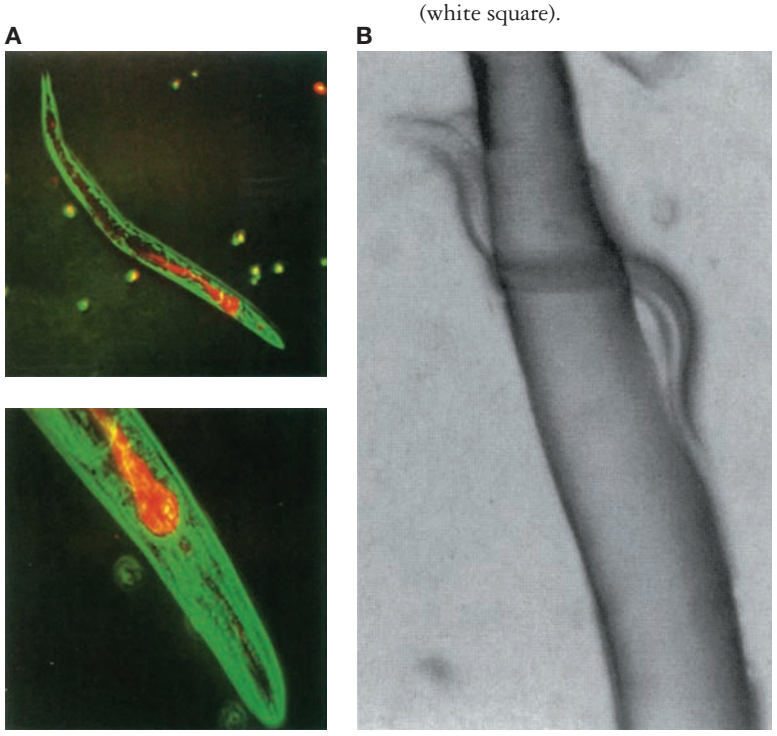

FIGURE 12.16 Slugs form a slime sheath that provides protection. **(A)** Micrographs of a nematode (*Caenorhabditis elegans*), shown in green, feeding on a *Dictyostelium* amoeba, shown in reddish orange. **(B)** A nematode (top center) wraps itself around a *Dictyostelium* slug but cannot ingest or harm the much larger slug (the slug is shown running from top to bottom of the image. This is an economy of scale: The group can make itself impervious to nematode predators in a way that a single individual cannot. From Kessin et al. (1996).

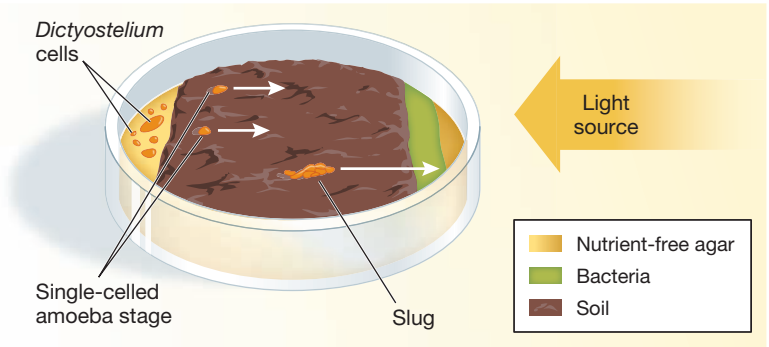

FIGURE 12.17 Slugs move more quickly than solitary cells. Strassmann and colleagues used the experimental setup shown here to test whether *Dictyostelium* slugs travel through their environment more efficiently than the single-celled amoeba. Indeed, slugs do. Adapted from Kuzdzal-Fick et al. (2007).

of *D. discoideum* (the wild-type strain) and a mutant strain (labeled CAP2 mutants) that was similar to the wild strain but did *not* form slugs in later stages of development—CAP2 mutants remained single-celled amoebas.

Wild-type cells or CAP2 mutant cells of similar ages to the wild-type cells were placed on the left side of a plate as shown in **Figure 12.17**. On the right side of the plate was a light and a bacterial food source. *Dictyostelium discoideum* typically migrate toward such resources. To get to the food and the light, however, the slime molds had to cross a soil barrier. When wild-type *D. discoideum* were placed on the plate, in 10 of 10 trials they formed a slug and successfully migrated across the soil barrier and toward the resources. But CAP2 mutants never formed slugs, and they were only able to migrate across the soil barrier in 2 of 10 trials, suggesting a selective advantage in terms of migration for the multicellular slug stage in slime molds. By aggregating to form a slug and coordinating their behaviors, the slime mold cells were able to benefit from an economy of scale, the ability to move more efficiently in a large, coordinated group (Figure 12.17).

Once a *D. discoideum* slug reaches the soil surface, the slug breaks apart into fruiting bodies, each of which consists of a multicellular stalk, made up of nonreproductive cells, and spores (reproductive cells) (**Figure 12.18**). The spores at the tip of the fruiting bodies are in a capsule that is raised from the soil surface on the stalks, and they are dispersed primarily by invertebrates that break the capsule when passing by. Being elevated from the soil increases the chances of dispersal by invertebrates—another economy of scale—and thus fruiting bodies provide additional selective advantages to multicellularity.

As we discussed earlier, one of the reasons that evolutionary biologists think that the staying together route to multicellularity is more common than the coming together route to multicellularity is that in the former, cells are genetic clones of one another (because mother and daughter cells fail to separate). Because of this high genetic relatedness, the fitness interests of cells are aligned, thus reducing conflict. If this is correct, how do we explain the division of labor in the fruiting bodies of slugs where cells have come together rather than stayed together? Why do some slug cells become part of the stalk—and forfeit the opportunity to reproduce—while other slug cells become spores?

The answer is that staying together is not the only way that genetic relatives can cluster together. In the case of slime mold cells in a slug's fruiting body, molecular genetic analysis has found that fruiting body cells—both stalk and spore cells—are highly genetically related to one another; indeed, when such aggregations are formed, cells may actively discriminate against other cells that are genetically dissimilar (Mehdiabadi et al. 2006; Gilbert et al. 2007, 2012b; Ostrowski 2008). While we don't know the mechanism by which genetically similar slime mold cells in slugs cluster together in fruiting bodies, by doing so, high genetic relatedness is built up, reducing the degree of conflict between cells.

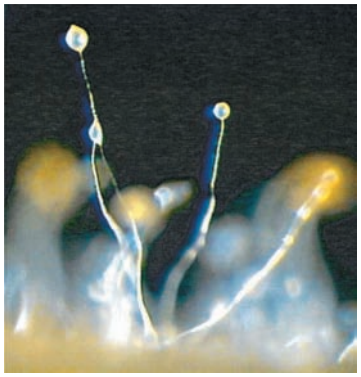

FIGURE 12.18 Fruiting bodies. A close-up photo of fruiting bodies. The stalks support a "spore head" that contains the spores.

12.4 Major Transition: The Evolution of Individuality

In this section, we will see that the evolution of multicellular *individuals* constituted another major transition. Before we look at this in more detail, we need to step back and ask a fundamental question: When does a group of cells become an individual? To answer this question, we need to have an evolutionary definition of "individual." Here, we adopt a definition suggested by Rick Michod, an evolution biologist who has studied multicellular individuality. Michod argues that individuals are "integrated and indivisible wholes" that can reproduce and pass on to their offspring heritable variations (Michod 2007). Michod immediately follows up on his definition with a point that we have emphasized a number of times now: Natural selection can facilitate transitions from one level of individuality to another by the same sort of gradual process with incremental improvements that Darwin proposed for the evolution of other complex traits.

How then did the evolutionary transition to a new level—multicellular individuals—occur? The answer entails an understanding of how fitness is transferred from one level of organization—the individual cell, for example—to a higher level of organization: the multicellular organism. In the case of the evolution of multicellular individuals, a critical component of the transfer of fitness from lower to higher levels of organization involves the differentiation of cell lineages into those specialized in reproduction (germ cells) and those specialized in maintenance and growth of the organism (somatic cells, or soma). This is a differentiation that is, by definition, impossible in single-celled organisms.

There are a number of different theories for how the division between somatic and germ cells evolved in multicellular organisms (Buss 1987; Michod 2000; Grosberg and Strathmann 2007; Bourke 2011). Many such theories share the following features: Early in the evolution of multicellularity, multicellular creatures were likely made up of a relatively small number of cells. With only a few cells making up the multicellular individual, it would be unlikely for one of these cells to acquire a mutation that allowed it to cheat and overrepresent itself in the cells of the next generation. Over evolutionary time, however, the number of cells per individual in many multicellular lineages grew. At some point, there would be enough cells in a single individual that the probability of a cheating mutation would become high. Natural selection would then start to favor a strategy whereby only a small number of cells in individuals retained reproductive capacity and became germ cells, while the remainder lost reproductive ability and became somatic cells. Somatic cells lost *totipotency*—the ability to differentiate into any type of cell in the body—because they could no longer switch to germ-line functions. This decrease in the number of cells involved in reproduction reduced (but did not completely eliminate) the problem of cheaters and led to the complete division between soma and germ lines.

Volvocine Algae and the Evolution of Individuality

To better understand the major transition to individuality via the differentiation of germ and soma lines, we now focus on volvocine algae. This group of green algae diverged from a unicellular ancestor about 230 million years ago (Herron et al. 2009). Volvocine algae are ideal for studying the evolution of individuality because of the exceptional variation found within this group. Some volvocine species are

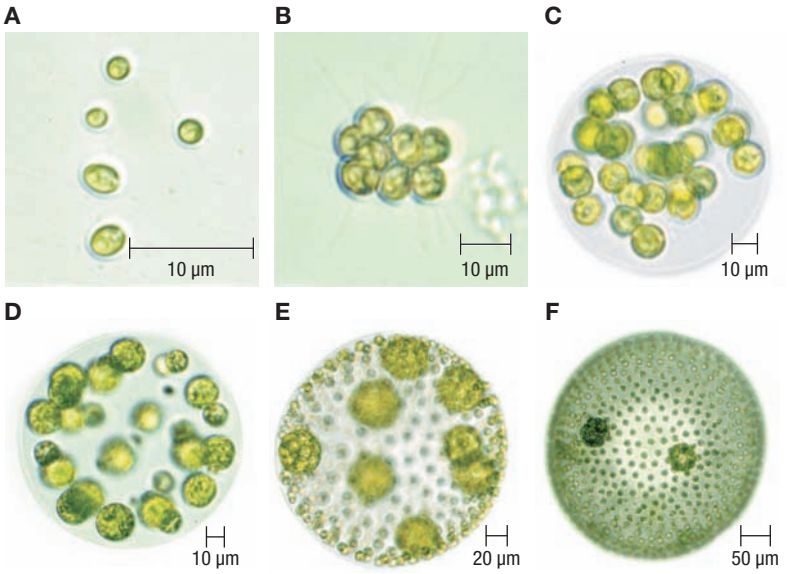

FIGURE 12.19 Cell number and germ-cell specialization in volvocine algae. Six species of volvocine algae that differ in cell number and germ-cell specialization. **(A)** Unicellular *Chlamydomonas reinhardtii*. **(B)** *Gonium pectorale*, a sheet of 8–32 undifferentiated cells. **(C)** *Eudorina elegans*, a colony of 16–64 undifferentiated cells. **(D)** *Pleodorina californica*, a colony with between 30% and 50% somatic cells. **(E)** *Volvox carteri*, with thousands of flagella-bearing somatic cells and only a handful of germ cells. **(F)** *Volvox aureus*. In panels D–F, when two cell types are present, the somatic cells are smaller and the reproductive cells are larger. From Michod (2007).

unicellular; some species are made of cells that live in groups but do not have specialized germ and soma lines; and some species, such as *Volvox aureus*, show well-differentiated germ cell and somatic cell lines (**Figure 12.19**). Indeed, the division of labor between germ and soma lines has evolved on at least three separate occasions in this group. But how?

To address this question, Michod and his colleagues focused on *Volvox carteri*, a species of volvocine algae in which there are both germ and somatic cells. Individual *V. carteri* are typically made up of about 2000 small somatic cells and as many as 16 large reproductive cells (Kirk et al. 1999). Each small somatic cell has two flagella, which are long, hairlike projections that produce motion. In *V. carteri*, movement by flagellar motion is critical to survival because most essential nutrients, such as phosphorus, as well as sunlight for photosynthesis, are found close to the water surface, and *V. carteri* uses flagellar motion to avoid sinking in the water. Flagellar motion also mixes the water around individuals, and it helps them to take up nutrients and to release waste. For example, experimental work with mutant strains of *V. carteri*, in which somatic cells do not produce flagella, shows that such mutants fare very poorly in terms of competition and reproduction (Solari et al. 2006a). Thus, the small somatic *V. carteri* cells specialize in survival and growth functions. They never reproduce to form new *Volvox*, but they are critical for the survival of a colony of *V. carteri*, in the same sense that our skin cells are critical for our survival.

The larger germ cells of *V. carteri* lack flagella and specialize in reproduction. Large cells are necessary for reproduction because of the unusual nature of cell divisions during reproduction. Rather than doubling in size and then dividing, germ cells in *V. carteri* undergo up to 13 rounds of cell division, with almost no cell growth during these divisions. As such, a reproductive cell has to be very large from the start.

How is the fate of a *V. carteri* cell—large germ or small somatic cell—determined? The answer to this question centers on the expression of a gene known as *regA* (Meissner et al. 1999; Short et al. 2006; Solari et al. 2006a,b). When this gene is expressed, it suppresses a number of nuclear genes that code for chloroplast proteins. Because cell growth is dependent on these chloroplast proteins, and cell division depends on cells reaching a critical size, cells in which *regA* is expressed remain small and produce flagella, becoming the somatic cells. If *regA* is not expressed, cells photosynthesize, grow larger, and lose the ability to produce flagella. These larger cells go on to form the germ line.

We can do more than link *regA* with the evolution of individuality. Evolutionary biologists have been able to trace the evolutionary history of *regA* by studying modern-day unicellular volvocine species, such as *Chlamydomonas reinhardtii*. In this species, a flagellated cell first grows in size and then absorbs its flagellum

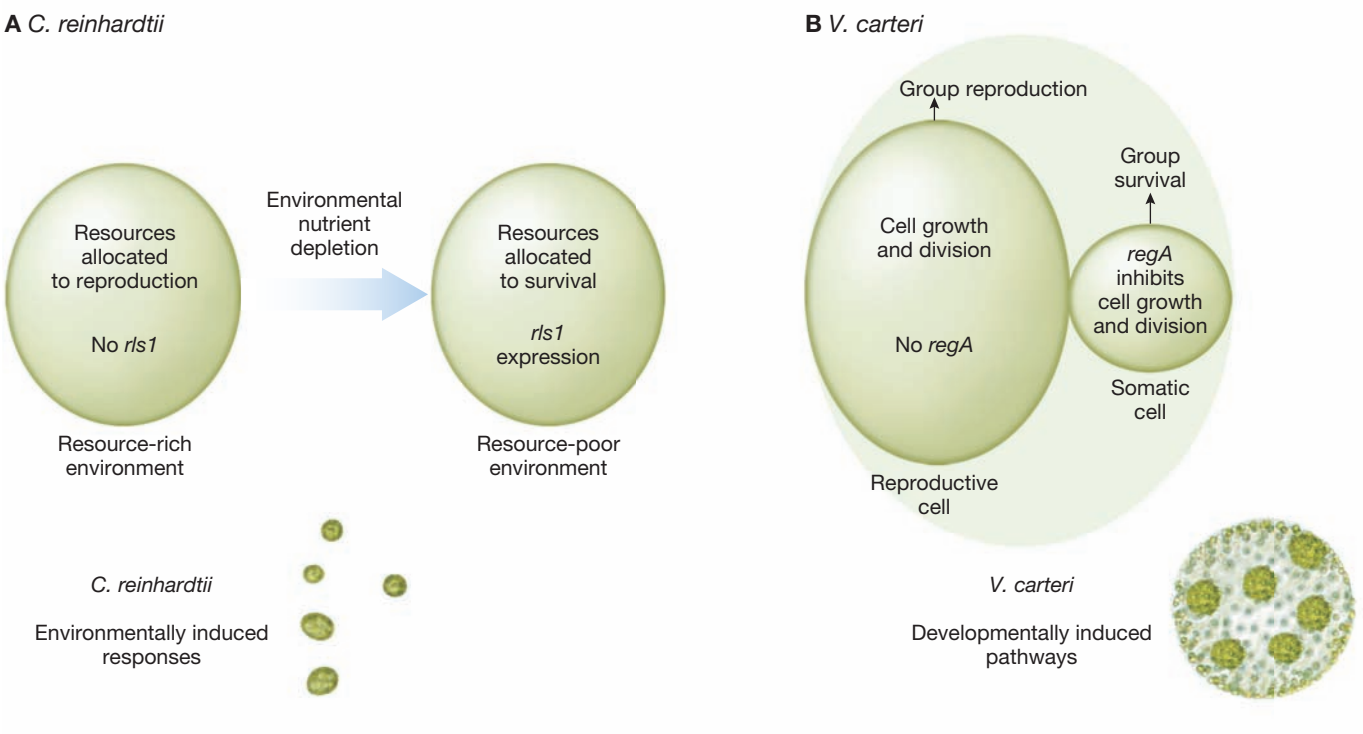

FIGURE 12.20 Soma, germ cells, and *rls1*. **(A)** In *Chlamydomonas reinhardtii*, *rls1* is expressed as a function of environmental cues such as resource depletion. Its expression inhibits cell reproduction. **(B)** Over evolutionary time, *rls1* has been co-opted and its homologue, *regA*, regulates the differentiation of cells into germ and soma in multicellular volvox species such as *Volvox carteri*. Adapted from Nedelcu (2009).

and produces daughter cells. A gene homologous to *regA* has been found in *C. reinhardtii:* This gene—*rls1*—is expressed as a function of environmental cues, and its expression inhibits the process of reproduction. In essence, this single cell switches from germ cell–like activity to somatic cell–like activity. It appears that over evolutionary time, the *rls1* gene, which regulates the timing of cell division in unicellular organisms (such as the unicellular ancestor of *C. reinhardtii*), was co-opted to become the *regA* gene, which regulates the differentiation of cells into germ and soma within a multicellular organism, such as *V. carteri* (Nedelcu and Michod 2006; Nedelcu 2009; Hanschen et al. 2014) (**Figure 12.20**).

We now turn to the major transition from solitary individuals to group-living individuals.

12.5 Major Transition: Solitary to Group Living

Another major transition occurred when individuals began living in groups, rather than solitarily. Group living provides a suite of benefits, many derived from economies of scale, including benefits associated with foraging and safety from predators. Living in groups requires a degree of sociality that is not required for solitary living, and this also often entails new levels of coordination and communication between individuals to obtain such benefits.

We define a group as a set of conspecific individuals who affect each other's fitness (Wilson 1980). Species vary tremendously in the extent to which individuals

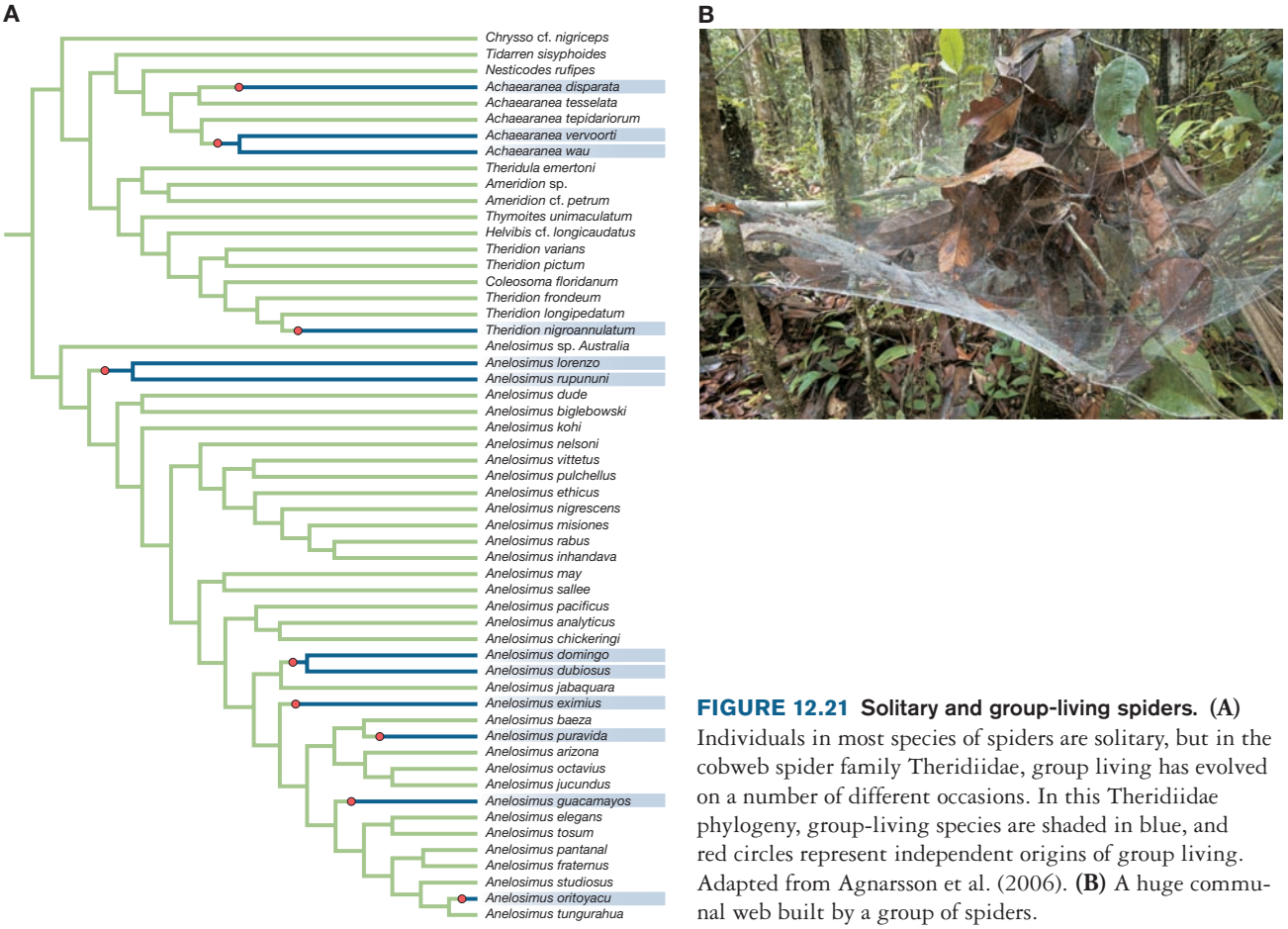

FIGURE 12.21 Solitary and group-living spiders. (A) Individuals in most species of spiders are solitary, but in the cobweb spider family Theridiidae, group living has evolved on a number of different occasions. In this Theridiidae phylogeny, group-living species are shaded in blue, and red circles represent independent origins of group living. Adapted from Agnarsson et al. (2006). **(B)** A huge communal web built by a group of spiders.

live in groups. In some ungulate species, such as the Japanese serow (*Capricornis crispus*), individuals spend the majority of their lives, aside from times of mating, living alone. In other species, such as the honeybee, individuals spend virtually all of their time in some sort of tightly coordinated group (Seeley 1985; Kishimoto and Kawamichi 1996). We need not compare such dramatically different creatures to see such variation in group size. Within spiders, for example, most species are solitary, but group living has evolved multiple times in this clade (**Figure 12.21**) (Agnarsson et al. 2006).

The Benefits to Group Living

A plot of the "time budget" of almost any animal would show that organisms spend most of their time either searching for food or engaging in some sort of antipredator behavior. As such, we will focus here on the foraging and antipredator-related benefits of group living.

Foraging in Groups

Living in groups provides individuals with numerous foraging-related benefits, many of which are linked to economies of scale. Consider the foraging behavior of the bluegill sunfish (*Lepomis macrochirus*). Bluegills feed primarily on small,

aquatic insects that live in underwater vegetation (**Figure 12.22**). Aquatic insect prey are difficult to catch in such vegetation, but when bluegills forage in groups, they are able to flush out many more prey from sediment and vegetation than can solitary individuals, and foraging success *per fish* often increases as a function of group size (Morse 1970; Bertram 1978; Mock 1980).

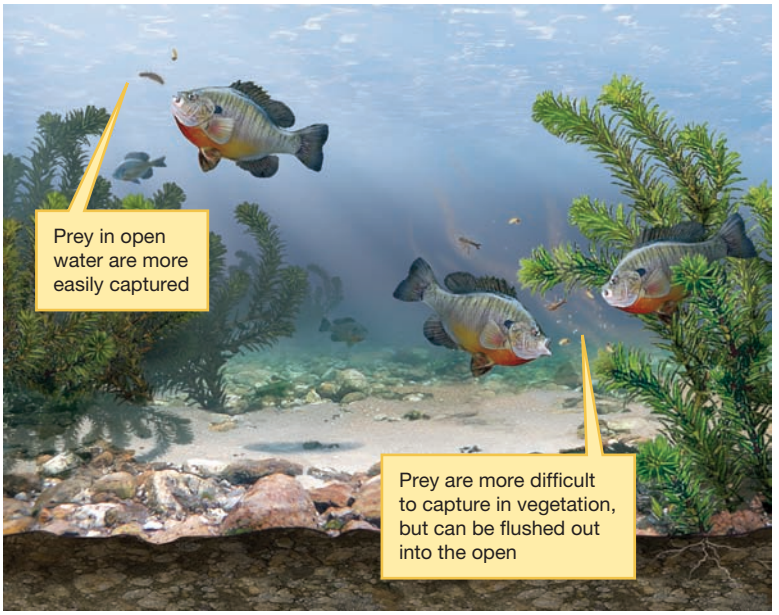

FIGURE 12.22 Foraging benefits of group living. Bluegills foraging in a lake feed on insects that are flushed from the vegetation. Flushing is more common in groups and leads to greater per-individual foraging success for members of a group. Adapted from Dugatkin (2009a).

Gary Mittlebach examined this benefit of group foraging by experimentally manipulating the group size of bluegills in a controlled laboratory setting (Mittlebach 1984). Mittlebach placed 300 aquatic prey in a large aquarium containing juvenile bluegill sunfish, and he recorded the feeding rates of bluegills that were foraging alone, in pairs, and in groups of three to six bluegills. He uncovered a positive relationship between foraging-group size and individual foraging success: The average amount of food that a fish received increased as its group size increased up to a certain number. This sort of relationship between group size and foraging success has been found in many different species (Creel 2001).

The bluegill example illustrates what we might call a "passive" benefit of group foraging. By passive, we mean that each bluegill in a group is foraging just as it would forage if it were alone. The fish are not behaving differently in groups; rather, the aggregate impact of their actions creates a flushing effect from which each animal benefits: an economy-of-scale benefit. In other species, the benefits of foraging in groups may go beyond passive benefits and involve much more coordination and communication—important processes involved in major transitions. For example, Christophe and Hedwige Boesch have found that groups of chimpanzees in the Tai Forest hunt for prey in a coordinated fashion (Boesch 2005; Gomes and Boesch 2009), and that four different hunting roles are sometimes involved in the capture of a single prey (**Figure 12.23**). After observing thousands of group hunts in the Tai Forest, Boesch describes the process:

> The *driver* initiates the hunt by slowly pushing the arboreal prey in a constant direction, *blockers* climb trees to prevent the prey from dispersing in different directions, the *chaser* may climb under the prey and by rapidly running after them try a capture, and the *ambusher* may silently climb in front of the escape movement of the prey to block their flight and close a trap around the prey. (Boesch 2005, p. 692)

FIGURE 12.23 Group foraging in chimps. In the Tai Forest (Ivory Coast), chimps cooperate in both capturing and consuming prey. Once the chimps have captured their prey, they follow subtle rules for distributing the food.

As chimpanzee hunting groups increase in size, group members increase their per capita food intake. In addition to these group-size effects, the Boesches have found clear evidence of cooperation in Tai chimp hunting behavior (Boesch 1994). Complex but subtle social rules regulate access to fresh kills, and they provide those that are involved in a hunt greater access to prey than those who failed to join a hunt.

We can also see the benefits associated with complex, coordinated group foraging in other species. Indeed, one of the most remarkable cases of such coordinated group-level foraging is found in the communication of information provided in the waggle dance of the honeybee.

On returning to the nest with food, a worker bee that has discovered a new source of food begins a waggle dance in which it "dances" up and down a vertical honeycomb within the hive, while other foragers in the hive make physical contact with the dancer as she moves. While waggling its body vigorously, the dancer is conveying important information about the food she has found. Her dance provides directional information for finding the food source from which she has just returned—the angle at which the forager dances shows the position of the food source in relation to the hive and to the Sun. In addition, the longer the waggle dance lasts, the farther away the food source. Every extra 75 milliseconds of dancing translates into the resource being approximately an additional 100 meters from the hive (**Figure 12.24**). The waggle dance thus provides bees living in groups with information about foraging sites that would not be available if they lived solitarily.

Increased Protection from Predators

Living in groups also provides economy of scale benefits with respect to detecting and avoiding predators. In species in which individuals scan their environments for predators, the more individuals in a group searching for predators, the less likely it is that a predator will be able to capture any member of the group. Consider a single bird that lifts its head and stops feeding every 5 seconds to scan for a predator. Now, imagine 10 such birds that are doing the same thing. Even if the scanning behavior of each bird is completely independent of the scanning behavior of the others in its group, the probability that a predator will successfully approach and capture any of the 10 birds is dramatically lower than the probability of capturing a solitary bird, because the odds are very high that one of the 10 birds will spot the predator and respond, perhaps by flying away from the danger and incidentally alerting the rest of the flock (Pulliam 1973). The bird in our group of 10 that has detected the

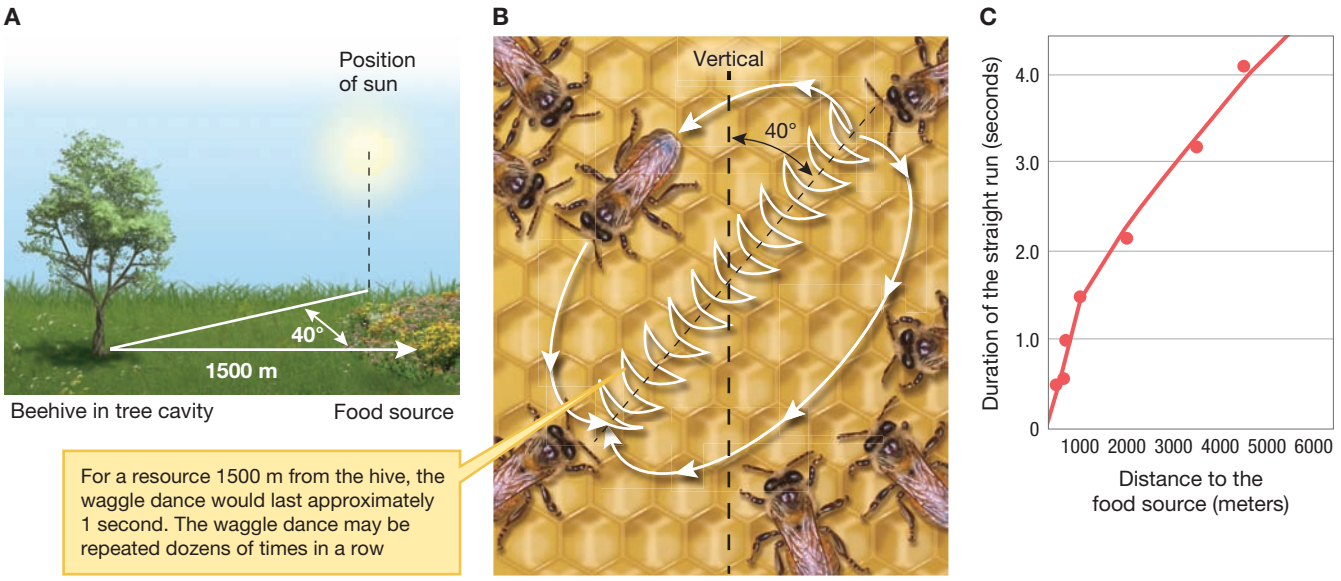

FIGURE 12.24 Honeybee waggle dances. **(A)** A patch of flowers that is 1500 meters from a hive, at an angle 40° to the right of the Sun. **(B)** When a forager returns, the bee dances in a figure-eight pattern. In this case, the angle between a bee's "straight run" (up and down a comb in the hive) and a vertical line is 40°. **(C)** The duration of the straight-run portion of the dance translates into the distance from the hive to the food source. Adapted from Dugatkin (2009a) and Seeley (1985).

predator is not responding any differently than it would if it were foraging alone, but its response produces a benefit for all group members. This idea has been dubbed the **many eyes hypothesis** (Powell 1974; Roberts 1996), but of course, it is not restricted to the case in which predators are detected visually: The same principle applies if predators are detected by sound, scent, or other sensory modalities.

Above and beyond the effects of a group having "many eyes," a transition to group living can be facilitated by other economy of scale benefits to group members (Hamilton 1971); for example, antipredator behaviors in schooling species of fish (Pitcher 1986). Swimming in a school produces a hydrodynamic effect that allows for faster movement than when swimming alone. This hydrodynamic effect alone can increase the chances of escaping from a predator. Fish in schools also use a number of antipredator tactics that are simply not possible for solitary individuals. In a "flash explosion," for example, individuals in a school swim off in all directions, confusing predators and facilitating escape (**Figure 12.25**).

Even in the absence of flash explosions, the very presence of a school of prey can confuse a predator by overloading the amount of information it must process and making it difficult for the predator to home in on a single target and follow it (Milinski 1979). Three-spined stickleback (*Gasterosteus aculeatus*) predators, for example, showed reduced foraging success as the group size of one of their prey—water fleas (*Daphnia*)—increased (Ioannou et al. 2008). When a model of the neural system of the stickleback was simulated using a computer, the results indicated that an increase in *Daphnia* group size caused a decrease in the ability of the stickleback to target any one specific prey item in its field of sight; that is, increased prey-group size confused the predator and increased the survival rates of the group-living prey (**Figure 12.26**).

FIGURE 12.25 Antipredator benefits of group living. During a flash explosion, fish in a school confuse predators by swimming off in many different directions. Adapted from Dugatkin (2004) and Pitcher and Wyche (1983).

The Costs of Group Living

When studying major transitions, evolutionary biologists are concerned not only with the benefits associated with the transition but also with the costs. One cost of group living is a simple proximity effect: When individuals live in a group, they are around other conspecifics who are natural competitors for food and other resources. A second type of cost is that of "cheaters" who attempt to usurp resources from others in a group. For example, consider a female mammal and her nursing offspring. If females do not live in groups, the only offspring that they nurse are their own. In group-living species, nursing females face a cheater problem: The young of other females may attempt to nurse from them. When this occurs, for example in elephant seals, females often respond by aggressively punishing such cheaters (Reiter et al. 1978, 1981; Clutton-Brock and Parker 1995). We will treat the problem of cheaters in detail in Chapter 17.

A third cost of group living is the transmission of parasites among group members, which we address below.

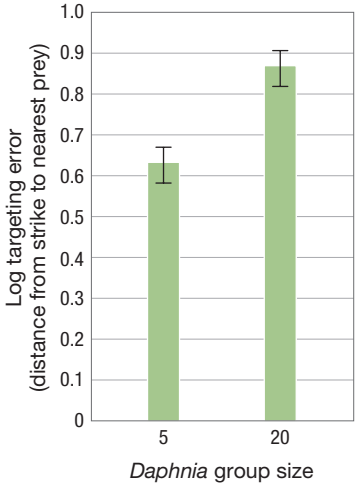

FIGURE 12.26 Group size and the confusion effect. Targeting errors by a predator (three-spined stickleback) increase as group size in prey (*Daphnia*) increases. Adapted from Ioannou et al. (2008).

Parasite Transmission as a Cost of Increased Group Size

Individuals in groups transmit information about foraging, predators, and so on, but they transmit something else as well to each other: pathogens and parasites. Because members of a group live in close proximity to one another, parasites can move from one group member to another much more easily than they can move between solitary-living hosts.

The cost of parasite transmission is nicely illustrated in Charles and Mary Brown's long-term field study of cliff swallow birds (*Petrochelidon pyrrhonota*). Cliff swallows build their nests in colonies that vary widely in size, and behavioral genetic work has found that the preference for small or large groups is a heritable trait (Brown and Brown 2000). Over the past two decades, the Browns have individually marked more than 160,000 cliff swallows in 239 different colonies by tagging their legs with identification numbers. They have recorded data on such critical variables as the probability that eggs will hatch and the survival probabilities of swallows of all ages. Overall, these data show a clear net positive effect of living in groups. As group size increases, the probability that eggs will hatch increases, as do the survival probabilities for birds of all ages (**Figure 12.27**).

Yet living in groups comes with a price for cliff swallows. Swallows are parasitized by a blood-sucking insect known as the swallow bug (*Oeciacus vicarius*). This

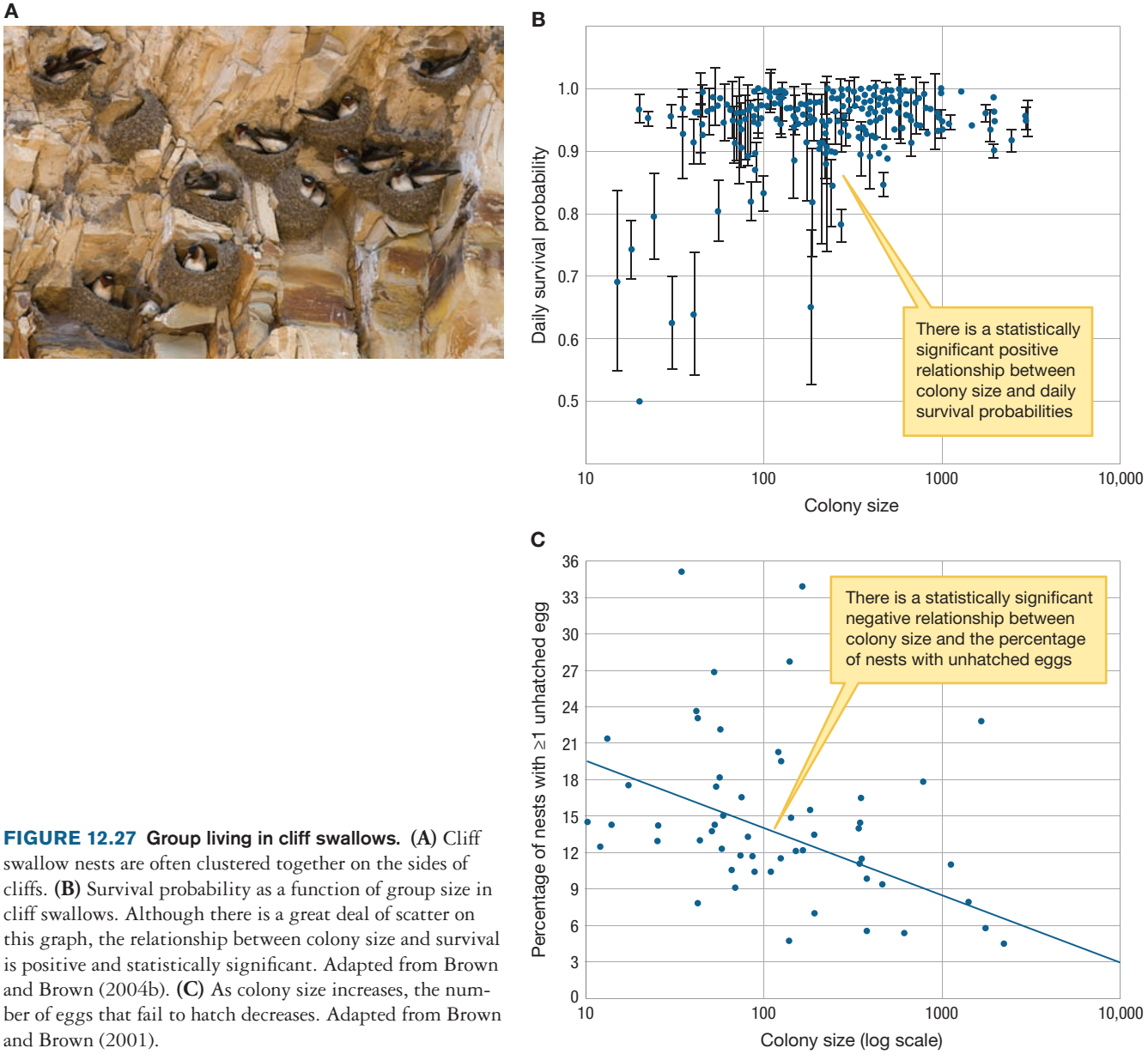

FIGURE 12.27 Group living in cliff swallows. **(A)** Cliff swallow nests are often clustered together on the sides of cliffs. **(B)** Survival probability as a function of group size in cliff swallows. Although there is a great deal of scatter on this graph, the relationship between colony size and survival is positive and statistically significant. Adapted from Brown and Brown (2004b). **(C)** As colony size increases, the number of eggs that fail to hatch decreases. Adapted from Brown and Brown (2001).

ectoparasite often clings to the feet of birds, can move from swallow to swallow within colonies, and is responsible for most of the nest failures and juvenile mortality in these birds (Brown and Brown 1996). The effects of swallow bugs can be experimentally measured by fumigating some swallow nests and leaving other nests untreated by pesticides. When the Browns did this, mortality was much higher in the unfumigated nests, providing strong experimental evidence for the costs of parasitism.

But it is not just the fact that parasites have negative fitness consequences that matters for our discussion of the evolutionary transition to group living. If we are interested in the costs of group living, we need to see evidence that as group size increases, the cost of parasitism increases. And, indeed, it does—as colony size increases, the number of swallow bugs *per nest* also increases (**Figure 12.28**). So, while the overall fitness effect of living in groups is positive for swallows, group living does not come cost free, and such costs are important to understand when analyzing the major transition from solitary to social living.

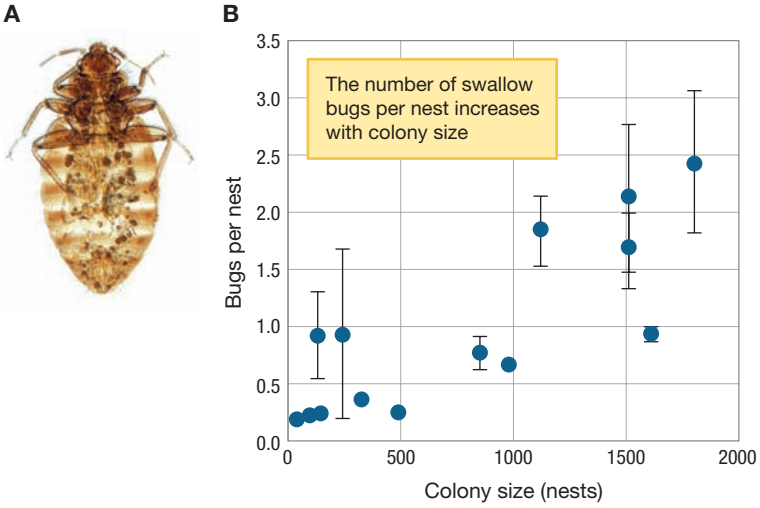

FIGURE 12.28 Cost of group living. **(A)** A swallow bug (*Oeciacus vicarus*). **(B)** The number of swallow bugs per nest per week increases with colony size. Panel B adapted from Brown and Brown (2004a).

Following the pathbreaking work of John Maynard Smith and Eörs Szathmary, we have outlined the framework biologists use to understand major evolutionary transitions—transitions such as the evolution of multicellular organisms from single-celled ancestors; the evolution of individuality, including the evolution of a specialized line of cells that become gametes; and the evolution of groups, including complex societies. We have already dealt with other transitions (the origin of self-replicating molecules, the transition from RNA to DNA, and the origin of the first cells) in earlier chapters, and we will return to additional examples of major transitions throughout the remainder of this book.

SUMMARY

1. Major transitions in evolution include (a) the origin of self-replicating molecules capable of heredity, (b) the transition from RNA as the catalyst and genetic material to protein as the catalyst and DNA as the genetic material, (c) the origin of the first cells, (d) the emergence of eukaryotic cells, (e) the evolution of sexual reproduction, (f) the evolution of multicellular organisms, (g) the evolution of developmental complexity within multicellular organisms, (h) the evolution of individuality, (i) the evolution of groups, including complex societies, and (j) the evolution of eusocial societies, with a division of labor and sterile workers.
2. Many of the major transitions in evolution share a common structure and lead to common consequences. Each transition possesses some of the following processes (some feature all of them): (a) individual agents give up the ability to reproduce independently, and they join together to form a larger aggregate ensemble with a shared reproductive fate; (b) once individual agents form these higher-level aggregations, they are able to take advantage of economies of scale and efficiencies of specialization; and (c) the processes of aggregation and specialization facilitate changes in information technologies.

3. Eukaryotes may have emerged from a fusion between an ancient bacterium and an ancient archaeal cell. This fusion likely involved some sort of endosymbiosis.
4. Endosymbiosis may also have been involved in the evolution of organelles, including mitochondria and chloroplasts, as well as some components of the cell nucleus.
5. Multicellularity can evolve from an ancestral population of solitary cells in two different ways. In the staying together route, which is thought to have been more common, cells remain together after cell replication. In the coming together route, formerly free-living cells join together.
6. The evolution of individuality involved the transfer of fitness from the individual cell to the multicellular organism. This transfer of fitness involved the differentiation of cell lines into those specialized in reproduction (germ cells) and those specialized in maintenance and growth of the organism (somatic cells).
7. Living in groups requires a degree of sociality that is not required for solitary living, and this also often entails new levels of coordination and communication between individuals to obtain such benefits. Group living typically imposes costs in addition to providing benefits.

KEY TERMS

economy of scale (p. 435)
efficiencies of specialization (p. 435)
endosymbiosis (p. 438)
genomic imprinting (p. 436)
germ cells (p. 432)
many eyes hypothesis (p. 457)
multicellularity (p. 445)
somatic cells (p. 432)

REVIEW QUESTIONS

1. What three processes characterize many major transitions?
2. Why don't higher-level organisms formed by a major transition succumb to the cheater problem we discussed?
3. What do evolutionary biologists think led to the major transition associated with the emergence of the eukaryotic cell?
4. How does phylogenetic analysis bolster the endosymbiotic theory of eukaryotic evolution?
5. What two paths do evolutionary biologists hypothesize led to multicellularity?
6. What is the evolutionary definition of *individual*?
7. Explain why a germ–soma distinction grows increasingly important as multicellular organisms grow larger.
8. What are two areas in which economy of scale benefits are associated with group living?
9. How have *rls1* and *regA* genes shed light on the major transition to individuality?
10. What is one evolutionary definition of a group?

KEY CONCEPT APPLICATION QUESTIONS

11. We dated specific major transitions in specific taxa, but we did not try to create a general timeline for major transitions. Why would we not expect to be able to generate a general timeline for all major transitions?
12. Based on the common themes that underlie most major transitions, why would we *not* include the following important evolutionary changes as major transitions: (a) the shift from aquatic to terrestrial life; (b) the evolution of flight?
13. In this chapter, we considered the antipredator and foraging benefits of group living. What other benefits of group living can you think of?
14. We will examine the evolution of sexual reproduction in detail in Chapter 16. Although we have not yet had an in-depth discussion about this topic, why is it clear that sexual reproduction represents a major transition?

SUGGESTED READINGS

Alvarez-Ponce, D., P. Lopez, E. Bapteste, and J. O. McInerney. 2013. Gene similarity networks provide tools for understanding eukaryote origins and evolution. *Proceedings of the National Academy of Sciences of the United States of America* 110: E1594–E1603. A nice review of the power of molecular genetics to help us understand one of the major transitions.

Kuzdzal-Fick, J. J., K. R. Foster, D. C. Queller, and J. E. Strassmann. 2007. Exploiting new terrain: An advantage to sociality in the slime mold *Dictyostelium discoideum*. *Behavioral Ecology* 18: 433–437. Experimental work on the benefits of the major transition to sociality.

Margulis, L. 1970. *Origin of Eukaryotic Cells*. Yale University Press, New Haven, Conn. An early presentation of the theory of endosymbiosis.

Maynard Smith, J., and E. Szathmary. 1997. *The Major Transitions in Evolution*. Oxford University Press, New York. One of the books in which Maynard Smith and Szathmary presented their view of the major transitions in evolution.

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13

Evolution and Development

◀ The intricate spiral structure of a nautilus (*Nautilus* sp.) shell.

Henry Bateson (1861–1926) was quite the fellow. When he was a young man, Henry announced that he wanted to become a naturalist, but, he added, that if he was not talented enough, "I suppose I shall have to be a doctor" (Cock and Forsdyke 2008). He was more than talented enough and became not only a natural historian, but also a geneticist, entomologist, evolutionary biologist, and developmental biologist. Indeed, Bateson translated Mendel's works into English for the first time, and he named the science of *genetics*. His own experimental work, in collaboration with Edith Saunders and Reginald Punnett (of Punnett square fame), led to the first published study of genetic linkage (Bateson et al. 1905).

In the latter part of the nineteenth century, Bateson observed a number of bizarre abnormalities in the insects and vertebrates he was studying at Cambridge University. He found cases in which one body part had replaced another during the developmental process; for example, one insect specimen had legs that developed where the antenna normally would be.

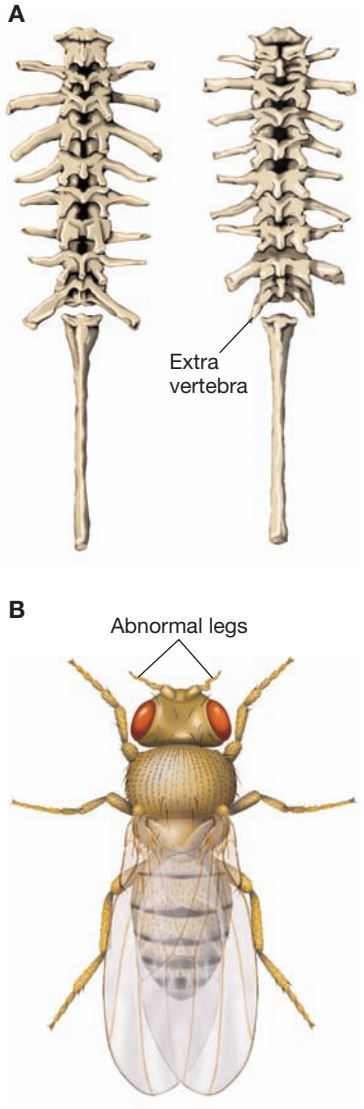

FIGURE 13.1 Homeotic transformations. (A) In *Materials for the Study of Variation*, Bateson showed the rib cage of a normal *Rana temporaria* frog (left) and that of an individual with an extra vertebra (right). Adapted from Bateson (1894). **(B)** A homeotic transformation in fruit flies. In fruit flies with the *Antennapedia* mutation, legs develop in place of antenna on the fly's head. Adapted from Exploratorium (2010).

Bateson found exceptions and abnormalities not only in fruit flies, but in vertebrates as well—cases where one vertebra had replaced another or where there were duplicate sets of ribs in the same individual. Bateson was among the first to recognize that these exceptions and abnormalities could tell us a great deal about the biology of properly formed organisms, advising young scientists, "If I may throw out a word of counsel to beginners, it is: Treat your exceptions!"

Bateson called the sorts of developmental changes he was seeing homeotic transformations (**Figure 13.1**) (Bateson 1894). It was just not the extraordinary appearance of the homeotic transformations that made them fascinating. Bateson was interested in what homeotic transformations could tell us about evolutionary change. Bateson noted that the homeotic transformations he observed seemed to be most common in parts of the body that were either repeated (appendages, ribs, and so on), segmented, or both. These structures are the building blocks used to construct animal bodies and so are important for an understanding of the immense and wonderful variation we see in animal body form. In his book, *Materials for the Study of Variation*, Bateson hypothesized that homeotic transformations would eventually allow scientists to decipher the evolution of animal body plans (Bateson 1894).

Bateson's homeotic "exceptions" served as a starting point for Edward Lewis, Christiane Nüsslein-Volhard, and Eric Wieschaus, who were awarded a Nobel Prize a century later for their discovery of **homeotic genes** (genes that determine the identity and positioning of anatomical structures during development; Lewis 1978) and **segmentation genes** (genes that are associated with patterning of the body segments during development; Nüsslein-Volhard and Wieschaus 1980). These researchers studied *Drosophila melanogaster* fruit flies and hypothesized that mutations to specific genes affected their body plan. They found that mutations of genes along the anterior-to-posterior (front-to-rear) body segments of the insect were responsible for many unusual phenotypes that could be observed in the mutant fruit flies. For example, through a series of targeted mutations in fruit flies, Nüsslein-Volhard and Wieschaus documented 15 loci that housed segmentation genes. Mutations to segmentation genes lead to many different types of changes to the body plan of *D. melanogaster*. Some mutations change the number of body segments in the fly embryo. Others change the internal structure of the segments. Yet others lead to the deletion of alternating body segments in flies.

This sort of work has led to hundreds of follow-up studies that together provide evolutionary biologists with a much deeper understanding of how the genetics of development sheds light on variation at the species level and beyond.

Today, we would call the approach described above **evo–devo** (short for **evolutionary developmental biology**), which incorporates developmental biology into evolutionary biology and is the fusion of the two disciplines (Raff and Kaufman 1983; Carroll et al. 2005; Carroll 2008).

In this chapter, we will delve more deeply into evolutionary developmental biology by addressing questions such as:

- How did the field of evo–devo emerge?
- How do homeotic genes map out a body plan, and how do changes in these genes lead to the evolution of new forms?

- How can an understanding of molecular genetics help us gain insight into the evolution of development and the variation of body shapes and forms that we see in nature?
- What is the role of gene duplication in the evolution of development?
- How does evo–devo help us to explain the evolution of novel, complex traits?

13.1 Evo–Devo: A Brief History

Although the sciences of evolutionary biology and developmental biology would not formally come into existence for millennia, the seeds of evo–devo-like thinking can be found in the work of ancient Greek philosophers such as Aristotle and Plato and their concept of what one day would be called the *scala naturae*, or the "great chain of being" (Bonnet 1769) (Chapter 2).

In the *scala naturae*, species can be classified from "lowest" to "highest," with humans at the summit. The ancient Greeks noted a parallel between the stages on the *scala naturae*—which involves the relationships between species—and the developmental stages of organisms. They argued that, like the simple to complex progression of the *scala naturae*, the development of an individual over its lifetime—its **ontogeny**—stepped through "simple" traits early in development to more complex traits later in the developmental process. All life, at all scales, it seemed to the ancient Greeks, moved from simple to complex.

This idea—that developmental stages mirror the *scala naturae* in moving from the simple to the complex—became known as parallelism, and its first major spokesman after the Greek philosophers was the German anatomist J. F. Meckel (1781–1833). Meckel added a critical evolutionary slant to the concept first suggested by the ancient Greeks, as he hypothesized that the developmental stages of the individual paralleled the evolutionary history of the species being studied (Meckel 1821). In particular, Meckel argued that the developmental stages of an organism step through all the animal species that came before it on the *scala naturae* (note that Meckel's ideas were pre-Darwinian, and so he didn't use the language of evolutionary biology in his writings).

Similar ideas were put forth by the French physician and embryologist Etienne Serres (1786–1868). The *Meckel–Serres law*, as it came to be known, was quickly modified in a subtle but important manner. While Meckel and Serres argued that embryos display characteristics of embryos from species that preceded them on the *scala naturae*, many people began to claim that the embryos of organisms step through the *adult stages* of species that preceded them. Again, although these ideas were pre-Darwinian, they clearly attempted to tie together developmental biology with what today we would call evolutionary history.

Karl Ernst von Baer (1792–1876), a German naturalist, biologist, and embryologist, rejected both the *scala naturae* and the Meckel–Serres law (von Baer 1828). Instead, what is known as *von Baer's law* states that when comparing developmental stages of closely related species, general traits develop before the specialized traits that allow us to distinguish different species from one another (**Figure 13.2**). This is a radically different approach to tying together developmental

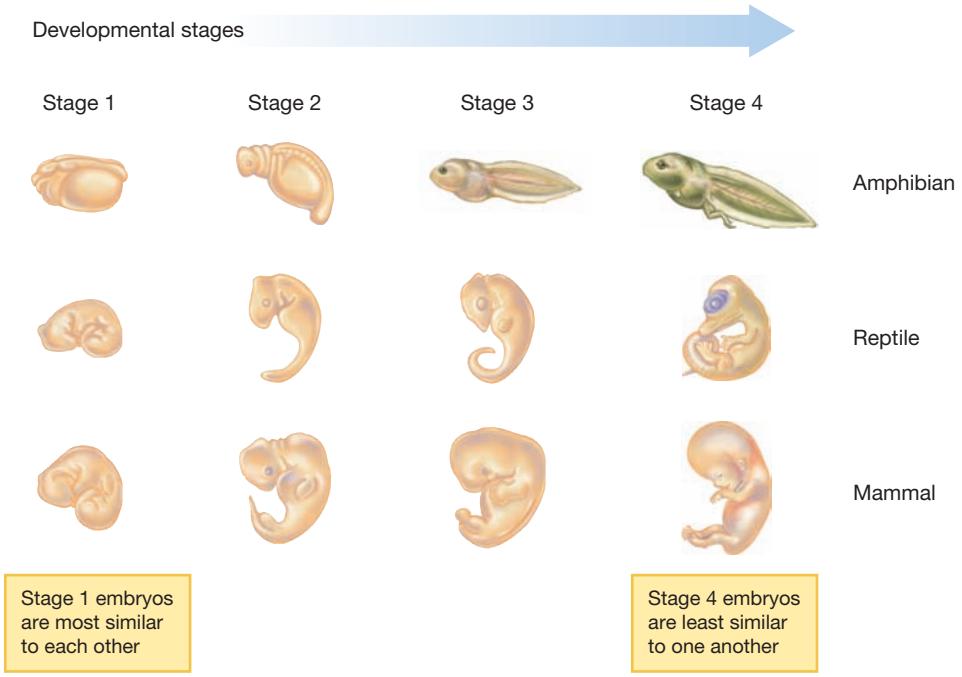

FIGURE 13.2 Von Baer's law. Karl von Baer argued that embryos in closely related species resemble each other and not the adult form of some ancestral species. He posited that the general characteristics that unite embryos from related species appear early in embryonic development, while specialized traits—those that start to distinguish embryos of different species from one another—appear later in development. Adapted from Horder (2006).

processes and evolutionary history than that proposed by Meckel and Serres. Von Baer argued that those traits that appear early in development are extremely resistant to evolutionary change (though again, von Baer was writing this in 1828, before Darwin, and so he did not use this language), and hence they are very similar across many taxa. Presumably, this is because changes at early stages in development have consequences that are enormous in magnitude and often fatal. It is only in the later stages of development, von Baer argued, that specific traits emerge that distinguish closely related groups of organisms. Although much later in his own life von Baer argued against Darwin's ideas on descent with modification, in modern terminology we would say that his ideas suggest that evolutionarily novel traits tend to appear late in development and are useful diagnostics for separating closely related species.

Ernst Haeckel (1834–1919), a German biologist and naturalist, disagreed with von Baer and further expanded on the Meckel–Serres law with his own *biogenetic law* (also known as Haeckel's theory of recapitulation). The biogenetic law proposes that "ontogeny is a precise and compressed recapitulation of phylogeny." Haeckel was arguing that the developmental progress of an organism (its ontogeny) replays (recapitulates) its evolutionary history (its phylogeny). This is the first theory that formally tied development to evolutionary theory by explicitly mentioning phylogeny.

For Haeckel, the evolutionary process produced new species by tacking on something new and novel to the terminal part of the development of an ancestral species, which was an idea first proposed by Fritz Müller (1821–1897). Today, Haeckel's biogenetic law has been rejected in evolutionary biology, largely because we now know that natural selection and other evolutionary processes act at all stages of development, including the embryonic stages. As such, these stages are not static replays of phylogenetic history, but a more subtle mixture of phylogenetic history and adaptation at the embryonic stage.

KEYCONCEPT QUESTION

13.1 Early mammalian development occurs in the uterus. What sort of selective pressures might operate in utero?

Timing of Development

Work in the area of evolution and development began to shift focus in the 1930s and 1940s. With the advent of the modern synthesis (Chapter 2), research began on the role of genes in shaping development. A major breakthrough occurred when this work revealed not only that genes code for physical traits, as researchers had known since Mendel's findings were rediscovered in 1900, but also that some genes control the *rate* of development, and thus the timing at which developmental stages occur (Morgan 1934; Goldschmidt 1938, 1940; de Beer 1930, 1940). The study of **heterochrony** focuses on the time in the developmental process at which a developmental trait is first expressed in a species, relative to when that same developmental trait is first expressed in the ancestor to the species being studied.

Gavin de Beer proposed a system for classifying four different types of heterochrony, a system which was later modified by evolutionary biologist Stephen Jay Gould (Gould 1977). Their system breaks developmental changes into two categories: (1) changes that affect the timing of the onset of reproductive traits, and (2) changes that affect the timing of the appearance of nonreproductive—*somatic*—traits (for example, wing or antennae development). The four types of heterochrony recognized today are acceleration, progenesis, neoteny, and hypermorphosis.

Consider a trait seen late in development in an ancestral species, but which appears earlier in development in a descendant species. This is referred to as **recapitulation**, and it can occur in two different ways. Genetic change can lead to (1) a somatic trait appearing earlier in development (this is called *acceleration*) or (2) a reproductive trait appearing later in development (this is referred to as *hypermorphosis*).

Conversely, suppose that we find that a trait that was formerly seen early in development in an ancestral species appears later in development in a descendant species. This is called **paedomorphosis**. Paedomorphosis, too, can occur in two very different ways: (1) reproductive traits appear earlier (*progenesis*) or (2) the onset of somatic traits is retarded (*neoteny*) (Table 13.1).

TABLE 13.1

Four Types of Heterochrony

Appearance of Somatic Traits	Appearance of Reproductive Traits	Type of Heterochrony
Accelerated	Unchanged	Recapitulation via acceleration
Unchanged	Accelerated	Paedomorphosis via progenesis
Retarded	Unchanged	Paedomorphosis via neoteny
Unchanged	Retarded	Recapitulation via hypermorphosis

Adapted from Gould (1977) and Raff and Kaufman (1983).

Before we look in more detail at an example of heterochrony, it is important to note that de Beer's (and then later Gould's) classification system was a significant step forward in development of a full-fledged field of evolutionary developmental biology because it (1) explicitly incorporated evolutionary history by comparing ancestor and descendant species, (2) focused on genetic change, and (3) recognized that traits associated with reproduction are fundamentally different than other traits.

KEYCONCEPT QUESTION

13.2 In general, when might natural selection favor progenesis?

The best-studied case of heterochrony is the neoteny seen in a suite of traits in the Mexican axolotl salamander, *Ambystoma mexicanum*. Most extant species of salamanders live in water during the juvenile stage and live on land as adults, and phylogenetic analysis suggests this is the ancestral state as well, but the axolotl remains in the water for its entire life (**Figure 13.3**). Developmentally, the axolotl matures into a normal, reproductively active adult, except that it never loses the traits associated with its aquatic existence, such as gills and a flattened tail. This represents an extreme form of neoteny in that *reproductive* traits appear at the same time in the axolotl as in most salamanders that metamorphose into land forms, but adult somatic traits (the loss of gills and the less flattened tail seen in other species) are so retarded in the axolotl that they never appear at all.

How can we explain the neoteny seen in axolotls? From a proximate perspective, we know that thyroid hormone (TH)—more specifically, the lack of TH—plays a

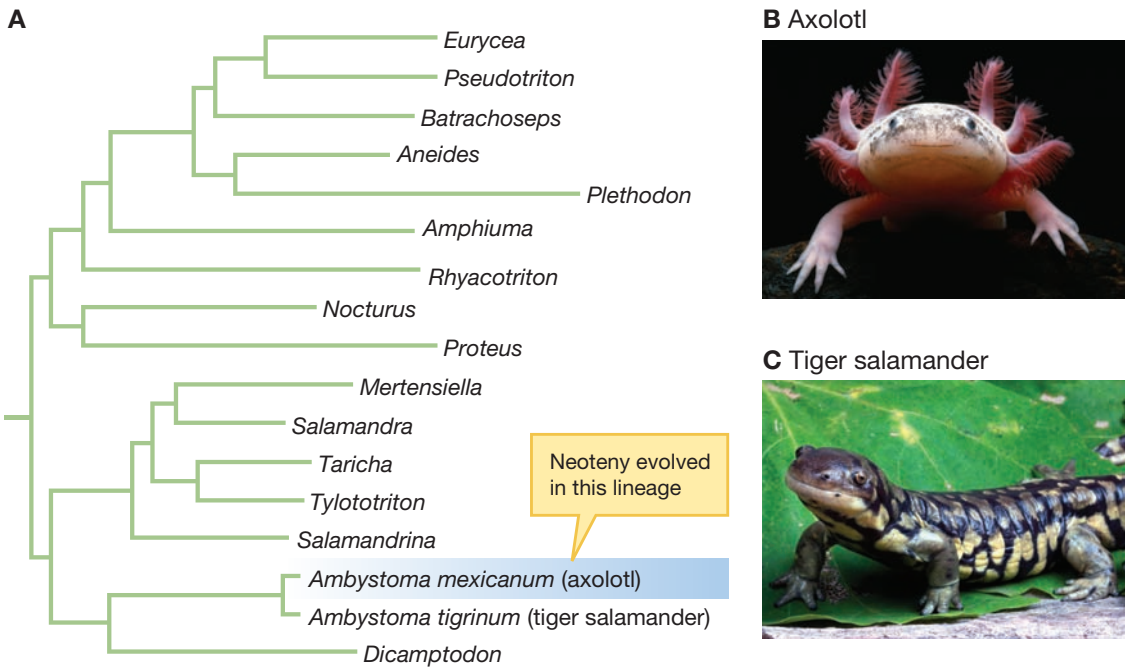

FIGURE 13.3 Neoteny in the axolotl. **(A)** A phylogeny of salamanders (suborder Salamandroidea) based on complete mitochondrial genomes. Adapted from Zhang and Wake (2009). **(B)** An adult Mexican axolotl salamander, *Ambystoma mexicanum.* Note the gills seen in this adult. **(C)** The tiger salamander, *Ambystoma tigrinum*, is the sister species to the axolotl. The larval stage of the tiger salamander is aquatic, but the adult stage (shown here) is terrestrial.

role (Johnson and Voss 2013). Most salamander species produce a burst of TH when they move from the water to the land. Axolotls never show this spike in TH. To test the hypothesis that the lack of TH production is linked to neoteny and to examine cause and effect, experimenters have added TH to the water in which axolotls live when they are juveniles. Axolotls maturing in such water metamorphose into a terrestrial form, suggesting a causal proximate relationship between the lack of TH and neoteny in this species (**Figure 13.4**) (Tompkins and Townsend 1977; Brown 1997).

Researchers are now beginning to understand the molecular genetics of TH production and thus neoteny in the axolotl. A genome-wide scan of both the axolotl and its sister species, the tiger salamander (*Ambystoma tigrinum*), found a large reduction in messenger RNA abundance across many loci, including genes that regulate the production of TH in the axolotl (Page et al. 2010). This tells us *how* neoteny is possible in the axolotl. But *why* has a neotenous developmental pathway evolved in the axolotl? One idea, called the *paedomorph advantage hypothesis*, suggests that neoteny may have been favored in the axolotl as a means for remaining in what is a relatively safe aquatic habitat, rather than undergoing metamorphosis and facing a new suite of terrestrial predators and a completely different environment (Wilbur and Collins 1973; Whiteman 1994; Denoel et al. 2005).

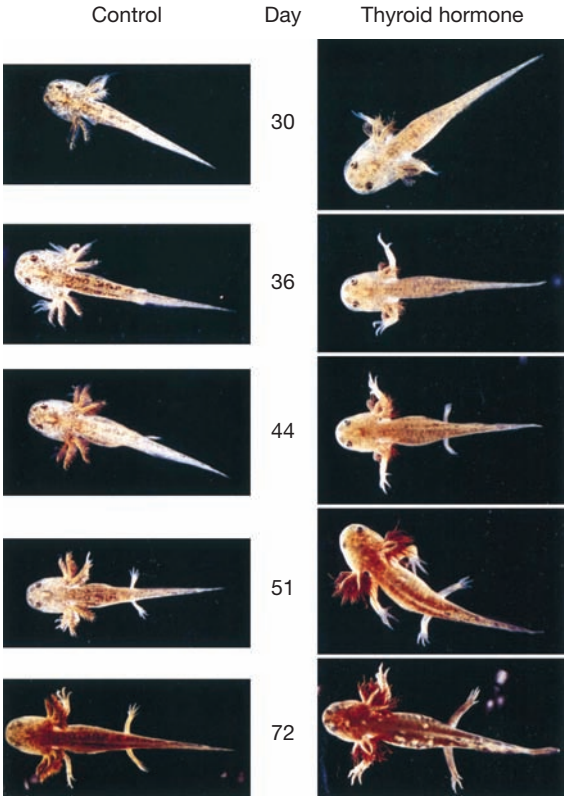

FIGURE 13.4 Thyroid hormone causes the normally neotenous axolotl to mature into an adult-like form not seen in nature. Addition of thyroid hormone (thyroxine; T4) to the water in which axolotl individuals were reared causes them to mature earlier and to develop into more "adult" forms than control individuals, as shown on these photographs at the specified days after fertilization occurred (Brown 1997).

Indirect evidence for the paedomorph advantage hypothesis in axolotls has been accumulating. This evidence comes from salamander species that are facultatively neotenous; that is, species in which some individuals, in some environments, exhibit neotenous development and remain in the water all their lives, while other individuals mature into terrestrial adult morphs. Experimental work has found that the proportion of neotenous individuals increased in facultatively neotenous salamanders when (1) pond levels were constant (as opposed to variable, with some ponds drying quickly), (2) there was a low density of conspecific competitors in ponds, and (3) predation rates were relatively low in aquatic environments relative to terrestrial ones (Harris 1987; Semlitsch 1987; Jackson and Semlitsch 1993). Such ecological factors may also have favored obligate neoteny in the axolotl lineage (Denoel et al. 2005).

After work on heterochrony, the next historical watershed in evolutionary developmental biology was the discovery of the genes responsible for the homeotic transformations that we mentioned earlier in the chapter and which we will discuss in more detail below.

KEYCONCEPT QUESTION

13.3 Stephen Jay Gould once wrote a playful essay on how Walt Disney kept making Mickey Mouse's features more and more paedomorphic, and how this seemed to increase the character's popularity (Gould 1979). Based on what you know about paedomorphosis, why do you suppose that strategy worked?

13.2 Regulation, Expression, and Switches

Consider two amazing facts about multicellular creatures: (1) every multicellular creature develops from a single cell, and (2) except for sperm and eggs, every cell in the body of a multicellular creature contains the same set of genes. Yet skin cells look, feel, and function very differently than the cells in muscles, cells in the liver, and so on. How cells function depends on the developmental pathways along which they progress. As we will see, this has important ramifications for the evolutionary process. Very early on in the developmental process, each cell in an embryo is *totipotent*; that is, it could, in principle, develop into any of the cell types that make up the adult organism. It could potentially function as a skin cell, a muscle cell, a liver cell, and so forth. Which of these it becomes depends on the fascinating ways that genes are regulated and expressed within the environment of a cell, a complex process we are just beginning to understand.

To understand the evolution of development, we need to recognize that the development of an organism is a dynamic process. During the process in which a single cell develops into a multicellular organism and then into an adult, cells receive information from the nearby cellular environment, and this information guides their development. What determines whether a cell will function as a liver cell or a skin cell or as any other specific type of cell depends on what is happening in the environment around that cell.

In the next two subsections, we will examine homeotic genes, as well as regulatory **enhancers** and **silencers**, DNA sequences that turn on and off, respectively, the expression of particular genes. Doing so will help us to understand how development of forms and structures in plants and animals unfolds from an evolutionary perspective. In an animal, this means studying the development of the animal from an egg into multiple cells, then into an embryo with various incipient organs and tissues, and finally into the adult form with full-grown internal and external structures such as legs and arms in primates or wings and antennae in insects. In a flowering plant, this means studying the development of the plant from a seed into multiple cells and ultimately into roots, stems, leaves, and other structures in the developing organism. We will see that development is guided by the turning on and off of genetic switches in a cascade that affects the production of proteins, the growth of cells, and the overall body plan of plants and animals. And we will also see that evolutionary changes in developmental pathways between species, genera, and so on, are largely a function of where and when these genetic switches are flipped on or off (Carroll 2005).

Homeotic Genes, Development, and Evolution

Homeotic genes play a key role in the developmental process. They encode proteins that control the switching on and off of a cascade of other genes in a set sequence and thereby affect cell size, shape, and division and the positioning of the cells within the organism's body plan. Researchers have found that gene products produced from combinations of homeotic genes act as gene activation signatures that create a sort of instructional map for where structures should develop. The signals occur locally and indirectly specify what structures other genes should form

in those particular local regions. As such, homeotic genes play a critical role in the construction of an organism's phenotype, and as we have learned, it is the phenotype on which natural selection acts.

Because so much is known about their genetics and their development, fruit flies have become a model system for examining the role of homeotic genes in evolution and development. In fruit flies, homeotic genes regulate the overall development of the insect's body regions, as well as segments within its body regions. Between 8 and 13 homeotic genes, called the ***Hox* genes**, affect the anterior-to-posterior positioning of structures on the embryo's body by encoding transcription factors, which are proteins that bind to DNA and that thereby influence gene expression.

Hox genes determine the fate of various cells in the head, thorax, and abdomen regions in a developing fruit fly and in other organisms (**Figure 13.5**). For example, the *Hox* gene called *labial* (*lab*) is expressed in cells that develop into mouth parts, while the gene *Abdominal B* (*Abd-B*) is expressed in abdominal body parts near the rear end of the fruit fly. Mutations in *Hox* genes can lead to the type of abnormality that Bateson found, in which antennae are replaced by legs, and as we shall see, even small changes in *Hox* genes can have large phenotypic effects.

Hox genes affect anterior-to-posterior development in myriad ways. In fish, for example, *Hox* genes play an important role in the development of what is called the lateral line (**Figure 13.6**). The lateral line runs along the side of a fish and is a cluster of sense organs that detect movements and vibrations in areas surrounding a fish. The sensory data provided by the lateral line is critical in helping the animal avoid predators and is used by fish when they form tight schools and move in unison. Lateral line development begins about 18 hours after embryos are formed, when a cluster of cells called the lateral line primordium moves down the anterior-to-posterior axis of the embryo. As the primordium moves, it lays down future sensory organ cells such as neuromasts as it goes. The movement of the primordium seems to be driven, in part, by expression of *Hox* genes. When Marie Breau and her colleagues studied gene expression during migration of the lateral line primordium, they found that a gene called *hoxb8a* was being expressed in the leading two-thirds of the cells in the migrating group. In a sense, *hoxb8a* was directing the primordium's march down the side of the embryo while putting

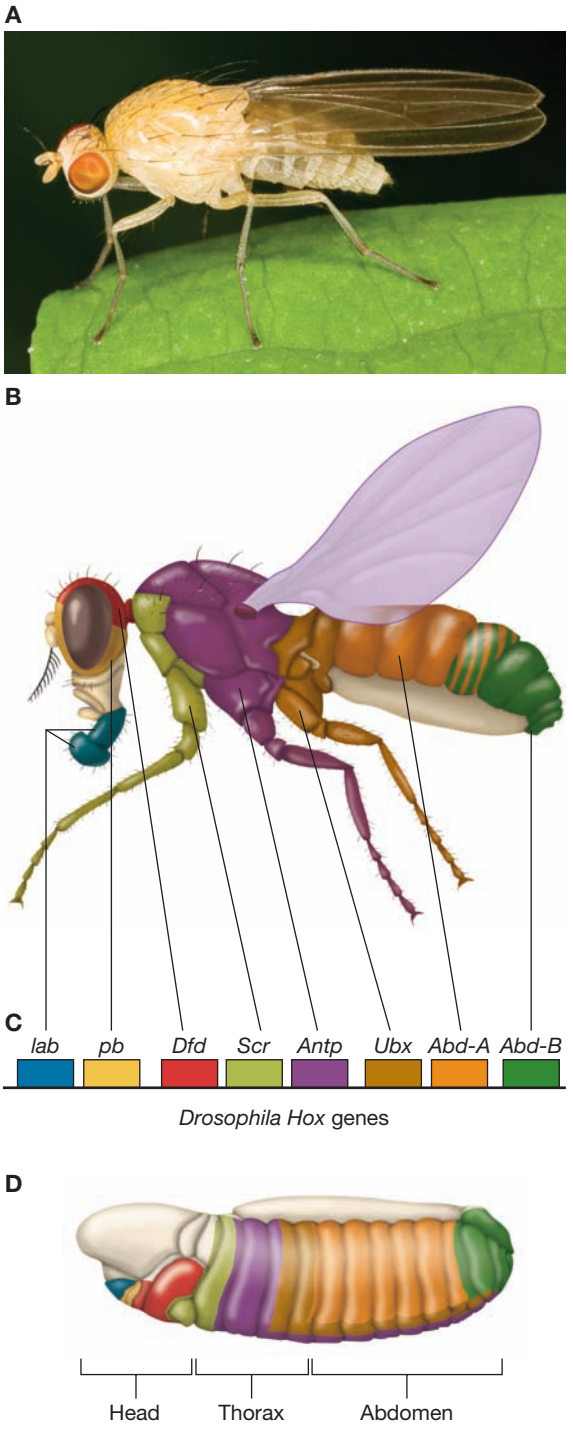

FIGURE 13.5 ***Hox* genes determine body segment identity.** At least eight different *Hox* genes are critical for the development of different body segments in (**A**) fruit flies (*Drosophila*). In the diagrams, *Hox* genes are color coded to show **(B)** the body segments in adults that are affected by each *Hox* gene, **(C)** the *Hox* genes as arrayed on a chromosome, and **(D)** the body segments in larvae that are affected by each *Hox* gene. Adapted from Carroll et al. (2005, p. 24).

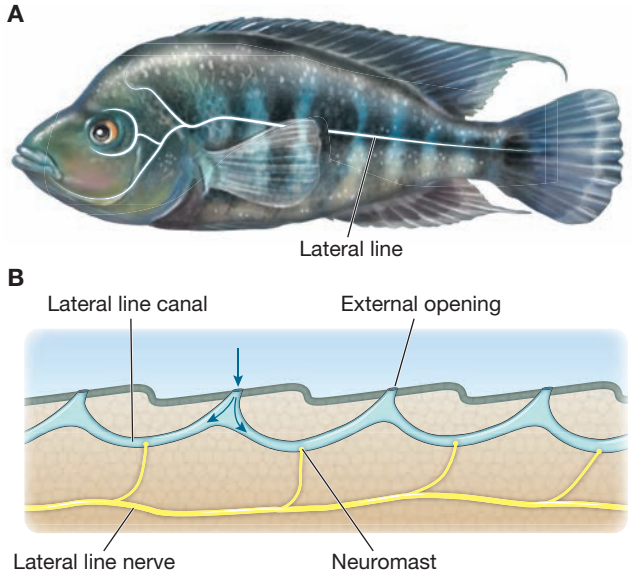

FIGURE 13.6 Lateral line in fish. (A) The lateral line (shown here in white for emphasis) runs along the side of fish and is used to detect motion. (B) A longitudinal section of the lateral line, which includes sensory detectors known as neuromasts.

into place the building blocks of the future lateral line system (Breau et al. 2013) (**Figure 13.7**).

Homeotic genes affect spatial development in plants as well as animals. Researchers have found that homeotic genes are involved in determining which cells become which structures—stamens, carpel, petals, and so forth—of flowering plants (Ng and Yanofsky 2001; Krizek and Fletcher 2005). Work on what are called *MADS-box* genes—homeotic genes that affect plant development—has shed important light on plant development and evolution (**Figure 13.8**). Because petals, carpels, and stamens play an important role in plant reproduction, a small change to the *MADS-box* genes underlying the development of these structures can have a large impact on the phenotype and reproductive success of an individual. Sufficiently large changes could, in principle, even drive speciation.

As new molecular genetic tools became available in the 1990s, evolutionary geneticists and developmental biologists began to search for homeotic genes across a wide spectrum of plants and animals and to study how these genes affected plant and animal development. They discovered that the system of building organisms dynamically, with homeotic genes as position-setters, is extremely powerful: This system not only concisely and robustly specifies how to build an organism but also allows for the creation of a vast diversity of body forms. The rich diversity of life that we have discussed throughout this book—the diversity that Darwin tried to explain in *The Origin of Species*—is largely a result of random mutations, subsequently acted on by natural selection, in these dynamic programs for assembling organisms. This is a remarkable statement, so let us examine the issue in a bit more detail.

The same 180-base-pair sequence, called the **homeobox**, is found in all homeotic genes in a wide array of animal species. Once molecular geneticists had discovered the homeobox, they were able to search for and identify additional homeotic genes, including *Hox* genes, in species from frogs to mice to humans. As in fruit flies, the expression of *Hox* genes is often associated with delineating which groups of cells become which body segments.

KEYCONCEPT QUESTION

13.4 Why do you think the homeobox is conserved across species that span a vast phylogenetic distance?

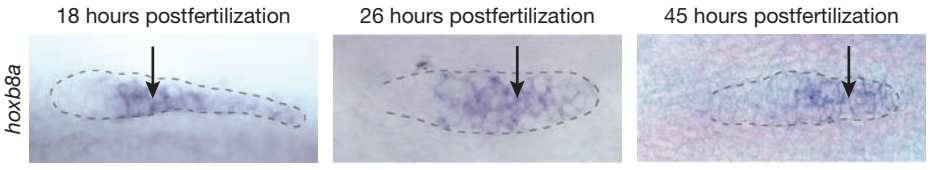

FIGURE 13.7 *Hox* gene expression during lateral line formation. The lateral line primordium, a cluster of cells that moves down the anterior-to-posterior axis of the embryo during development, is outlined by dashed lines. As the primordium moves, it lays down future sensory organs such as neuromasts. The movement of the lateral line primordium seems to be driven, in part, by expression of the *hoxb8a* gene. The expression of *hoxb8a*, is shown at the arrows. Adapted from Breau et al. (2013).

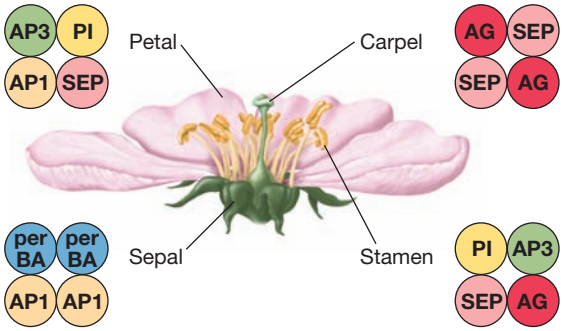

FIGURE 13.8 ***MADS-box* genes and flowering plants.** Expression of homeotic *MADS-box* genes helps explain the developmental pathways of different sections of flowering plants. The *MADS-box* transcription factor proteins (colored circles) are hypothesized to form complexes as shown, which jointly determine which structure—sepal, petal, carpel, or stamen—is formed in which location. Other *MADS-box* genes may also be involved in the development of these sections of flowering plants. Adapted from Thieben and Saedler (2001).

As our understanding of *Hox* genes increased, researchers began to notice that in many *Hox* gene complexes, the position of each *Hox* gene on a chromosome corresponds to the relative position of the body part that the *Hox* gene regulates. This phenomenon is known as **colinearity**. For example, in fruit flies, the genes associated with the development of mouth parts and eyes are found on the near end of the chromosome; genes associated with the thorax are found in the middle section of a chromosome; and genes associated with development of abdominal sections are found on the far end of the chromosome. We do not yet fully understand why colinearity is observed.

As in the case of fruit flies, we see colinearity when we study *Hox* genes in vertebrates. Perhaps most remarkably, the ordering of *Hox* genes on vertebrate chromosomes parallels the ordering of *Hox* genes on fruit fly chromosomes. This means that homologous *Hox* genes in invertebrates and vertebrates not only have similar DNA sequences, but they are also ordered on chromosomes in a similar way in both vertebrates and invertebrates (**Figure 13.9**) (Tarchini and Duboule 2006; Noordermeer et al. 2011; Papageorgiou 2012).

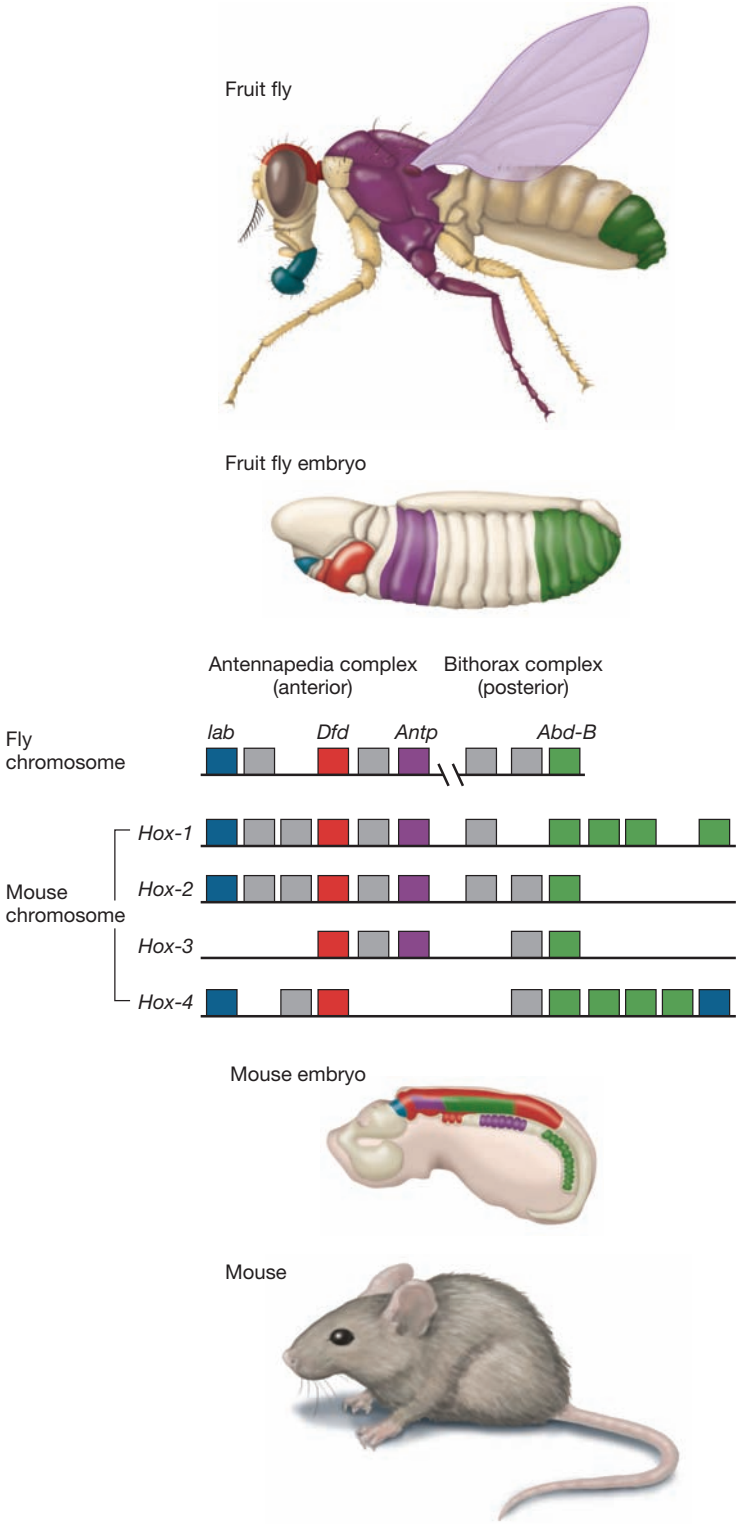

FIGURE 13.9 **Hox genes in mice and flies.** Homologous *Hox* genes in invertebrates and vertebrates are not only similar in DNA sequence, but also in the way they are ordered along the chromosome. Here we see the colinear arrangement of *Hox* genes in both fruit flies and mice. Adapted from Taubes (2010).

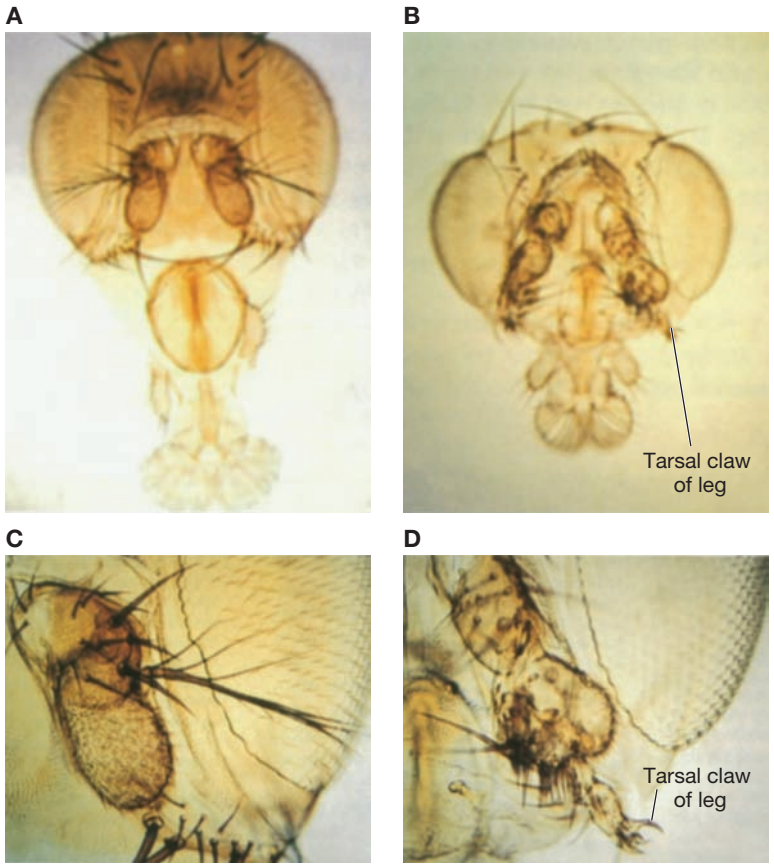

FIGURE 13.10 A *Hox* gene swap. When the mouse *Hox-2.2* gene is experimentally inserted into the fruit fly genome and expressed in the head of developing fruit flies, adults produce legs in place of antennae, just as they do when *Antp* is expressed in the head area (Malicki et al. 1990). **(A)** Wild-type head phenotype of an adult fruit fly. **(B)** Head of a fruit fly with the transplanted mouse *Hox-2.2* gene. **(C)** Close-up of the wild-type antenna. **(D)** Close-up of the thoracic leg parts that develop in place of the antennae in fruit flies with the transplanted mouse *Hox-2.2* gene. From Malicki et al. (1990).

Researchers have developed a suite of molecular genetic techniques that allow evolutionary biologists to test hypotheses about homeotic genes and developmental pathways. For example, a specific gene can be deactivated, allowing researchers to test hypotheses about what sorts of changes in development should then occur. A second technique is to transfer homologous *Hox* and *Hox*-like genes experimentally from one species to another. For example, the mouse *Hox-2.2* gene is structurally very similar to the fruit fly *Antennapedia* (*Antp*) gene. Both encode highly similar homeobox domains, and in a remarkable 1990 experiment, Bill McGinnis and his colleagues tested the hypothesis that the mouse *Hox-2.2* gene would have the same developmental effects as *Antp* on *Drosophila.* Recall that mutations in *Antp* cause adult fruit flies to develop legs in place of antennae (see Figure 13.1B). When *Hox-2.2* from mice was experimentally inserted into the fruit fly genome and expressed in the head area of developing flies, adults produced legs in place of antennae (**Figure 13.10**), just as they do when *Antp* is expressed in the head area (Malicki et al. 1990; McGinnis et al. 1990; Akam 1991).

Because some *Hox* genes are so highly conserved evolutionarily, a *Hox* gene from one species can sometimes substitute for that from another in normal development as well. In a 1997 study, Lutz and his colleagues found that by inserting a *Hox* gene from chickens into fruit flies (*Drosophila)* that had a defective labial *Hox* gene, they could enable the normal phenotype of the fly to develop (Lutz et al. 1997). In other words, the corresponding *Hox* gene from a chicken worked in regulating development in a fruit fly that originally lacked the appropriate *Hox* gene. This is all the more surprising in that the researchers found that the fruit fly and chick proteins expressed in this experiment differed widely *except* in their homeobox domains. The key to understanding how this could be is to recognize that the homeobox domains of *Hox* genes encode the DNA binding regions of the transcription factor proteins. Thus, the strong conservation of the homeobox domains is sufficient to allow the transcription factor of one species to function in the other species, as it switches on the expression of genes in that species.

Both the comparison of mouse and fruit fly *Hox* genes and the ability to transfer *Hox* genes between species have important evolutionary implications. These results suggest that homeotic genes display deep (ancient) homologies. Homologous *Hox* genes have been uncovered in groups as diverse as jellyfish, mollusks, earthworms, and octopuses, and, in each case, these genes are involved in constructing the anterior, central, and posterior body parts of these creatures.

Deep homology of homeotic genes is also seen in plant *MADS-box* genes. As we have seen, *MADS-box* genes play a role in flower development, but they

also are instrumental in nonflowering plant species, where they are involved in developmental pathways in leaf and root systems (Kim et al. 2004; Frohlich and Chase 2007) (**Figure 13.11**).

Why should we see such deep homology in homeotic genes? Why do we see *Hox* genes affecting anterior-posterier developmental patterning across the whole animal kingdom? Why are similar *MADS-box* genes important in the early developmental pathways of both flowering and nonflowering plants? One hypothesis, first suggested by von Baer more than 175 years ago, is that while developmental changes can and do lead to radical new body plans, the dynamic programs that underlie the *early stages of development* are extraordinarily resistant to change. Mutations that change the structure of genes that affect early development are very likely to be lethal, and homeotic genes appear to be fundamental in establishing body plans early in development. As a result, we expect homeotic genes to be highly conserved over evolutionary time, and indeed they are.

Regulatory Enhancers as Switches

We have seen that homeotic genes, including *Hox and MADS-box* genes, encode transcription factor proteins that guide development. But transcription factors do not act in isolation; rather, they operate by binding to stretches of DNA known as

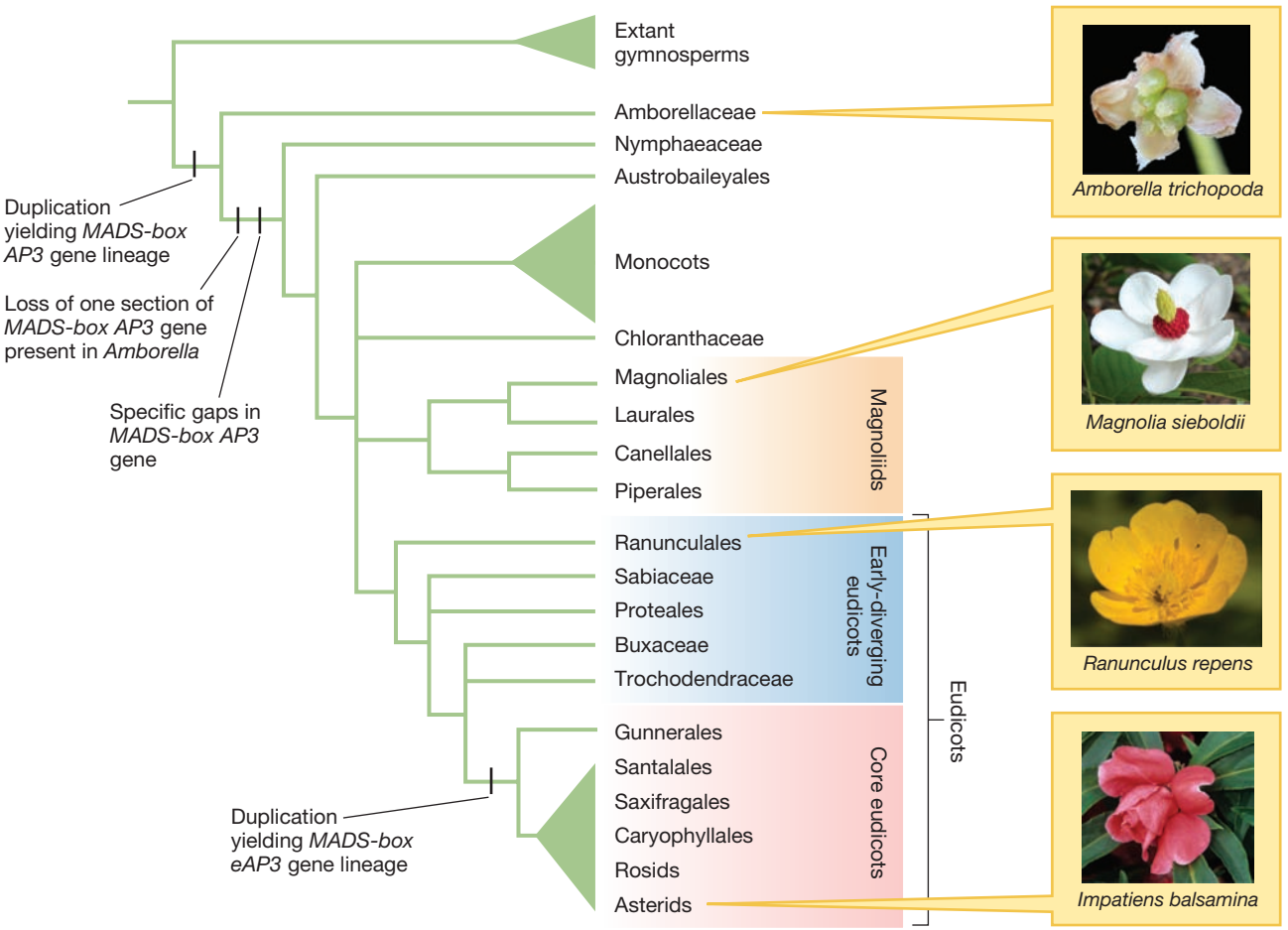

FIGURE 13.11 *MADS-box* genes and flowers. Some examples of the diverse flowering morphology seen in angiosperms and a phylogenetic tree showing their relationships. *MADS-box* genes, such as *AP3,* have been used to work out phylogenetic relationships within flowering species of plants, though the phylogeny shown here is not based strictly on *MADS-box* genes (Ng and Yanofsky 2001; Kim et al. 2004; Frohlich and Chase 2007). Adapted from Kim et al. (2004).

regulatory enhancers (**Figure 13.12**). A regulatory enhancer of a gene is a section of DNA that lies outside of that gene but is involved in regulating the timing and level of that gene's expression.

Regulatory enhancers are one type of *cis*-regulatory element: a noncoding stretch of DNA that controls the spatial and temporal expression of nearby genes. By *noncoding*, we mean that these stretches of DNA do not code for protein sequences. To a large extent, it is these noncoding regions of DNA that allow the cells of a multicellular organism to do such different things despite containing the same set of genes. (Peter and Davidson 2011; Lagha et al. 2012; Wittkopp and Kalay 2012; Rubinstein and de Souza 2013).

We now know that *cis*-regulatory elements are also a potentially powerful generator of the diversity of life. Whole-genome sequencing demonstrates that closely related species show very high levels of genetic similarity. At the same time, closely related species may also look and act very differently. For example, whole-genome comparisons of human and chimp DNA found an average difference of only 1.3%—a remarkable degree of similarity at the nucleotide level (Mikkelsen et al. 2005; Khaitovich et al. 2006). Yet chimps and humans *look* and *act* very differently from one another. How can these sorts of findings be reconciled? *Cis*-regulatory elements provide one possible solution (there are others, as discussed in Hoekstra and Coyne 2007). Though closely related species show very high levels of genetic similarity, because *cis*-regulatory elements can diversify over time to affect the timing (and spatial location) of gene expression differently, even closely related species may look and act very differently.

Cis-regulatory enhancers act as switches that turn genes on and off, and they affect the amount of product (primarily proteins) produced by a gene. A single gene can have numerous regulatory enhancers associated with it, and these regulators can operate independently of one another on that gene. Indeed, a gene affected by multiple regulatory enhancers can be expressed differently in different parts of

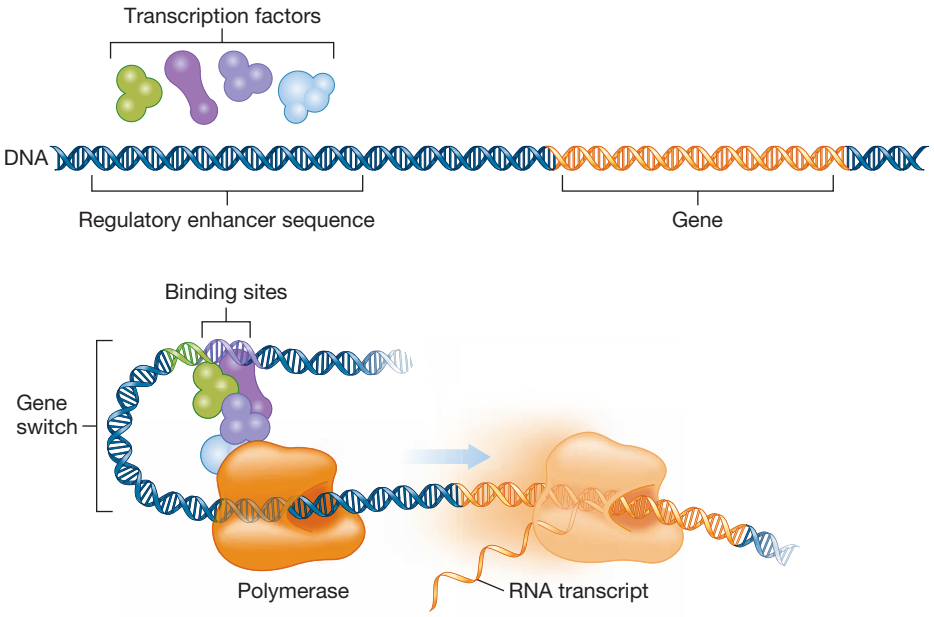

FIGURE 13.12 Gene switches. Gene expression is controlled in part by regulatory enhancer sequences in DNA. Transcription factor proteins bind to the regulatory enhancer, and the result is like a switch being turned on—the switch triggers RNA polymerase to start transcribing an RNA copy of the gene. Adapted from Carroll et al. (2008).

the body and at different points in time. Variation in the expression of regulatory enhancers, in other words, can increase morphological variation and hence the amount of variation that natural selection has to act on.

As with homeotic genes, regulatory enhancers have been a major focus for researchers interested in understanding the construction and patterning of animal bodies. Much of this work has concentrated on traits such as size, shape, and color, as these traits are so fundamental both to the process of development and to the phenotypic variation we see around us in nature.

Extensive work has been done on the regulatory enhancers that affect the development of pigmentation patterns in insects. In some species of fruit flies, males have black spots on the edges of their wings, and these spots are used in their visual displays during courtship dances with females. In other fruit fly species, the black wing spots are completely absent. Why is there a difference between species? At one level of analysis, the difference between spotted and nonspotted species can be attributed to a gene called *yellow* and the protein it codes for, which is referred to as yellow protein. In species that have black spots on the edges of their wings, the yellow protein is produced at high levels, but only in the wing cells that produce black spots. In species of fruit flies that lack black spots, the yellow protein is produced in all wing cells, but at levels much lower than those found in the black-spot cells of spotted fruit flies.

But that answer only gets us so far. We want to know why the *yellow* gene is expressed differently across different fruit fly species. The key to the differences in the amount of yellow protein and the spatial distribution of wing spots across different species of fruit flies lies in the effects of regulatory enhancers on the *yellow* gene. Sean Carroll and his colleagues uncovered the role of a regulatory enhancer in this system by examining the genetic sequence around the area of the *yellow* gene. They found that in fruit fly species without spots, there is a regulatory enhancer that causes the *yellow* gene to express the yellow protein at low levels all over the wing. This same enhancer in spotted species of fruit flies was associated with both high expression of the yellow protein in the black-spot area on the wing and low expression of yellow protein in the other areas of the wing. Carroll and his team also found that in the spotted species, new binding sites for transcription associated with the yellow protein have evolved, and these new binding sites allow for greater expression of black wing spots. The changes to the wing-spot enhancer were very specific; that is, new binding sites did not affect the expression of the *yellow* gene in any other cells in fruit fly species that have black wing spots. The enhancer of the *yellow* gene, then, specifically affects the development of black wing spots in males. Later, when males mature, these black wing spots play a role in obtaining mates, and so this work helps illustrate how an understanding of development can uncover the causal chain underlying an evolutionary process: Genetic differences in a regulatory enhancer lead to differences in developmental patterns, which in turn lead to differences in traits associated with mating (**Figure 13.13**).

In addition to differences in the presence of black wing spots, fruit fly species also differ in whether males have dark black coloration on the abdomen. And just as with black wing spots, coloration of the abdomen both is affected by a regulatory enhancer during development and plays a role in mate choice during courtship in fruit flies. But it is a second, and separate, regulatory enhancer that affects the expression of the *yellow* gene and appears to be associated with the

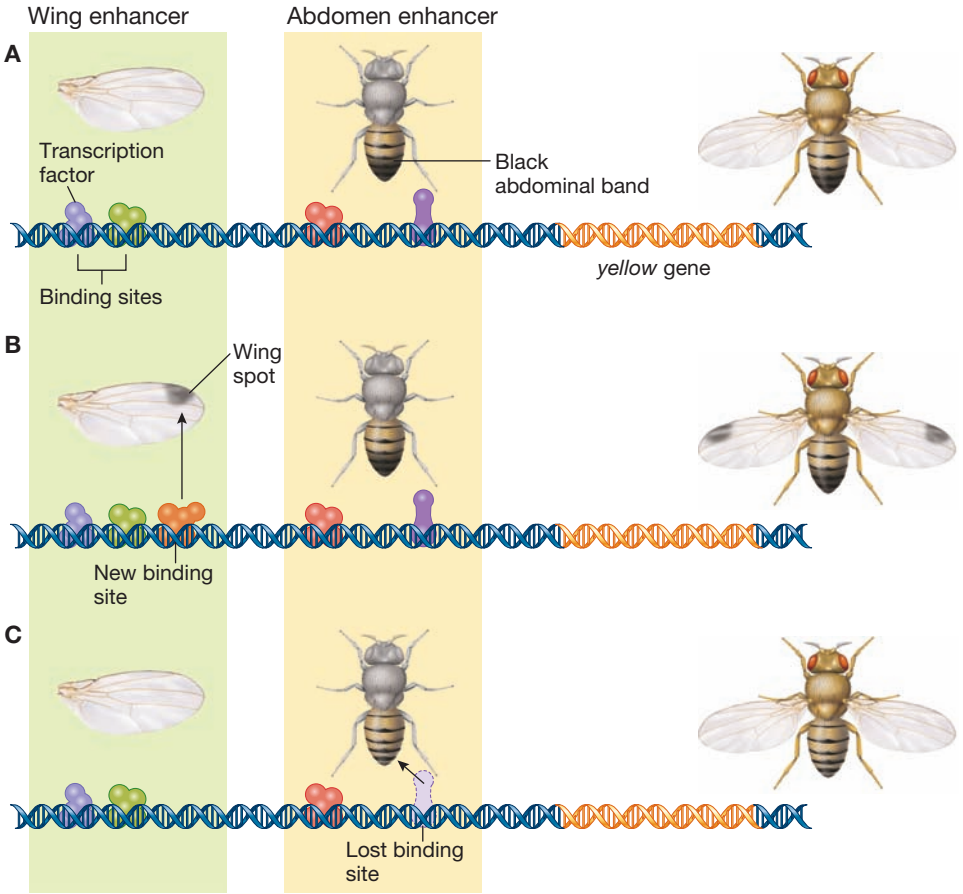

FIGURE 13.13 Gains, losses, and multiple enhancers. Multiple enhancers control color expression in different parts of a fruit fly's body. **(A)** A hypothetical case of a DNA sequence leading to light wings and a dark abdomen. **(B)** A new binding site produces dark spots on wings. **(C)** A lost binding site leads to the loss of black coloration on the abdomen. Adapted from Carroll et al. (2008).

presence or absence of black abdomens in fruit flies. The evolutionary history of this enhancer differs from that of the wing-spot enhancer, where new binding sites facilitated dark wing spots. In the case of black abdomens, the key developmental change associated with the yellow protein is that binding sites *were lost* (as opposed to gained) in fruit fly species that lack abdomen coloration.

Earlier in the chapter, we looked at an example of how a *Hox* gene from one species can sometimes substitute for that from another species and how such transplant experiments can shed light on the evolution of diversity. Similar sorts of gene swap experiments have been undertaken with regulatory enhancers. For example, Chris Cretekos and his colleagues were interested in whether regulatory enhancers might help explain why, unlike other mammals, bats develop wings rather than typical mammalian appendages. Cretekos and his team replaced a regulatory enhancer at a locus called *Prx1* that is associated with limb development in mice with the homologous regulatory enhancer found in short-tailed fruit bats (*Carollia perspicillata*). If the bat regulatory enhancer that was inserted into the mice was critical for wing development, then they should see mice with appendages that looked more bat-like than usual (Cretekos et al. 2008). And, indeed, that is what Cretekos found: Not only were transcription levels of this gene higher in the appendages of mice that had the transplanted bat regulatory enhancer, but also these mice had longer, more "bat-like" limbs than control animals. At a mechanistic level, one way to think about these results is that regulatory enhancers

help explain how it is that we have flying mammals: Differences in regulatory enhancers help bats to develop wings rather than typical mammalian appendages.

13.3 Evo–Devo and Gene Duplication

Gene duplication—the establishment of multiple copies of genes within the genome—plays an important role in the evolution of developmental pathways. Once a gene duplication event occurs, a number of different fates can befall the duplicate copy of a gene. It may be lost by the process of natural selection if the duplication comes at a cost or it may evolve into a functionless copy known as a pseudogene, as described in Chapter 8. Yet, what makes **gene families** so important for work in evo–devo is that not all duplicate genes are lost or converted into pseudogenes. Duplicate genes that continue to be expressed are known as **paralogs**. Paralogs of genes involved in developmental patterning can create new developmental pathways, as over time, two paralogs will undergo different mutations and so may follow different evolutionary trajectories. These new pathways contribute to the diversity of form that we see in nature. For example, duplications of *Hox* genes have contributed to the complexity of vertebrate body plans (**Figure 13.14**).

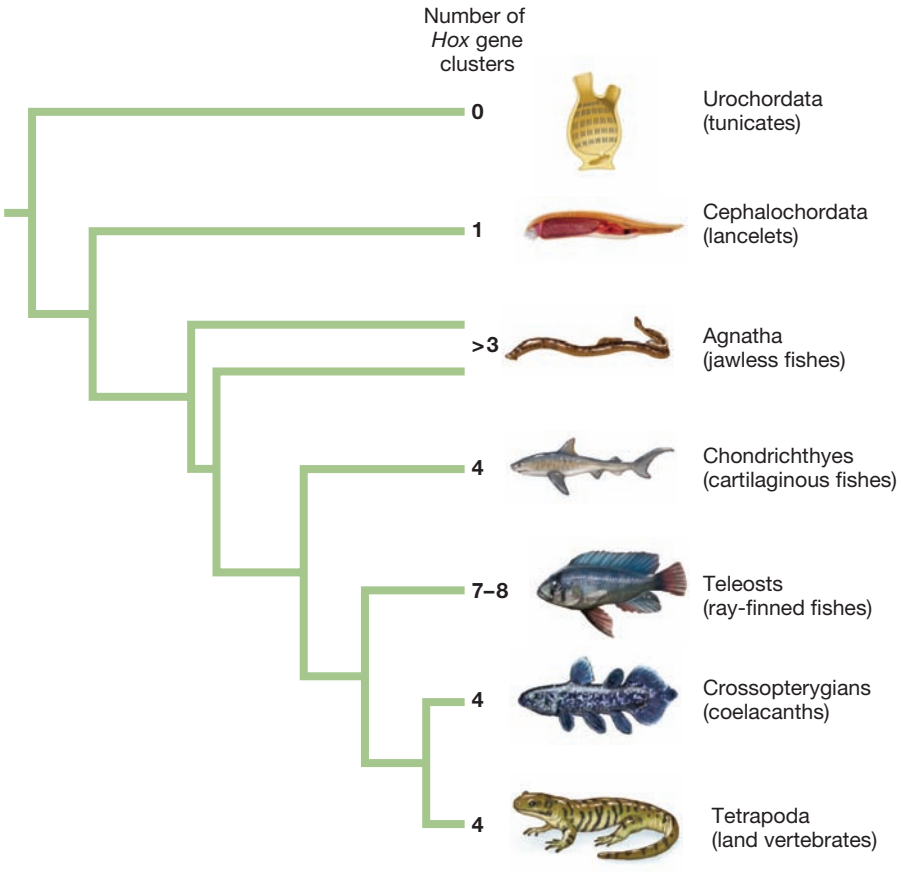

FIGURE 13.14 *Hox* gene clusters and chordate phylogeny. The number of *Hox* gene clusters is mapped onto a chordate phylogeny. The increase in some clades is due in part to *Hox* gene duplications. Because *Hox* genes are so fundamentally tied to the development of body plan, the increase in *Hox* gene clusters may be partly responsible for the diversity in body plans seen here. Adapted from Wagner et al. (2003).

Duplicate genes—both the original gene and the duplicate copy—may be maintained in a population for at least three different reasons:

1. Duplicate genes may influence gene expression levels by increasing production of histones, ribosomal RNA, and other products.
2. After duplication, paralogs may diverge because they divide the work initially undertaken by the gene before duplication. This is referred to as **subfunctionalization**.
3. Duplicated genes may diverge, with one of them taking on a new, but related, function. This process is called **neofunctionalization**.

KEYCONCEPT QUESTION

13.5 How does an evo–devo approach to gene duplication support and strengthen the argument that evolution is like a tinkerer, building new gadgets from whatever is available at the time?

Let's examine evolution, development, and neofunctionalization in a bit more detail. The effects of gene duplication on the developmental process have been implicated in the evolutionary diversification of plants. For example, the gene *OEP16* has been identified in all major lineages of land plants. OEP16 proteins are involved in activating enzyme reactions in the presence of light. Phylogenetic analysis suggests that a duplication event involving *OEP16* took place in the ancestral lineage leading to land plants. This duplication produced two genes, labeled *OEP16L* and *OEP16S*.

In flowering plants (angiosperms), *OEP16L* and *OEP16S* have diverged from one another by the process of neofunctionalization. *OEP16L* is expressed primarily in leaves, and its expression is very sensitive to temperature change. *OEP16S*, which in flowering plants appears to have gained between 20 and 27 amino acids after the gene duplication event, operates in a very different manner than *OEP16L. OEP16S* is expressed during the maturation period of seeds and pollen grains, and its primary function appears to be associated with tolerating desiccation (Drea et al. 2006). By providing additional protection to developing seeds, the neofunctionalization of the *OEP* gene may have been partly responsible for the explosion of plant diversity associated with the evolution of flowering land plants.

13.4 Evo–Devo and Neural Crest Cells

Neural crest cells are a group of embryonic stem cells that give rise to many different cell types in vertebrates and whose development is controlled by a set of developmental regulatory genes, such as *Hox, snail,* and *Dlx*. These cells are initially positioned near the neural tube during early development and then migrate to new locations during subsequent embryological stages. After neural crest cells migrate during ontogeny, they form or contribute to critical tissues and organs, including the blood vessels and heart, the brain and nervous system,

the thymus, adipose tissue, the craniofacial region of the skull, and the teeth (**Figure 13.15**) (Trainor et al. 2003).

To look at the dramatic effects that neural crest cells have on vertebrate craniofacial development, we begin with an observation made by Darwin in *On the Origin of Species.* Darwin noted that the beak proportions of birds are often constant throughout life and that these proportions "appeared at an extremely early period [during development] … from causes of which we are wholly ignorant." **Figure 13.16** provides an example: quail have narrow short beaks, ducks have long flat beaks, and these differences originate in the embryo. In both species, the shape of the beak makes a major contribution to fitness by influencing foraging ability, aggressive interactions, mate choice, and other aspects of performance. Richard Schneider and Jill Helms hypothesized that beak proportions were determined early in ontogeny by the development of neural crest cells and that this development differed between species with different beak proportions. Schneider and Helms ran an elegant tissue transplant experiment involving ducks and quail to test their hypothesis (Schneider and Helms 2003; Fish et al. 2014). When they transplanted embryonic neural crest cells from a duck into a quail embryo, the quail developed a duck-like beak. The reciprocal transplant resulted in the development of a duck with a quail-like beak (**Figure 13.17**).

Neural crest cells were once thought to have evolved during the early stages of vertebrate evolution (Santagati and Rijli 2003; Trainor et al. 2003). Indeed, it was

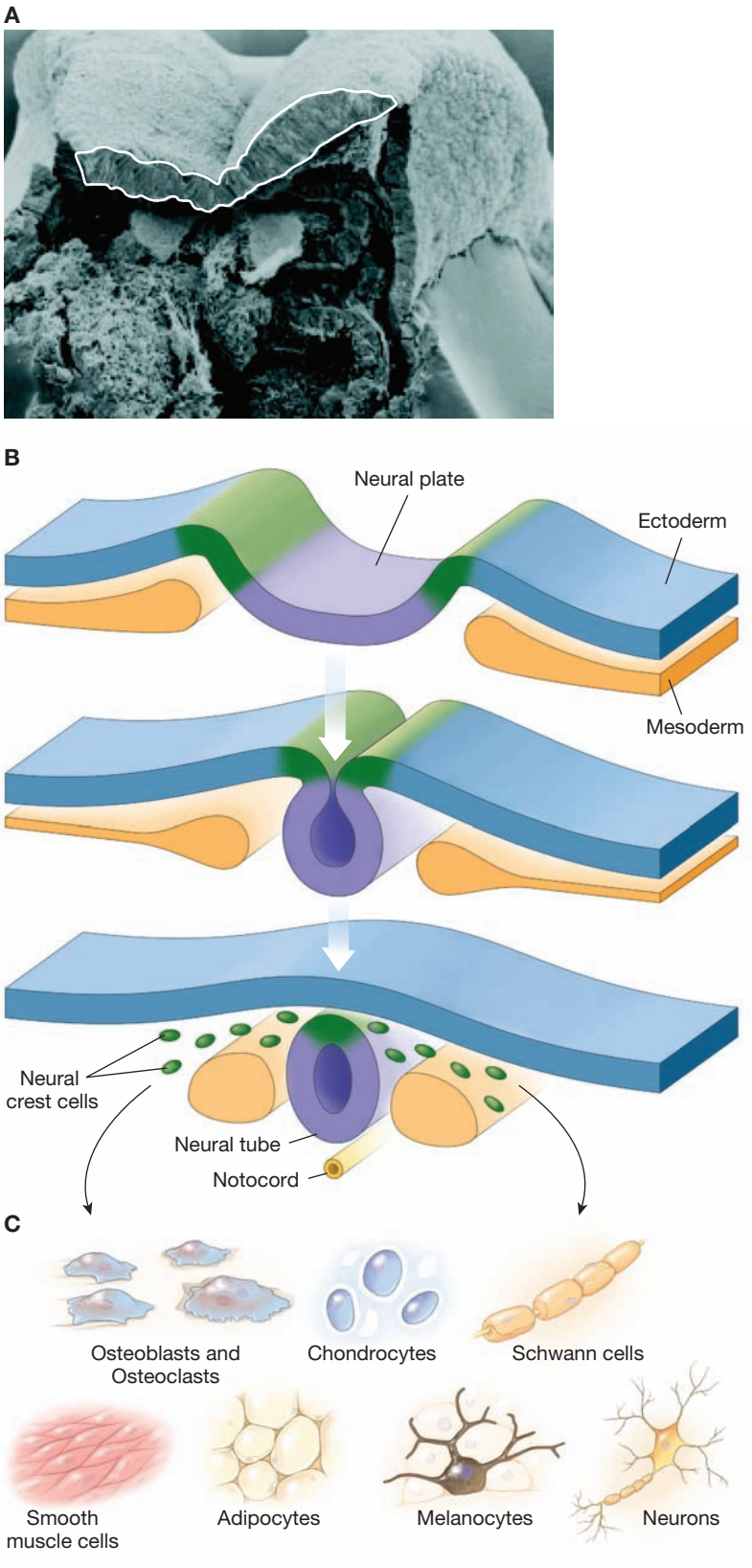

FIGURE 13.15 Neural crest cells. (A) Neural crest cells first appear at the neural plate (indicated by the white outline) and then migrate along the vertebrate central nervous system during early development. From Trainor et al. (2003) **(B)** After neural crest cells migrate, **(C)** they develop into a wide range of cell types. In panel B, the blue regions represent ectoderm, and the tan region represent mesoderm. From Gammill and Bronner-Fraser (2003).

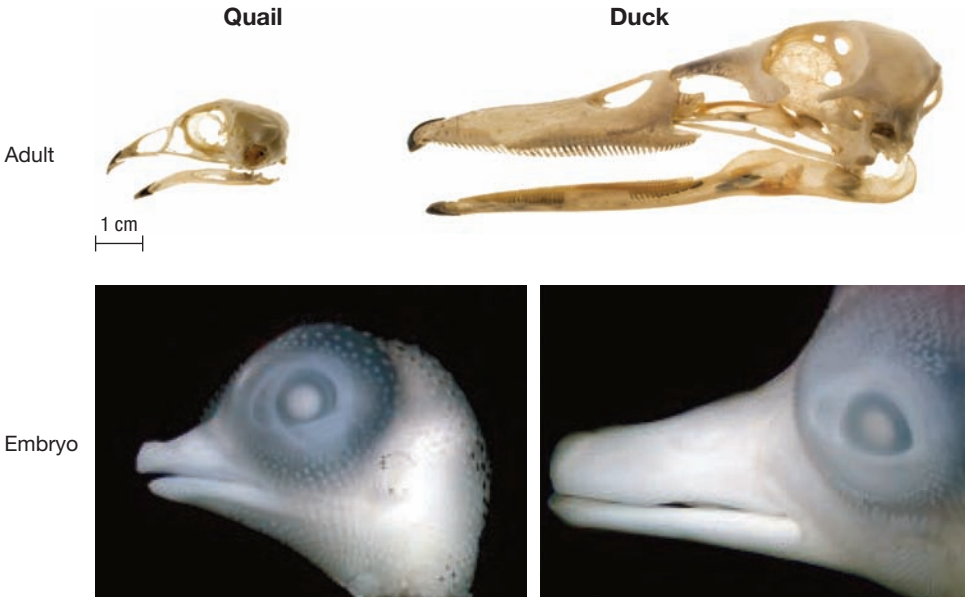

FIGURE 13.16 Beak shape in an adult and an embryonic quail and duck. The skulls of an adult quail and adult duck exhibit a dramatic difference in beak length. These differences are seen early in embryonic development. From Fish et al. (2014) and Schneider and Helms (2003).

long thought that because early vertebrate evolution coincided with the emergence of the neural crest, neural crest cells represented a fundamentally new vertebrate cell type. Work on *Amphioxus* (the closest living relative to vertebrates), however, indicates that this group, too, possesses cell types that are similar to neural crest cells in that they migrate over the neural tube during development and, like neural crest cells, are under the control of a series of homeotic genes (Holland et al. 1996). In addition, cells similar to these have been found in the ascidians, a close outgroup to the vertebrates (Mackie 1995; Powell et al. 1996; Shimeld and Holland 2000; Holland and Holland 2001; Wada and Satoh 2001). Based on this and other evidence, evolutionary biologists have hypothesized that neural crest cells evolved from ancestral cells similar to those found in ascidians and *Amphioxus*, perhaps through a series of gene duplications (Wada and Satoh 2001; Green and Bonner, 2013) (**Figure 13.18**).

As a second example of both the importance and complexity of neural crest cell development, let's briefly consider the role these cells play in marsupials.

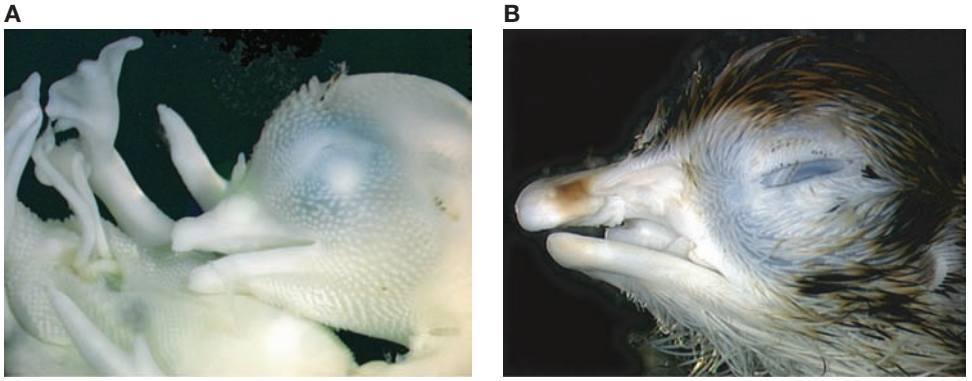

FIGURE 13.17 Neural crest cell transplants. Grafting quail neural crest cells into a duck results in a duck with a short quail-like beak **(A)**, whereas grafting duck neural crest cells into a quail results in a quail with a long and somewhat malformed duck-like beak (**B**).

Marsupials are born after a relatively short gestation period, and hence they require the ability to suck their mother's milk at an earlier developmental stage than eutherian mammals. The jaw structure in mammals is primarily under the control of neural crest cells. Analysis of marsupial embryos found that neural crest cells begin their migration much earlier in marsupials than in other mammal groups (Vaglia and Smith 2003; Smith 2006), allowing marsupials to develop the needed jaw structures for nursing much earlier in the developmental process. As with our other examples, here again we see how a deeper understanding of changes in developmental processes helps us explain the myriad forms of phenotypic diversity we see in the world around us.

Evo–devo is a broad field that covers many aspects of evolution and organismal development. In this chapter, we have seen that homeotic genes and regulatory enhancers control much of the development process. We have also learned that small changes in timing or spatial positioning during ontogeny can lead to large-scale phenotypic effects, and so these changes may be under strong selection. Some of these changes may be involved in the formation of new species, a subject to which we turn in the next chapter.

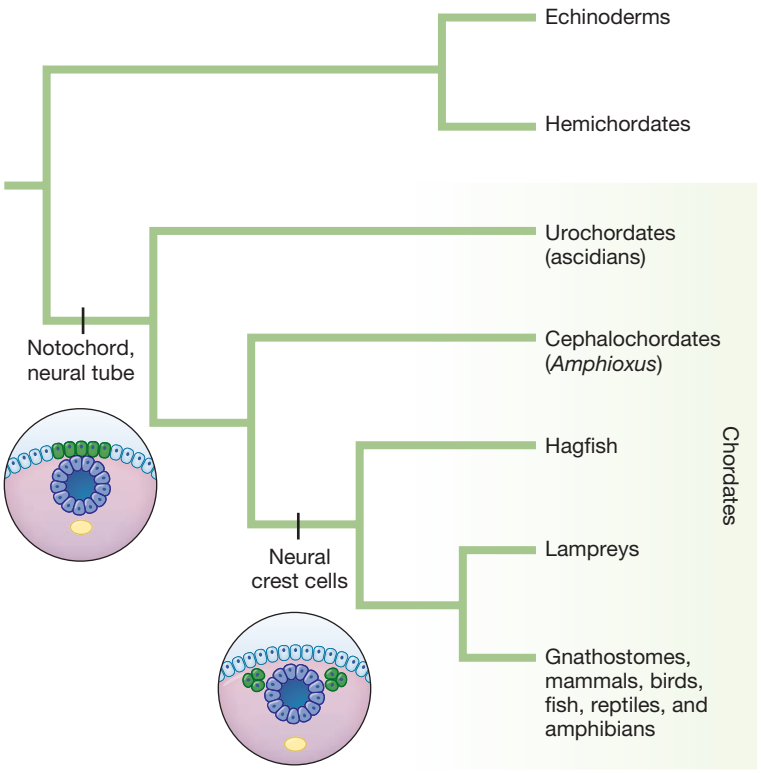

FIGURE 13.18 Phylogeny of neural crest cells. A phylogenetic tree mapping the emergence of neural crest cells and their possible progenitors (cells that migrate over the neural tube, shaded green). Adapted from Trainor et al. (2003).

SUMMARY

1. Early ideas on what today is called evo–devo can be found in the work of the ancient Greek philosophers and the concept of the "great chain of being." They noted a parallel between what would one day be called the *scala naturae*, which involves the relationships between species, and the *developmental stages* of organisms.
2. J. F. Meckel hypothesized that the developmental stages of an organism step through all the animal species that came before it on the *scala naturae.* Ernst Haeckel expanded on Meckel's ideas with his *biogenetic law*, which states "ontogeny is a precise and compressed recapitulation of phylogeny."
3. Von Baer's law posits that embryos in closely related species resemble each other, and not the adult form of some ancestral species. The most general characteristics that are shared among embryos from closely related species appear early in embryonic development, while specialized traits appear later in development.
4. Gavin de Beer coined the term *heterochrony* to describe changes in the rate of development, and he focused on whether the time at which a trait was first expressed in a given species was accelerated or decelerated relative to that of an ancestral species.
5. One of the key players associated with the dynamic rules that govern development are the *homeotic genes* that specify position within an embryo.
6. The position of *Hox* genes on a chromosome corresponds to the position on the anterior–posterior axis of the body part that the *Hox* gene regulates. This phenomenon is known as colinearity. Homologous *Hox* genes are often ordered on chromosomes in a similar way across vertebrates and invertebrates.
7. The expression patterns of regulatory genes (such as *Hox* genes) in their local cellular environment are responsible for the very different sorts of body plans that we see in vertebrates and invertebrates.
8. Homeotic genes display deep homologies. *Hox* genes have been uncovered in jellyfish, mollusks, earthworms, and octopuses. In each case, the genes are involved in constructing the anterior, central, and posterior body parts of these creatures.
9. Development is also guided by regulatory enhancers. A regulatory enhancer of a gene is a section of DNA that lies outside of the coding region but is involved in *regulating* the timing and level of that gene's expression.
10. A gene can have numerous regulatory enhancers associated with it. A gene with multiple regulatory enhancers can be expressed differently in different parts of the body and at different points in time. Regulatory enhancers increase morphological variation, and hence the amount of variation that natural selection has to act on.
11. Duplicated genes may evolve into *paralogs*. Paralogs of homeotic genes allow new developmental pathways to emerge, and these new pathways may help explain the diversity of forms that we see in nature.
12. A set of cells known as neural crest cells plays a critical role in vertebrate development. These cells arise from ectoderm early in the developmental process and migrate throughout the body, where they help form important organs including the brain, heart, and craniofacial structure. Differences in gene regulation within neural crest cells result in different morphology, as demonstrated by neural crest cell transplant experiments using ducks and quail.

KEY TERMS

colinearity (p. 473)
enhancers (p. 470)
evo–devo (evolutionary developmental biology) (p. 464)
gene family (p. 479)
heterochrony (p. 467)
homeobox (p. 472)
homeotic genes (p. 464)
Hox genes (p. 471)
neofunctionalization (p. 480)
neural crest cells (p. 480)
ontogeny (p. 465)
paedomorphosis (p. 467)
paralogs (p. 479)
recapitulation (p. 467)
segmentation genes (p. 464)
silencers (p. 470)
subfunctionalization (p. 480)

REVIEW QUESTIONS

1. What is the *scala naturae*?
2. What is a parallelism?
3. What does von Baer's law suggest about the evolution of novel traits?
4. What is the study of heterochrony?
5. What are homeotic genes? Name two sets of homeotic genes and what they do.
6. What is the homeobox?
7. What is colinearity?
8. What do studies that successfully transplant *Hox* genes across phylogenetically distant species tell us?
9. What are regulatory enhancers, and why are they important from an evolutionary perspective?
10. With respect to gene duplication, what is neofunctionalization?

KEY CONCEPT APPLICATION QUESTIONS

11. Why was the discovery of so-called rate genes so important for the study of evo–devo?
12. How does the work on marsupial jaw development and neural crest cells show the power of the evo–devo approach to understanding development?
13. Why are "model species"—extraordinarily well-studied species—such as fruit flies (*Drosophila*) especially useful for studies on genes involved in development?

SUGGESTED READINGS

Carroll, S., J. Grenier, and S. D. Weatherbee. 2005. *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design*. Blackwell, Malden, Mass. An accessible, book-length treatment of basic evo–devo concepts.

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Pearson, J. C., D. Lemons, and W. McGinnis. 2005. Modulating *Hox* gene functions during animal body patterning. *Nature Reviews Genetics* 6: 893–904. A technical paper on the importance of *Hox* genes in the study of evo–devo.

Raff, R., and T. C. Kaufman. 1983. *Embryos, Genes, and Evolution*. Macmillan, New York. Although somewhat dated now, this was one of the first modern books on the subject of evo–devo and is full of interesting material.

Rubinstein, M., and F. S. J. de Souza. 2013. Evolution of transcriptional enhancers and animal diversity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 368. DOI: 10.1098/rstb.2013.0017. A technical review of regulatory enhancers and their role in promoting diversity.

14

Species and Speciation

◀ Butterflies congregate on the mineral-rich river bank in Peru's Manú National Park.

During Nazi bombing raids in World War II, the citizens of London were often forced to take refuge in the tunnels of the Underground—the city's subway system. In addition to all of the other discomforts associated with spending long periods of time in an underground labyrinth, Londoners complained of the mosquitoes, *Culex pipiens*, that continually harassed them (Shute 1951). This was an especially irksome problem because above ground in England, mosquitoes preferred to bite birds rather than humans, while in the tunnels of the Underground, they showed a strong inclination to bite mammals, including humans. This underground population has the rather ominous scientific name of *Culex pipiens molestus*.

When biologists began examining the aboveground and underground forms of *Culex pipiens*, they found that mosquitoes from these populations look remarkably similar. But, in many ways, their life histories are dramatically different (**Figure 14.1** and **Table 14.1**). Not only do the mosquitoes in these populations prefer to bite different sorts of animals, but their breeding patterns differ as well. The aboveground populations breed only seasonally,

FIGURE 14.1 Speciation in mosquitoes in the London Underground. A large, deep shelter built alongside London's Underground subway system. This is an ideal breeding ground for the mosquito *Culex pipiens molestus*.

but in the moister, warmer underground setting, *Culex pipiens molestus* populations breed all year round.

The difference between these populations spurred evolutionary biologists to ask whether the populations might in fact be different species. But how could they test this? As we will see throughout the course of this chapter, especially when we discuss what is called the biological species concept, one way that evolutionary biologists diagnose whether two populations are different species is based on gene flow: the movement of genes between populations. When gene flow is absent, two populations are often diagnosed as being members of different species—or on their way to becoming such. Given the dramatic life history differences between aboveground and belowground London mosquito populations, Katharine Byrne and Richard Nichols had reason to suspect these were different species or, at least, that they were on the evolutionary path toward becoming separate species. Byrne and Nichols hypothesized that when they examined these populations, they would find little if any gene flow between them (Byrne and Nichols 1999).

Byrne and Nichols examined 20 populations, and they found *no* gene flow between the aboveground and belowground populations. This held true when comparing an aboveground population to a distant underground population (many kilometers away), but there was also no gene flow even when the comparison was between aboveground and underground populations that were very close to one another (just 100 meters apart). And, when Byrne and Nichols undertook laboratory breeding experiments, they found that *all* mating crosses between mosquitoes from underground populations produced viable and fertile offspring, while crosses between aboveground and underground individuals produced *no* offspring at all. The aboveground and belowground forms are genetically distinct, and they fail to produce viable offspring when crossed. Using gene flow as a diagnostic, the aboveground and belowground populations are different species or, at the very

TABLE 14.1

Differences in the Biology of *Culex pipiens* and *Culex pipiens molestus*

Trait	*C. pipiens molestus*	*C. pipiens*
Breeding site	Underground	Aboveground
Mating	In confined spaces	Open spaces
Host preference	Bites mammals	Bites birds
Egg production	No blood meal needed to lay eggs	Blood meal needed to lay eggs
Life cycle	Active all year	Dormant in winter

Adapted from Byrne and Nichols (1999).

least, on the path to becoming such. Although the exact origin of the underground populations is still a matter of debate (Fonseca et al. 2004), this work on *C. pipiens* demonstrates that evolutionary biologists can study speciation in real time, and they can develop and test hypotheses about speciation using tools that are readily available.

In this chapter, we will examine the following:

- What is a species?
- How does speciation occur?
- What creates reproductive isolation among populations?
- What do evolutionary biologists know about the genetics of speciation?

14.1 The Species Problem

Charles Darwin chose the title of his classic book, *On the Origin of Species*, with some care. It is sometimes easy to forget that Darwin developed his theory of evolution by natural selection largely in an effort to understand what is often referred to as the "species problem": How can we account for the vast array of different life-forms that have inhabited Earth for the past 4 billion years? This requires us to answer two separate questions: what is a species, and how do we identify species and delineate species boundaries in nature (Coyne and Orr 2004; de Queiroz 2007; Wilkins 2009)?

What Is a Species?

When evolutionary biologists refer to a group of organisms as a species, the fundamental underlying notion is that this group forms a lineage that has a distinct evolutionary fate from that of other lineages.

The **evolutionary species concept**, first proposed by George Gaylord Simpson and then modified by E. O. Wiley (Simpson 1961; Wiley 1978), characterizes a species as: "a lineage of . . . populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley 1978, p. 17). This definition puts evolution front and center. The key attributes that make a group of populations into a species are their shared past evolutionary history and their common future evolutionary fate—at least until this species itself bifurcates to form new descendant species. Notice that this definition is inherently phylogenetic: A species is a group of populations that have had a shared past and will have a shared future on a phylogenetic tree.

KEYCONCEPT QUESTION

14.1 Consider a number of different purebred dog breeds. Using the evolutionary species concept, how might assumptions you make about the degree of control humans will have on these breeds in the future affect whether you define these breeds as different species or at least on their way to becoming different species?

The evolutionary species concept defines what a species is and what role species play in evolutionary history, but it does not offer particularly useful practical advice on how we should go about identifying species and drawing species boundaries in the study of natural populations. To that end, evolutionary biologists have developed a number of diagnostic approaches to decide whether populations are or are not members of the same species. These approaches include the phenetic species concept, the biological species concept, the ecological species concept, and the phylogenetic species concept.

Identifying Species

If you sampled a large number of big cats, you would find many individuals that look like what we call lions and many that look like what we call tigers but few, if any, that look like something midway between the two. As a general empirical observation, organisms are clustered together in phenotypic space. The **phenetic species concept** takes advantage of this fact, drawing species boundaries around clusters of phenotypically similar individuals or populations (**Figure 14.2**) (Michener and Sokol 1957; Cain and Harrison 1960; Sokol and Sneath 1963). A similar process can be applied at higher levels of taxonomic organization to delineate genera, families, orders, and other taxonomic levels.

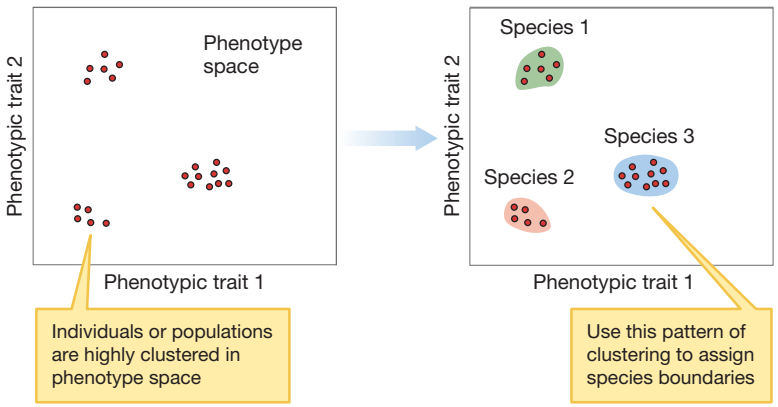


FIGURE 14.2 The phenetic species concept. The phenetic species concept uses the clustering of individuals or populations in phenotype space to draw species boundaries between clusters. The vertical and horizontal axes may each represent a single phenotypic trait or multiple phenotypic traits.

Historically, the phenetic species concept has been used by *numerical taxonomists*—biologists who use statistical analyses of multiple traits to classify organisms (Gilmour 1937; Sturtevant 1939; Rogers and Tanimoto 1960; Sokol 1985). At the most basic level, numerical taxonomists examine large data sets composed of measurements of many traits in many individuals, over many populations, and search for patterns in these data. In particular, they use computational algorithms to search for statistically meaningful groupings or clusters, and then they use such clusters to delineate species boundaries. **Figure 14.3** shows an example in which this approach was used to classify shrubs from nine populations into three species.

The phenetic species concept is still commonly used today, especially in the classification of plants and microorganisms (Sneath 1995). Paleontologists, who primarily work with fossil remains, also use this method when analyzing their data.

One of the challenges associated with the phenetic species concept is how to weigh the relative importance of the characters or traits used to delineate species boundaries. Should all traits be viewed as equally important in classifying organisms or should some traits be weighed more heavily because they are particularly important? Early numerical taxonomists tended to assign equal weights to all characters they measured, but this approach was quickly abandoned by some in favor of weighing certain characters more heavily than others (Cain and Harrison 1960).

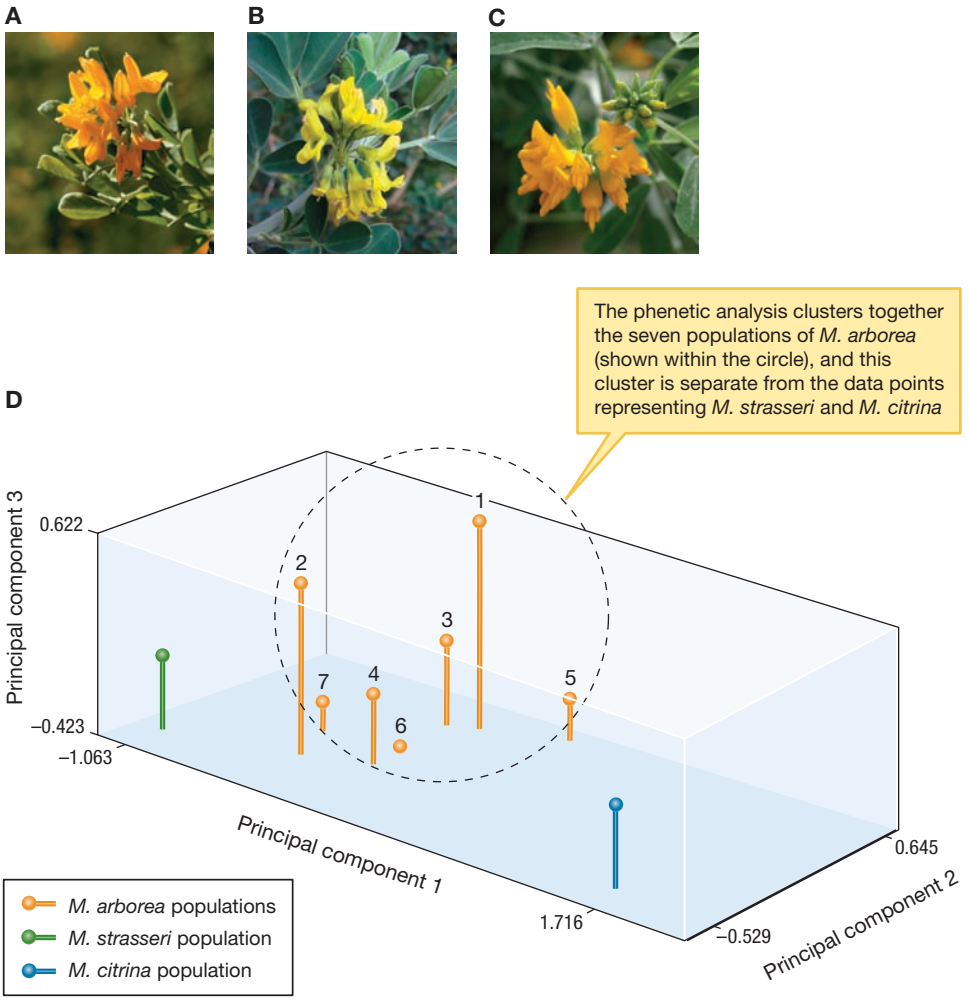


FIGURE 14.3 Applying the phenetic species concept. Researchers measured 12 traits on individual plants from nine populations of shrubs from the genus *Medicago:* seven populations of what were thought to be **(A)** *M. arborea* (a widespread species) and a single population from each of the less widely distributed **(B)** *M. citrina* and **(C)** *M. strasseri*. **(D)** They then distilled the 12 traits into what are called principal components—a statistical measure that groups a large number of different traits into a small number of variables. The *x*, *y*, and *z* axes are the principal components. The analysis found support for delineating these populations into three species: *M. arborea*, *M. citrina*, and *M. strasseri.* Panel D adapted from González-Andrés et al. 1999.

The process of convergent evolution poses a deeper problem for the phenetic species concept. By definition, the phenetic species concept uses phenotypic traits to create clusters (species), but it makes no assumptions about what *causes* phenotypic clustering. As we saw in Chapter 3, convergent evolution also can produce similar phenotypes, but not because of common ancestry.

KEYCONCEPT QUESTION

14.2 More and more work on the phenetic species concept uses molecular genetic data rather than anatomical or morphological data. How does this help to minimize the convergent evolution problem?

The **biological species concept** was first introduced by Ernst Mayr. Under the biological species concept, a species is composed of "groups of actually or potentially interbreeding populations which are reproductively isolated from other such groups" (Mayr 1942, 1982, 2002; Beurton 2002). Thus, according to the biological species concept, it is the pattern of gene flow that determines species boundaries (**Figure 14.4**). In diagnosing what constitutes a species, the biological

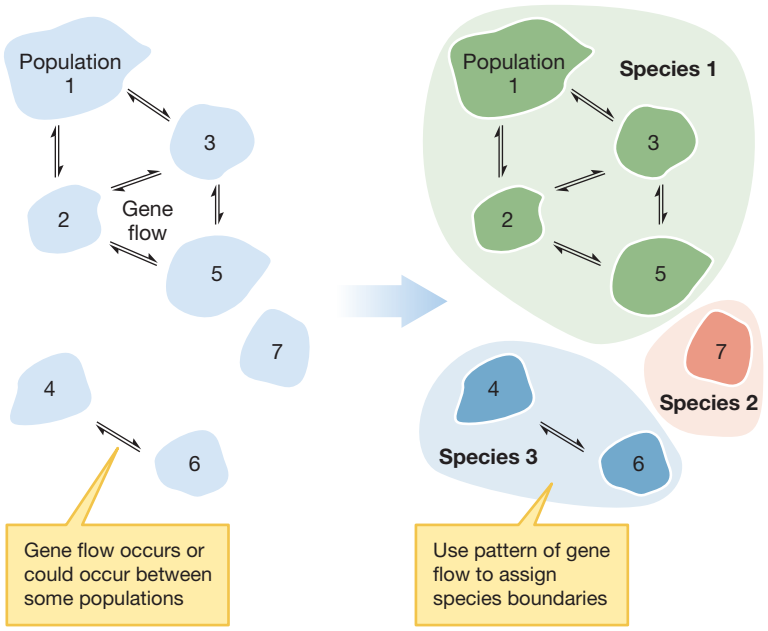


FIGURE 14.4 The biological species concept. The biological species concept uses the presence or absence of gene flow among populations to delineate species boundaries.

species concept looks directly to the evolutionary mechanism—gene flow—responsible for the "shared evolutionary fate" that is fundamental to the concept of species. As a result, the biological species concept is not based on attributes of the individuals, but rather it delineates species by properties possessed by *populations*.

If individuals in one population are capable of mating with individuals in another population, then individuals in both populations are part of the same species, and they are said to share the same gene pool. If populations are reproductively isolated from one another—recall our discussion of the mosquito populations in London—then the individuals in such populations are not considered to be part of the same species.

A practical difficulty with the biological species concept is that it is very hard to apply this concept to extinct species that are known only from paleontological evidence. Although reproductive isolation can sometimes be inferred from the distribution and form of fossils, this is not often the case.

Another problem for the biological species concept is the occasional hybridization events between individuals in populations that are, for all practical purposes, reproductively isolated. If individuals in population 1 consistently mate with those in population 2, individuals in these populations are classified as part of the same species. But even if matings between individuals in different populations are rare or nonexistent, the two populations might still be part of a common species because the biological species concept allows the populations to be *potentially interbreeding*. Consider another problem with the biological species concept: If the offspring produced by cross-population matings are nonviable or infertile, then we clearly have two species. But what if the offspring merely have reduced viability or reduced fertility? How rare do cross-population matings have to be and how poorly must the hybrid offspring fare before we can say that the two populations are two separate species? The answers to questions such as these are murky—indeed, the questions themselves are sometimes murky—and no clear consensus exists on this issue.

Perhaps the most severe limitation to the biological species definition is that it is restricted to sexual species. With its emphasis on the reproductive isolation of populations, the biological species concept makes little sense as a species concept for asexual organisms. As Ernst Mayr notes, "[i]n an asexually reproducing species every individual and every clone is reproductively isolated. It would be absurd to call each of them a separate species" (Mayr 1982, p. 283).

Another species concept is known as the **ecological species concept** (Van Valen 1976; Ridley 1996; Rice et al. 2011; Shafer and Wolf 2013). Here, a species is defined as a cluster of individuals that occupy the same niche; If two lineages have evolved adaptations allowing them to occupy two different niches, they are designated as different species (**Figure 14.5**). As an example, consider the aboveground and belowground populations of mosquitoes we discussed at the start of this chapter.

The two populations live in different places, specialize on different hosts, and have different reproductive timing—they occupy two different niches. As such, they constitute two different species under the ecological species concept.

Like the biological species concept, the ecological species concept relates species boundaries to the underlying processes involved in creating these boundaries. While the biological species concept focuses on the evolutionary processes of gene flow between populations, the ecological species concept focuses on ecological processes that allow two populations to coexist. According to the ecological species concept, members of the same species compete with one another more directly than individuals of different species compete with one another. Why isn't one population replaced entirely by members from the other? If two populations have evolved to thrive in different niches, neither will be able to invade the other's niche and replace it. Then and only then we will call them separate species. Unlike the biological species concept, this approach applies readily to asexual species as well as sexual ones (Cohan 2002).

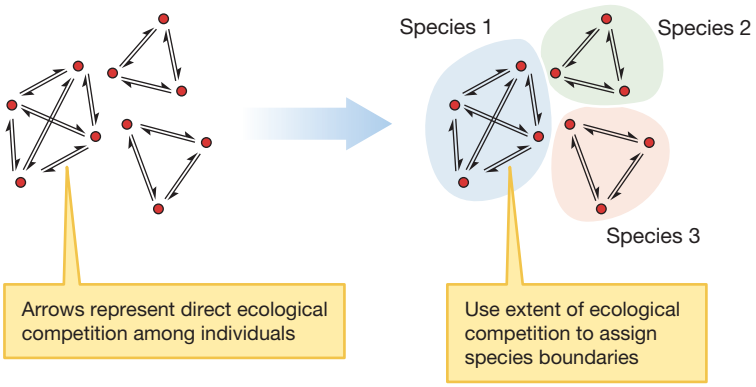


FIGURE 14.5 The ecological species concept. The ecological species concept uses the scope of ecological competition to delineate species boundaries. Individuals or populations that compete closely and whose descendants can potentially replace one another are said to be members of the same species.

One problem in applying the ecological species concept is that *niche* is a broad term that is often defined as a multidimensional space that incorporates all of the biotic factors (for example, predation, competition, parasitism, life history) and abiotic factors (for example, temperature, salinity, humidity, acidity) in a particular habitat. As such, unlike the fairly clear-cut case of the aboveground and belowground niches of the mosquitoes in the London subway system, identifying a niche and the subsequent adaptations to it can often be very difficult.

The **phylogenetic species concept** is the final species concept we will consider here. Like the phenetic species concept, this approach looks to character differences in order to distinguish among species, but it does so in a different way. The basic problem in distinguishing species remains the same: How do we determine whether two groups are acting as evolutionary species that are able to maintain distinct identities so that they have their own evolutionary histories? If two groups have been separated long enough to have diverged and produced distinguishing characters, they must have already experienced unique evolutionary histories.

But what characters are the right characters to use in making such distinctions? The phylogenetic species concept proposes that we look to phylogeny to answer this question. According to this approach, we draw species boundaries using shared derived characters that are unique to one monophyletic group and absent from all other populations in the phylogeny. These characters can then be used to distinguish among species. In particular, we define a phylogenetic species as *the smallest monophyletic group distinguished by a shared derived character*. **Figure 14.6** illustrates the basic way in which shared derived characters can be used to distinguish among species.

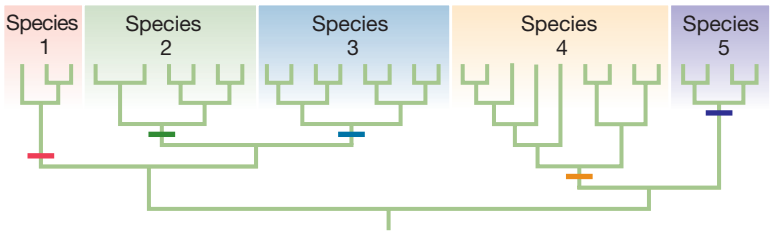


FIGURE 14.6 The phylogenetic species concept. The phylogenetic species concept uses shared derived traits to draw species boundaries between monophyletic groups. Branch tips represent populations and each colored tick mark represents a trait that is a shared derived character—this allows us to diagnose five different species labelled 1–5 here.

By looking at shared derived characters that distinguish monophyletic groups, the phylogenetic species concept suggests appropriate

characters for classifying species. Characters that are polymorphic within a population will not form monophyletic clades, and therefore they should not be used to define species boundaries under the phylogenetic species concept (**Figure 14.7**). In contrast, characters that are unique to a population or set of populations and that are also ubiquitous within those populations are ideal for drawing species boundaries; these characters will define monophyletic groups, and thus are used by the phylogenetic species concept in assigning species boundaries.

By requiring that a species be the *smallest* distinguishable monophyletic clade, the phylogenetic species concept also determines an appropriate taxonomic level at which to draw species boundaries. The trait "has fur and mammary glands" is a shared derived trait of the monophyletic clade of mammals, but we certainly would not want to say that all mammals are members of the same species. Instead, we can look for shared derived traits that distinguish smaller monophyletic groups; for example, spoken language and a dearth of body hair distinguish the monophyletic clade of humans from other primates.

Whereas the biological species concept requires information about gene flow to diagnose species, the phylogenetic species concept has no such requirement. In most cases, we would expect that a breakdown of gene flow would have occurred in populations that diverged enough that we can identify shared derived characters, but in some instances this may not yet have occurred (Cracraft 1989).

One of the major critiques of the phylogenetic species concept is that the *shared derived* traits it uses to distinguish among species can be of minimal significance with respect to natural selection. As a result, the phylogenetic species concept often divides up organisms into more fine-grained species categories than may seem appropriate, resulting in a far greater number of species than would be delineated by other species concepts. Moreover, the phylogenetic species concept does little to ensure that species considered separate at present will have separate evolutionary fates in the future. Because there is no requirement of restricted gene flow, members of two distinct phylogenetic species may be able to interbreed readily, which would enable the two species to fuse back into one species at some point in the future. Such events run strongly counter to our intuitions about what a species is under the evolutionary species concept.

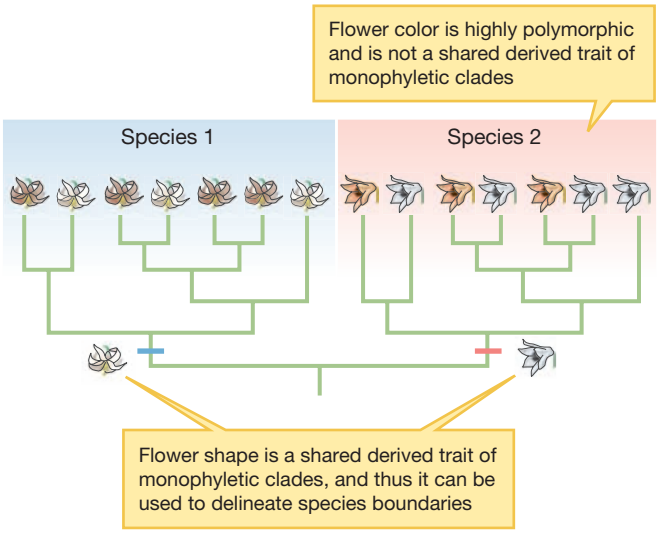


FIGURE 14.7 Polymorphic characters are not used by the phylogenetic species concept. Characters that are polymorphic within populations are not used by the phylogenetic species concept because they are not shared derived characters of monophyletic clades.

Clearly, no one species concept will work for all organisms. But it is important to recognize that once we adopt the evolutionary species concept to define what a species fundamentally is, we can then use the phenetic, biological, ecological, and phylogenetic species concepts to delineate species in nature. Each takes a somewhat different diagnostic approach: The phenetic species concept looks for clusters of phenotypic characters, the biological species concept looks at the presence or absence of gene flow, the ecological species concept focuses on the niche, and the phylogenetic species concept relies on shared derived traits of monophyletic groups. But most of the time, all four species concepts will readily agree on species boundaries. Populations

that belong to different species typically show large phenotypic differences, absence of gene flow, adaptations to different niches, *and* shared derived traits. These species concepts will give different answers only in relatively special cases; for example, when populations have had time to move into different niches and diverge in characters but have not yet evolved mechanisms that prevent gene flow. In these cases, phenetic, ecological, and phylogenetic species concepts will tend to classify the populations as separate species, while the biological species concept will classify them as a single species. But, as we will see shortly, such cases should be transient; there are numerous reasons to expect that barriers to gene flow between such populations will evolve relatively quickly.

KEYCONCEPT QUESTION

14.3 Imagine that researchers have been studying two populations of a hypothetical creature, *Darwinius huxlianus*. These two populations are geographically separated by 3000 miles, and individuals look and act quite differently across these two populations and appear to be well adapted to their respective habitats. Geological and molecular genetic evidence suggests that these populations have been separated for at least 20 million years, with no gene flow during that time. Yet, when individuals from each population are brought into the lab, they readily mate with members of the other population. Make the case that individuals in these two populations are members of the same species, and then make the case that they are not.

14.2 Modes of Speciation

All around us we see an astonishing array of different life-forms. How could such a diversity of different species come to be? This question of the origin of species and process of **speciation** occupied and even tormented Darwin for many years. In this section we will explore the models of speciation that evolutionary biologists have since developed. What predictions do these models make, and how have they been tested (Otte and Endler 1989; Coyne and Orr 2004)?

We answer these questions by examining three models of speciation—allopatric, parapatric, and sympatric speciation. These three models are distinguished from one another by the relative geographic positions of populations undergoing speciation. In **allopatric speciation**, the process of speciation takes place in populations that are geographically isolated from one another. In **parapatric speciation**, incipient species—diverging populations on the path to speciation—have distributions that abut one another. In **sympatric speciation**, populations diverge into new species while in the same location (**Figure 14.8**).

Allopatric Speciation

The central premise underlying allopatric speciation is that **reproductive isolating mechanisms** evolve between populations while they are geographically isolated. This geographic isolation may be the result of a physical barrier such

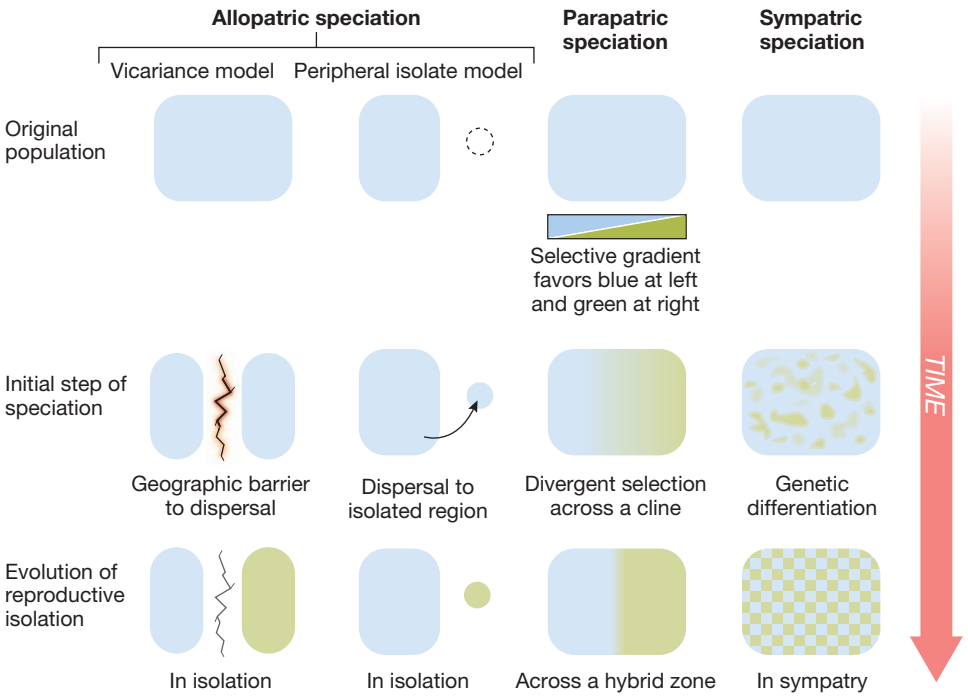


FIGURE 14.8 Different types of speciation. Two forms of allopatric speciation are shown in the first and second columns, parapatric speciation is shown in the third column, and sympatric speciation is represented in the fourth column. Blue and green represent incipient—and eventually distinctive—species.

as a mountain range, a river, an ocean, a desert, or some other barrier. In a moment, we will subdivide allopatric speciation into two related models, but for now let us examine one feature that *all* allopatric models of speciation have in common.

In all allopatric speciation models, genetic drift and natural selection cause populations to diverge from one another. In Chapter 8, we saw how genetic drift leads to divergence between two populations as different alleles are fixed by chance in each one. In addition, no two geographically isolated populations will experience exactly the same selective conditions, and thus the populations may diverge by natural selection. In the long run, drift and selection may lead to multiple forms of reproductive isolation between these populations. This is because gene flow between geographically isolated populations may be permanently eradicated when the members of one population lose the ability to breed successfully with members of the other population due to differences in geographic range, genetics, behavior, or reproductive physiology. In Section 14.3, we will look at some of the mechanisms by which reproductive isolation occurs. For now, the key point is that once gene flow becomes impossible, the populations no longer share a common evolutionary fate, and thus this process can result in the formation of new species.

Allopatric speciation is often subdivided into the **vicariance model** and the **peripheral isolate model**. In the vicariance model of allopatric speciation, an initially large population is subdivided into new populations that are themselves still relatively large. In the peripheral isolate model, the populations that are geographically isolated from one another differ in size, with one large population and one or several smaller populations. A classic example of peripheral isolate allopatry would be a mainland and surrounding islands, when islands are populated

by individuals who have dispersed from the mainland across some barrier like a body of water. One of the most important differences between the vicariance and peripheral isolate models pertains to the role of genetic drift in driving divergence between the populations. In the vicariance model, the descendant populations are each relatively large in size, making it unlikely that drift dramatically affects divergence. By contrast, in the peripheral isolate model, a peripheral population may be founded by a relatively small number of individuals, resulting in strong founder effects. Moreover, the net population size in the peripheral population may be much smaller than that of the progenitor population, resulting in accelerated genetic drift.

Allopatry via the Isthmus of Panama

The vicariance model of allopatric speciation has been studied in a genus of shrimp called *Alpheus*. Approximately 3 million years ago, the Isthmus of Panama formed and isolated populations of aquatic organisms in the Caribbean Sea from those in the Eastern Pacific (**Figure 14.9**). In a series of studies, Nancy Knowlton and her colleagues studied pairs of sister species of *Alpheus* snapping shrimp (recall from Chapter 4 that sister species share an immediate common ancestor on a phylogenetic tree) (Knowlton 1993; Knowlton et al. 1993; Knowlton and Weigt 1998; Hurt et al. 2009). In each of these sister species, members of one species lived on the Caribbean side of the isthmus, while members of the other of the species lived on the Pacific side, and so we refer to these as *trans-isthmus sister species*.

Knowlton's team used two different molecular genetic estimates of the divergence time for sister species, and they found that sister species varied widely in their divergence times—ranging from 18 million years ago (before the Isthmus of Panama began forming) through 9 million years ago (when terrestrial mammals first began crossing from North America to South America) to 3 million years ago (when the isthmus was complete) (**Figure 14.10**).

If reproductive isolation is linked to how long sister species have been geographically isolated, then we would expect to see a greater degree of reproductive

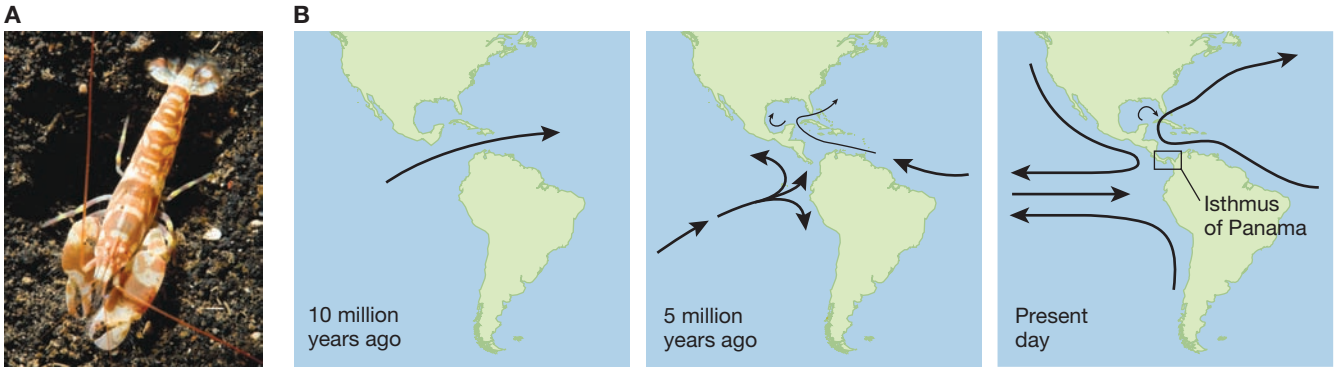


FIGURE 14.9 Allopatric speciation in shrimp. **(A)** A shrimp from the genus *Alpheus*. **(B)** The Isthmus of Panama: 10 million years ago, 5 million years ago, and in the present. Arrows indicate ocean currents. The Isthmus of Panama separated sister species of shrimp on the Caribbean and Pacific sides of the Isthmus of Panama, leading to allopatric speciation. Panel B adapted from Haug et al. (2004).

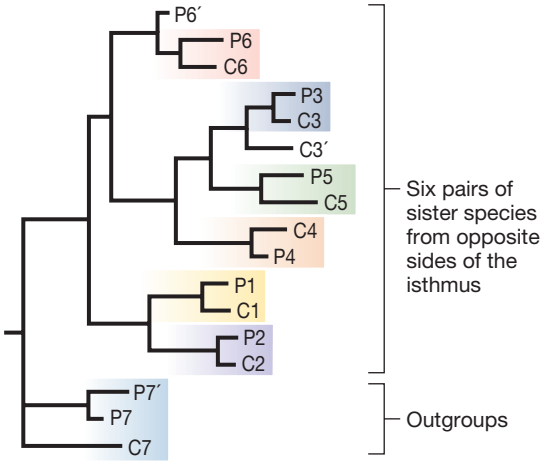


FIGURE 14.10 Sister species of shrimp. A phylogenetic tree of the genus *Alpheus* based on mitochondrial DNA (mtDNA) data. Species P1–P7 are from the Pacific side of the isthmus; Species C1–C7 are from the Caribbean side of the isthmus. Adapted from Knowlton et al. (1993).

isolation in sister species that had been separated for 18 million years than in those that had been separated for 3 million years. To test this, Knowlton's team used a series of aggressive behaviors as an indicator of reproductive isolation: the more aggression displayed between males and females, the more reproductive isolation was assumed. Similarly, the more males and females from sister pairs "tolerated" one another, the less reproductive isolation between populations was assumed. They found that tolerance decreased and aggression increased in trans-isthmus sister pairs as a function of how long they had been geographically isolated from one another by the Isthmus of Panama. Equally or perhaps even more important, they found that, although sister species were phylogenetically closely related, only 1% of matings between trans-isthmus pairs produced viable clutches of offspring, while 60% of matings between control pairs—with both partners of the same species—produced viable clutches of offspring.

These findings strongly suggest that speciation in snapping shrimp has largely occurred among populations in allopatry—the longer that sister species had been geographically isolated from one another, the greater the extent of behavioral and genetic divergence.

The Peripheral Isolate Model in Black Spruce and Red Spruce Trees

In Chapter 8, we examined Isabelle Gamache's work on genetic variation and founder effects *within* populations of black spruce (Gamache et al. 2003). In the original study that we discussed, Gamache and her colleagues examined mitochondrial DNA (mtDNA) migration via wind-dispersed seeds, and they found that mtDNA distribution was more restricted and localized than nuclear DNA migration via pollen (and seed) dispersion. Here, we will look at the same research group's follow-up study, which examined allopatric speciation and genetic variation in black spruce (*Picea mariana*) and red spruce (*Picea rubens*) (Jaramillo-Correa and Bousquet 2003).

The peripheral isolate model of allopatric speciation can lead to so-called progenitor–derivative species pairs (Crawford 2010). The derivative species forms when a small subset of the progenitor species becomes geographically isolated and begins to diverge from the original population (Gottlieb 1973; Gottlieb et al. 1985; Witter 1990). In such pairs, the progenitor species typically does not change very much through time, but the derivative species changes substantially.

A number of lines of evidence led evolutionary biologists to hypothesize that black spruce and red spruce form a progenitor–derivative pair (**Figure 14.11**). Red spruce, the derivative species, seems to have arisen from a southern population of black spruce, the progenitor species. This population became geographically isolated from other black spruce populations at some point during the Pleistocene glaciations. There are a number of lines of evidence for the progenitor-derivative relationship between black and red spruce. First, black spruce has a much broader geographic distribution than the derivative species, red spruce. Second, both nuclear and mitochondrial DNA studies show that red spruce has low genetic diversity compared to black spruce (Hawley and Dehayes 1994; Jaramillo-Correa and

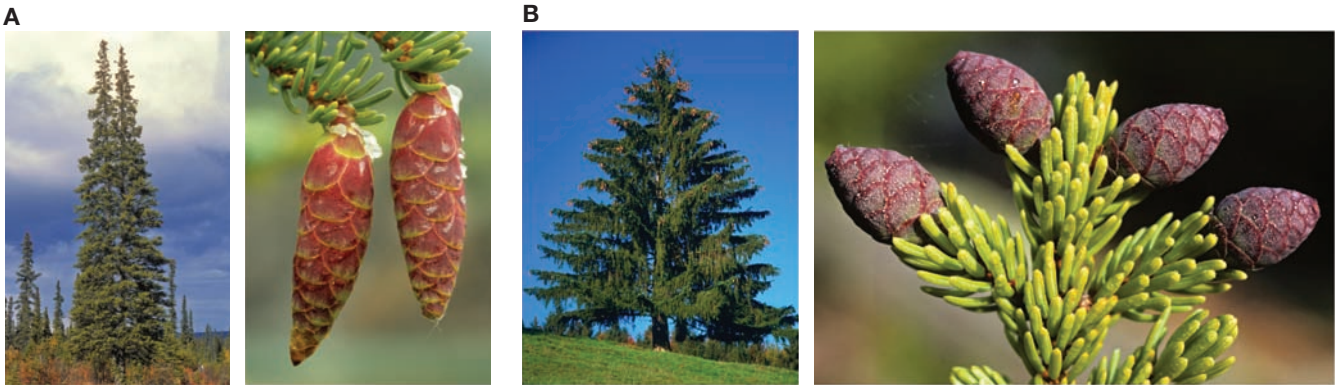


FIGURE 14.11 Progenitor–derivative species. The closely related black spruce **(A)** and red spruce **(B)** are thought to be a progenitor–derivative pair, with black spruce the progenitor species and red spruce the derivative species.

Bousquet 2003). Third and most critically, researchers found no unique mitochondrial haplotypes in red spruce—all mitochondrial genetic variation in red spruce is a subset of that found in black spruce. This is what we would expect if red spruce evolved from a geographically isolated population of black spruce (Perron et al. 1995, 2000; Jaramillo-Correa et al. 2003) (**Figure 14.12**).

KEYCONCEPT QUESTION

14.4 Some evolutionary biologists have suggested that the peripheral isolate model should lead to speciation at a faster rate than that of the vicariance model. Why might this be the case?

Parapatric Speciation

Parapatric speciation occurs when two adjacent populations diverge into separate species in the absence of a geographic barrier to dispersal (Mayr 1970). The core concept underlying the parapatric speciation model is that some sort of **cline**—a spatial gradient in the frequency of phenotypes or genotypes—is formed when adjacent populations experience different selective conditions. A **hybrid zone** where there is gene flow between diverging populations exists somewhere along this cline (Harrison and Rand 1989; Hewitt 1989).

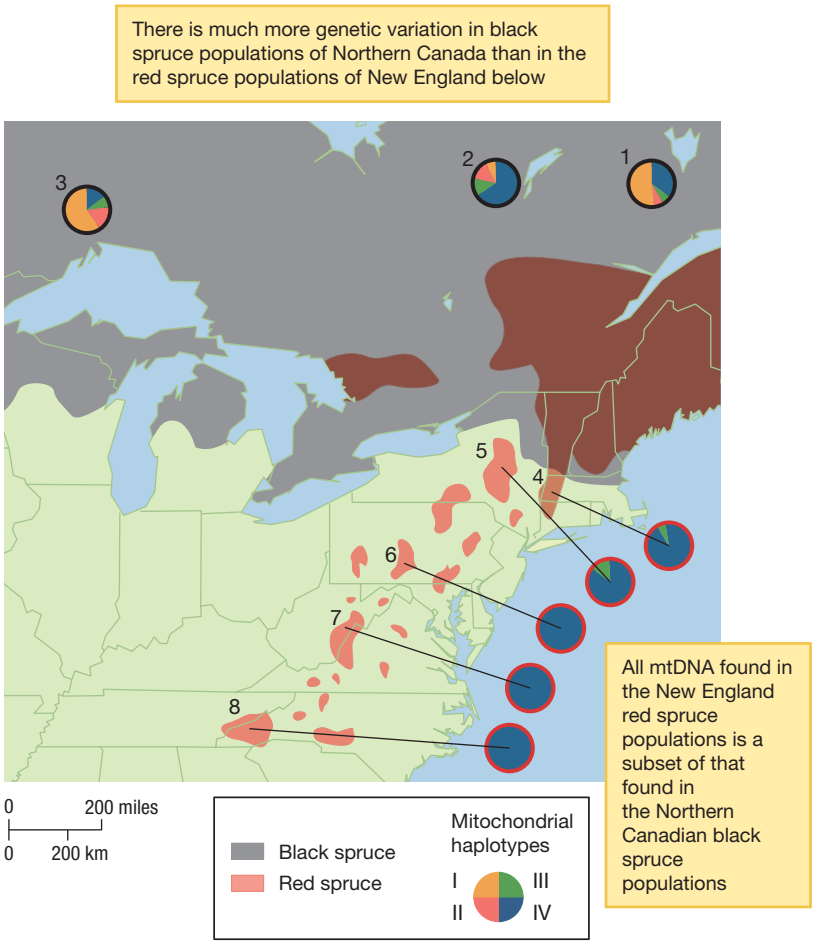


FIGURE 14.12 Progenitor–derivative species in spruce. Black spruce populations (1–3, with black rim on circles) show much more genetic variation than red spruce populations (4–8, with red rim on circles). All mtDNA variation in red spruce is a subset of the mtDNA variation in black spruce. Gray indicates the range of black spruce and red the range of red spruce; brown represents regions where both species are present. Adapted from Jaramillo-Correa and Bousquet (2003).

The Hybrid Zone in Parapatric Speciation

In most parapatric speciation models, it is assumed that the hybrid zone between populations will eventually disappear, completing the speciation process (Bayzkin 1969; Moore 1977; Barton and Hewitt 1985). This can occur for many different reasons, the most common of which is that hybrid offspring may be at a selective disadvantage compared to offspring that come from within-population matings. This is because hybrid offspring often possess a suite of traits that is not particularly well suited to life in any section of the cline, while offspring from within-population matings are typically well adapted to their respective environments. This generates selection for genetic, physiological, or behavioral reproductive isolating mechanisms that reduce hybridization between the two populations and which, once in place, may lead to the completion of the speciation process.

Hybrid zones can vary dramatically in size, depending upon the geographic scale at which speciation is occurring. In some cases, like that of parapatric speciation in the carrion crow *Corvus corone* and the hooded crow *Corvus cornix* across Europe and Asia, the hybrid zone runs about 2100 kilometers north to south and ranges from 50 to 150 kilometers east to west. (Meise 1928). In other cases, such as parapatric speciation in grass species living on and adjacent to contaminated metal mines, the hybrid zone may be only tens of meters wide.

For the past 50 years, Janis Antonovics and his colleagues have been studying what may be the first stages of a parapatric speciation event in the sweet vernal grass (*Anthoxanthum odoratum*) living on and near a contaminated lead and zinc mine at the Trelogan Mine complex in Wales (Antonovics et al. 1971; Antonovics 2006) (**Figure 14.13**). The soil at this mine has very high levels of zinc and lead, heavy metals often toxic to plants. Antonovics and his team have studied sweet vernal grass populations in an area of the mine that has been in operation since the mid-1850s. They compared these sweet vernal grass populations to other populations in a pasture that is adjacent to the mine but has soil that is relatively uncontaminated by lead and zinc. Tolerance to heavy metals is much higher in the mine populations than in the pasture populations. Moreover, common garden experiments, in which sweet vernal grass from both areas is grown in both high and low heavy-metal-concentration treatments, indicate that this difference in tolerance is genetic in origin.

While gene flow between the mine and pasture populations still exists, a number of lines of evidence suggest that over the past 160 years of mining, natural selection has acted against hybrid matings across these populations. In addition to differences in tolerance to heavy metals, grasses in the mine and pasture populations also show genetic differences in key reproductive traits. Sweet vernal grass can either self-fertilize or outcross with other individuals. Grass on the mine self-fertilizes more often than pasture grass, and grass on the mine flowers much earlier than grass in the pasture: Both of these traits have the effect of breaking down gene flow between mine and pasture populations. Equally important for the parapatric model is the finding that, on average, differences in most reproductive traits were greatest in the area closest to the mine/pasture, contaminated/uncontaminated boundary—the part of the hybrid zone where gene flow is most common—and decreased with distance from this boundary.

Not all parapatric models assume that hybrid individuals are at a disadvantage. To see this, let's examine the work of Han Wang and his team on hybrid zones and

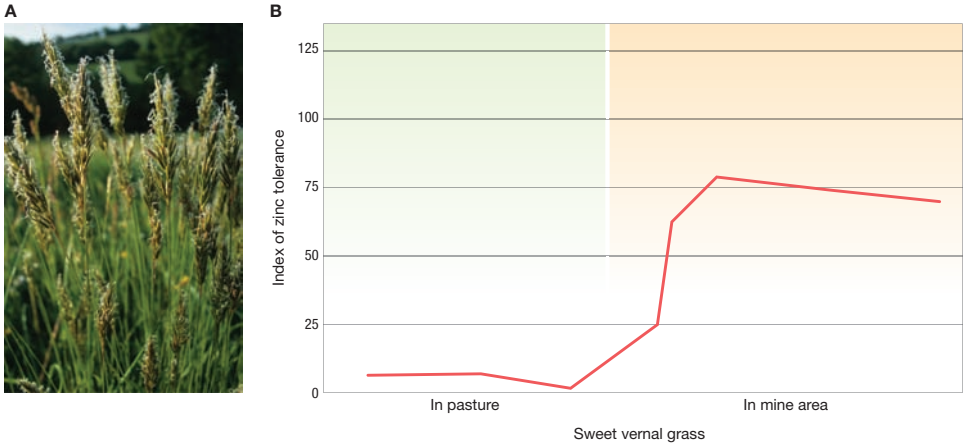


FIGURE 14.13 Parapatric speciation and heavy metal resistance. Populations of sweet vernal grass, *Anthoxanthum odoratum* **(A)**, can be found in the soil on contaminated lead and zinc mines at the Trelogan Mine, while other populations live in a pasture that is adjacent to the mine but where the soil is relatively uncontaminated. Tolerance to heavy metals is much higher in the mine populations than in the pasture populations **(B)**.

parapatric speciation in big sagebrush (*Artemisia tridentata*) (McArthur et al. 1998; Wang et al. 1998, 1999; Byrd et al. 1999). Wang and his colleagues attempted to distinguish between two theories associated with hybrid zones. The *ecologically neutral dynamic equilibrium model* suggests that hybridization produces hybrids that are always inferior to nonhybrids. In contrast, the *ecologically dependent bounded hybrid superiority model* assumes a genotype-by-environment interaction, such that *in hybrid zones*, hybrids may have superior fitness to nonhybrids.

Wang and his team studied two parapatric subspecies of big sagebrush (Wang et al. 1997). In the mountains of Utah, basin big sagebrush (*Artemisia tridentata tridentata*) grows up to elevations of about 1800 meters, while mountain big sagebrush (*Artemisia tridentata vaseyana*) can be found at elevations above 1900 meters. Between 1800 and 1900 meters, the two subspecies form a narrow hybrid zone. To distinguish between the dynamic equilibrium and the bounded hybrid superiority models, Wang and his colleagues ran a series of reciprocal transplant experiments. In these experiments, mountain big sagebrush, basin big sagebrush, and hybrid sagebrush were each raised in three different environments—below 1800 meters, above 1900 meters, and in the hybrid zone between 1800 and 1900 meters.

Wang and his team found strong support for the bounded hybrid superiority model. In experiments on seed survivorship, size, and flower number, they found a fascinating genotype-by-environment interaction. While hybrid individuals generally fared poorly in environments below 1800 meters and above 1900 meters, they had a higher fitness than that of either subspecies when all types were raised in the *hybrid zone* (**Figure 14.14**).

Although it is difficult to pinpoint why hybrids have higher fitness in the hybrid zone, it may in part be related to the fact that soil in the hybrid zone is not just a simple blend of soils from the mountain and basin areas; rather, this soil has

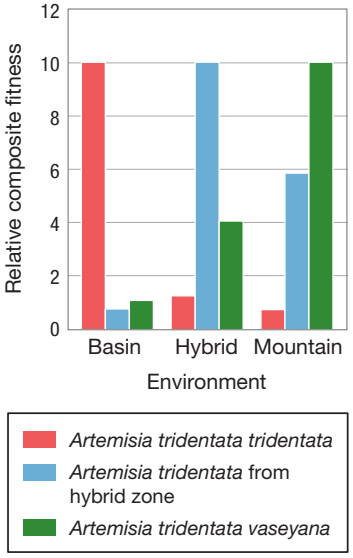


FIGURE 14.14 Bounded hybrid superiority. Mountain big sagebrush, basin big sagebrush, and hybrid sagebrush were each raised in three different environments: below 1800 meters, above 1900 meters, and in the hybrid zone between 1800 and 1900 meters. A relative composite fitness measure shows that each subspecies is better than the other two species when raised in its home environment.

its own unique, novel characteristics, suggesting that selection may have favored hybrids that have been produced in such soil for many, many generations (Wang et al. 1998). The parapatric speciation process under way in the big sagebrush may, over evolutionary time, result in three species rather than two—a basin species, a mountain species, and an intermediate-elevation species, where we currently see a hybrid zone.

Ring Species

It is sometimes difficult to make a clear distinction between allopatric and parapatric speciation. A case in point is when individuals live in one of a series of populations that are connected to one another in a ringlike fashion, forming what is known as a **ring species** (Stebbins 1949; Dobzhansky 1958; Irwin et al. 2001; Monahan et al. 2012) (**Figure 14.15**).

More than 60 years ago, Theodosius Dobzhansky and Robert Stebbins described a beautiful example of a ring species of *Ensatina eschscholtzii* salamanders. This species of lungless salamanders can be found in a series of populations that range from British Columbia in Canada to Baja California in Mexico (Stebbins 1949). These populations have subsequently been studied in great depth by David Wake and his colleagues (Wake 1997; Kuchta et al. 2009).

Ensatina eschscholtzii originated in northern California and southern Oregon and then, approximately 21.5 million years ago, they began to spread south along two separate but parallel fronts. One group of populations moved south along the coastal mountain ranges; further inland, a second group expanded south along the Sierra Nevada range. These two groups were separated from one another by the hot, dry Sacramento and San Joaquin valleys, resulting in the ring distribution that we see today (**Figure 14.16**). Along these two fronts, salamanders show an impressive degree of phenotypic variability in skin coloration—differences in hue, blotchiness, the number of colored stripes, and other characters.

To measure gene flow across salamander populations, David Wake and his colleagues collected skin samples from individuals and extracted DNA from the samples to compare mitochondrial DNA sequence data. They compared DNA from 385 individuals collected from 224 different populations along both fronts that the salamanders inhabit (Kuchta et al. 2009). Their results suggest that while there is some gene flow between populations near one another along either front, gene flow is not continuous along the ring. Indeed, as one would expect if the ring originated at the northern tip, the amount of gene flow decreases along both fronts as one moves south, leading to southern populations at the end of each front being more genetically distinct from one another than from other populations in the ring (Wake and Yanev 1986; Wake et al. 1986; Kuchta et al. 2009, Devitt et al. 2011; Monahan et al. 2012). When the DNA results are mapped onto a phylogeny, separate coastal and inland clades emerge.

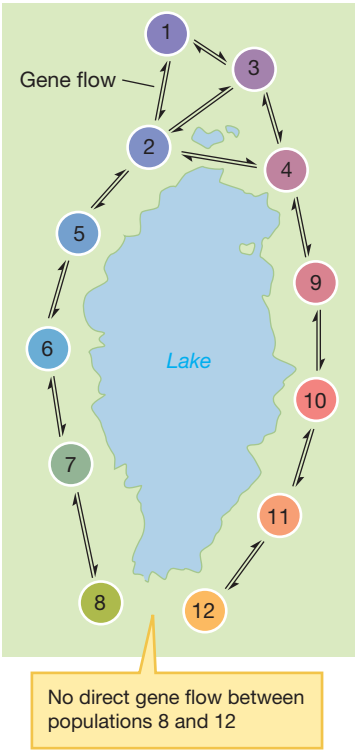


FIGURE 14.15 Ring species concept. An ancestral population (1) spreads down along both the east and west shores of a vast lake, resulting in a series of descendant populations (2–12). As a result of both selection and drift, populations along the west shore diverge from those along the east shore. Gene flow occurs between adjacent populations on each shore, but at the southern edge of the lake where west-shore population 8 comes into contact with east-shore population 12, these two populations have diverged so much that no direct gene flow occurs between them.

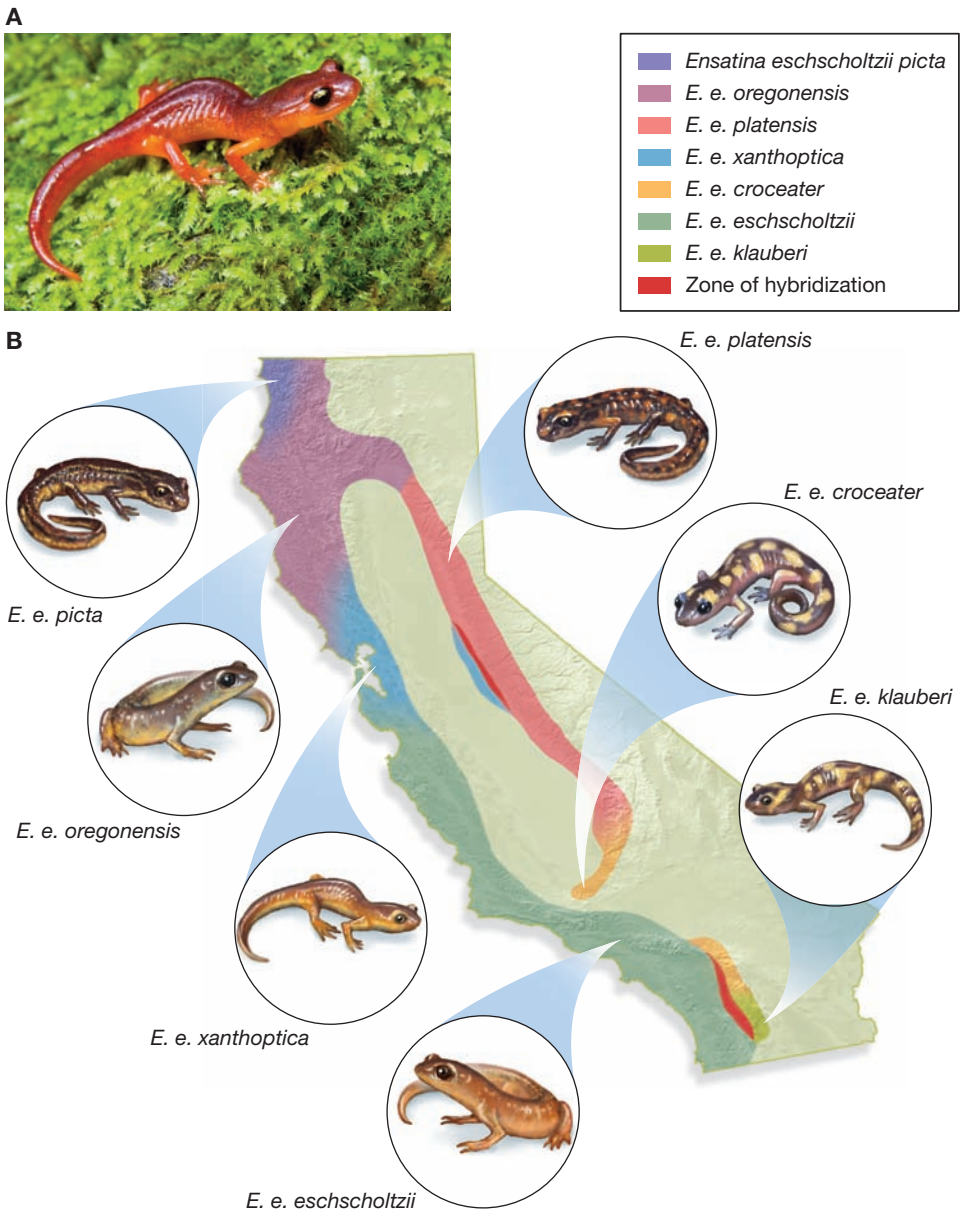


FIGURE 14.16 *Ensatina eschscholtzii* salamanders have been studied as a ring species. **(A)** *Ensatina eschscholtzii*. **(B)** Along the two fronts they inhabit in California, these salamanders show a tremendous amount of phenotypic variability in skin color and blotch pattern. Panel B adapted from Thelander (1994).

Sympatric Speciation

Sympatric speciation occurs when no geographic boundary separates diverging populations. For evolutionary biologists, sympatric speciation is the most difficult of the three forms of speciation to understand (Bolnick and Fitzpatrick 2007). The difficulty stems from the fact that without some sort of geographic barrier (as in allopatric speciation) or some sort of gradient in selective conditions (as in parapatric speciation), some other mechanism must drive a single species to split into two species. One possibility is that speciation may be driven by resource competition; we explore this mechanism later in Box 14.1. Other alternatives involve some form of reproductive isolation that arises without geographic separation. We consider some of these possibilities below.

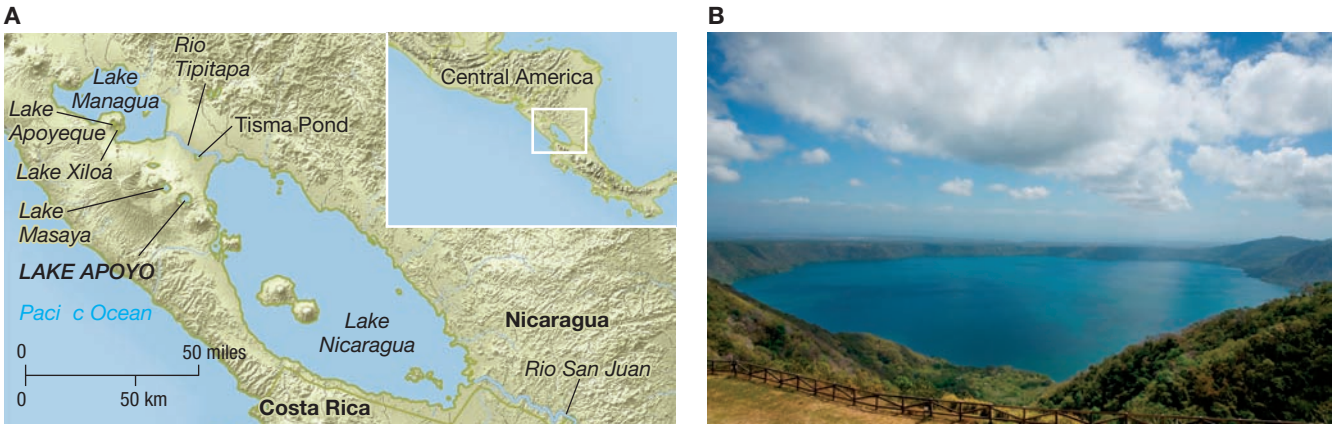


FIGURE 14.17 Lake Apoyo. **(A)** Map of the Nicaraguan lakes (including Lake Apoyo, Lake Managua, and Lake Nicaragua). Adapted from Barluenga et al. (2006). **(B)** A photograph of Lake Apoyo showing its steep wall.

Sympatric Speciation in Cichlids

Here we will examine two species of cichlid fish found in Nicaragua's Lake Apoyo (**Figure 14.17**). Lake Apoyo is a small lake with a diameter of about 5 kilometers and is fairly shallow and quite homogeneous in appearance throughout. Geological data suggest that this lake is also young—it originated about 23,000 years ago.

Lake Apoyo contains two species of cichlids—the Midas cichlid (*Amphilophus citrinellus*) and the Arrow cichlid (*Amphilophus zaliosus*). Whereas the Midas cichlid is found in many Nicaraguan lakes, the Arrow cichlid is only found in Lake Apoyo.

Marta Barluenga and her colleagues hypothesized that the Arrow cichlid arose sympatrically from an ancestral population of the Midas cichlid at Lake Apoyo (Barluenga et al. 2006). To test this hypothesis, they used an array of phylogeographic, population genetic, ecological, and morphological tools to examine whether the two species diverged sympatrically. They began by comparing 840 base pairs of mitochondrial DNA in hundreds of Arrow and Midas cichlid fish. This comparison revealed two remarkable bits of information: (1) The Midas and Arrow cichlids form a monophyletic clade, suggesting that the Arrow cichlid arose in Lake Apoyo, and (2) not even one mitochondrial haplotype was found in cichlids in any other Nicaraguan lake that was the same as the mitochondrial haplotypes found in the two Lake Apoyo species. This suggests that there was most likely a single colonization of Lake Apoyo and no further contact between fish in this lake and other lakes.

A number of lines of evidence have converged to suggest that the speciation event responsible for forming these two species occurred in sympatry rather than allopatry. First, as mentioned earlier, Lake Apoyo is small, shallow, and homogeneous. Taken together, these geological and geographic characteristics of the lake make it unlikely that there is a physical boundary that Barluenga and her colleagues did not observe. Because Lake Apoyo is young, it is unlikely that such a boundary once existed but has since disappeared. In addition, population genetic data provide evidence that these two species were somehow dividing up Lake Apoyo in ways that were not obvious, even after Barluenga and her team observed these two species across the entire lake. But how? If there is no physical boundary to prevent gene flow between these two species, what does prevent gene flow?

The answer appears to center on habitat and ecological specialization (**Figure 14.18**), similar to that described in **Box 14.1**. The Midas cichlid is "high-bodied" and relatively short in length. This species shows the morphology classically associated with living at the bottom of lakes (known as a *benthic* body form). Diet analysis

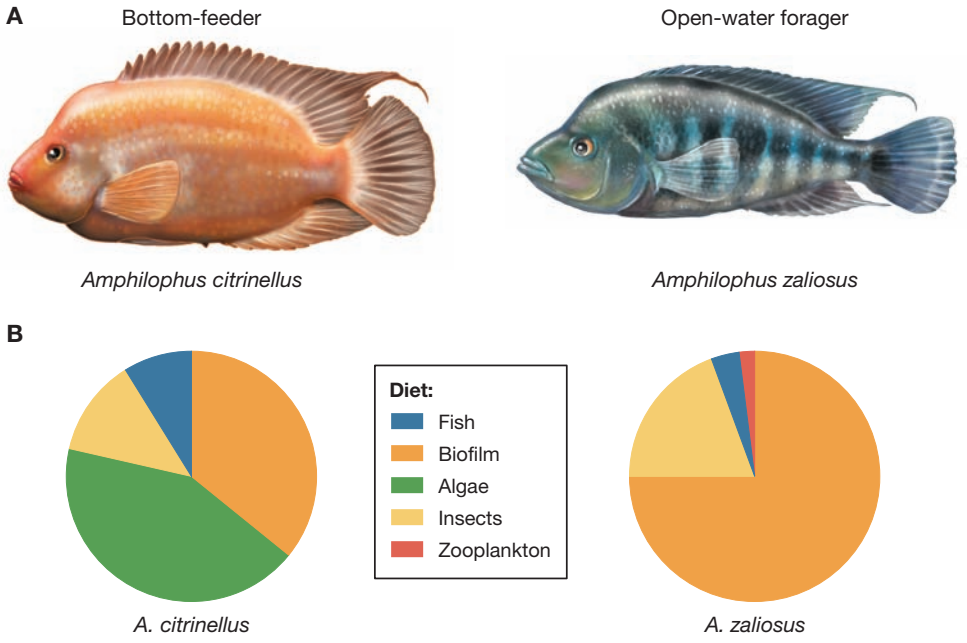


FIGURE 14.18 Sympatric speciation, habitat specialization, and ecological specialization in two species of Lake Apoyo fish. (A) The Midas cichlid (*Amphilophus citrinellus*) and the Arrow cichlid (*Amphilophus zaliosus*) are morphologically very different from one another. **(B)** These two species of cichlids have different diets as a function of their different habitat preferences. Adapted from Barluenga et al. (2006).

of the Midas cichlid supports the idea that this species spends its time primarily near the bottom of the lake. The Arrow cichlid is low-bodied and longer, and it has a morphology associated with foraging in open water (known as a *limnetic* body form). Again, diet analyses support this contention. Moreover, Barluenga and her colleagues used behavioral experiments to demonstrate that both Arrow cichlids and Midas cichlids prefer mates from their own species, suggesting reproductive isolation between the Arrow and Midas cichlids. This partitioning of the lake into bottom-of-the-lake and open-water areas appears to be the mechanism by which sympatric speciation occurred in Lake Apoyo.

Sympatric Speciation in the Apple Maggot Fly

Perhaps the best-studied case of sympatric speciation is that of the apple maggot fly, *Rhagoletis pomonella.* Just 5 years after Darwin published *On the Origin of Species*, Benjamin Walsh suggested that sympatric speciation was common in insects such as *R. pomonella* (Walsh 1864, 1867). Walsh noted a dramatic shift in the host species of *R. pomonella* from downy hawthorn shrubs/trees (*Crataegus mollis*) to both hawthorn shrubs/trees and domesticated apple trees (*Malus pumila*) (**Figure 14.19**). These different forms of *R. pomonella*—known as the hawthorn "race" and the apple

FIGURE 14.19 Divergence in apple maggot flies. (A) The apple maggot fly, **(B)** a hawthorn tree, and **(C)** an apple tree.

BOX 14.1 Sympatric Speciation: A Resource Competition Model

Jon Seger hypothesized that resource competition may play an important role in the process of sympatic speciation (Levene 1953; Maynard Smith 1966; Rosenzweig 1978; Bengtsson 1979; Gibbons 1979; Seger 1985). Consider a phenotypic trait that is important in resource competition—following Seger's lead, let's imagine that this trait is beak size in birds, and let's assume that the size of seeds consumed by birds is a function of beak size. If beak size is heritable and controlled by many genes, we might expect beak size to be normally distributed. If we assume that the distribution of seed sizes follows a similar normal distribution, individual birds get about the same amount of food irrespective of their beak sizes (**Figure 14.20A**).

But what if the *seed distribution* is much flatter rather than the distribution of beak sizes (**Figure 14.20B**)? In this case, birds with either very large or very small beaks will get more food, and so both very large and very small beaks will be favored by natural selection. This is because there are similar numbers of small, medium, and large seeds. But because beak size is normally distributed, there are fewer large-beaked and small-beaked birds than birds with medium-sized beaks.

Large-beaked and small-beaked birds then get more food per bird than that acquired per bird by birds with medium-sized beaks, and disruptive selection will favor the extreme beak phenotypes. Our population will then start to diverge into large-beaked and small-beaked individuals. This is the first step toward sympatric speciation in the Seger model. The second step involves the emergence of reproductive isolation between large-beaked and small-beaked birds. For speciation to occur, large-beaked birds need to mate with large-beaked birds, and small-beaked birds need to mate with small-beaked birds; that is, there must be some sort of *positive assortative mating*, where like mates with like. This assortative mating could complete the process of disruptive selection and result in sympatric speciation. The result would then be a large-beaked species and a small-beaked species of birds.

The problem with the above scenario is that it requires assortative mating to emerge at just the right time; that is, after disruptive selection has begun to pull apart our two types of birds (small-beaked and large-beaked birds). The odds of assortative mating coming about at just this time are quite small. There is, however, a much simpler process that can allow sympatric speciation.

Suppose that large and small seeds are spatially segregated. For the sake of argument, imagine that plants producing large seeds grow in shady habitats, whereas plants that produce small seeds grow in habitats with more sunshine. Birds would then distribute themselves according to where the food is: Large-beaked birds would spend their time in shady habitats and

"race"—may be different species or, at the very least, diverging and on the path to becoming different species.

Incipient speciation in the apple maggot fly may be occurring sympatrically, as a function of which trees these insects use as hosts. The domestic apple tree was introduced into North America about 400 years ago, and it occurs sympatrically with the downy hawthorn tree: In the eastern region of North America, both trees have been hosts to apple maggot fly larvae. Because of their economic importance, apple trees have been closely monitored, and so we know that *R. pomonella* only began using the apple tree as a host about 150 years ago—before that period it was only found on downy hawthorn trees (Bush 1969, 1975; Berlocher and Feder 2002).

Evolutionary biologists wanted to test whether differences between the races of *R. pomonella*—in particular, differences in the breeding seasons of the different races of flies—resulted from different selective conditions associated with their host trees. Over the years, a series of experiments has addressed this question. The key to understanding both the differences between the races of these flies and how these differences are tied to sympatric speciation in *R. pomonella* is the different fruiting times of their hosts—apple trees produce fruit 3 to 4 weeks earlier than downy hawthorn trees.

Researchers hypothesized that the difference in the host trees' fruiting times causes fly maggots in apples and downy hawthorn fruit to emerge at different times, which reduces the gene flow between the two populations. This in turn produces significant

small-beaked birds would prefer sunny habitats. In this case, even if birds don't have a preference to mate with others with similar beak sizes to their own, and instead they simply choose mates randomly from those individuals around them, assortative mating occurs. This allows the sympatric speciation process to proceed.

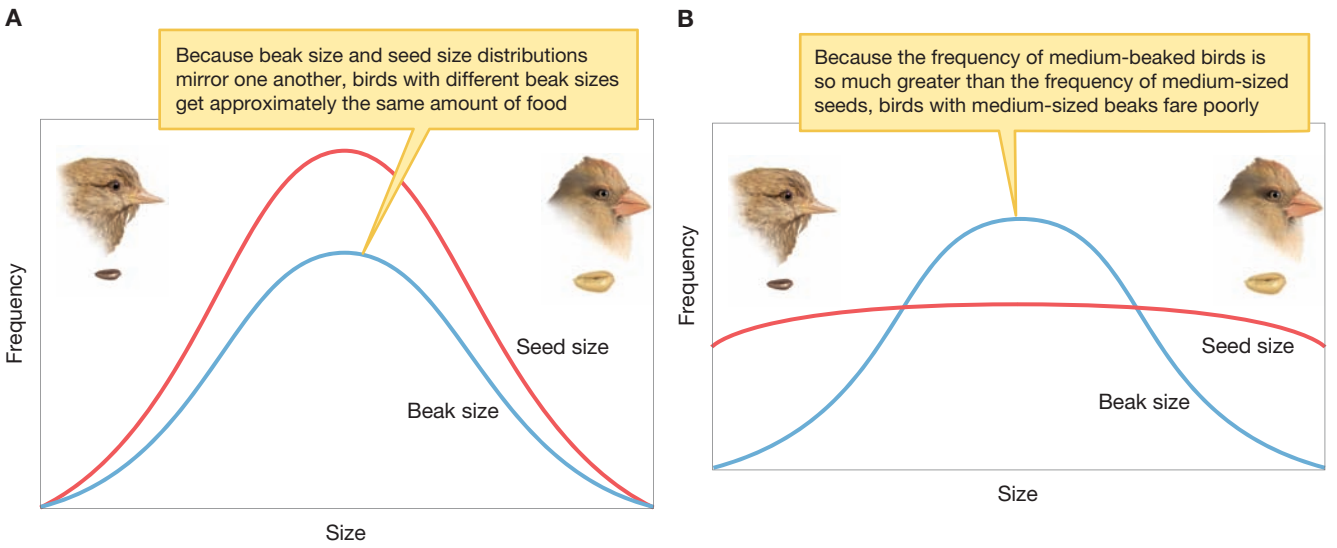


FIGURE 14.20 Two distributions of seed size and beak size. **(A)** Both seed size (red) and beak size (blue) are normally distributed, and all birds receive approximately the same amount of food. **(B)** Beak size (blue) is normally distributed, but seed size (red) has a flatter distribution. Birds with large or small beaks get more food *per bird* than birds with average-sized beaks.

genetic differences between the downy hawthorn and apple races of the apple maggot, including differences not only in when flies emerge but also in their fruit preferences (Boller and Prokopy 1976; Feder et al. 1997; Feder and Filchak 1999; Filchak et al. 2000; Schwartz et. al. 2009).

Behavioral tests in both the laboratory and the field show that flies prefer the odors associated with the fruit of their respective hosts (Linn et al. 2003; Dambroski et al. 2005). This host specificity in emergence time and fruit preference reduces gene flow between the apple and downy hawthorn races to 4% to 6% each generation (Feder et al. 1994), suggesting that sympatric races of *R. pomonella* are indeed diverging and potentially on the path to becoming separate species.

A recent study has found that the genetic changes associated with the shift from hawthorn to apple trees may have occurred very quickly. Scott Egan and his team carried out a genome-wide comparison of differences between contemporary apple and hawthorn races of the flies (Egan et al. 2015). They then conducted a single-generation selection experiment using individuals from a contemporary population of hawthorn-population flies, exposing them to a developmental period that mimicked the developmental period associated with living on apple trees. Comparing the results of their selection experiment to the genome-wide differences that they documented in contemporary apple and hawthorn populations, they found evidence that selection had acted on least 154 loci, spread across the fly genome. Moreover, Egan and his

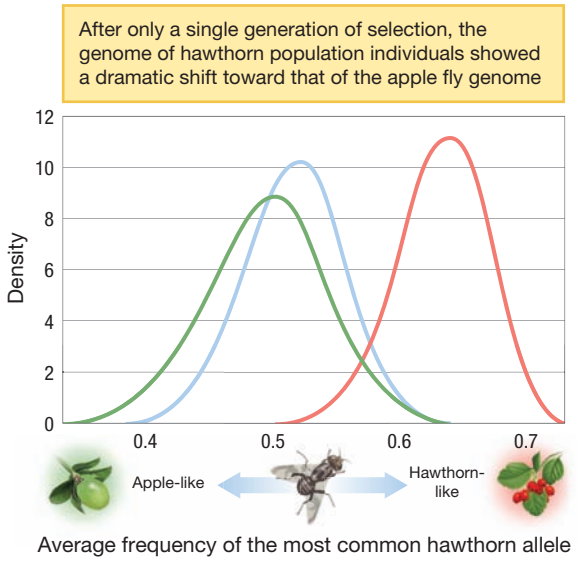


FIGURE 14.21 Sympatric speciation in *R. pomonella*. Egan and colleagues exposed a population of the hawthorn race of the apple maggot fly *R. pomonella* to selection mimicking the conditions associated with living on apple trees. After a single generation, Egan and colleagues found that selection had acted on least 154 loci. At these loci, the allele frequencies in the selected population (blue) shifted dramatically from the allele frequencies of the founding hawthorn race (red) toward the allele frequencies of the apple race (green).

team were able to determine that after just a single generation of selection, the genomes of hawthorn-population individuals had become significantly more similar to the genomes of individuals from apple populations (Figure 14.21).

In addition, in what may be additional instances of incipient sympatric speciation in *R. pomonella,* recent work on gene flow, behavior, and emergence times suggests that other races of *R. pomonella* have specialized on blueberry hawthorn

BOX 14.2 Secondary Contact

What happens when two populations have been diverging from one another in allopatry but are then reunited before reproductive isolation is complete? For example, imagine that population 1 and population 2 have been geographically isolated for many generations, during which time natural selection and genetic drift have caused significant, but not complete, reproductive isolation between the individuals in these populations. When the geographic isolation comes to an end, will the two populations complete the divergence process or remain part of a single species?

The answer depends on the extent of the reproductive isolating mechanisms that have evolved during allopatry. If the reproductive isolating mechanisms are sufficiently weak as to allow free interbreeding across the reunited populations, and such matings produce offspring that are not at some fitness disadvantage compared to offspring derived from matings between individuals from within either population, then the process of speciation halts and a single species remains. But if the reproductive isolating mechanisms that developed during allopatry put offspring from matings between population 1 and population 2 individuals at a selective disadvantage, the speciation process may continue, and over time we may end up with two different species. This process is referred to as **secondary reinforcement** (Figure 14.22).

One clue that evolutionary biologists can use to infer that secondary reinforcement has occurred is **reproductive character displacement (RCD)**. RCD is defined as the case in which a reproductive trait is less similar when two incipient species overlap (in areas of sympatry) than when these two species do not overlap (areas of allopatry). The basic premise underlying RCD is that if hybrids are at a disadvantage, natural selection should act more intensely on the ability to mate with conspecifics in areas of sympatry than in areas of allopatry (Brown and Wilson 1956).

A fascinating case of RCD has been documented in two Japanese species of land snails, *Satsuma eucosmia* and *Satsuma largillierti*. In some areas on Okinowa Island, these two species live sympatrically; in other areas, populations of the species

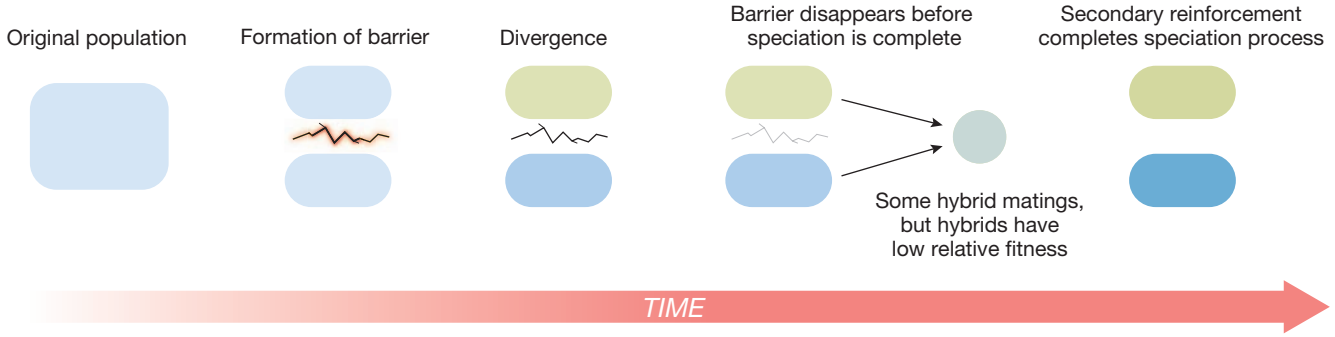


FIGURE 14.22 Secondary reinforcement. The process of secondary reinforcement in populations that diverge in allopatry may complete the speciation process.

(*C. brachyacantha*), southern red hawthorn (*C. mollis* var. *texana*), and green hawthorn (*C. viridis*) in the southern part of the United States and on black hawthorn (*C. douglasii)* and the English ornamental hawthorn (*C. monogyna*) in the western United States (Linn et al. 2012; Powell et al. 2014).

14.3 Reproductive Isolating Mechanisms and the Genetics of Speciation

Evolutionary biologists and ecologists have devoted considerable effort to understanding how reproductive isolation can arise and lead to the origin of new species (Du Rietz 1930; Mayr 1942; Dobzhansky 1970; Widmer et al. 2009). What drives reproductive isolation between populations? Why don't two separate species merge back together if they overlap in range (**Box 14.2**)? In his classic book, *Genetics of the Evolutionary Process*, Theodosius Dobzhansky divided reproductive isolating mechanisms into two categories: **prezygotic isolating mechanisms** and **postzygotic isolating mechanisms** (**Table 14.2**) (Dobzhansky 1970).

live in allopatry. Prior work has suggested that areas of modern sympatry in these species appear to represent secondary contact between *S. eucosmia* and *S. largillierti* (Kameda et al. 2007). Because of its obvious implications for mating, Yuichi Kameda used penis length as the reproductive trait of interest, and he measured this character in individual snails who lived in either sympatric or allopatric *Satsuma* populations. Average penis length showed greater differences between these species when they lived in areas of sympatry, suggesting RCD in this trait, and indicating that penis length may have been a key trait in the process of secondary reinforcement in *S. eucosmia* and *S. largillierti* (Kameda et al. 2009) (**Figure 14.23**).

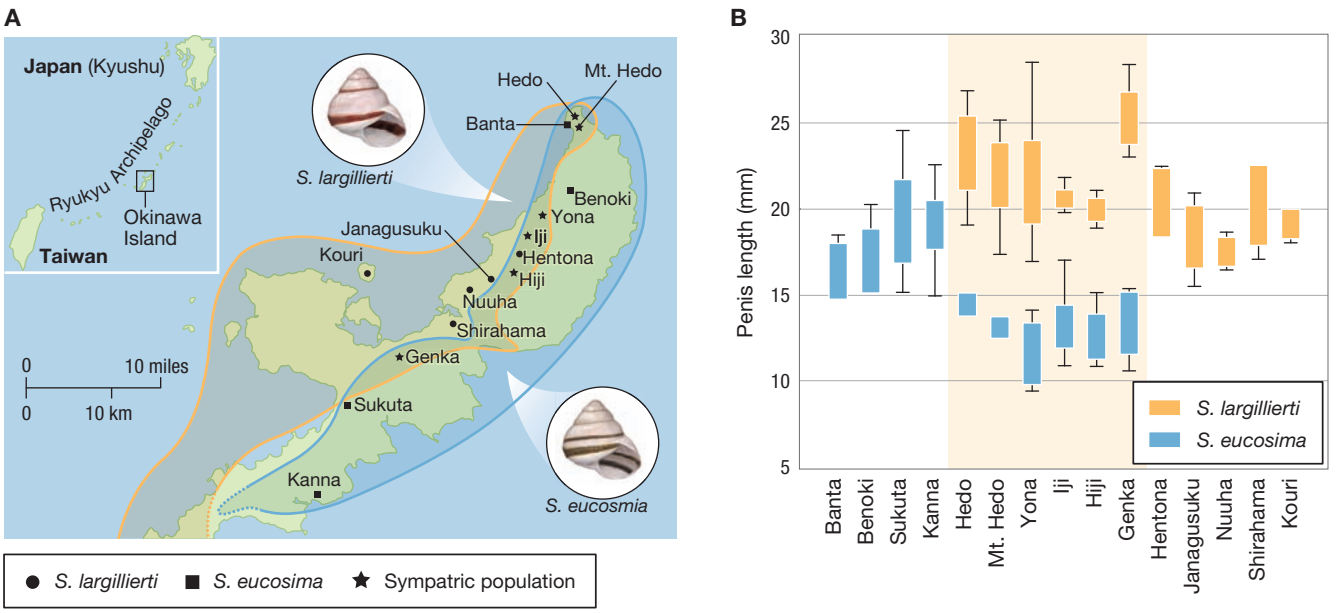


FIGURE 14.23 Reproductive character displacement in snails. **(A)** The geographic distributions of *Satsuma* snails on Okinawa Island, in Japan. The orange border indicates the area occupied by *Satsuma largillierti*, and the blue border indicates the area occupied by *Satsuma eucosmia*. Particular localities inhabited by *S. eucosmia* (squares) and *S. largillierti* (circles) are also shown on the map. Sympatric populations of the two species are depicted by stars. **(B)** Penis length of *S. eucosmia* (blue) and *S. largillierti* (orange) across study sites in Japan. Differences in penis length are greater in areas of sympatry (shaded on the graph) than in areas of allopatry, suggesting reproductive character displacement. Adapted from Kameda et al. (2009).

TABLE 14.2

Dobzhansky's Reproductive Isolating Mechanisms

Prezygotic Isolating Mechanisms
Potential mates live in the same place but do not encounter one another, due to either: habitat isolation and/or temporal isolation (by time of day or time of year).
Potential mates interact but do not mate (behavioral isolation).
Individuals copulate, but male gametes (sperm or pollen) are not transferred.
Male gametes are transferred, but the egg is not fertilized (gametic incompatibility).
Postzygotic Isolating Mechanisms
Zygote dies early in embryogenesis.
F_1 hybrids are inviable.
F_1 hybrids survive but are sterile.
Backcross or F_2 hybrids are inviable or sterile.

Adapted from Barton et al. (2007).

BOX 14.3 The Role of Symbiotic Bacterial Communities in Speciation

Hologenome is a relatively new term that refers to the complete genome of a host species and the genomes of all the microorganisms that reside within it (Zilber-Rosenberg and Rosenberg 2008). Some of these microbial species are parasites, while others are symbionts. In our own species, in each individual there are approximately 10 bacterial cells for every human cell and about 100 bacterial genes for each human gene. All these genes and the genes of other microorganisms we host make up the human hologenome.

Evolutionary biologists have long suspected that what we now call the hologenome may play a role in speciation (Wallin 1927), but only recently have we developed the tools to test the idea that symbiotic species can promote reproductive isolation. Insects are especially good model systems for examining such questions, not only because of the relative ease of running experimental evolution studies on model species such as fruit flies, but also because insects house huge communities of microbial symbionts. In addition, prior work has shown that diet affects the composition of microbial symbiotic communities in flies, and that symbiotic communities are transmitted vertically from parent to offspring (this can occur in many ways, including when a mother's feces contaminates the surface of a developing egg [Matos and Leulier 2014]).

To test the hypothesis that symbiotic species can promote reproductive isolation in *Drosophila melanogaster,* Gil Sharon and colleagues ran a 37-generation experimental evolution study (Sharon et al. 2010). They began by dividing an inbred line of *D. melanogaster* into two treatments. Flies in one treatment were raised on a medium in which the main source of nutrients was a cornmeal–molasses–yeast mix (CMY flies). In a second treatment, flies were raised on a medium with starch as the sole nutrient (starch-raised flies). For 37 generations, all flies in both treatments were raised on their respective food sources. Periodically, flies were tested to see if they had a preference for mates from their own treatment, the other treatment, or showed no preference for mates from one treatment over the other. During the generations when mate choice was assessed, all flies in *both* treatments were raised on the CMY mix to make sure that food source per se, rather than changes in the microbial symbiotic communities that were in present across food treatments, was not responsible for any mate choice preferences that were uncovered.

Sharon and colleagues found that CMY flies preferred CMY flies as mates, and starch-diet flies preferred starch-diet flies as mates (**Figure 14.24**). This preference for mating with individuals from one's own treatment—a precursor for the reproductive isolation associated with speciation—was present after

Prezygotic isolating mechanisms prevent or deter individuals from different populations from mating with one another—or prevent fertilization from occurring if such a mating does take place. Postzygotic isolating mechanisms operate after fertilization and conception. With postzygotic mechanisms in place, a mating between individuals from different populations may lead to successful fertilization, but the embryo may not survive. If it does, it may either be sterile or have dramatically reduced fitness. From an evolutionary perspective, even though matings can occur across these populations, the populations in question are functionally reproductively isolated from one another (Sobel et al. 2010).

To understand reproductive isolating mechanisms better, let us look at two examples: one involving delivery isolation in plants, and one a study of reproductive isolation and shell coiling patterns in snails (we present a third example, involving the role of symbiotic bacterial communities in the speciation process of their hosts, in **Box 14.3**).

Reproductive Isolating Mechanisms: Isolation through Pollinators

The tight linkage between feeding morphology of animal pollinators and plant traits associated with pollination has long been of interest to evolutionary biologists. Darwin himself was fascinated with this subject. For example, on January 25, 1862, he received a box of plant samples from an orchid grower in Madagascar. The samples included a long-spurred orchid, *Angraecum sesquipedale,* a species with

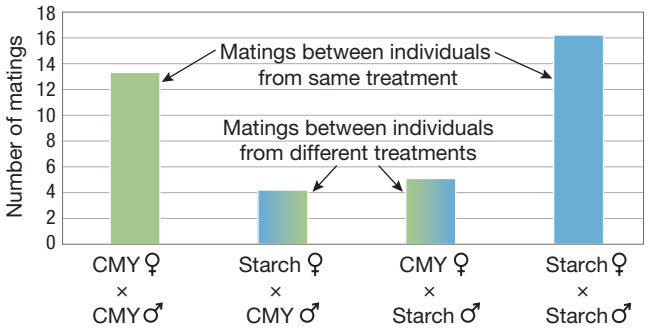


FIGURE 14.24 Symbiosis and reproductive isolation. In each trial of the experiment, four flies—one male and one female from the CMY treatment and one male and one female from the starch treatment—were placed together and matings were recorded. Flies in both the CMY and starch treatments preferred to mate with individuals from their own treatment. During generations when mate choice tests were done, all flies in both treatments were raised on the CMY mix to control for the effects of the immediate food source. Adapted from Sharon et al. (2010).

a single generation and was maintained for the 37 generations of the experiment. Molecular genetic analysis found that the number of symbiotic *Lactobacillus* species differed across the two fly treatments by almost an order of magnitude, suggesting that *Lactobacillus* may be important in mate preference. More specifically, flies in the starch-diet treatment had almost 10 times more *Lactobacillus* cells than that of flies in the CMY treatment. Flies with heavy loads of *Lactobacillus* may have been favored in the starch treatment because *Lactobacillus* produces an enzyme called amylase, which breaks starch down into sugar. Exactly how differences in *Lactobacillus*, and potentially in other symbiotic microbes, leads to differences in mate preference in the flies is not known, but there is some evidence that *Lactobacillus* affects the production of many cuticular hydrocarbons, which are critical components of sex pheromones in flies.

Additional evidence that microbial symbiotic communities can lead to reproductive isolation was uncovered: Sharon and colleagues found that when flies in their two treatments were administered antibiotics that eliminated the difference in microbial communities within individuals, they showed no preference for mates from their own treatment, and differences in the cuticular hydrocarbons between treatments decreased significantly.

a very long tubule that leads to a nectary at its end (**Figure 14.25A**). That same day, Darwin wrote his colleague, J. D. Hooker, "I have just received . . . [an] astounding *Angraecum sesquipedalia* [*sic*] with a nectary a foot long. Good Heavens what insect can suck it." This led Darwin to predict that there must be a moth species with a proboscis long enough to pollinate this orchid, though such a pollinator had never been observed (Darwin 1862; Arditti et al. 2012). In 1903, a candidate moth, *Xanthopan morganii praedicta*, was discovered in Madagascar (**Figure 14.25B**), and at long last in 1992 this species was photographed pollinating *A. sesquipedale*. (Wasserthal 1996, 1997, 1998).

Over time, the effects of pollinators on reproductive isolation in plants have been documented for many different plant species (Grant 1994; Hodges et al. 2004; Whittall and Hodges 2007; Kay and Sargent 2009; Widmer et al. 2009; Rosas-Guerrero et al. 2014). In many plant taxa, such as the genus *Aquilegia* (columbine) and the genus *Ipomopsis* (skyrocket and its relatives), closely related species are pollinated by very different organisms (**Figure 14.26**). In *Aquilegia*, six species are primarily pollinated by hummingbirds, and four species are primarily pollinated by eastern hawkmoths; in the *Ipomopsis* group, seven species are pollinated by hummingbirds, and seven species are pollinated by hawkmoths (Grant 1992).

A

B

Species pollinated by hummingbirds have markedly different floral structures than those pollinated by hawkmoths. In species pollinated by hummingbirds, floral tubes (spurs) are trumpet shaped and fairly long (16–24 millimeters); the mouth parts of hummingbirds used during pollination average about 23 millimeters in length. In contrast, the floral tube of species pollinated by hawkmoths is longer and more slender, ranging in length from 30 to 70 millimeters, which corresponds to the length of the proboscis of the species of hawkmoths involved in pollination (Grant 1992; Whittall and Hodges 2007) (**Figure 14.27**). These differences in floral structures between hawkmoth-pollinated and hummingbird-pollinated species minimize gene flow across plant species that rely on different pollinator groups.

The difference between pollinators need not be as dramatic as the difference between insects and birds. In many closely related species of orchids, for example, reproductive isolation occurs because, although each is pollinated by insects, different species of orchids are associated with different species of insect pollinators (Van der Pijl and Dodson 1966; Nilsson et al. 1987; Armbruster et al. 1992).

Reproductive Isolation and Shell Coiling Patterns in Snails

Although evolutionary biologists do not expect that reproductive isolation will typically be attributed to a single point mutation, there is evidence that reproductive isolation is sometimes linked

FIGURE 14.25 Darwin's orchid. (A) Upon receiving a specimen of the orchid *Angraecum sesquipedale* from Madagascar in 1862, Charles Darwin predicted that there must be a moth with a proboscis long enough to pollinate it. **(B)** In 1992, the moth *Xanthopan morganii praedicta* was finally photographed doing exactly that.

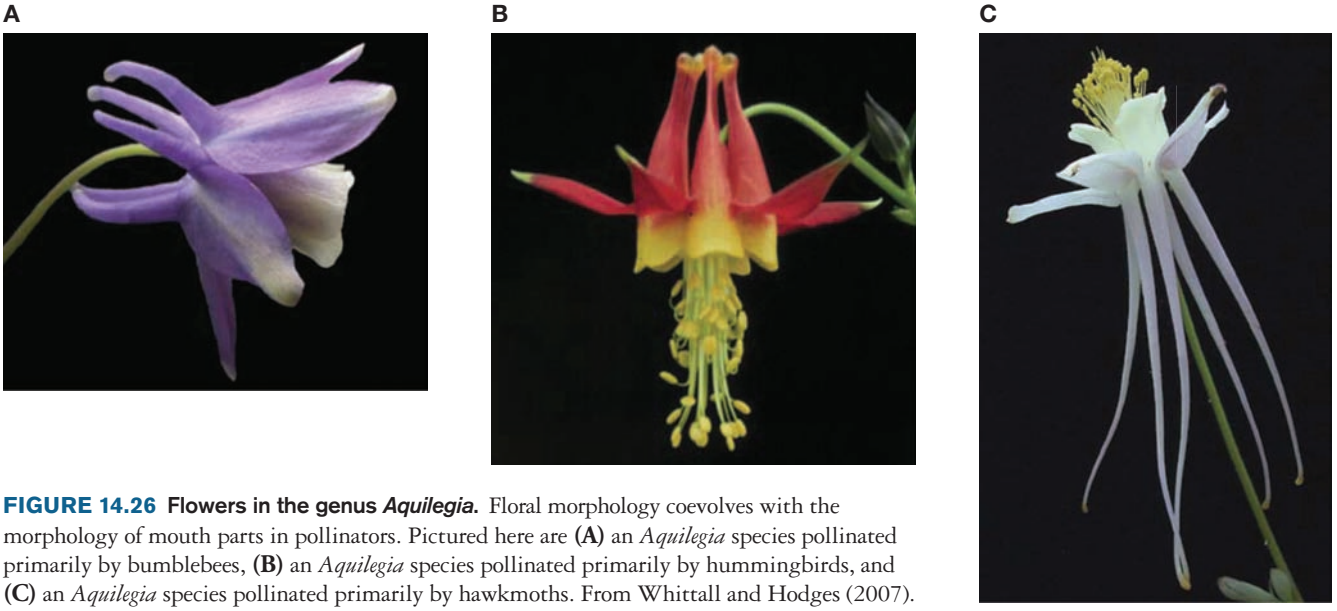


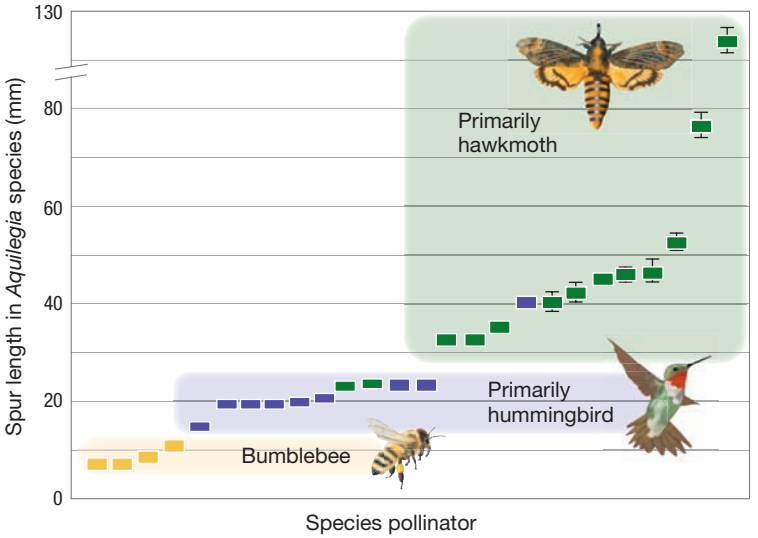
FIGURE 14.26 Flowers in the genus *Aquilegia*. Floral morphology coevolves with the morphology of mouth parts in pollinators. Pictured here are **(A)** an *Aquilegia* species pollinated primarily by bumblebees, **(B)** an *Aquilegia* species pollinated primarily by hummingbirds, and **(C)** an *Aquilegia* species pollinated primarily by hawkmoths. From Whittall and Hodges (2007).

to evolutionary change in a single gene. Recall the case of land snails that have shells that either coil to the right or to the left, which we first discussed in Chapter 7. In some snail species, because of physical constraints, mating can only take place between individuals whose shells coil in the same direction: Individuals whose shells coil to the right cannot mate with individuals whose shells coil to the left.

The directionality of the coil (chirality) in snails is controlled by a single gene with *delayed inheritance*: The phenotype of each offspring is determined by the genotype of the mother, not by its own genotype. The dextral (right-handed) allele is dominant, so that homozygous dextral and heterozygous individuals produce offspring with right-handed coiling, while homozygous sinestral (left-handed) individuals exhibit left-hand coiling (Ueshima and Asami 2003). Because coil direction determines whether or not individuals are physically capable of mating with one another, individuals of the more common chirality will have more mating opportunities. As we discussed in Chapter 7, the result is positive frequency dependent selection. This should lead to rapid fixation of the more common type in a local population (Asami et al. 1998)—though some mechanism is needed to allow new variants to reach high enough frequencies to be favored (Hoso et al. 2010; Utsuno et al. 2011). By this process, populations of different chiralities can be established and these populations will be reproductively isolated from one another. In this way, changes in a single locus can drive sympatric speciation (Ueshima and Asami 2003).

Phylogenetic analysis of *Euhadra* snails reveals numerous instances in which speciation is associated with a reversal of coil directionality. **Figure 14.28** illustrates a mitochrondrial DNA phylogeny for a number of *Euhedra* species

FIGURE 14.27 Differences in floral structures. The distribution of spur lengths (floral tube lengths) among *Aquilegia* species, ranked by size and color coded by pollinator (bumblebees in gold, hummingbirds in purple, and hawkmoths in green). Adapted from Whittall and Hodges (2007).



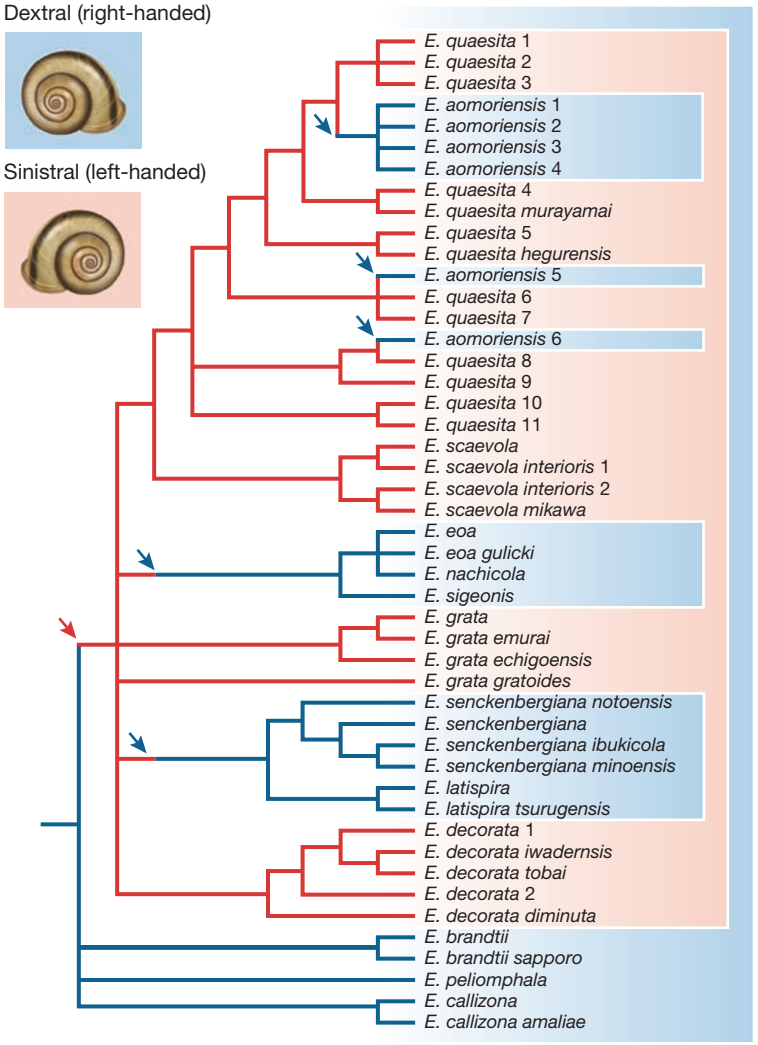


FIGURE 14.28 A phylogenetic view of coil direction in *Euhadra* snails. This maximum likelihood phylogeny is based on mtDNA data and includes multiple individuals (numbered) from some species. Blue and red shading indicate right-hand coiling and left-hand coiling, respectively. Arrows indicate possible points at which coil direction was reversed. Adapted from Ueshima and Asami (2003).

(Ueshima and Asami 2003). This phylogeny includes multiple individuals of some species, as indicated by the numbering, and in doing so informs us about the frequency of chirality reversals. Notably, the phylogeny reveals that members of *E. aomoriensis* are derived from at least three separate reversals by *E. quaesita* ancestors. This is one of the interesting cases in which the biological species concept and the phylogenetic species concept draw different species boundaries. The sinestral *E. quaesita* is reproductively isolated from the dextral *E. aomoriensis* by shell geometry, and thus according to the biological species concept these are two different species. But neither *E. quaesita* nor *E. aomoriensis* is a monophyletic group on its own, and thus these two groups should be placed together in a single species according to the phylogenetic species concept.

The Genetics of Reproductive Isolation

In the subsections that follow, we will expand our discussion of genetic and genomic mechanisms that can cause reproductive isolation. In particular, we will explore

- reproductive isolation via changes in chromosome number;
- reproductive isolation via chromosomal rearrangement;

- reproductive isolation via Dobzhansky–Muller incompatibility;
- Haldane's rule, sex chromosomes, and reproductive isolation.

Reproductive Isolation via Changes in Ploidy

Change in the number of complete sets of chromosomes that an organism possesses—also known as a change in ploidy—can lead to reproductive isolation. For example, imagine a diploid organism with three pairs of chromosomes. Suppose that during gamete production, a breakdown in the normal process of meiosis produces offspring that have six pairs of chromosomes. In plants, two individuals with different ploidy numbers may successfully fertilize one another and produce hybrids, but such hybrids are often sterile. In animals, mating between individuals with different ploidy numbers almost always produces infertile offspring. This is because fertilizations that result from gametes produced by individuals with different ploidy numbers produce embryos that cannot properly undergo meiosis (Fowler and Levin 1984; Rodriguez 1996). If, however, the polyploid individuals with six pairs of chromosomes are viable and can either self-fertilize or mate with another polyploid individual that possesses six pairs of chromosomes, then the change in chromosome number will result in instant reproductive isolation for the polyploid individuals with six pairs of chromosomes.

In what sorts of organisms should we expect to see this form of reproductive isolation? One critical prerequisite is the ability to survive a dramatic change in chromosome number. In plants, for reasons that are not completely understood, changes in ploidy can often be tolerated more commonly than in animals (Muller 1925; Orr 1990; Ainouche and Jenczewski 2010; Mayrose et al. 2011). In addition, because self-fertilization is common in plants, once a change in ploidy has occurred, an individual need not find a mate with the same number of chromosomes as itself (Stebbins 1938; Bell 1982; Ramsey and Schemske 1998). Self-fertilization is not a prerequisite for this type of reproductive isolation, but it should increase the likelihood that sympatric speciation can occur by a change in ploidy.

Given that self-fertilization is common in plants, as is the ability to survive changes in chromosome number, it is not surprising that speciation via changes in chromosome number is common in plants (Ramsey and Schemske 1998; Otto and Whitton 2000; Levin 2002; Soltis et al. 2004). At the phenotypic level, polyploidy in plants is often associated with increased cell volume, larger pollen grains, and larger seed sets, and sometimes, but not always, larger plant size.

The frequency of polyploid speciation in many plant lineages is quite high. For example, approximately 31% of all fern species have originated as a direct result of polyploid speciation, and about one-third of certain clades of grass (Andropogoneae) arose from polyploidy (Wood et al. 2009; Estepa et al. 2014) (**Figure 14.29**).

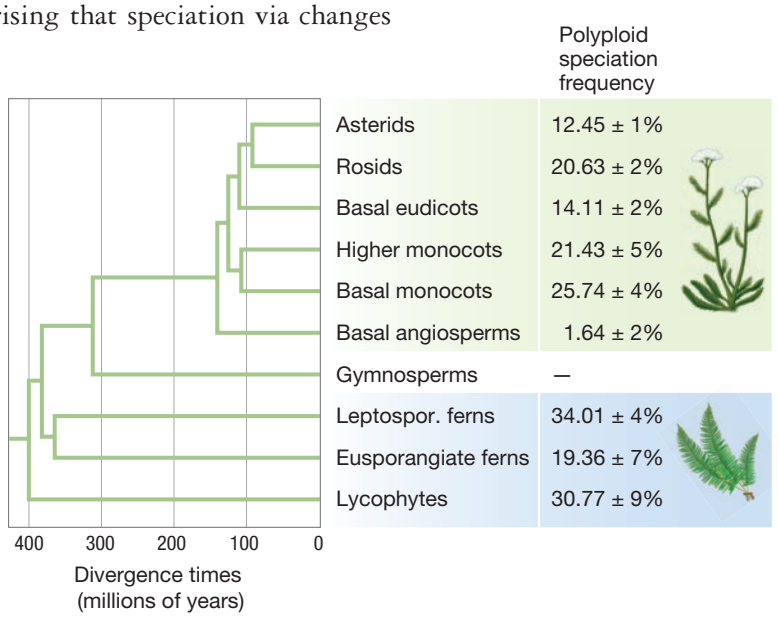


FIGURE 14.29 Polyploid speciation in plant lineages. Polyploid speciation frequencies—measured as the fraction of phylogenetic branching events that involve a shift in ploidy—vary across major groups of vascular plants. Less is known about polyploid speciation in gymnosperms than in other taxa. Adapted from Wood et al. (2009).

Before leaving the subject of reproductive isolation via changes in ploidy, it is important to mention that while this phenomenon is most often seen in plants, it does occur in animals, albeit at a much reduced rate. In certain cases, and for reasons that we do not yet understand, changes in chromosome number in some species of animals do not cause death or sterility; for example, speciation by changes in chromosome number may have occurred in some species of shrimp, frogs, insects, fish, bivalves, and coral (Otto and Whitton 2000).

Reproductive Isolation via Chromosomal Rearrangement

Reproductive isolation may be initiated when genes or clusters of genes become rearranged on a chromosome. Such rearrangements include chromosomal fusion (the joining together of chromosomes or parts of chromosomes), chromosomal fission (the splitting of chromosomes), and chromosomal inversions and translocations. For example, chromosomal inversions have been found in the sympatric populations of the apple maggot fly that we discussed earlier in the chapter, and these inversions have been linked to differences in the time at which eggs hatch in these populations (Feder et al. 2003).

When some individuals in a population possess the original chromosome arrangement and others have the rearranged chromosome set, these *individuals* may not be able to successfully reproduce with one another. More important, when individuals in one *population* have the original chromosome arrangement and individuals in another population have the rearranged version, reproductive isolation between these *populations* may ensue.

The reproductive isolation that results from chromosome rearrangement is thought to emerge for at least two reasons (Rieseberg 2001; Ayala and Coluzzi 2005). First, hybrids formed by individuals with different chromosome arrangements will often produce dysfunctional gametes, and, as such, these hybrids will have reduced rates of genetic recombination and fewer or no offspring. And so selection will lead to the production of fewer hybrids. The theoretical problem with this argument involves how the chromosomal rearrangement becomes common in the population in the first place. A chromosomal rearrangement will first appear as a mutation in a single individual, who will have to mate with another population member who has the original chromosomal arrangement. Yet, because selection acts against the resulting hybrid offspring, the mutation should quickly disappear.

A second way that chromosomal rearrangement may lead to reproductive isolation is that the reduced rates of genetic recombination found in hybrids will lead to an increase in linkage disequilibrium in their descendants. Researchers hypothesize that if linkage disequilibrium occurs between traits involved with mating behaviors in hybrids and their descendants, differences between populations with respect to such mating behaviors may increase. This, in turn, can result in reproductive isolation over evolutionary time (Rieseberg 2001; Ayala and Coluzzi 2005; Hoffmann and Rieseberg 2008). Evidence for this is seen in the sympatric pair of fruit flies *Drosophila pseudoobscura* and *Drosophila persimilis.* These species are distinguished by chromosomal inversions associated with prezygotic and postzygotic isolation, and the hybrids show relatively low rates of recombination (Noor et al. 2001).

Reproductive Isolation via Dobzhansky–Muller Incompatibility

Dobzhansky and Muller independently developed an elegant conceptual model for how hybrid incompatibility, and hence postmating reproductive isolation, might evolve as a result of epistatic interactions among loci (Dobzhansky 1937; Muller 1942). Imagine an ancestral population, and consider two loci in members of this population. The population is fixed for allele A_1 at one locus and allele B_1 at the other locus (A_1B_1/A_1B_1). Suppose now that the ancestral population splits into two geographically isolated populations. In population 1, a mutation from A_1 to A_2 occurs. On the B_1 background, the A_2 allele is selectively favored and sweeps to fixation, so that individuals in this population have genotypes $A_2A_2\,B_1B_1$. In population 2, a mutation from B_1 to B_2 occurs. On the A_1 background, the B_2 allele is selected and goes to fixation. Thus, the populations are made up of A_1A_1 B_2B_2 genotypes (**Figure 14.30**).

If geographic barriers are removed and population 1 and population 2 can again interbreed, the hybrids will have a previously untested gene combination: A_2 with B_2. If there are negative epistatic interactions between the A and B loci, there may be fitness costs associated with this combination, despite the fitness advantages of having A_2 on a B_1 background or B_2 on an A_1 background. The hybrids will then be selected against. This, in turn, can select for mechanisms of reproductive isolation between the two populations. Furthermore, if additional substitution differences occur at other loci with similar patterns of epistasis—for example, an allele C_2 could replace C_1 in population 1, while D_2 could replace D_1 in population 2—the fitness costs of hybridization can be compounded.

Studies in the yeast *Saccharomyces cerevisiae* provide empirical support for the Dobzhansky–Muller model (Dettman et al. 2007; Anderson et al. 2010). Using the type of experimental evolution approach we described in Chapter 3, James Anderson and his team have been selecting for yeast strains that are well adapted

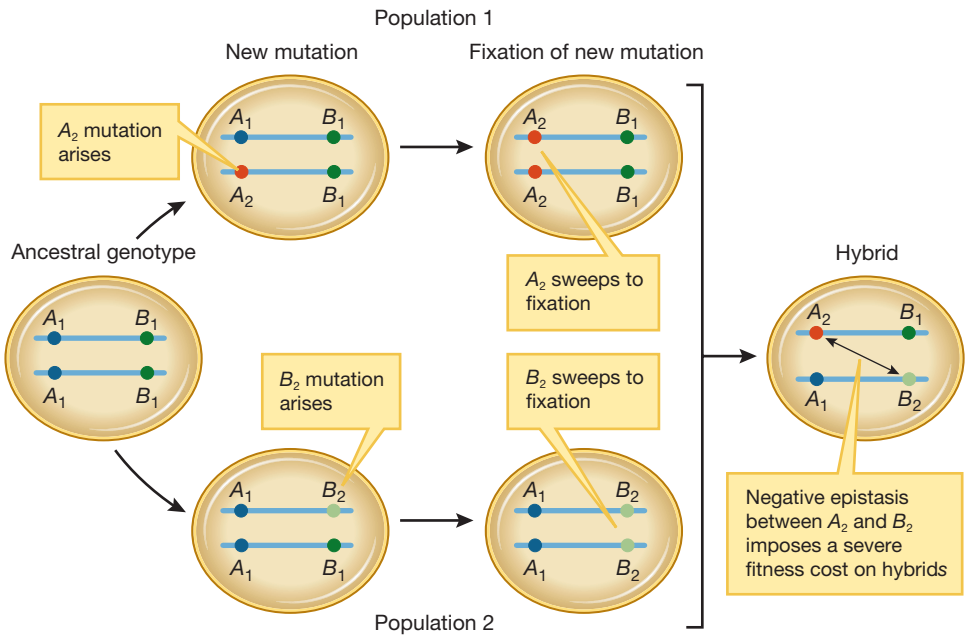


FIGURE 14.30 A schematic of the Dobzhansky–Muller model. Allele A_2 emerges and sweeps to fixation in population 1, while allele B_2 emerges and sweeps to fixation in population 2. Epistatic interactions between the A and B loci result in a fitness cost to individuals with both the A_2 and B_2 alleles. As a result, hybrids are selected against, driving reproductive isolation between individuals in population 1 and population 2. Adapted from Wu and Ting (2004).

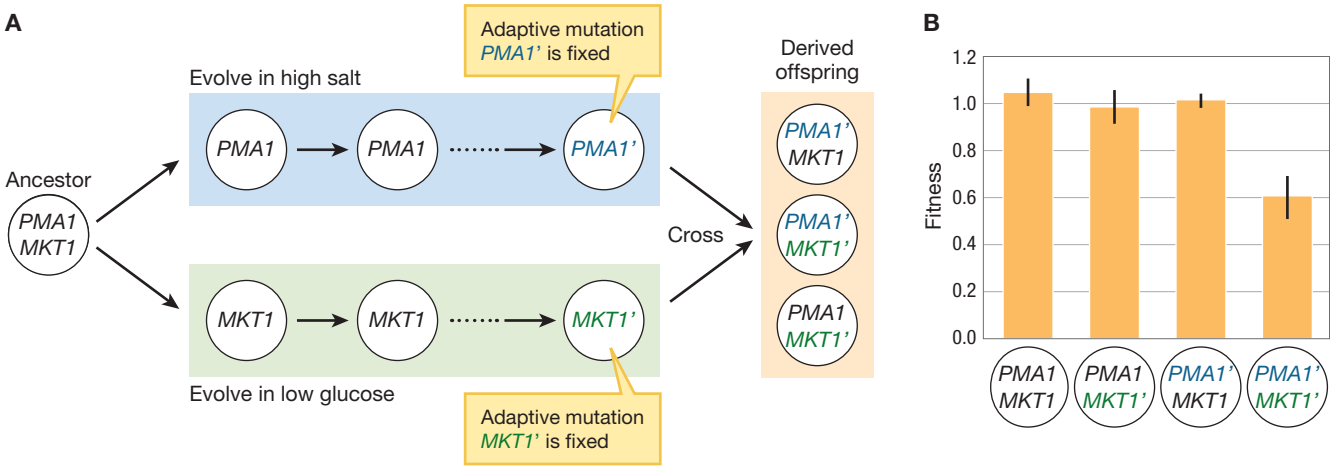


FIGURE 14.31 Dobzhansky-Muller incompatibility in yeast experimental evolution. (A) Starting from a common ancestor, two yeast populations were evolved in high salt and low glucose environments, respectively. The high salt population acquired one adaptive mutation at the *PMA1* locus, which we call *PMA1'*. The low glucose population acquired a different adaptive mutation, *MKT1'*, at a different locus *MKT1*. These populations were then crossed to produce derived offspring varying at the *PMA1* and *MKT1* loci. **(B)** Measured in a low-glucose environment, derived offspring carrying both new mutations had substantially lower fitness than offspring carrying neither or just one of the new mutations. Error bars indicate one standard error. Panel B adapted from Anderson et al. (2010).

to either high-salt or low-glucose environments. Molecular genetic analysis has documented many mutations that have occurred in populations in these high-salt and low-glucose environments. For example, a mutation in the proton efflux pump gene, *PMA1*, has positive effects on reproductive success in strains growing in the high-salt environment. In the low-glucose environment, a mutation in the *MKT1* gene, which regulates mitochondrial protein production, has positive effects on reproductive success. In these two different environments, *PMA1* and *MKT1* have diverged from ancestral strains used in the laboratory. Crossing the two evolved strains, Anderson and colleagues found that hybrids carrying derived alleles at both the *PMA1* and *MKT1* loci have reduced reproductive success relative to hybrids carrying neither derived allele or only one of the derived alleles (**Figure 14.30**). These observations reveal negative epistatic interactions of the sort predicted by the Dobzhansky–Muller model between the derived alleles at the *PMA1* and *MKT1* loci (**Figure 14.31**).

Haldane's Rule, Sex Chromosomes, and Reproductive Isolation

In the early part of the twentieth century, J. B. S. Haldane suggested that sex chromosomes may play a special role in the genetics of reproductive isolation (Haldane 1922). In discussing hybrids that are formed between incipient species, Haldane made an observation about hybrid viability and fertility that is now known as Haldane's rule. He noted that if among hybrid offspring "one sex is absent, rare, or sterile, that sex is the heterozygous one." By heterozygous—or what today we would call *heterogametic*—Haldane meant the sex that has two different sex chromosomes, as, for example, human males do with their XY sex chromosomes.

In mammals and fruit flies, males (XY) are heterogametic and females are homogametic, and so Haldane's rule predicts that if one sex formed from the hybridization of incipient species is at a fitness disadvantage, it will be males. In birds and butterflies, in contrast, females are heterogametic and males are

TABLE 14.3

Percent of Hybrids Conforming to Haldane's Rule

	STERILITY		INVIABILITY	
	# hybrids	% conforming	# hybrids	% conforming
Heterogametic males				
Drosophila sp.	114	98%	17	76%
Mammals	34	100%	5	100%
Heterogametic females				
Lepidopterans	18	94%	39	85%
Birds	27	89%	247	99%

Adapted from Coyne and Orr (2004) and Schilthuizen et al. (2011).

homogametic, and so female hybrids should be at a disadvantage. In terms of the genetic underpinnings of reproductive isolation, Haldane's rule suggests that the decreased fitness of the heterogametic hybrids is what creates the basis for reproductive isolation.

The evidence that has amassed over the past 75-plus years overwhelmingly supports Haldane's rule (Coyne and Orr 2004; Schilthuizen et al. 2011). Across birds, butterflies, mammals, and fruit flies, if one sex is absent, rare, or sterile, that sex is the heterogametic sex an impressive 97.6% of the time (**Table 14.3**). For example, black howler monkeys (*Alouatta caraya*) and southern brown howler monkeys (*Alouatta guariba clamitans*) sometimes live in mixed-species groups and occasionally hybridize. But of the hybrids that survive to adulthood, almost all are female—the homogametic sex (Aguiar et al. 2008).

But why is it the heterogametic hybrid sex that is at a disadvantage? While a number of theories have been put forth to explain this, what is known as the dominance theory is the most widely accepted (Turelli and Orr 1995). The idea behind the dominance theory is straightforward. Consider mammals, where males are the heterogametic (XY) sex. Suppose that alleles on the X chromosome from one parent species interact negatively with autosomes from the other parent species. If these problematic alleles on the X chromosome are recessive, their effects will be present in males (which have an X chromosome from only one parent species) but not in females (which have an X chromosome from each parent species). Thus males will suffer greater fitness consequences than females. A similar argument can be made for why females fare poorly when they are the heterogametic sex, as in birds and butterflies.

Evolutionary biologists today are just as interested in "the species problem" and the process of speciation as Darwin was when he published *On the Origin of Species.* While Darwin laid out many of the questions to be addressed and provided some of the answers to these questions, extensive progress has been made over the past 150-plus years. In this chapter, we have touched on some of the major advances that have been made in the study of speciation. With a clearer understanding of how evolutionary biologists conceptualize what a species is and how speciation occurs, we now move on to the topic of extinction.

SUMMARY

1. Evolutionary biologists have long struggled with how to classify organisms. What makes for a species? How different do two groups have to be before they are considered different species?
2. The evolutionary species concept provides an answer to what a species fundamentally *is*: a set of populations with their own distinct evolutionary history and a shared future evolutionary fate.
3. The phenetic species concept, the biological species concept, the ecological species concept, and the phylogenetic species concept each provide different diagnostic criteria for how species boundaries can be drawn in practice.
4. Major models of speciation include allopatric, parapatric, and sympatric speciation. Allopatric speciation includes a vicariance model of speciation and a peripheral isolate model.
5. Ring species live in a series of populations that are connected to one another in a ringlike fashion. In ring species, we expect gene flow across adjacent populations, but gene flow between populations that are not adjacent should be minimal and decrease as a function of distance.
6. Evolutionary biologists have identified and studied many types of prezygotic and postzygotic reproductive isolating mechanisms; that is, mechanisms that restrict or prevent gene flow.
7. Work on the genetics of speciation includes studies of reproductive isolation via changes in chromosome number, reproductive isolation via chromosomal rearrangement, reproductive isolation via Dobzhansky–Muller incompatibility, and reproductive isolation via Haldane's rule.

KEY TERMS

allopatric speciation (p. 495)
biological species concept (p. 491)
cline (p. 499)
ecological species concept (p. 492)
evolutionary species concept (p. 489)
hybrid zone (p. 499)
parapatric speciation (p. 495)
peripheral isolate model (p. 496)
phenetic species concept (p. 490)
phylogenetic species concept (p. 493)
postzygotic isolating mechanisms (p. 509)
prezygotic isolating mechanisms (p. 509)
reproductive character displacement (p. 508)
reproductive isolating mechanisms (p. 495)
ring species (p. 502)
secondary reinforcement (p. 508)
speciation (p. 495)
sympatric speciation (p. 495)
vicariance model (p. 496)

REVIEW QUESTIONS

1. What four species concepts can be used to delineate species boundaries in practice?
2. What is the evolutionary species concept?
3. What is the key measure used to determine species boundaries under the biological species concept?
4. How does the phylogenetic species concept distinguish between species?
5. What characteristic is common to both the vicariance and peripheral isolate models of allopatric speciation?
6. What is the major conceptual hurdle associated with sympatric speciation?
7. What is a cline?
8. What are the two most general categories of reproductive isolating mechanisms?
9. What is secondary reinforcement during the process of speciation, and how can it be detected in nature?
10. What is Haldane's rule?

KEY CONCEPT APPLICATION QUESTIONS

11. Why is it important for evolutionary biologists to separate the question "What is a species?" from the question "How can we distinguish among species in nature?"
12. What might be one conservation biology implication of the phylogenetic species concept's tendency to produce more species than that by other species concepts?
13. The Bdelloid rotifers are a clade of largely asexual invertebrate species.
    a. Name one species concept/definition that would *not* be suitable for identifying species boundaries among the Bdelloid rotifers, and briefly explain why it would not work.
    b. Name one species concept/definition that would be suitable for identifying species boundaries among this group, and briefly explain why it would work.
14. For a given locus, which species is likely to have the more recent coalescent time: a progenitor species or a derivative species? Explain.
15. Explain how linkage disequilibrium can contribute to reproductive isolation.

SUGGESTED READINGS

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15

Extinction and Evolutionary Trends

◀ Dead camelthorn trees (*Acacia erioloba*) in the Namib-Naukluft National Park in Namibia.

Over the course of four decades in the mid to late eighteenth century, the world's leading naturalist of the day, Count Buffon (Georges-Louis LeClerc), published his massive natural history encyclopedia, *Histoire Naturelle* (Buffon 1749–1804). *Histoire Naturelle* was a huge undertaking—Buffon wanted to provide readers with "the exact description and the true history of each thing," and it revolutionized the field of natural history. Unfortunately, in places in his text, Buffon made some rather sweeping, unsubstantiated claims. One such claim was that all life in the New World—the Americas—was small, weak, and feeble compared to life in the Old World (Gerbi 1973; Dugatkin 2009b). Not surprisingly, many in the New World disagreed, and the protests were spearheaded by none other than Thomas Jefferson.

One major area of contention in the argument between Buffon and Jefferson was extinction: not its cause, but whether it ever took place at all. The very notion that animals could go extinct was a matter of heated debate in the eighteenth century (and even in the early part of the nineteenth century). Buffon and Jefferson sparred over whether mammoths

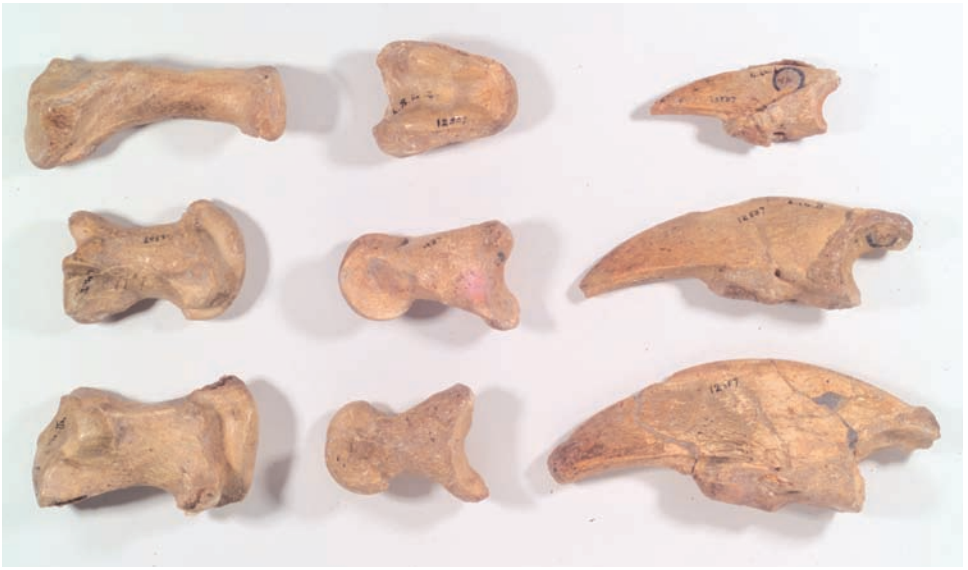


FIGURE 15.1 Jefferson's ground sloth (*Megalonyx jeffersonii*). Fossil remains **(A)** and artist's reconstruction **(B)** of Jefferson's ground sloth (*Megalonyx jeffersonii*). Thomas Jefferson thought that the bones were likely those of a lion-like creature that still existed in the unexplored area of the western United States. Subsequent work found the remains to be of a giant sloth that has been extinct since the most recent ice age, approximately 11,000 years ago.

and mastodons, known from fossil remains, were extinct. In volume 9 of *Histoire Naturelle*, Buffon—the man who would mentor Jean-Baptiste Lamarck (Chapter 2)—wrote of his "astonishment" at the size of these creatures that "no longer exist[ed]" and asked, more generally, "how many smaller, weaker, and less remarkable species must likewise have perished, without leaving any evidence of their past existence?"

For his part, Jefferson thought such creatures still roamed the unexplored West of the fledgling United States. In a speech he gave to the American Philosophical Society, Jefferson noted that "In the present interior of our continent there is surely space and range enough for . . . mammoths . . . who may subsist there" (Jefferson 1797). When Jefferson eventually sent out the Lewis and Clark expedition, he told them to look for mammoths, mastodons, and even *Megalonyx* ("giant claw"), an animal that Jefferson initially thought was a lion-like creature. We now know that *Megalonyx* was a giant sloth, not a lion, and went extinct about 11,000 years ago (Kurtén and Anderson 1980; Schubert et al. 2004) (**Figure 15.1**).

Jefferson was certain that these giant creatures roamed the unexplored West. *They had to*, not only because Jefferson believed the Native American legends he had heard of encounters with these behemoths, but also because he believed that extinction was impossible. Jefferson had come to think that extinction was

incommensurate with nature's laws. "Such is the economy of nature," Jefferson wrote, "that no instance can be produced of her having permitted any one race of her animals to become extinct" (Jefferson 1785). Extinction would be evidence of imperfection, and Jefferson's deist views held no room for imperfection. He was not alone: At the time, many philosophers, religious leaders, and even scientists (though that word was not used then) ruled out the possibility of extinction, as this would suggest a less-than-perfect world, and hence an imperfect supernatural creator of that world.

Over the course of the next hundred years, the evidence for extinction became so abundant that such arguments over "imperfection" were placed on the scrap heap of history. Today, evolutionary biologists and paleontologists study extinction using the same tools we use to study any biological phenomenon. Indeed, we can even address, in detail, the extinctions that Buffon and Jefferson debated. More than that, we can generate and test hypotheses regarding why these extinctions occurred.

Fossil evidence suggests that 50,000 years ago, individuals from more than 150 genera of megafauna (large animals) roamed the Earth—short-faced bears weighing 2500 pounds, saber-toothed cats, 500-pound kangaroos, and the giant ground sloths, mammoths, and mastodons of which Jefferson and Buffon wrote, to name just a few (**Figure 15.2**). But, by 10,000 years ago, two-thirds of the 150 genera that were present just 40,000 years earlier had gone extinct in what is known as the Pleistocene megafauna extinction (Barnosky et al. 2004). They were gone forever, leaving only fossil remains—and on rare occasion, a well-preserved frozen carcass—to alert us to the fact that they had ever existed.

What caused these extinctions? Evolutionary biologists continue to pursue this question, putting forward a number of possible explanations for the megafauna extinction. The evidence on islands, for example, strongly suggests that intense hunting by humans as well as less direct human effects, such as fire, habitat fragmentation, and the introduction of exotic species, played a large role in the Pleistocene megafauna extinction (Martin and Kelin 1984; MacPhee 1999).

The disappearance of megafauna on continents is less well understood. Dramatic changes in average temperature in certain regions are correlated with many of these extinctions. For example, during an ice age about 18,000 years ago, temperatures were 2°C to 5°C colder than modern temperatures at low altitudes and 10°C to 20°C colder at higher latitudes and altitudes (Kutzbach et al. 1998). These temperature changes would have had direct and indirect effects on survival

FIGURE 15.2 Pleistocene megafauna. A few examples of the megafauna that existed 50,000 years ago. **(A)** Giant short-faced kangaroo (*Procoptodon goliah*). **(B)** American mastodon (*Mammut americanum*). **(C)** Saber-toothed cat (*Smilodon gracilis*).

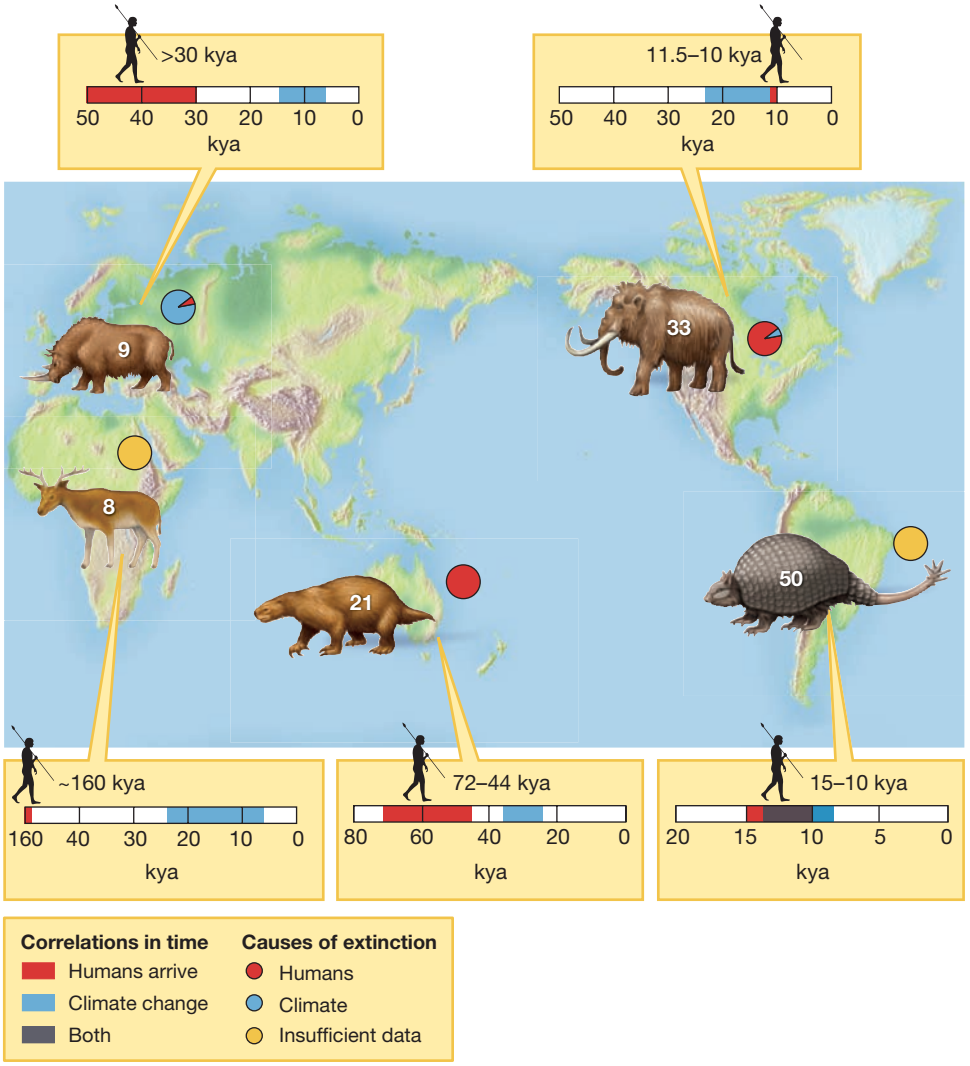


FIGURE 15.3 Map of Pleistocene megafauna extinction. Pleistocene megafauna extinction on each continent with reference to the timing of human arrival and climate change (kya = thousands of years ago; the last ice age ended 11.7 kya). Dates above each timeline refer to the arrival of humans. The number of extinct genera for each continent is listed on each megafauna icon. Colored circles indicate the causes of extinction. The blue circle with a red slice indicates mostly climate change with some human influence; the red circle with a blue slice indicates the converse. Adapted from Barnosky et al. (2004).

by changing the food chain, and hence the diet of megafauna. As on islands, human hunting again seems to have played a role in some extinctions. Archaeological remains suggest that human hunters in this period had superior weapons compared to those of their predecessors (Bar-Yosef 2002). Human hunting and climate change are not mutually exclusive explanations. Indeed, while the most recent evidence strongly links humans to the extinction of Pleistocene megafauna on islands, it may be that intense human hunting precipitated extinction in Pleistocene megafauna that were already on the decline as a result of environmental change (**Figure 15.3**) (Barnosky et al. 2004; Koch and Barnosky 2006; Sandom et al. 2014).

KEYCONCEPT QUESTION

15.1 What sorts of evidence do you think could be used to infer that human hunting was a factor in the extinction of Pleistocene megafauna?

More work is needed to better understand the precise causes of the Pleistocene megafauna extinction. But one thing is certain: These species are gone forever. All of the exquisite adaptations that natural selection produced in those creatures over millions of years, all of the unique genetic variation that they possessed, all of this is lost. What's more, all of the complex and often subtle ways that these species affected and interacted with the biotic communities in which they lived are gone as well (Johnson 2009).

As we will see, many biotic and abiotic factors have been responsible for extinction over the past 600 million years. In this chapter, we will examine how evolutionary biologists address the following questions:

- What is background extinction and how does it differ from mass extinction?
- How do processes such as competition, predation, host–parasite interactions, and even the impact of asteroids sometimes lead to the extinction of species and contribute to shaping the diversity of life?
- How does a better understanding of extinction inform us about large-scale changes in the history of life—massive pruning of the tree of life and the subsequent growth of newer, often quite different, branches?
- Are there certain attributes of a species or taxa that make them more or less prone to going extinct?
- Is the rate of evolutionary change steady and gradual or punctuated by periods of rapid change?
- Are there evolutionary "trends" both at the microevolutionary and macroevolutionary scales? If so, what are they, and what explains them?

15.1 The Concept of Extinction

When we say that a species has gone extinct, we mean that all individuals in that species have died out and left no living descendants. If all species in a genus are extinct, then that genus is extinct, and similarly for all genera in a family, and so on. Today, we know that most species that have ever lived have gone extinct.

When we study extinction as a process that is occurring right now, in contemporary times, we can search for the last living representatives of a species. When we look at extinction in evolutionary time, we most often must use evidence from the fossil record to determine if, when, and how a species has gone extinct.

Extinction and Phylogenetic History

As we attempt to slow the rate of *anthropogenic* (human-caused) extinctions, we often have to make difficult choices about which species and habitats to save. Historically, conservation biologists have tried to preserve the largest total number of species, but recently some experts have argued that instead we should try to maximize a quantity known as phylogenetic diversity (Faith 1992; Rolland et al. 2012; Davies et al. 2013; Diniz et al. 2013; Winter et al. 2013). Phylogenetic diversity is a measure of the total length of the branches of a phylogenetic tree.

FIGURE 15.4 The tuatara *Sphenodon guntheri*. The tuatara resembles a lizard superficially, but it is not a lizard. Rather, the tuatara is a representative of a deep phylogenetic lineage parallel to the squamate reptiles (lizards and snakes).

If one aims to preserve phylogenetic diversity in some clade, then loss of a deep branch of the tree for that clade is a much worse outcome than loss of one or even several very shallow branches.

The endangered tuatara provides a striking example (May 1990; Vane-Wright et al. 1991) (**Figure 15.4**). Superficially, tuataras look rather like iguanas or any number of other lizard species, but phylogenetically they are very different. The two living species of tuataras diverged from the snakes and lizards—their closest living relatives—more than 220 million years ago (Hay et al. 2008). If these two species go extinct, a huge loss in phylogenetic diversity will result. Almost everywhere on this tree, loss of a pair of sister species would correspond to only a tiny snip off the smallest branch tips of the tree of life. But if the two tuatara species were lost, this would be a devastating cut at the base of the tree. An entire order, Rhynchocephalia, would disappear (**Figure 15.5**). We would irreversibly lose the deepest branch on this phylogeny, and with it a piece of evolutionary history older than the divergence of snakes from other squamate groups.

Above and beyond its role in decisions relating to human activity and extinction, the notion of phylogenetic diversity highlights a fact: Not all extinctions are equal with respect to how they affect phylogenetic

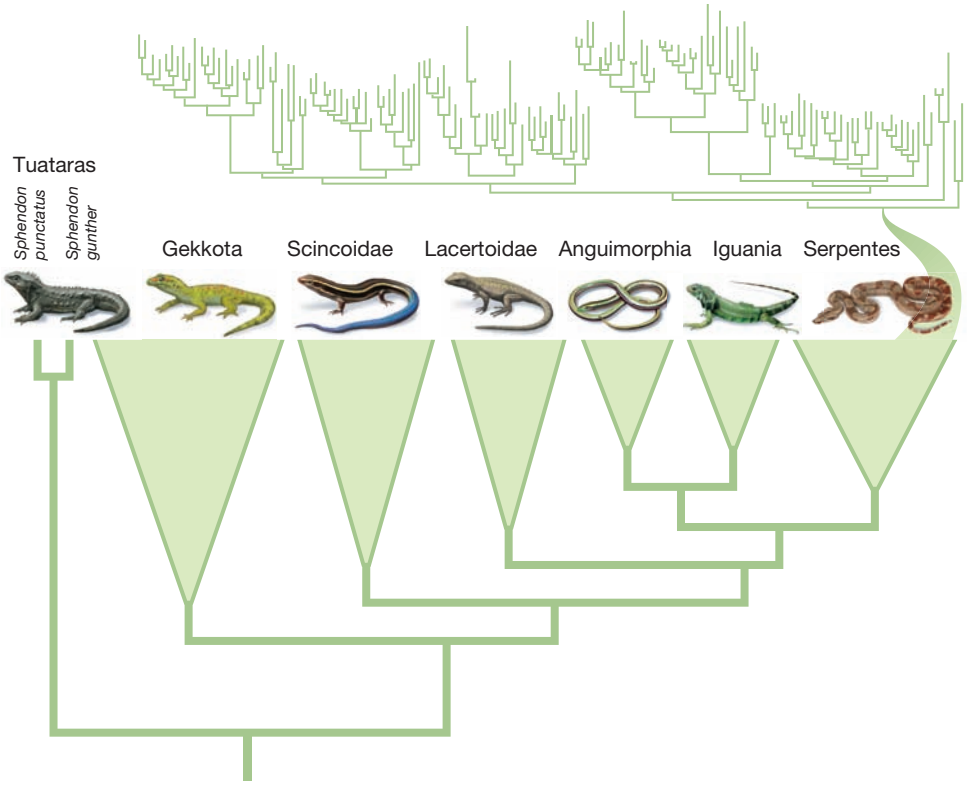


FIGURE 15.5 Extinctions vary greatly in their effect on phylogenetic history. This figure depicts a phylogeny of the Lepidosauria—squamate reptiles and tuataras—based on a phylogeny constructed by Pyron et al. (2013). The fine-scale phylogeny at top represents a small portion of the total Serpentes phylogeny. If two sister species of snake from this species-rich portion of the phylogeny were lost to extinction, this would prune the tree only slightly and would cause only a minimal loss of phylogenetic history. But if the two species of tuatara were to go extinct, this loss would prune away the deep branch at the left of the figure, and a piece of phylogenetic history dating back to well before the divergence of snakes and lizards would be lost forever.

history (Erwin 2008; Purvis 2008). For example, in a 1997 study, Sean Nee and Robert May ran computer simulations of large-scale extinction events. They found that in a clade that has been growing exponentially in size, about half of the evolutionary history—measured as phylogenetic branch length—is lost in a mass extinction that kills 80% of all species. But in a clade with many members that has been about the same size for a long time, the losses are less severe: Even in a mass extinction that eliminates 95% of all species, 80% of the evolutionary history of the tree can be preserved (Nee and May 1997).

Extinctions and the Fossil Record

Fossil evidence is the key to understanding the history of extinction on Earth. Paleontologists define a **fossil** as the remains or traces of a past-living organism, and the term is usually reserved for remains or traces that are more than 10,000 years old (Prothero 2003; Larsen 2008). Fossil remains of living organisms are slowly transformed over time into rock. Minerals such as calcium and phosphorus, once part of a living organism, are slowly replaced by minerals such as iron and silica (on occasion, organisms are fossilized within a gooey tree resin called amber) (**Figure 15.6**). Fossils vary in the extent to which this replacement process has occurred: The longer the fossilization process has been going on, the more rocklike the fossil. In fossils formed fairly recently, bones, skin, and even remains in the organism's digestive tract are sometimes uncovered. In some instances, the biochemical substances, including DNA, can be extracted and analyzed.

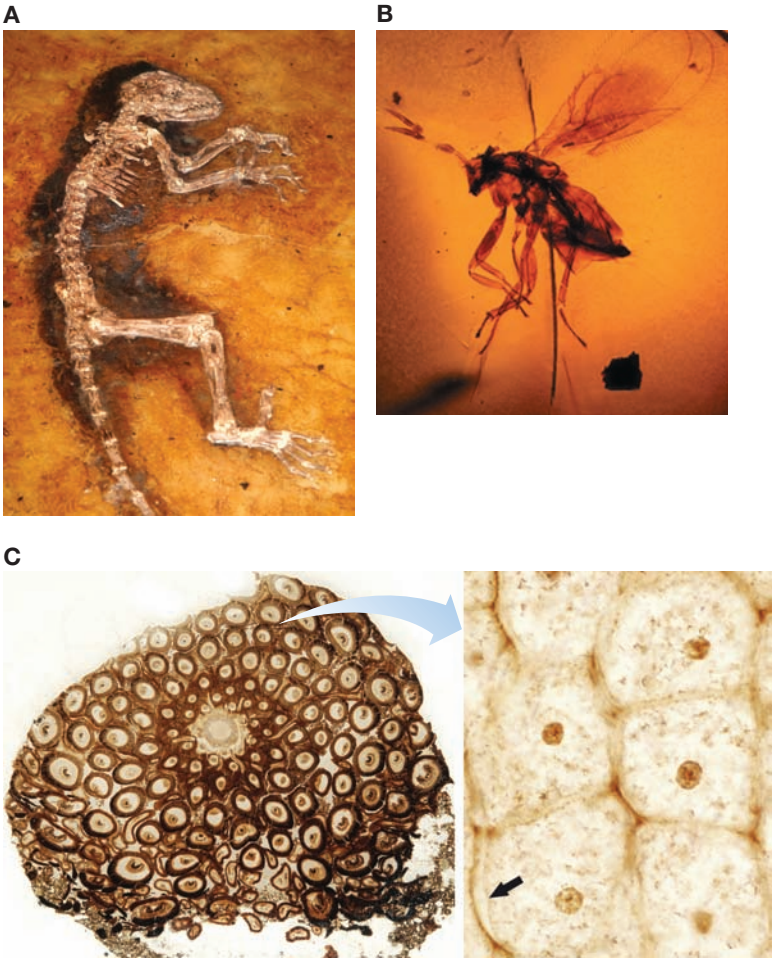
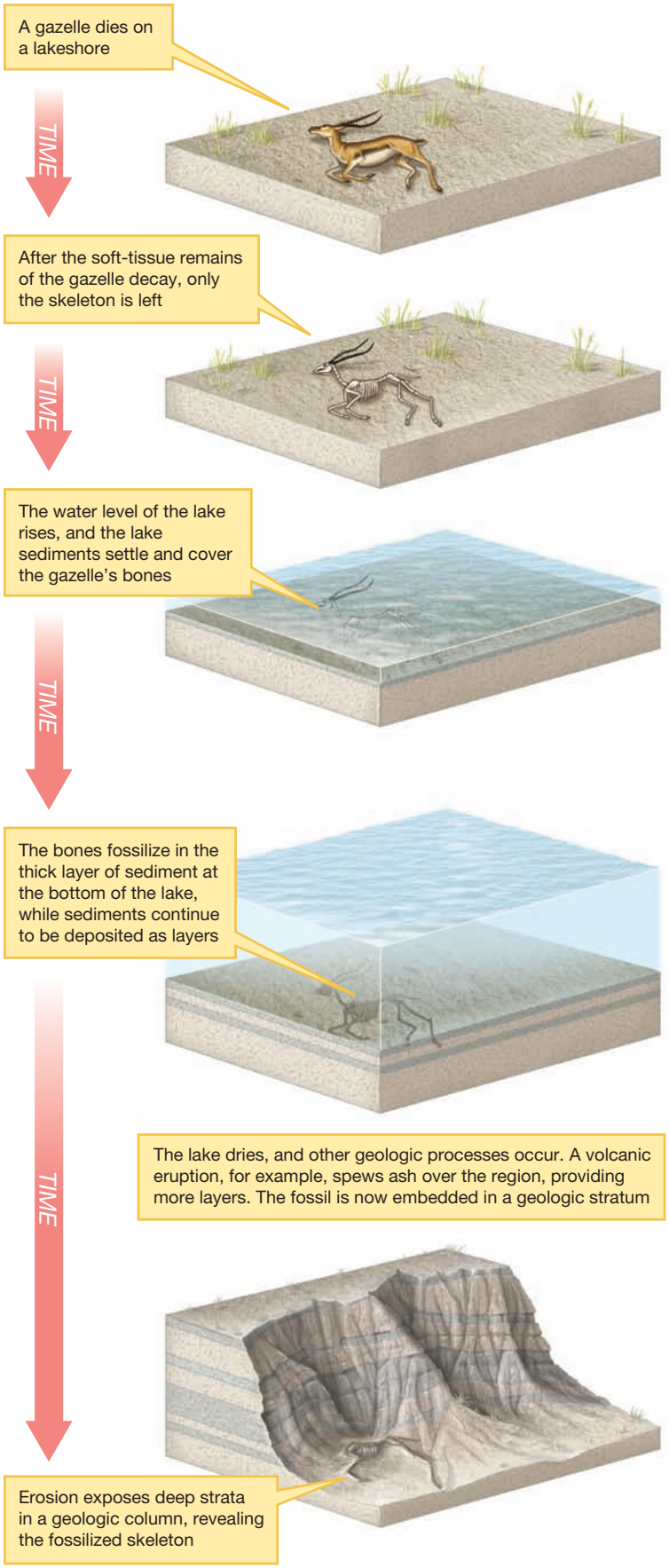


FIGURE 15.6 Fossils. **(A)** The beautifully fossilized remains of *Darwinius masillae*, a primate species found recently in Messel, Germany. This fossil is approximately 47 million years old. **(B)** A fossil of a hymenopteran insect in amber found in Ethiopia. This fossil has been dated at approximately 95 million years old. **(C)** Cytological cross section of the rhizome (left) of a well-preserved, 180-million-year-old royal fern (Osmundaceae): A magnification (right) shows cells with cell membranes (black arrow), cytoplasm, and nuclei in the interphase stage. Of course, most fossilized remains are not nearly as complete and detailed as the examples shown here.

Organic remains that have been fossilized into rock are not the only way to tap into what we call the **fossil record**, by which we mean the history of life on Earth as recorded by fossil evidence. Sometimes, water seeps into fossils and breaks down a fossil that has formed (this is referred to as dissolution), but the *shape* of the fossil is preserved in the sediment around it, providing a rough outline of the organism. In other cases, organisms fossilize as layers of thin carbon spread on sandstone and shale (this is referred to as **carbonization**), a process that is particularly common with plants (**Figure 15.7**). Through geological analyses of oxygen content, acidity,

FIGURE 15.7 Carbon-layer plant fossil. A flower of *Porana oeningensis* fossilized as a thin carbon layer on rock.



and other properties of fossils and their surrounding substrates, we can also use the fossil record to gain knowledge about the environment that organisms lived in—and hence the selective conditions they faced.

The process of fossilization requires highly specific conditions (**Figure 15.8**). A dead organism, or at least parts of it, must be buried—often by soil deposited by flowing water or by volcanic ash—and the remains must stay in an anoxic (oxygen-free) environment. Although fossilization can occur in many types of rock, it most often occurs in sedimentary rocks (such as chalk, limestone, sandstone, and shale), which make up only about 5% of all rocks and occur mostly as a thin layer on Earth's surface.

Even when geological and abiotic conditions are right for fossilization to occur, many factors can disrupt the process. When an organism dies in areas conducive to fossilization, predators and scavengers often leave little of the organism behind. Soft tissues rarely remain long enough to fossilize, which is why so much of what we see in the animal fossil record consists of hard substances that were once teeth, bones, shells, exoskeletons, and so on. Wind, water currents, and other abiotic processes break down even those parts of an organism that could fossilize. It is not surprising, then, that paleontologists typically find evidence of only a single bone, tooth, shell, or exoskeleton of an organism, and they rarely find anything as complete as what we see in Figure 15.6. On occasion, however, much more complete fossils—multiple bones, even full skeletons—are uncovered. On even rarer occasions, geological and biotic conditions in the past have, by chance, been such that huge numbers of fossils are found together—these are referred to as *Lagerstatten*. Examples include the exquisite fossils of the Ediacaran period from 635 million to 541 million years ago—fossils that tell of a huge burst of new multicellular organisms—and the Burgess Shale fossils dating from about 520 million years ago.

FIGURE 15.8 The process of fossilization. Fossilization can occur in many ways. Here, a dead gazelle lies on the shore. Soft tissues quickly decay, and only skeletal remains are left. After the water level rises, sediments settle on the remains of the gazelle, producing anoxic conditions needed for fossilization. Adapted from Larsen (2008).

Lagerstatten have also been found in the relatively recently formed La Brea tar pits in California, where paleontologists have attempted to extract DNA from the fossil remains of a Columbian mammoth (*Mammuthus columbi*) that was trapped for tens of thousands of years in the natural tar that has seeped up to the surface (Akersten et al. 1983; Xiao and Laflamme 2009; Gold et al. 2014) (**Figures 15.9** and **15.10**).

Paleontologists use many factors when deciding where to search for fossils of the organisms they study. Take the case of *Tiktaalik roseae*, which we discussed in Chapter 5 (Daeschler et al. 2006; Shubin et al. 2006). Researchers chose the Canadian Arctic region of Ellesmere Island in part because geological data suggest that during the Devonian period 375 million years ago, the area was subtropical and replete with the shallow stream systems that evolutionary biologists hypothesized would be associated with the transition from water-based to land-based life. Serendipity also played a role here. Not long after the researchers began work on Ellesmere Island, they found a *Tiktaalik* fossil skull literally jutting out of a stone on an icy bluff. Naturally, they then intensified their search as a result. And, of course, while paleontologists are searching for fossil evidence from one species, they may come upon fossilized remains of other species. Indeed, rather than a particular species being the focus of a paleontological excavation, an area is often targeted as a potential hotbed of fossil remains.

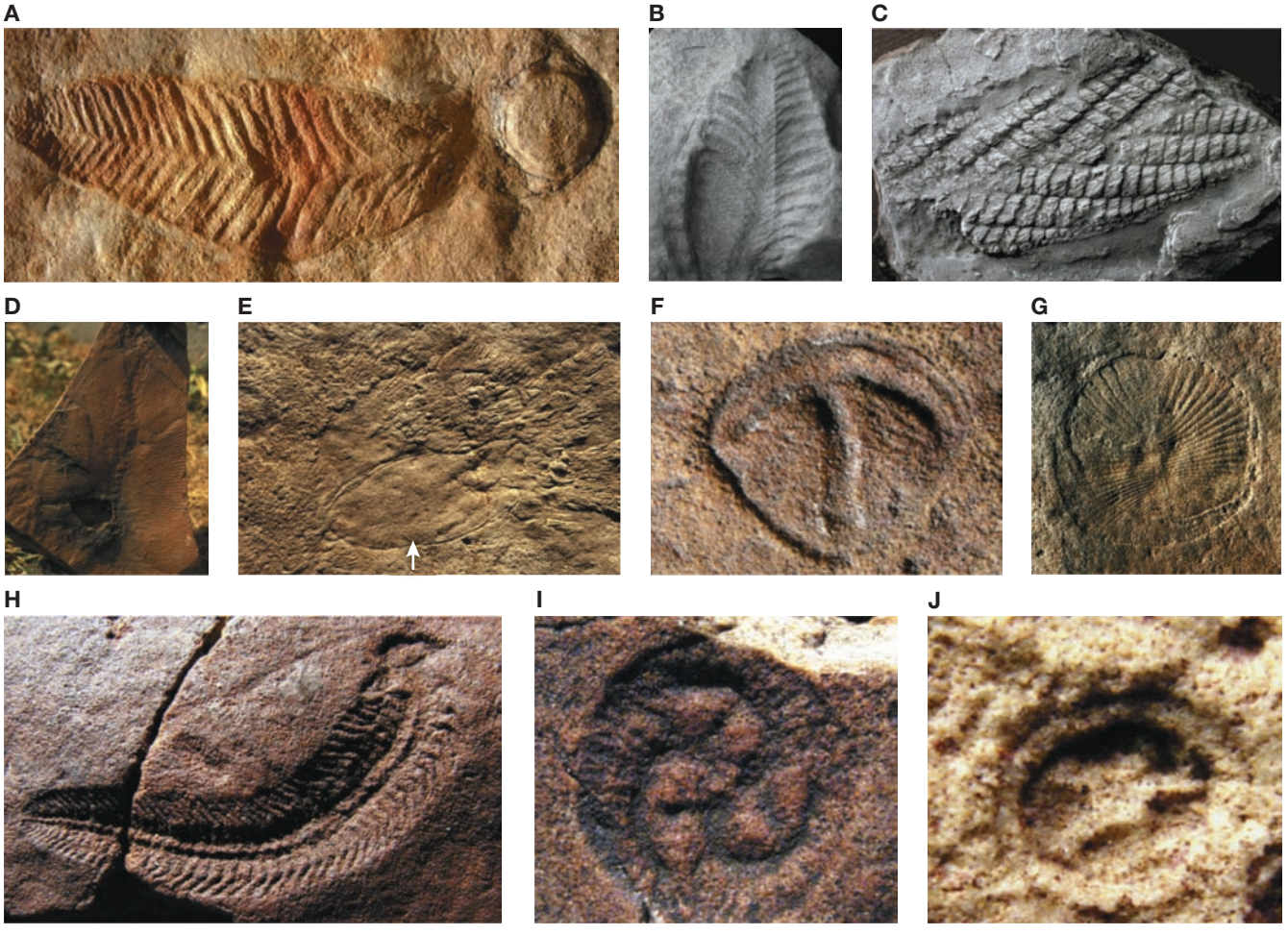


FIGURE 15.9 Ediacaran fossils. Some of the body plans represented in the Ediacaran biota. **(A)** *Charniodiscus* frond. **(B)** *Rangea*. **(C)** *Charnia* frond. **(D)** *Swartpuntia* frond. **(E)** *Kimberella* (white arrow). **(F)** *Parvancorina*. **(G)** *Dickinsonia*. **(H)** *Spriggina*. **(I)** *Tribrachidium*. **(J)** *Arkarua*.

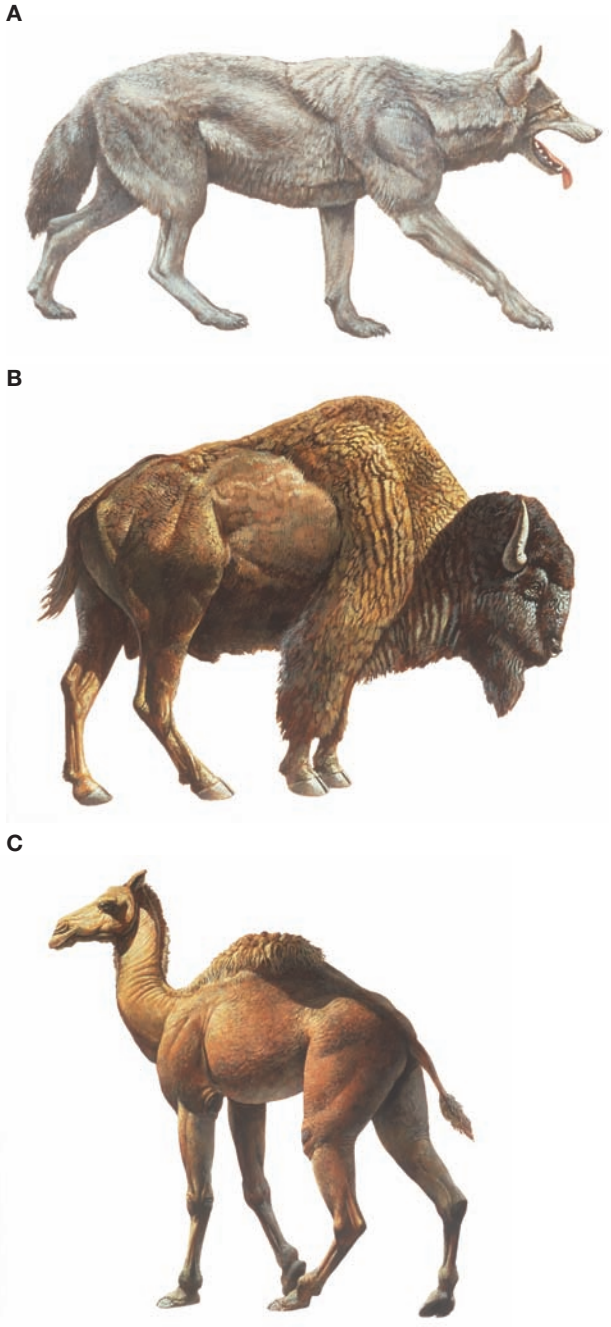


FIGURE 15.10 Animals from the La Brea tar pits. Fossilized remains of **(A)** extinct dire wolves (*Canis dirus*), **(B)** ancient bison (*Bison antiquus*), and **(C)** extinct camels (*Camelops hesternus*) have been found in the La Brea tar pits.

Some factors that paleontologists use when choosing sites include the following:

1. Paleontologists focus on the sites that best match the geological and abiotic conditions in which fossilization is likely to have occurred; for example, in areas where volcanoes have erupted in the past. In addition, they search for sites that have been sufficiently weathered to expose fossils.
2. In most instances, a paleontologist is not the first researcher to be searching for fossils from his or her organism of interest. In such cases, researchers often begin at or near sites where others—be they other professional paleontologists, amateur paleontologists, or people indigenous to the area—have already uncovered related fossils.
3. Predictions derived from phylogenetic reconstruction, biogeography, and/or molecular genetics might guide paleontologists to a particular area; for example, a site with rocks of a particular geological age or one where ancestral species of interest may have lived.

These three factors are not mutually exclusive, and they often work in concert. In many cases, it is a combination of all three of these factors that leads paleontologists to choose their sites for excavating the fossil record.

Paleontologists have many techniques for determining the age of a fossil. Some of these techniques provide a measure of relative age, and others provide a measure of absolute age. An example of the way relative age is gauged is the **law of superposition**, which states that fossils found lower down in the sediment at a particular locality are older than those found closer to the surface. Certain types of chemical dating also provide information on the relative age of a fossil. For example, fluorine is found in some types of soil, and it builds up in bone remains as they fossilize. At a given site, the older a bone is, the more fluorine it will have in it.

To estimate the absolute age of a fossil, paleontologists use techniques such as **radiocarbon dating** and **radiopotassium dating**. In 1949, Willard Libby found that one form of carbon, isotope carbon-14 (^{14}C), decays into nitrogen-14 (^{14}N), at a constant rate. Every 5730 years, half of the ^{14}C in a substance will decay into ^{14}N. This rate of decay is known as the **half-life** of an element. All living plants and animals absorb small amounts of ^{14}C from Earth's atmosphere, fixing it directly by photosynthesis or acquiring it through the food chain. Once an organism dies, however, the intake of ^{14}C ceases. Carbon-14 then begins to decay, and so, knowing that the half-life of ^{14}C is 5730 years, we can measure the age of a fossil by looking at the $^{14}C/^{14}N$ ratio in its remains. Because

^{14}C has a short half-life, radiocarbon dating is a useful tool for measuring absolute age for about 50,000–75,000 years into the past; after this point, there is usually not enough ^{14}C remaining to use the technique.

The half-life of other elements can also be used to date fossils. Potassium-40 (^{40}K) has a very long half-life of approximately 1.3 billion years. Paleontologists also use the half-life of elements such as uranium-235 (which decays to lead-207 with a half-life of 700 million years) to date fossil beds and the fossils within them. Many fossils are found in sedimentary rock, which can directly be dated using radiocarbon dating (if organic material is incorporated in the rock), and the age of a fossil in sedimentary rock older than 50,000–75,000 years can be estimated by dating the igneous rock layers above and below it.

Paleomagnetic dating can estimate the age of a fossil by measurement of changes in Earth's magnetic field (the position of magnetic north). Over the course of Earth's history, the magnetic polarity of the planet has flipped on numerous occasions. Crystals of magnetic minerals in Earth's crust align with the existing magnetic field on Earth when they form or when they settle out of water into sediments. By measuring the alignment of magnetic minerals in nearby layers and/or in the substrate in which a fossil was found (and thus the polarity of Earth's magnetic field when the rock layers formed), paleontologists can estimate a relative date for that fossil.

Finding high-quality fossilized remains is grueling and painstaking work. Despite the fact that evolutionary biologists and paleontologists have developed hypotheses for where it is best to search for fossils, and despite the fact that we now have good techniques for dating strata (layers of rock) in Earth's surface, the last fossilized remains of an organism that we find will not be from the last actual survivor of that species. And the further back in time we go, the fewer the fossils that can be recovered. This time lag between the last known fossil and actual extinction is called the **Signor–Lipps effect**, named after Jere Signor and Philip Lipps, who developed this idea (Signor and Lipps 1982). The Signor–Lipps effect is a form of *backward smearing*: Its effect is to make us date an extinction earlier than it actually occurred.

Another problem associated with dating extinction from the fossil record is called *forward smearing*, which causes us to date an extinction later than it actually occurred. A common cause of forward smearing is the fact that burrowing animals move fossilized remains up through layers of earth and distort the fossil record. Worms and shrimp, for example, stir up sand and sediment, and, in so doing, they push fossilized remains into a strata that is more recent than the one in which the now-extinct species perished. This then makes it appear that an extinct species shows fossilized remains well after its extinction. One way that paleontologists minimize forward smearing effects is to search for evidence of extensive burrowing in the strata from which they obtain their fossils (Jin et al. 2000).

Magnitude of Extinction: Background Extinction versus Mass Extinction

The fossil record shows that rates of extinction vary over time, and that extinction rates sometimes spike in what are referred to as **mass extinctions**. When extinction occurs outside a period of mass extinction, it is referred to as part of **background extinction**.

Although there is no hard-and-fast definition adopted by all evolutionary biologists, a mass extinction usually refers to a series of events that causes large-scale loss—at least 50% to 75% of all species in many major taxa—over a broad geographic range (Benton 2003a; Barnosky et al. 2011). Depending on how exactly mass extinction is defined, we have evidence for at least five and perhaps as many as eight mass extinctions over the course of the past 600 million years. Some of these extinctions wiped out 90% of all species alive at the time. But mass extinctions are few and far between, and of all the extinctions that have ever occurred, about 95% have *not* been associated with a mass extinction; rather, they represent background extinction (Raup 1986, 1992; Pimm et al. 1995). And so it is with background extinction that we begin to delve more closely into the phenomenon of the extinction of species.

KEYCONCEPT QUESTION

15.2 How might the Signor–Lipps effect mask the occurrence of a large-scale or even mass extinction?

15.2 Background Extinction

Our discussion of background extinction will focus on testing hypotheses about extinctions caused by predation, competition, disease, and climate change. Many of our examples involve species that are **endemic**; that is, native to only one area (**Figure 15.11**). The reasons for focusing on endemic species are (1) extinctions are

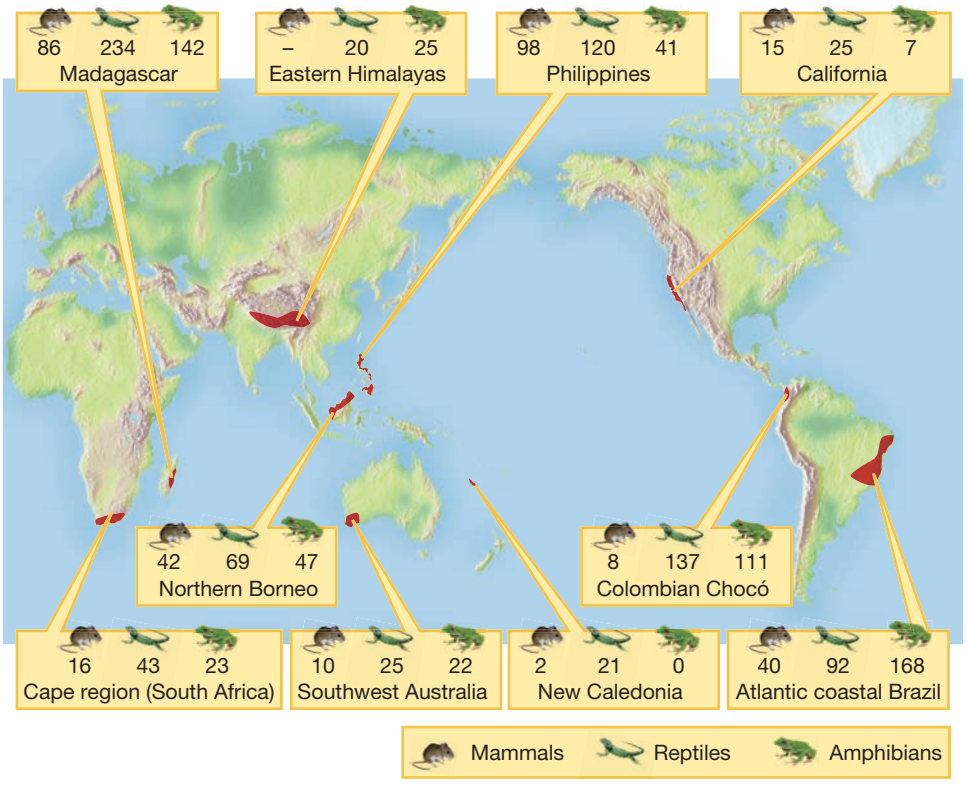


FIGURE 15.11 Endemic hot spots. Endemic hot spots for vertebrates around the world. The number of endemic mammal, reptile, and amphibian species in each location is indicated. Adapted from MHHE (2010) and based on data from Myers (1988) and World Conservation Monitoring Center (1992).

common in such species (IUCN 2001; Jansson 2003), and (2) it is much easier to study extinction in endemic species because local extinction becomes synonymous with global extinction.

Extinction and Predation

Populations face many threats, including predation. As a result, many organisms show both morphological and behavioral antipredator adaptations. For example, squirrels will often mob a snake predator, biting and harassing it until the snake is forced to leave the area (Owings and Coss 1977; Coss and Owings 1985; Coss 1991). Their antipredator behavior also includes kicking dirt and rocks at predators, as well as emitting alarm calls that specifically signal that snakes, as opposed to other predators, are present (Owings and Leger 1980) (**Figure 15.12**).

Natural selection operates not only on prey to favor behaviors that help them avoid predators but also on predators, improving the ability of predators to capture their prey. We will examine this sort of *coevolutionary arms race* further in Chapter 18. For now, our point is that natural selection can favor traits in predators that make them very efficient at capturing their prey. Such efficient foraging behavior may result in the extinction of the prey species. In addition, when new predators enter an area—either through migration or some form of human introduction—they may cause extinction of the prey species that they feed on. Here, we will look at two examples of predator-induced extinction: one from the fossil record and one a contemporary example in which human-introduced predators have led directly or indirectly to the near-extinction of native fauna.

Evidence of Predation-Induced Extinction from the Fossil Record

For much of the Cretaceous period, from about 145 million years ago to 66 million years ago, clams from the bivalve family Inoceramidae were among the most prominent bottom-dwelling members in aquatic communities. But toward the end of the Cretaceous, inoceramid species began to decline, and by about 67.5 million years ago, virtually all inoceramid species were extinct. What happened? Researchers initially focused on abiotic changes such as cooling temperatures and changing water chemistry (MacLeod 1994; MacLeod and Huber 1996), but more recent work points to predation as a possible cause for the extinction of many inoceramid species (Ozanne and Harries 2002).

A detailed analysis of fossilized shells provides evidence that predation on inoceramid species increased dramatically in the period just before these extinctions took place. Certain shell deformities, known as wedges, double wedges, and scabs, are indicative of predator attacks on bivalves, and these shell deformities increased dramatically in the periods before the inoceramid species became extinct (**Figure 15.13**). What makes these marks especially informative is that only one group of shell-crushing predators—the brachyuran (or "true") crabs—were capable of producing the force necessary to create them. The brachyuran crabs underwent an **evolutionary radiation**—a rapid burst of speciation—at just the same time that the inoceramid clams were declining toward extinction. Though other factors, for example increased parasitism, may have played a role in the extinction of the inoceramids, the fossil record paints a picture in which predation by brachyuran crabs played a large part in this process (Ozanne and Harries 2002).

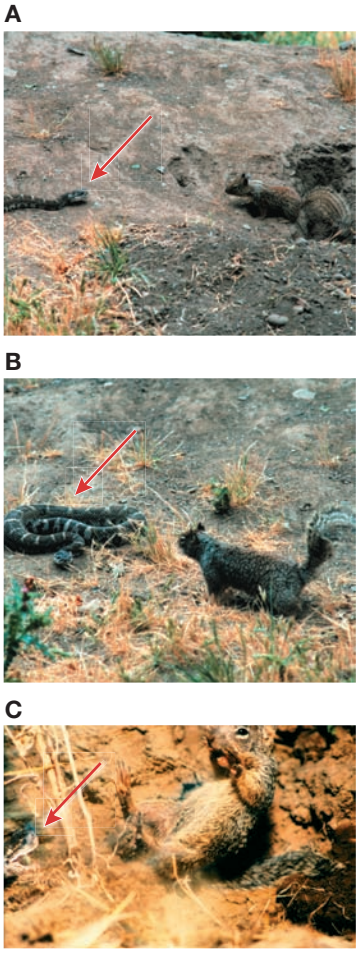


FIGURE 15.12 Antipredator behavior in squirrels. **(A)** Ground squirrels emerging from their burrow recognize snakes (red arrow) as predators. **(B)** Confrontations with rattlesnakes (red arrow) are common, and **(C)** they sometimes lead a squirrel to kick dirt and rocks at the snake (note the snake's head at the red arrow) to defend itself.

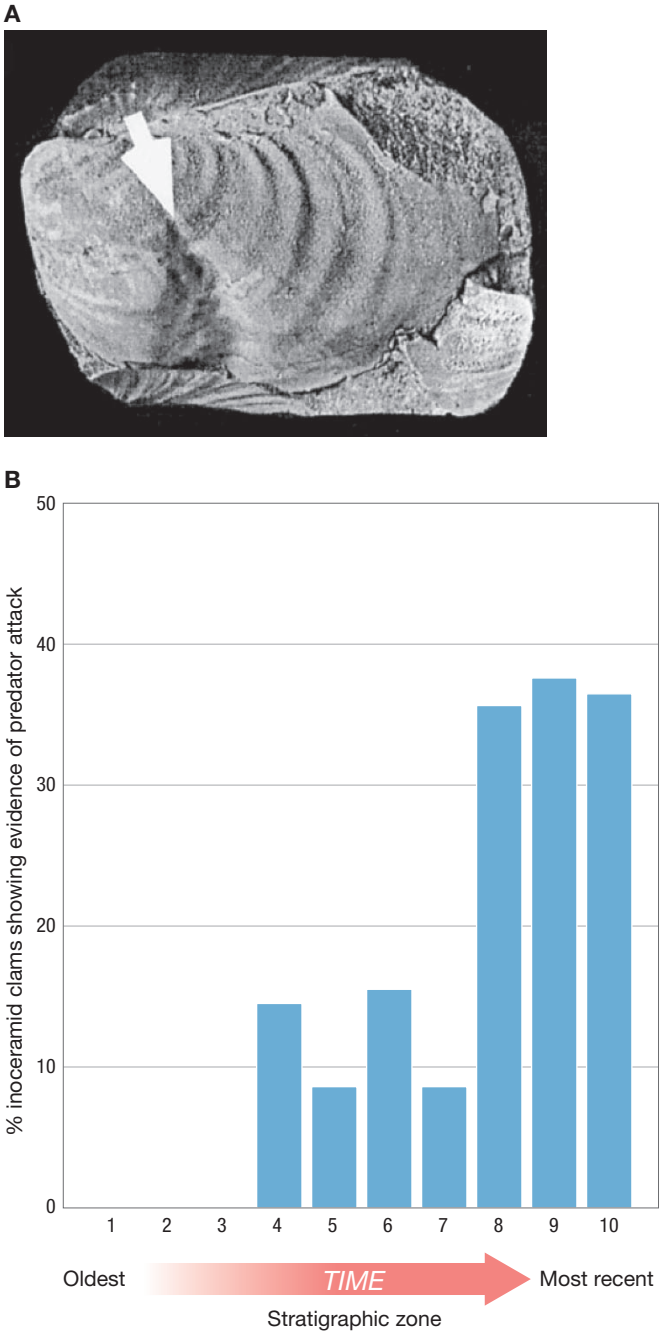


FIGURE 15.13 Predation and the extinction of inoceramid clams. **(A)** A wedge mark (at the arrow) in the shell of an inoceramid clam is indicative of a predator attack. **(B)** The percentage of inoceramid clams showing evidence of predatory attack increased before species in this family went extinct 67.5 million years ago, toward the end of the Cretaceous period. The stratigraphic zone illustrated here was deposited approximately 80 million to 70 million years ago. Panel B from Ozanne and Harries (2002).

Predation, Extinction, and Indirect Effects

Next we turn to California's Channel Islands, a set of islands in the Pacific off the West Coast of North America (**Figure 15.14**), to examine the near-extinction of a mammalian predator there (Roemer et al. 2002). Six of the Channel Islands are inhabited by an endemic fox species, the island fox (*Urocyon littoralis*)—a predator that feeds primarily on mice, insects, and fruit (Roemer et al. 2001b). Two of these six islands are also inhabited by the endemic Western spotted skunk (*Spilogale gracilis*) (Crooks 1994). In addition, one island—Santa Cruz—was home to a human-introduced population of feral pigs (*Sus scrofa*). Until the early 1990s, when foxes and skunks co-occurred on some of the islands, foxes outcompeted skunks for prey, and the foxes were found in high numbers. But then, in about 1992, a series of indirect events led to the near-extinction of foxes by a new predator—the golden eagle (*Aquila chrysaetos*).

What happened is illustrated in **Figure 15.15**. First, after being introduced to the island by humans, the feral pig population on Santa Cruz grew large enough in the early 1990s to attract a colony of golden eagle predators. Before this, golden eagles had been seen in the Channel Islands, but no established colonies of golden eagles had existed in these islands since the 1950s (Roemer et al. 2001a). The eagles then began feeding on pigs, which became a staple of their diet. At the same time, once they became established, the golden eagles also began attacking foxes. As a result, the fox populations were driven to near-extinction. That is, initial colonization of the eagles occurred as a result of an increased population size of pigs, but once the colonization occurred, it also caused the near-extinction of the island fox.

Not only did the increase in the number of pigs lead to more eagles and then a drastic decline in the fox population, but at the same time, the decrease in the number of foxes led to an increase in the number of skunks (Roemer et al. 2002; Coonan et al. 2005; Knowlton et al. 2007). While the National Park Service has stepped in and removed the introduced pig population over the past 15 years (National Park Service 2002), before that point human introduction (of pigs) led to the establishment of a new predator population (of eagles), a near-extinction (of foxes), and an increase in the number of another species (skunks, once fox numbers declined). Extinction often

occurs as a result of such tangled ecological and evolutionary interactions.

Predation may be particularly likely to lead to extinction in cases like this. When a predator relies heavily on a single prey species, a decline in the prey population can restrict the predator's food source enough to reduce or eliminate the predator population before the prey species is driven to extinction. But when a predator relies on one food source (in this case, pigs) and incidentally catches another food source (foxes), the decline in the number of foxes will have relatively little impact on predator numbers because the predator can always forage on its primary food source.

FIGURE 15.14 The Channel Islands. The Channel Islands lie in the Pacific Ocean off the coast of California.

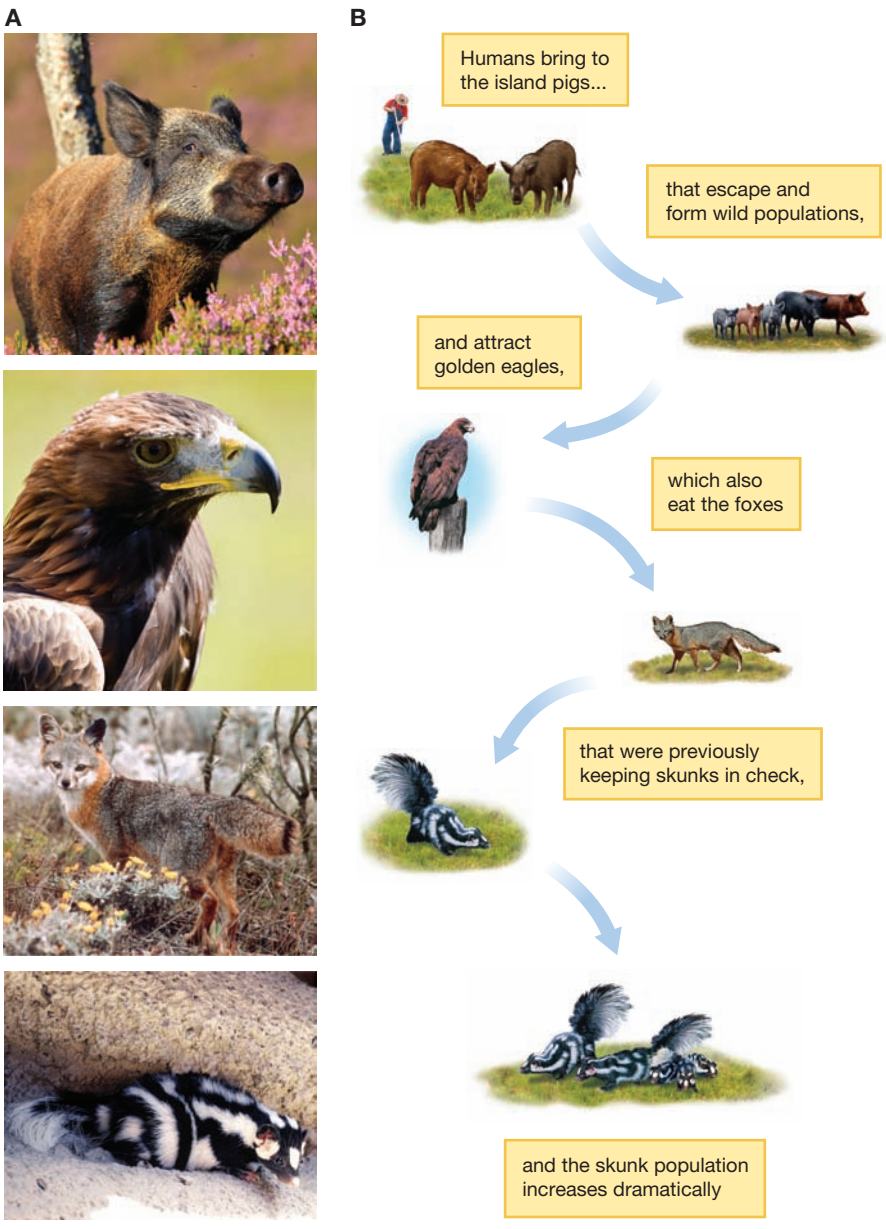


FIGURE 15.15 Interspecific interactions on Santa Cruz Island. **(A)** On Santa Cruz Island, feral pigs, golden eagles, island foxes, and Western spotted skunks interact. **(B)** A schematic of indirect effects, predation, and extinction on Santa Cruz Island. Large numbers of introduced pigs attracted golden eagle predators to the island. These eagles nearly eradicated the native foxes. The declining fox population relaxed predation pressure on skunks, which increased in population size.

Extinction and Competition

In the case of California's Channel Islands, we observed how predation can lead to near-extinction with extensive downstream effects on other species in the ecosystem. Next, we look at work that uses patterns uncovered in the fossil record to examine whether competition itself can lead to extinction in plants. Andrew Knoll analyzed published studies on 391 fossil plant communities dating from 410 million years ago to 1.6 million years ago (Knoll et al. 1984; Knoll 1986a, b). Although a number of patterns emerged in these data, we focus here on one: Over and over, Knoll found evidence that in herbaceous plant communities, a single species—or at most a few—dominated. Tracking backward through time, Knoll found that those species that came to be dominant were found at relatively low abundances in the community early on. In one sense, this is hardly surprising: After all, if we begin by focusing on a dominant species and move back in time, we might expect that species, and indeed any species in that community, to be fairly rare when it first becomes part of that community. But there is more to this situation than that.

Knoll found that there was always overlap between a dominant species that was in decline and the new species that would, over time, replace it as the dominant species when it went extinct, which hints that competition may play a part in this process. A similar pattern can be observed at higher taxonomic levels as well (**Figure 15.16**). Plants are a particularly good group in which to study competition as it relates to extinction: At a basic level, virtually all plant species make their living in the same way, in that they all require water, CO_2, and simple nutrients. Moreover, evolutionary biologists and botanists have a fairly good understanding of how plants transport water, CO_2, and simple nutrients and how these are used for growth and reproduction. What Knoll saw when he looked at the morphologies of the flora in the fossil record was that species that became dominant often had new morphological adaptations that allowed them to gather light in more efficient ways than others in their communities, and that they often showed evidence of systems that were better at both absorbing and transporting water and key nutrients. All of this together has led Knoll to hypothesize that competition was the driving force that led to extinction in these communities.

Extinction and Disease

Over the course of the past 30–40 years, there has been a major worldwide decline in amphibian populations, including the extinction of many amphibian species (**Figure 15.17**). Recent analyses suggest that the current rate of extinction in amphibians is much higher than typical background extinction rates in this taxon, and some researchers have argued that these rates approach those seen in mass extinctions (Houlahan et al. 2000; Collins and Storfer 2003; Storfer 2003; McCallum 2007; Wake and Vredenburg 2008; Blaustein et al. 2012). Although there are many factors that contribute to this steep increase in extinction rates in amphibians, here we will focus on one of the major culprits: infectious disease (Hero and Gillespie 1997; Berger et al. 1998; Daszak et al. 1999). We focus on 14 species of frogs native to the Australian rain forest

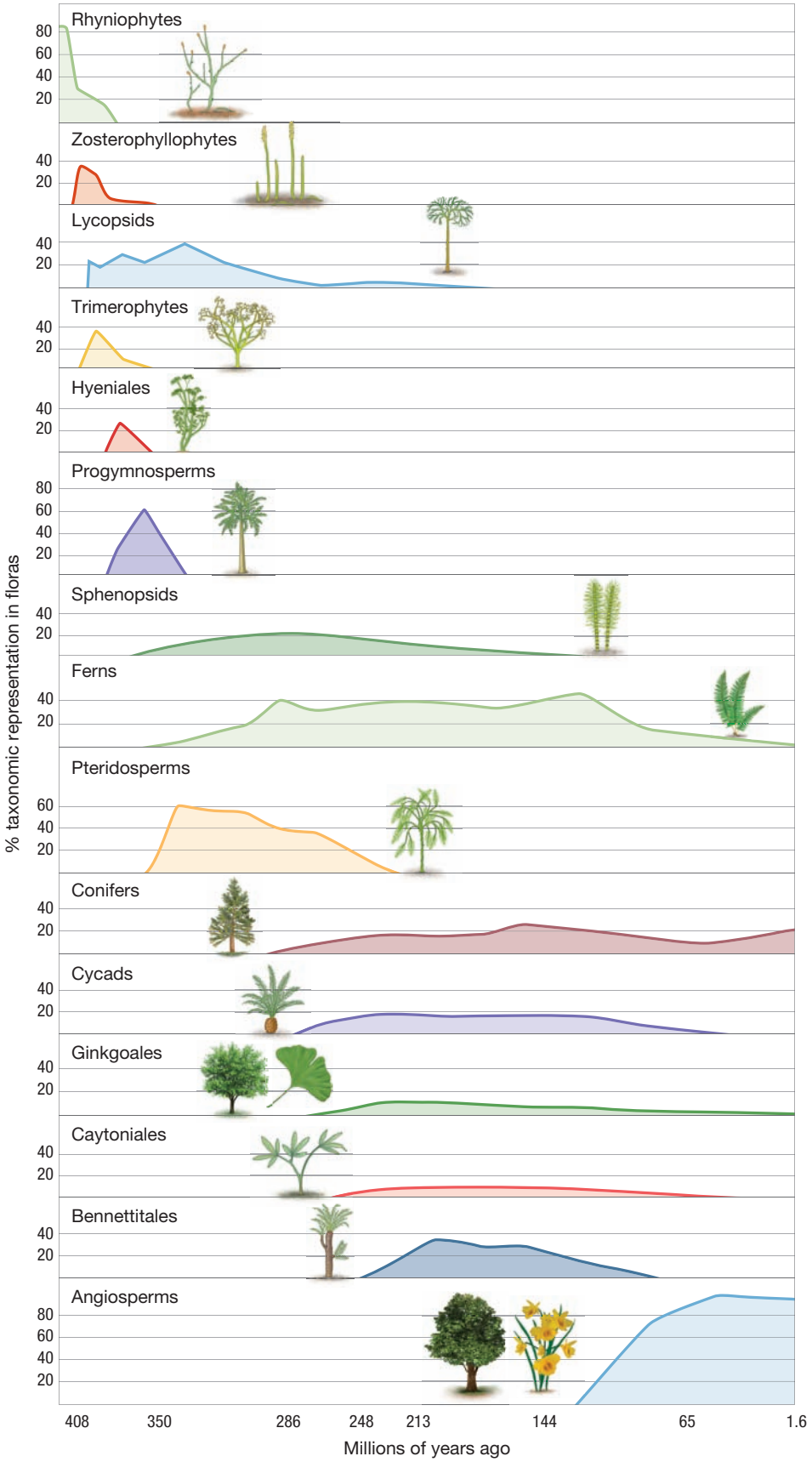


FIGURE 15.16 Abundance of major plant taxonomic groups over 400 million years. Andrew Knoll estimated the relative abundance of major plant taxa on the basis of evidence from the fossil record. When a new taxon arises, it often increases sharply in frequency and drives other taxa to lower abundance.

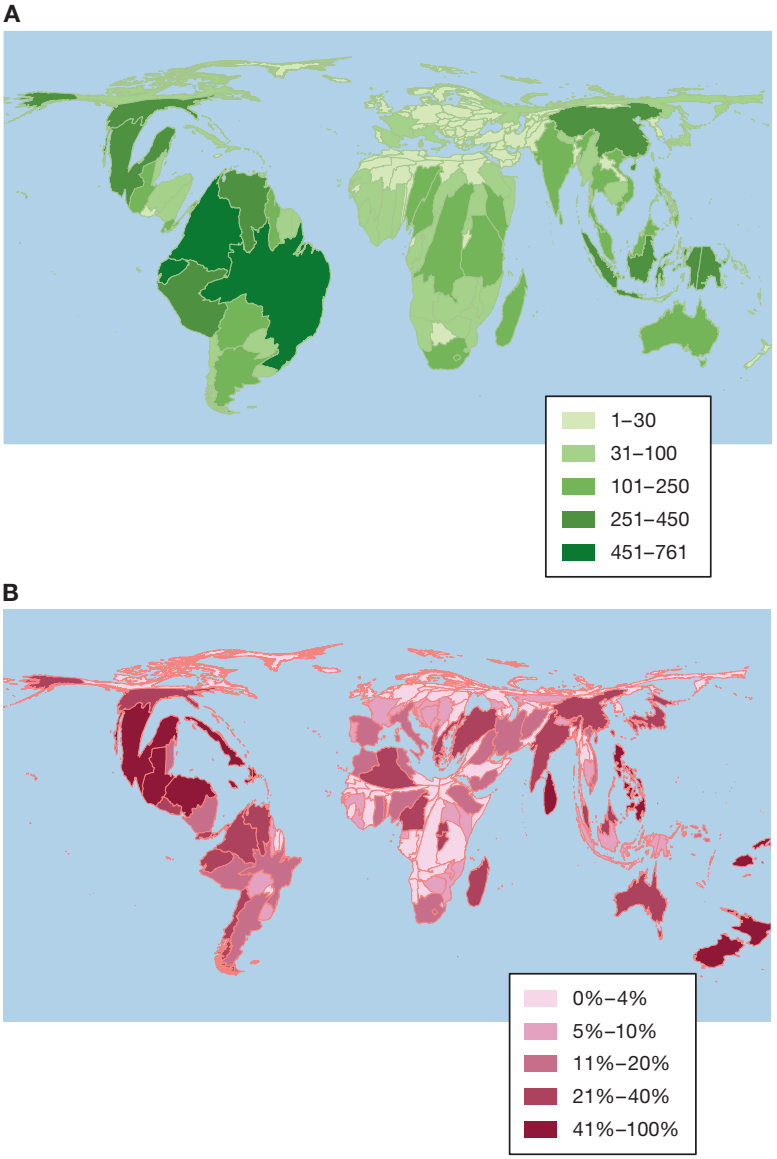


FIGURE 15.17 Amphibians throughout the world.
(A) Amphibian diversity by country. Country size has been scaled in proportion to the total number of amphibian species occurring in that country relative to its size. **(B)** Percentage of amphibians in each country in the top three categories of threat (critically endangered, endangered, and threatened). Country size has been scaled in proportion to the density of threatened species. Adapted from Wake and Vredenburg (2008).

(Tyler 1991; Ingram and McDonald 1993; Laurance et al. 1996). Since the late 1970s, as many as seven of these species may have gone extinct in the wild, and others have experienced a dramatic decrease in their population size.

In 1996, William Laurance and his colleagues suggested that infectious disease might play a major role in the decline of the Australian rain-forest frog species (Laurance et al. 1996). A number of lines of evidence suggest infectious disease as a major cause of the Australian rain-forest extinctions. To begin with, the dramatic declines occurred in a specific order, and quite rapidly. At first, populations in the southern part of the rain forest began to decline. Next, populations farther north were affected—a map of the populations in decline shows a wavelike pattern moving north, at a rate of about 100 kilometers per year (**Figure 15.18**). Once a population began to decline in size, it dropped precipitously—often by 80% or more—in a matter of months. Both the manner in which populations declined in a wavelike pattern from south to north and the speed by which populations declined are classic signatures of a virulent infectious disease as the cause.

Researchers found that individuals in affected populations were lethargic and demonstrated motor dysfunction and anemia. Histological analysis of dead individuals showed widespread damage to the kidneys, liver, skin, and other organs (Speare 1994). Another observation is also indicative of an infectious cause: When healthy individuals from populations that were not in decline were introduced into populations that were in decline, the healthy individuals often soon began to display the symptoms and pathologies described above (Laurance et al. 1996). While Laurance and his colleagues suggested a number of possible diseases as the culprit behind the population decline and extinction of many of the Australian rain-forest frog species, a definitive diagnosis of which specific disease was responsible was not made for another 2 years, when the disease was identified as chytridiomycosis (caused by a chytridiomycete fungus, *Batrachochytrium dendrobatidis*) (Laurance et al. 1996; Berger et al. 1998). *B. dendrobatidis* interferes with the ability of amphibians to transport chemicals across the epidermis and has been found not only in

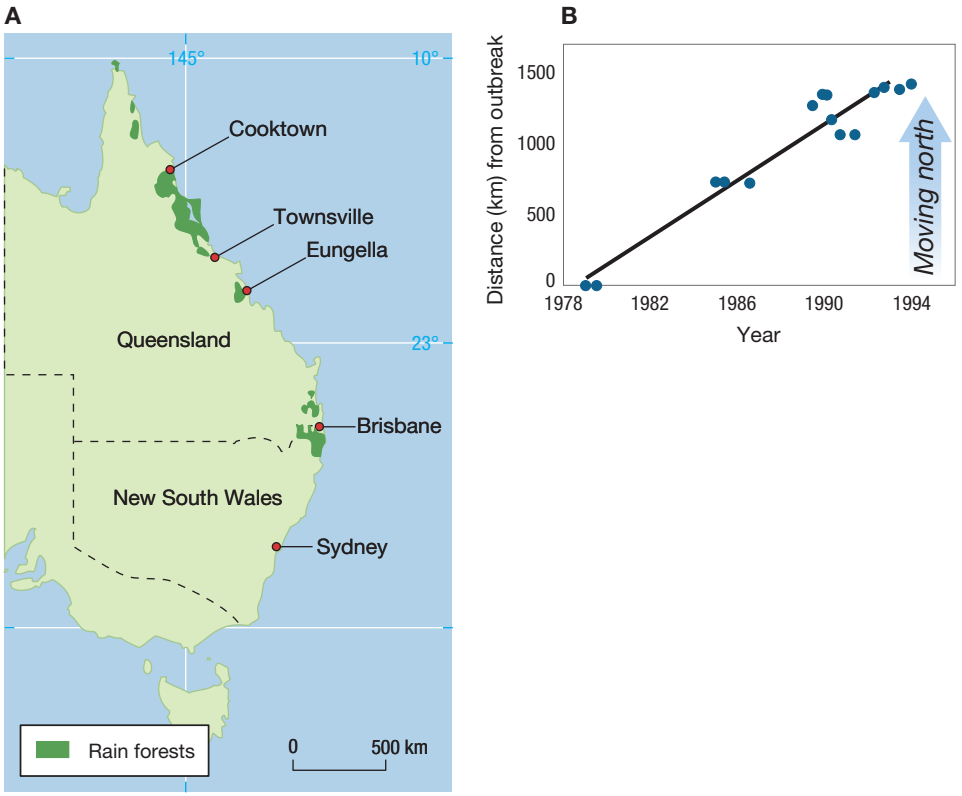


FIGURE 15.18 Disease and amphibian decline. **(A)** Tropical rain forests in eastern Australia. **(B)** Spread of amphibian population declines in eastern Australia measured from the distance of the outbreak of disease. Circles indicate where population size declined dramatically in less than 12 months. Declines spread across eastern Australia at a rate of roughly 100 kilometers (km) per year. Adapted from Laurance et al. (1996).

frogs from the Australian rain forest but also in sick individuals in declining populations of Panamanian frogs (**Figure 15.19**).

Multiple Causes of Background Extinction

Predation, competition, and disease are not mutually exclusive explanations for background extinction. Indeed, in many cases, two or all three of these causes may be connected to background extinction. To see this, let's consider the bird extinctions on the Hawaiian Islands.

The Hawaiian Islands are home to a diverse array of plants and animals, many of which are native to the islands. But a majority of the bird species that existed on the Hawaiian Islands just a few thousand years ago have gone extinct, and most of the species we see today are the result of human introduction (**Figure 15.20**).

The islands of the Pacific Ocean, including the Hawaiian Islands, have gone through at least two waves of human colonization (Diamond 1984a,b; Milberg and Tyrberg 1993; Smith et al. 1993; Pimm et al. 1995). From about 4000 to 1500 years ago, people in the first wave of human colonization emigrated from the East Indies and settled on the Hawaiian Islands. The second wave, which was primarily led by European explorers, began with Magellan and essentially came to an end when Captain Cook died on the Hawaiian Islands in 1779 (Pimm et al. 1995). Stuart Pimm and his colleagues found evidence that 30 species of land-dwelling birds went extinct during the first wave of human colonization (Pimm et al. 1994, 1995; Boyer 2008). And just since 1800, at least 19—and probably many more—Hawaiian bird

FIGURE 15.19 Chytridiomycosis and amphibian decline. Consequences of a chytridiomycosis outbreak in the Sixty Lake Basin of California.

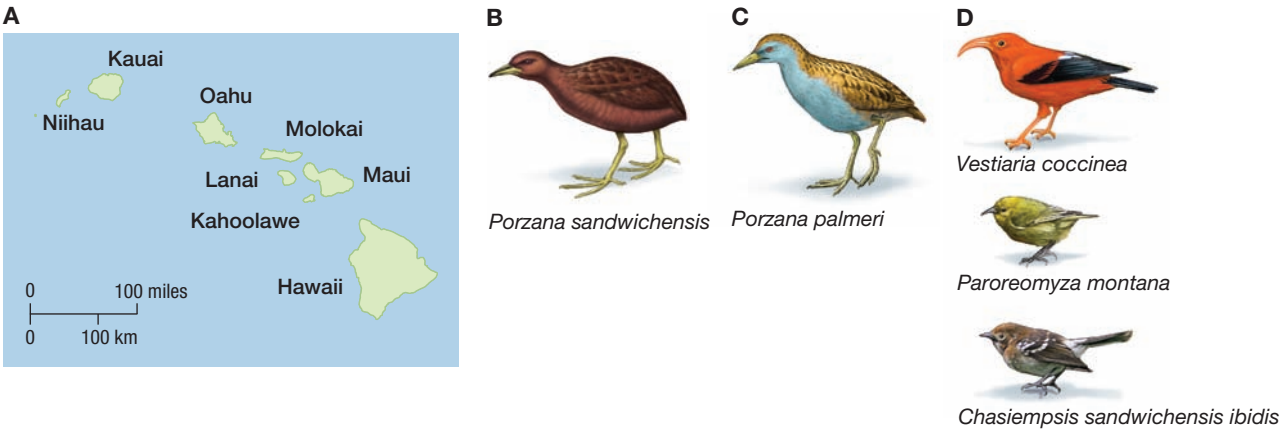


FIGURE 15.20 The Hawaiian Islands and their birds. **(A)** The Hawaiian Island chain. **(B)** The flightless Hawaiian rail (*Porzana sandwichensis*) went extinct in the 1890s. **(C)** The flightless Laysan rail (*Porzana palmeri*) became extinct in the 1940s. **(D)** An assortment of surviving bird species endemic to the Hawaiian Islands.

species have gone extinct. Similar patterns have been found across many Pacific archipelagoes (Steadman 2006; Duncan et al. 2013; Boyer and Jetz 2014).

Of the approximately 125–145 bird species that once inhabited the Hawaiian Islands before human colonization, 90–110 are now extinct, many as the result of direct and indirect interactions with humans. Disease introduced by humans, predation by humans who hunted birds for food and for decorative feathers, predation by human-introduced species such as snakes and rats, competition with human-introduced species, and destruction of the native habitat by humans have acted together to lead to the extinction that has occurred on these islands (Pimm et al. 1995, 2006).

Alison Boyer analyzed the data on the Hawaiian Islands extinctions and uncovered some fascinating patterns about which species were most likely to survive human colonization (Boyer 2008). During the first round of colonization—the prehistoric colonization from about 4000 to 1500 years ago—bird species that were large, flightless, and that nested on the ground suffered much higher rates of extinction than other bird species. Evidence suggests that this was largely a result of humans who were hunting the less mobile targets. In the second human colonization wave, many large species of birds were already extinct (**Figure 15.21**).

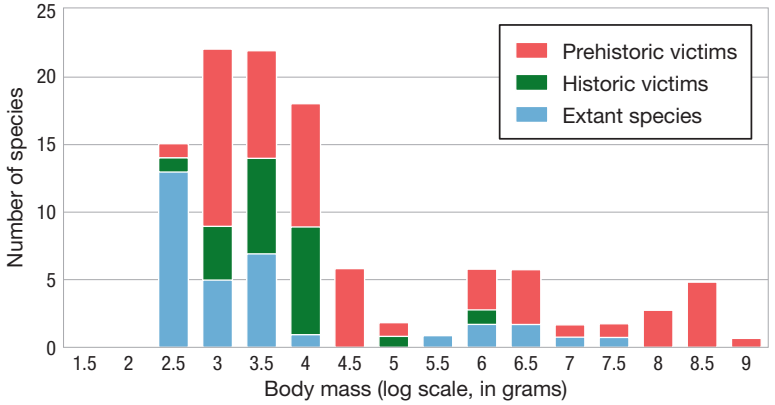


FIGURE 15.21 Hawaiian bird extinctions. Distribution of body size in Hawaiian bird species. Larger species tend to have been victims of prehistoric extinction or historic extinction. Adapted from Boyer (2008).

At that time, bird species that fed on insects and nectar were especially susceptible to extinction. Why? The evidence suggests that habitat destruction by humans and human-introduced predators devastated the lowland forests of the Hawaiian Islands during the second wave of colonization, and those forests were home to many birds that fed on insects and nectar.

15.3 Mass Extinction

If there had not been a mass extinction about 65 million years ago, reptilian dominance of the land would likely have continued for some amount of time, which in turn might have kept mammals as they were—small, mouse-sized, nocturnal creatures. There might have been no mammalian evolutionary radiation, no primates, and no humans. As paleontologist David Jablonski has written:

> To the conservation biologist, there is little positive to be said about extinction. From an evolutionary perspective, however, extinction is a double-edged sword. By definition, extinction terminates lineages and thus removes unique genetic variation and adaptation. But over geological time scales, it can reshape the evolutionary landscape in more creative ways, via the differential survivorship of lineages and the evolutionary opportunities afforded by the demise of dominant groups and the postextinction sorting of survivors. (Jablonski 2001, p. 5393)

A mass extinction typically refers to the wholesale loss of many groups of organisms over a broad geographic range. Although no precise technical definition exists, some paleontologists suggest that when more than 75% of species are lost over a relatively short geological time period, a mass extinction has occurred (Barnosky et al. 2011). At least five, and perhaps as many as eight, such mass extinctions have occurred over the past 600 million years—at the end of the Ordovician, in the Late Devonian, the Late Permian, at the end of the Triassic, and at the Cretaceous–Paleogene boundary. Though the numbers are not yet near this 75% figure, some scientists have argued that we are in the midst of, or at the very least heading toward, a human-induced mass extinction in the next few hundred years (Wake and Vredenburg 2008; Dunn et al. 2009; Barnosky et al. 2011; Dirzo et al. 2014).

Current estimates are that extinction rates today are close to 1000 times higher than background extinction rates (de Vos et al. 2014). If we consider (a) not just the number of species that have gone extinct recently and the rate of extinction but also (b) the number of species that are seriously endangered, (c) the fact that we know very little about many species that may be in danger of extinction, (d) the massive human-caused deforestation, defaunation, and habitat fragmentation that is occurring right now, (e) the rapid and major climate change, driven by CO_2 emissions, that now appears inevitable, and (f) our current understanding of how extinction of one species can have profound direct and indirect effects on ecosystem functioning, the case for a possible human-caused sixth mass extinction becomes much stronger (**Figure 15.22**).

The numbers associated with mass extinctions can be staggering—estimates for the late Permian mass extinction have somewhere from 80% to 96% of all marine species going extinct (Raup and Sepkoski 1979; Stanley and Yang 1994)

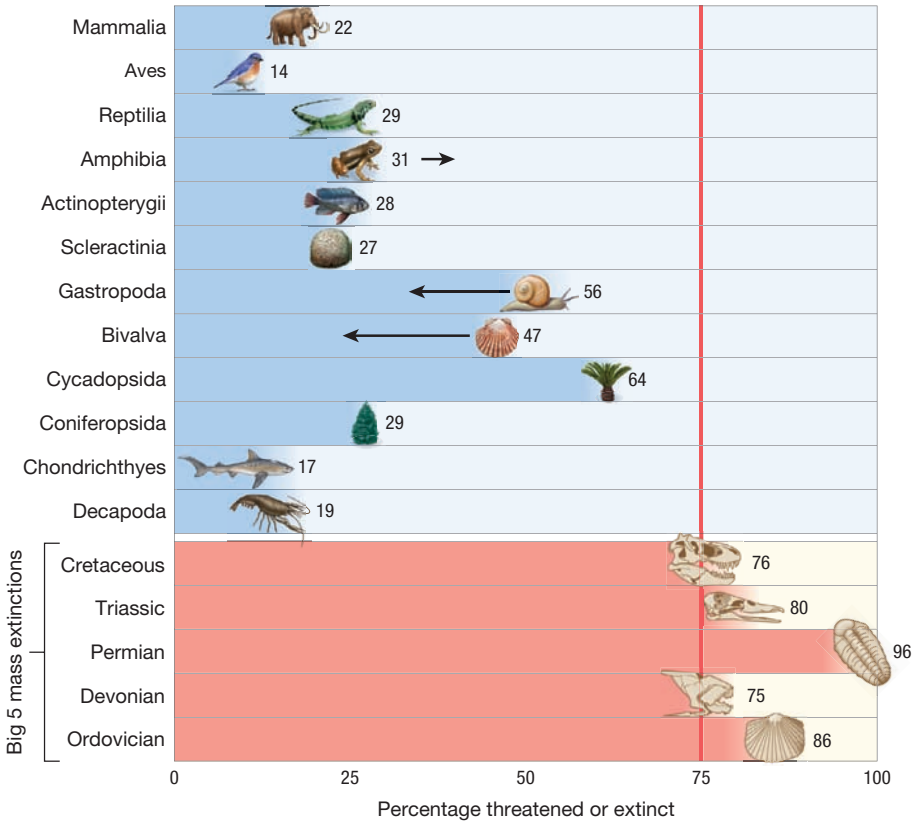


FIGURE 15.22 Is a sixth mass extinction under way? The upper portion of the graph indicates the fraction of species in each taxon that is currently threatened or extinct according to the International Union for Conservation of Nature (IUCN). The lower portion of the graph indicates the fraction of species lost in each of the Big Five mass extinctions. Adapted from Barnosky et al. (2011).

(**Figure 15.23**). And it isn't just the sheer numbers of species that are lost that make the effects of mass extinction so dramatic. Douglas Erwin found that mass extinction not only is associated with decrease of the total number of surviving species and genera (loss of taxonomic diversity) but also decreases diversity with respect to morphology (form and structure of organisms), behavior (measured in the fossil record by "traces" left by burrowing organisms, herds of animals, and so on, indicating movement of organisms), the number of different niches occupied, and developmental patterns (for example, number of body parts) (Erwin 2008).

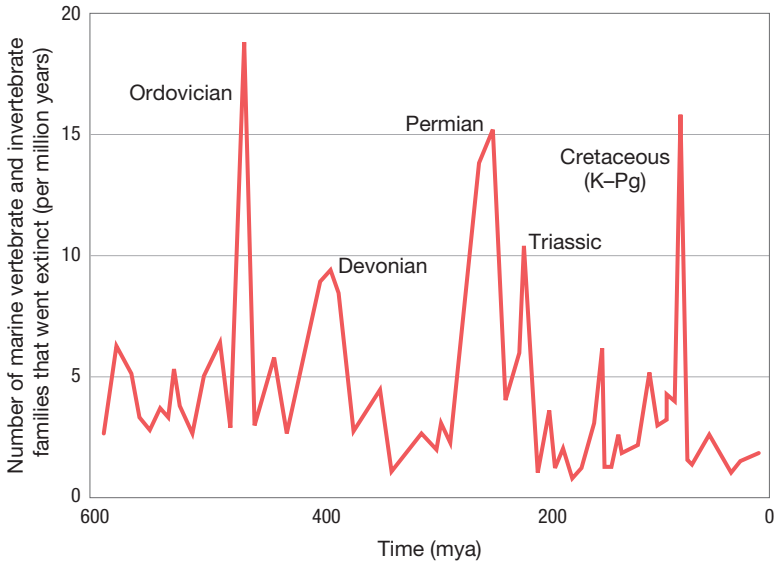


FIGURE 15.23 Extinction rates of marine families over time. Spikes represent mass extinctions. Mya = millions of years ago.

The effects of mass extinction can be far-reaching in time. Jablonski has coined the phrase "dead clade walking" to describe clades that survived a period of mass extinction, only to go extinct some time in the following geological time period (Jablonski 2002).

Jablonski found that the geological time periods immediately following four of the five mass extinctions he examined were marked by the subsequent loss of 10% to 20% of the orders of marine invertebrates that had made it through the mass extinction (Jablonski 2002). This rate of extinction was significantly greater than that seen in the geological time period immediately preceding the mass extinction, providing evidence that the post–mass extinction losses

were higher than normal background extinction in the marine invertebrate orders in question (Figure 15.24).

Evolutionary biologists have gathered data of various sorts for a number of the mass extinctions that have occurred over the past 600 million years, but for a whole suite of reasons, including access to fossil beds and clues to causation, much of the work on mass extinctions has focused on the Permian and Cretaceous–Paleogene extinctions.

The Cretaceous–Paleogene (K–Pg) Mass Extinction

The most well known and well studied of the mass extinctions occurred approximately 65 million years ago, close to the boundary between the Cretaceous and Paleogene periods (Figure 15.25). This most recent of mass extinctions—often called the **K–Pg mass extinction** after the German words for Cretaceous and Paleogene—had profound effects on many different taxa both in the water and on the land, flora and fauna, invertebrate and vertebrate. Conservative estimates report that half of all the genera alive before the end of the Cretaceous period died off during this mass extinction. The most famous victims were the dinosaurs.

Geologists and evolutionary biologists first assumed that although the K–Pg extinction was a mass extinction in terms of its effect on diversity, this mass extinction occurred gradually over the course of millions of years, likely as a result of gradual changes in temperature, humidity, sea level, and other environmental properties. This initial assumption of slow, gradual change makes good sense.

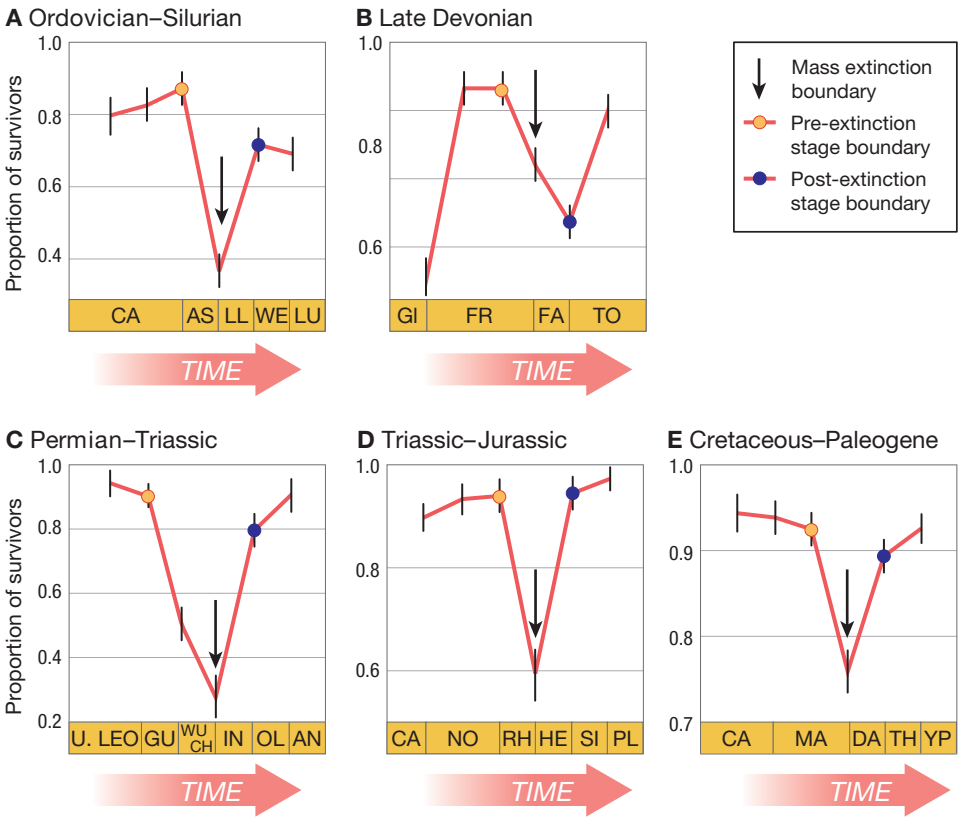


FIGURE 15.24 Dead clades walking. Many clades survive periods of mass extinction, only to go extinct in the following geological time period. In four **(A, B, C, E)** of the mass extinctions shown here, the survival rate for genera during the period before the mass extinction (yellow dot) was higher than the survival rate during the period that followed the mass extinction (blue dot). Only **(D)** shows (slightly) higher survival after the mass extinction. The abbreviations on the *x* axis indicate geological time periods going forward in time from left to right. Adapted from Jablonski (2002).

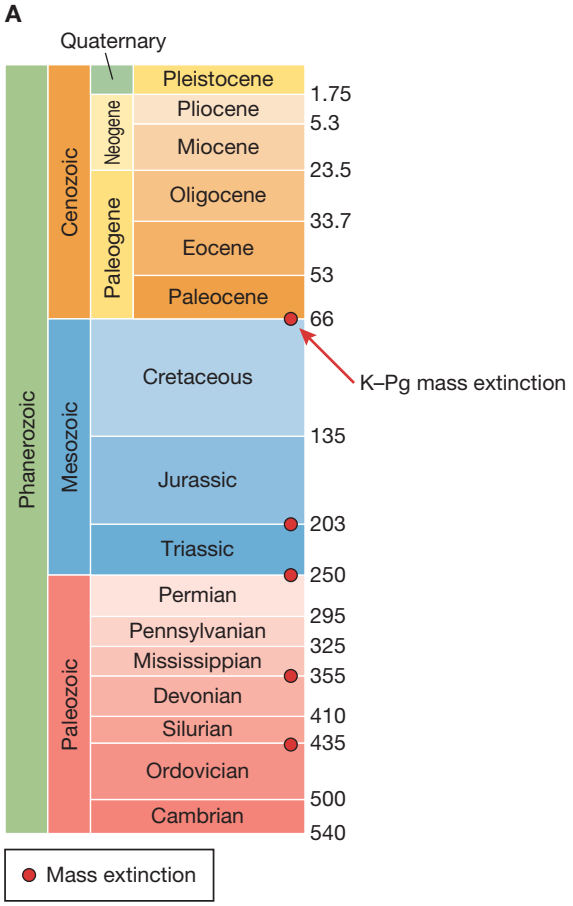


FIGURE 15.25 The K–Pg mass extinction. **(A)** Timetable with red arrow at K–Pg extinction. Adapted from Kring (2010). Red dots indicate mass extinction events. **(B)** The arrow points to the K–Pg boundary claystone interval.

Recall our discussion of Lyell's theory of uniformitarianism in Chapter 2. Geologists and evolutionary biologists since Lyell's day have gathered enormous amounts of data suggesting that change—both geological and biological—is often slow and gradual. But in the case of the K–Pg extinction, the more data that were collected, the less it looked like the mass extinction was the result of slow, gradual processes. The alternative—some sort of catastrophic event—had to be reconsidered.

The first serious attempt to do so was made by Dale Russell and Walter Tucker, who suggested that if a large supernova had exploded near Earth, the radiation produced and the climate changes triggered by such an explosion would be massive enough to cause extinction on the scale seen in the K–Pg mass extinction (Russell and Tucker 1971). This extraterrestrial hypothesis for the K–Pg mass extinction has not stood the test of time, but another closely related idea—that the K–Pg mass extinction was the result of a large asteroid colliding with Earth—has.

Walter Alvarez, a paleontologist, began discussing the idea of an extraterrestrial cause for the K–Pg mass extinction with his father, physicist Luis Alvarez, in 1976. If this mass extinction was the result of some large extraterrestrial event, the evidence should indicate that the mass extinction happened rapidly—on the order of decades or centuries—rather than over millions of years as in the gradualist theories. They started examining the K–Pg boundary—the rock strata that denote the end of the Cretaceous period and the start of the Paleogene period—to see if there was evidence that events surrounding the K–Pg mass extinction had occurred on the timescale of decades. What they found stunned them.

At sites in Italy and Denmark, Alvarez and his colleagues examined a 1-centimeter-thick layer of clay that demarcates the boundary near the Cretaceous and Paleogene periods. They measured the concentration of 28 elements (Alvarez et al. 1980). At an Italian site where they worked, 27 of the 28 elements examined were found at similar concentrations above and below the layer of clay demarcating the K–Pg boundary. The one exception was iridium, which showed a dramatic 30-fold increase above the boundary. Similar results were found at a Danish test site, with iridium levels measured in sediments that were just above the K–Pg boundary being 160 times greater than baseline levels. Subsequent work has confirmed the findings from the sites in Italy and Denmark, finding increased levels of iridium near the K–Pg boundary at 50 other sites around the world (**Figure 15.26**) (Ganapathy 1980; Kyte et al. 1980; Smit and Hertogen 1980; Orth et al. 1981; Alvarez et al. 1990).

So what? What does it matter if the amount of one element in the crust increased, even if the change was dramatic? The answer is that iridium is extraordinarily rare in Earth's crust. Geological work has shown that much of the iridium found in Earth's crust is extraterrestrial in origin, brought to Earth on comets, meteors, and asteroids. Perhaps the spike in iridium found in the K–Pg clay was evidence

that the mass extinction was the result of the collision of such an object with Earth 65 million years ago. As Alvarez and his colleagues methodically ruled out alternative explanations for a spike in iridium—for example, under very rare circumstances, iridium might have built up in seawater and been deposited at the K–Pg boundary—they began to think that the iridium indeed was extraterrestrial in origin (Alvarez 1983; Alvarez et al. 1980, 1984a,b, 1990; Kastner et al. 1984).

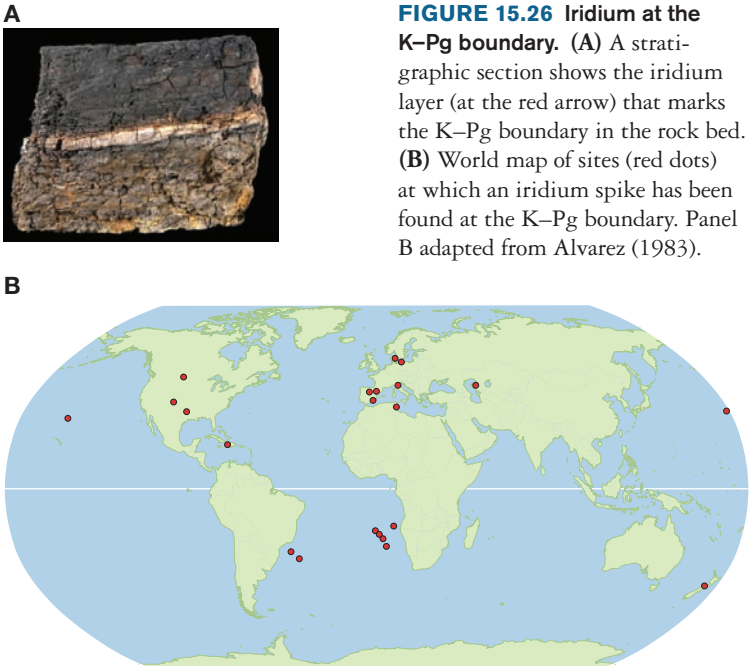


FIGURE 15.26 Iridium at the K–Pg boundary. **(A)** A stratigraphic section shows the iridium layer (at the red arrow) that marks the K–Pg boundary in the rock bed. **(B)** World map of sites (red dots) at which an iridium spike has been found at the K–Pg boundary. Panel B adapted from Alvarez (1983).

Alvarez and his team hypothesized that an asteroid, approximately 10 kilometers in diameter, struck Earth 65 million years ago. Models and computer simulations (which were initially built to examine the impact of the use of nuclear weapons) indicate that if a 10-kilometer asteroid struck the surface of Earth, it would form a huge crater (**Figure 15.27**). Giant tsunamis would follow. Huge amounts of particulate matter from the crater would shoot into the atmosphere, spread around the globe, and block out sunlight. Photosynthesis would plummet, causing a collapse of the food chain and mass extinction.

Are there asteroids out in space large enough to cause such an impact? Astronomers have calculated that at any given time, there are about seven asteroids 10 kilometers in diameter or larger that are orbiting Earth (Chapman et al. 1978; Wetherill 1979), and that, on average, one of these asteroids collides with Earth approximately every 30 million years. The shutdown of photosynthesis that Alvarez and his colleagues predicted would follow the impact of such an asteroid is consistent with what we know from the huge eruption of the Krakatoa volcano in 1883 (Symons 1888). That eruption shot 18 cubic kilometers of dust into the stratosphere, causing a significant decrease in the average temperature of Earth for more than 2 years and a subsequent drop in photosynthesis. Simulations indicate that the impact of a 10-kilometer asteroid would have dwarfed the effects of the Krakatoa eruption. The decreased primary productivity seen in at least some taxa, such as plankton, at the K–Pg boundary may have been a consequence of the particulate matter that was shot into the atmosphere when an asteroid hit Earth about 65 million years ago (Stuben et al. 2002).

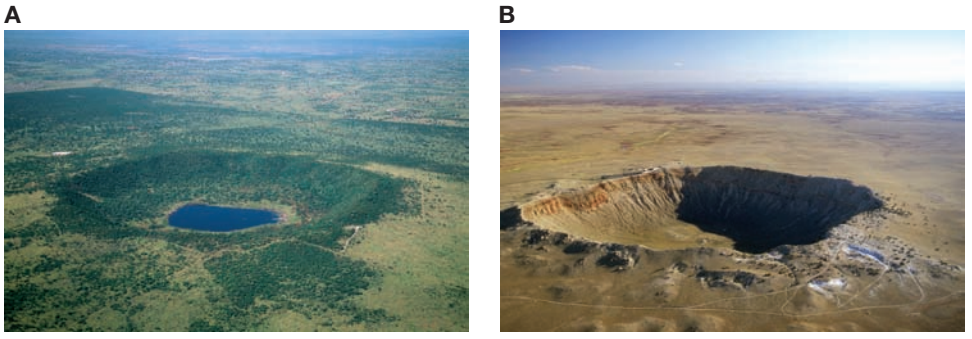


FIGURE 15.27 Asteroid impact craters. **(A)** The 1.13-kilometer-diameter Pretoria Saltpan impact crater. **(B)** Arizona's Barringer Meteor crater. The meteor that produced this crater was only 150 feet in diameter, which is *much* smaller than the asteroid that likely was the major cause of the K–Pg extinction.

Iridium in the clay near the K–Pg boundary is only one of the many sources of evidence suggesting that an asteroid collision was the primary cause of the K–Pg mass extinction. Subsequent work shows the following:

- There are amino acids of extraterrestrial origin in the clay near the K–Pg boundary (Bada et al. 1986; Zhao and Bada 1989). Researchers have uncovered both alpha-aminoisobutyric acid and racemic isovaline—two amino acids that are very rare on Earth but that are major amino acids found on comets, meteors, and asteroids—near the K–Pg boundary.
- Hard, glassy minerals called spinels are found near the K–Pg boundary. These may have been crystallized from the vapor associated with the impact of a large asteroid (Smit and Kyte 1984).
- Tiny diamonds known as "impact diamonds" were formed as a result of a large collision approximately 65 million years ago (Carlisle and Braman 1991; Carlisle 1992).
- Evidence from more than 3000 fossils from 340 genera of bivalves (clams, oysters, scallops, and others) indicates that extinction occurred on a global level, as predicted from an impact of a huge asteroid (Raup and Jablonski 1993).
- Recent work from astrophysics indicates that the breakup of a huge asteroid in the Solar System's inner asteroid belt would have produced large fragments, a shower of which would likely have struck Earth about 65 million years ago (Bottke et al. 2007). Nonetheless, there remains an active debate as to whether this event was associated with the K–Pg mass extinction (Reddy et al. 2009).

The asteroid hypothesis for the K–Pg mass extinction suggests that this extinction happened over a very short geological time period—of the order dozens to thousands of years. And, indeed, there is evidence that the decline in biodiversity was not gradual, at least not for pollen-producing plants, which showed a dramatic decrease that coincides almost exactly with the iridium spike. But is this pattern of rapid decline that is seen in pollen-producing plants near the K–Pg boundary observed in other taxa?

To examine this question, Alvarez and his colleagues examined the fossil record data on four groups that were common during the Paleozoic era—ammonites (extinct organisms whose closest living relatives are cephalopods like octopuses, squids, and cuttlefish), bryozoans (small aquatic invertebrates), brachiopods (marine invertebrates that have hard shells on their upper and lower surfaces and use a fleshy, stalk-like structure to burrow), and bivalves. They found clear evidence for the rapid disappearance of these four groups, very close to the K–Pg boundary, as expected from the asteroid hypothesis. But Alvarez and his colleagues also found that a gradual decline was under way in *some* of these groups *before* the K–Pg boundary. Ammonites, for example, often went through cycles of abundance and decline, and they were likely in decline 65 million years ago. Nonetheless, ammonites had also gone through a dramatic decline in diversity earlier in their evolutionary history, and they had rebounded at that time. The impact of an asteroid, then, may have precipitated a mass extinction of ammonites that likely would not have occurred otherwise. Indeed, Alvarez argues that the groups that were in a period

of decline, perhaps in response to normal environmental fluctuations, were generally those that were most susceptible to extinction when an asteroid hit (Alvarez et al. 1984a,b).

If there was a mass extinction as the result of an asteroid colliding with Earth 65 million years ago, where is the huge crater that would have resulted from the impact? Alvarez and his team recognized the importance of finding this crater, but they were not particularly optimistic about discovering it when they first hypothesized the asteroid theory in the early 1980s. After all, a substantial portion of pre-Paleogene ocean had long ago disappeared under Earth's surface, which could have made it impossible to find the crater (Alvarez et al. 1980, 1990).

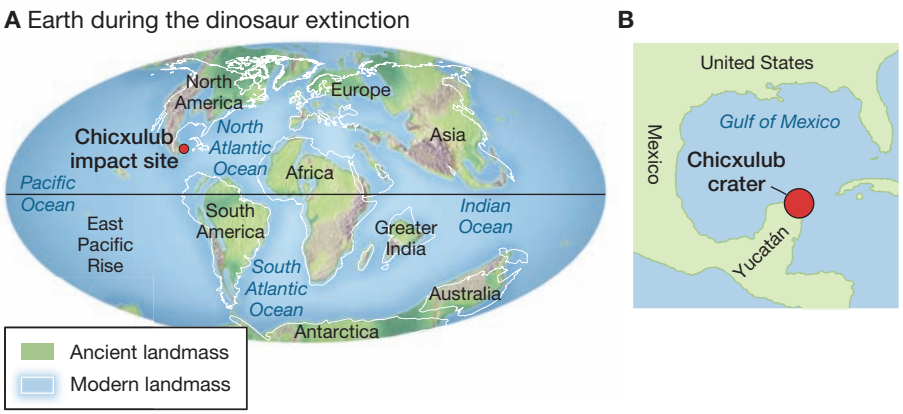


FIGURE 15.28 The Chicxulub impact site. **(A)** Location of the Chicxulub crater on a map of what the continents looked like at the time of the K–Pg extinction. From National Geographic (2010). **(B)** Location of the Chicxulub crater in the Yucatán Peninsula, Mexico.

In 1980, when the asteroid hypothesis first came to light, there were about 100 large craters identified around the world. Alvarez and his team immediately encountered a problem—they estimated that the K–Pg crater should be about 150–200 kilometers in diameter, and yet the available data did not suggest any crater that matched that description. A few of the 100 craters were large enough, but they were the wrong age.

An important discovery by Jody Bourgeois put the Alvarez team on the path that would eventually lead to the discovery that some would come to call the "crater of doom." The Brasos River, which empties into the Gulf of Mexico, was one of the many sites showing high levels of iridium near the K–Pg boundary. At that site, Bourgeois found sedimentary evidence that a giant tsunami had struck approximately 65 million years ago (Bourgeois et al. 1988). Recall that the asteroid hypothesis predicts that just such a tsunami would be produced if the impact occurred in water.

Working with data from geology, oceanography, and many other disciplines, in 1991 Alan Hildebrand and his group published the first account of the location of the crater associated with the K–Pg mass extinction (Hildebrand et al. 1991; Sharpton et al. 1992; Urrutia-Fucugauchi et al. 1996). The so-called Chicxulub crater was discovered in the Yucatán Peninsula of Mexico (**Figure 15.28**). In fact, scientists at PEMEX—the state oil company of Mexico—had known of the existence of this crater for many years, but it took Hildebrand and his group to recognize that this might be the crater that Alvarez and his team were looking for. The Chicxulub crater had very high levels of iridium, it was the right size and the right age, it was located near the site of a giant tsunami that would have been generated by an asteroid impact, and it was composed of rock with mineralogical characteristics that showed evidence of a massive collision (Izett 1991; Sigurdsson et al. 1991; Smit et al. 1992; Swisher et al. 1992).

Active debate continues about whether the asteroid that produced the Chicxulub crater was *the* asteroid that set the K–Pg mass extinction into motion, whether it was only one of series of such asteroids, and whether the asteroid collision(s) interacted with other biotic and abiotic factors associated with this mass extinction (Keller et al. 2004a,b, 2010; Archibald et al. 2010). Regardless

of which of these is correct, the Chicxulub crater provides us with evidence that an asteroid collision occurred at the K–Pg boundary and had profound effects on the worldwide environment and on a large number of life-forms that existed at the time of the impact.

The Permian Mass Extinction

Dramatic as the K–Pg mass extinction was, the largest mass extinction on record occurred much earlier, at the end of the Permian period at its boundary with the Triassic period, approximately 250 million years ago (mya). In the Permian mass extinction, an astonishing 90% of all species went extinct (**Figure 15.29**). Although there is some debate as to whether many of these species were in decline prior to the mass extinction (Marshall 2005; Ward et al. 2005a,b), what we know is that many major groups of plants, animals, fungi, and so on, went extinct—in essence, the slate of life was *almost* wiped clean. In his book *When Life Nearly Died: The Greatest Mass Extinction of All Time*, Michael Benton suggests that if we think of the diversity of life as a tree, then during the Permian extinction, "vast swathes of the tree are cut short, as if attacked by crazed, axe-wielding madmen. . . . After such a severe attack, the great tree of life, with over 3000 million years of history behind it at the time, might have withered away and died completely" (Benton 2003a, p. 10).

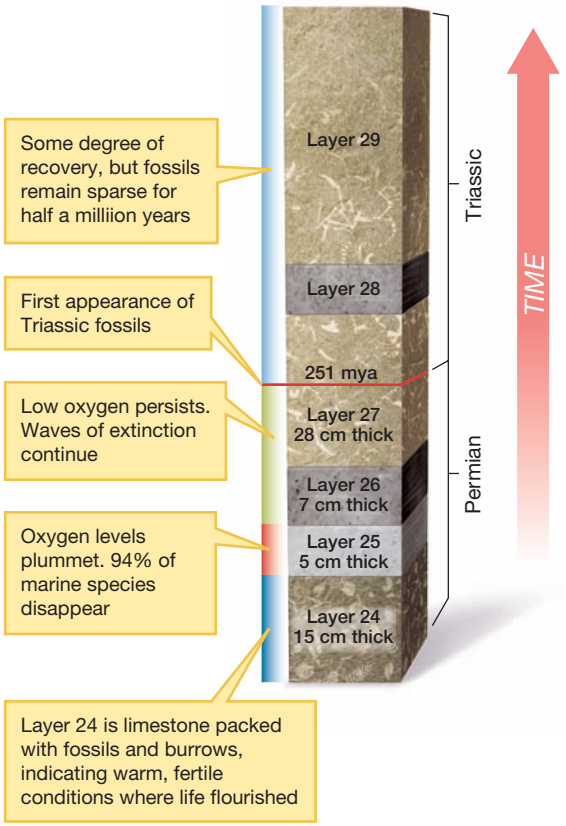


FIGURE 15.29 The Permian mass extinction. A schematic of the Permian extinction as depicted in a rock bed from China. Adapted from Benton (2003b).

Damage in the Sea

Rather than try to categorize the approximately 90% of aquatic and 70% of terrestrial species that went extinct during the Permian mass extinction, we will discuss some of the taxa that were most devastated. Then we will examine which, if any, characteristics were shared by the species that survived. This will then lead us into an examination of the possible causes of the Permian mass extinction.

Plankton—small, often microscopic creatures that drift in water currents—were hit hard during the Permian mass extinction. One group of protozoan plankton called the radiolarians was nearly annihilated, despite the fact that its extinction rate prior to the end of the Permian period was relatively low (Rampino and Adler 1998; Rampino 1999). Another plankton group called the fusulinids, which were first thought to have gradually declined before the end of the Permian period, now seem to have died off en masse about 251 million years ago—5000 species of plankton existed prior to the Permian mass extinction, and scarcely any of them remained after it (Kozur 1998; Bragin 2000; Isozaki et al. 2004). Because plankton are at the base of the aquatic food chain, their destruction during the Permian mass extinction would have had effects on the survival of many organisms higher in the food chain (**Figure 15.30**).

Fossil evidence suggests that hundreds of species inhabited the many coral reefs that thrived during the Permian period: Mollusks, starfish, shrimp, fish, and so on, abounded near these often massive

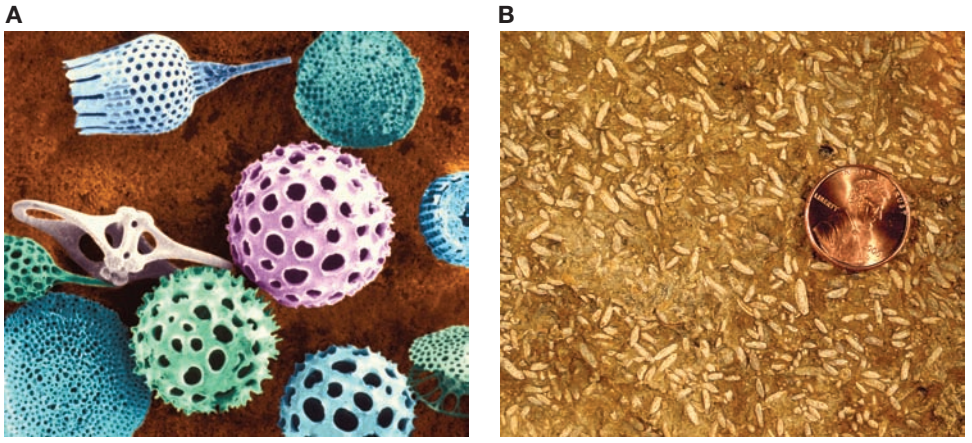


FIGURE 15.30 Two groups affected by the Permian extinction. **(A)** Radiolara. **(B)** Fossilized fusulinids cover the surface of this rock slab collected in Kansas (see coin for scale).

structures. And then, 251 million years ago, the fossil record shows a "reef gap"; that is, for the 7 million to 8 million years that followed the Permian mass extinction, reef life was remarkably depleted (**Figure 15.31**). Moreover, the reefs that evolved subsequent to the Permian extinction looked very different from the vibrant communities that had existed before. These reefs appear to have been strung together by the few surviving species that managed to make it through the extinction, and they took millions of years more to develop the diverse communities of organisms associated with them. Other aquatic creatures—such as fish and bivalve mollusks—were also hit by the Permian mass extinction. Some were hit harder than others, but all suffered significant losses.

Damage on the Land

Work on the Permian extinctions on the land has centered in two areas: the Karoo Basin of South Africa and the Ural Mountains. Here, we will focus on the Karoo Basin studies, but the patterns of change found in South Africa were also mirrored in the Ural Mountains (Benton et al. 2000).

Fossil evidence from the Karoo Basin before the Permian extinction shows that the area teemed with centipedes, spiders, cockroaches, and beetles (Benton 2003a). There is also fossil evidence that many species of fish swam in the lakes and rivers, as well as fossil remains of two species of fish-eating amphibians and 72 species of reptiles. Herbivorous reptiles and carnivorous reptiles, small and large, were plentiful before the end of the Permian period. Indeed, the remains from the Karoo Basin show evidence for a complex ecosystem. At the end of the Permian period, this Karoo ecosystem all but disappeared.

As many as 72 of the 74 sampled species of vertebrates present before the end of the Permian period went extinct. At the global level, 36 of the 48 families of amphibians and reptiles vanished (Maxwell 1992; Benton 1993, 1997). There was also a massive die-off of plant species. Many species of woody trees and bushes went extinct, leaving mostly low-lying mosses and lycopsids (small vascular plants) on an otherwise fairly barren landscape (Eshet et al. 1995; Retallack et al. 1996; Retallack 1999; Looy et al. 2001; Smith and Ward 2001; Twitchet et al. 2001).

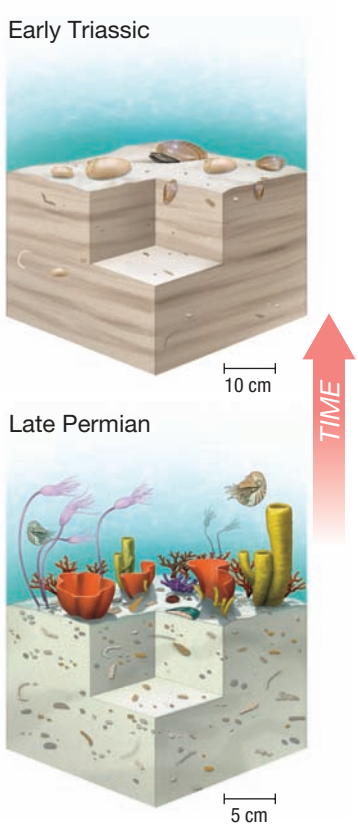


FIGURE 15.31 A diagram of the ocean seabed off China before and after the Permian extinction. The *diversity* of life dramatically decreased as a result of the Permian mass extinction. Whether the *density* of creatures that existed before and after the extinction changed remains unclear. The "cutouts" in both diagrams depict life beneath the seafloor. Adapted from Benton (2003a).

FIGURE 15.32 The Siberian Traps. A map of the world during the Permian period shows the area of the massive volcanoes in the Siberian Traps. Adapted from Benton (2003b).

What Caused the Greatest Mass Extinction of All Time?

Paul Wignall has tried to piece together what caused the Permian mass extinction. He begins with a well-established geological fact—251 million years ago, there was a series of huge volcanic eruptions in Siberia in an area known as the Siberian Traps (Campbell et al. 1992) (**Figure 15.32**). It is estimated that these eruptions spewed between 2 million and 3 million cubic kilometers of lava into the air, and that this lava covered almost 4 million square kilometers of Siberia to a depth of somewhere between 400 and 3000 meters. The resulting cycle of extreme cooling and extreme heating of the planet that these eruptions produced may have brought about the greatest mass extinction ever recorded.

This cycle began with a brief period of global cooling caused by debris in the atmosphere followed by global heating of the planet. The huge amounts of carbon dioxide released from the volcanic eruptions produced a massive *greenhouse effect*, raising the temperature around the planet by as much as 6°C and probably causing an increase in ocean acidity. Vast quantities of sulfur dioxide and chlorine were also spewed into the atmosphere. These gases—carbon dioxide, sulfur dioxide, and chlorine—created an atmosphere that was very low in free oxygen. This has led Wignall and collaborator Anthony Hallam to hypothesize that creatures well adapted to low-oxygen environments may have been predisposed to survive the Permian mass extinction. Indeed, their detailed analysis of the survivors and victims of the mass extinction at the end of the Permian period found that the better that individuals in a species were adapted to low oxygen levels (hypoxia), the more likely that species was to survive the Permian mass extinction (Hallam and Wignall 1997). And it wasn't just a low-oxygen environment that was at play during this time: sulfur dioxide and chlorine also created a worldwide acid-rain problem that devastated plant life. In addition, one consequence of the greenhouse effect was the melting of polar ice caps, releasing large quantities of methane gas buried around these ice caps.

This combination of increased temperature, global hypoxia, massive amounts of acid rain, and the release of methane gas may have combined to produce the Permian mass extinction (**Figure 15.33**).

15.4 Factors Correlated with Extinction

Evolutionary biologists are interested in whether certain attributes of a species or taxon make it more or less prone to going extinct, both in periods of mass extinction and during background extinction. In general, we hypothesize that any factor that allows a species to endure environmental perturbations better—changes in temperature, oxygen availability, and so on—should reduce the probability of extinction. We have seen one example of this during the late Permian mass extinction and the differential survival of species that fared well in low-oxygen

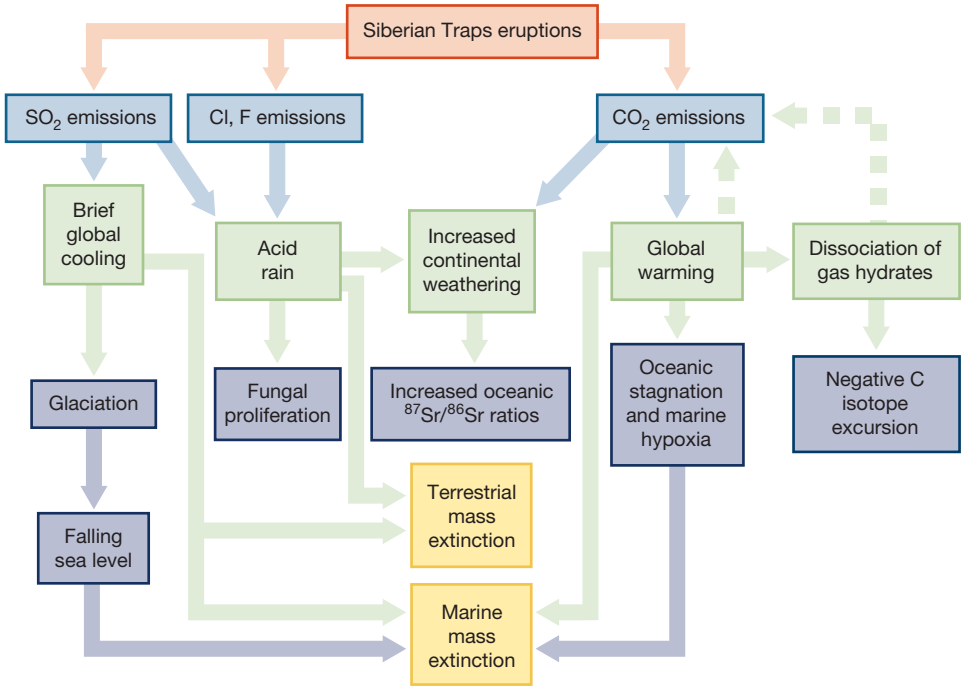


FIGURE 15.33 Direct and indirect effects of the Siberian Traps eruptions on the Permian extinction. A complex combination of direct and indirect effects resulted from the Siberian Traps eruptions. Adapted from Benton (2003a).

environments. A second example comes from a 2009 study of 4536 species of modern mammals, which found that species in which individuals hibernated or used burrows or hiding places of some sort—all means of escaping environmental perturbations—were at lower risk of extinction. They were less likely to be classified as "endangered" on the IUCN Red List of Threatened Species than species that did not hibernate or use burrows or hiding places (Liow et al. 2009; Kronfeld-Schor and Dayam 2013; Hanna and Cardillo 2014).

In the next section, we will look at two other factors that have been examined as possible correlates with the probability of extinction: (1) species' longevity and (2) species' geographic range.

Species' Longevity and Extinction Probability

What might we expect when we compare the length of time a taxon has existed and its probability of going extinct in a subsequent time interval? One hypothesis is that the longer a taxon has existed, the less likely it is to go extinct at any given point in time in the future, because long-lived taxa have demonstrated the ability to adapt to their environments. An alternative hypothesis is that a taxon might have some constraints on its life span: for example, the longer a species has existed, the more we might expect local conditions, both biotic and abiotic, to have changed, making it less likely that the species will survive much longer. Or perhaps the age of a species is irrelevant to its chances of extinction; maybe extinction has nothing to do with how long a species has existed.

To distinguish among these possibilities, Leigh van Valen plotted the probability of extinction as a function of species' longevity in a wide array of different taxa. He found that species' longevity had no effect on the probability of extinction

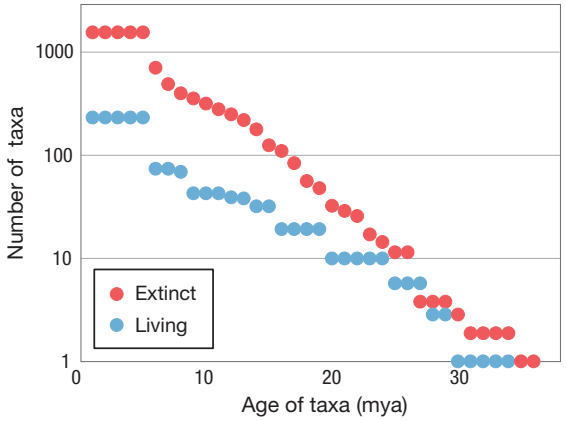


FIGURE 15.34 The probability of extinction is not a function of how long a taxon has already existed. Here, we see data on mammalian genera. An extinct genus is one in which all species are extinct, while a living genus contains both extinct and living species. Number of surviving taxa is plotted on the *y* axis, and age of a taxon is plotted on the *x* axis. The vertical axis is measured on a logarithmic scale. Because of the semi-log scaling, when the probability of extinction is unrelated to the age of taxa, we expect a linear relationship such as the one seen here. Adapted from van Valen (1973).

in virtually any of the taxa he examined (**Figure 15.34**). Why? Van Valen suggested that this was because how well species had adapted to their environments in the past was irrelevant to the probability of extinction in the future (van Valen 1973). That is, the biotic and abiotic environments are always changing, and extinction is a function of how well individuals in a species adapt to the *current* environment, not how well they adapted to *past* environmental conditions.

KEYCONCEPT QUESTION

15.3 Figure 15.34 plots the number of species of various ages for both extinct species (red) and living species (blue). Explain why the series of red points representing extinct species is above the series of blue points representing living species on the graph. Does this mean that species used to persist longer in the past?

Species' Geographic Range and Extinction Probability

Whatever the cause or causes of extinction may be, evolutionary biologists hypothesize that the broader the geographic range of a species, the less likely that species will go extinct (Manne et al. 1999; Foote 2003; Jones et al. 2003). The logic here is straightforward. The broader a species' geographic range, the less likely that each and every population of which it is composed will be extirpated (Harnik et al. 2012).

Jonathan Payne and Seth Finnegan analyzed fossil data from 12,300 marine invertebrate genera spanning the Middle Cambrian (about 500 million years ago) through the Middle Miocene (about 14 million years ago), and they subdivided their samples into 10-million-year periods (Payne and Finnegan 2007). They found strong support that broad geographic ranges reduced rates of extinction of species within genera (**Figure 15.35**). Such an effect was statistically significant in forty-four of forty-seven 10-million-year time periods analyzed by Payne and Finnegan.

The exceptions to the rule that wide geographic range is positively correlated with the probability of survival were clustered around times of mass extinctions, at which times the correlation was weaker. This makes some sense, as mass extinctions themselves are very broad geographically, which would dampen the generally positive effect that a species' geographic range would normally have on survival. But for some taxa, geographic range is correlated with species' survival even around periods of mass extinction. Jablonski and colleagues' work on geography and extinction in gastropods (slugs and snails) provides a good example of this.

Jablonski and his coworkers found that for gastropods of the Late Cretaceous period, the key to a broad geographic range at the species level—and hence to increasing the chances of surviving the mass extinction at the K–Pg boundary—was a specific type of larval development. Planktotrophic larvae feed in the open water on very small prey (zooplankton and phytoplankton), and they develop into

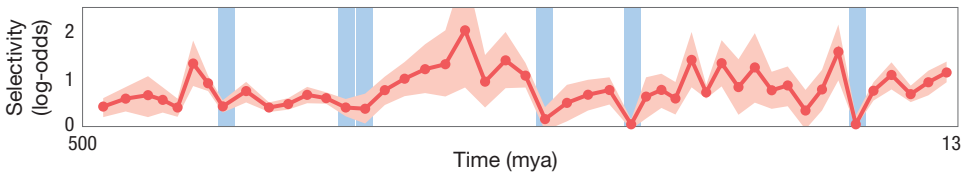
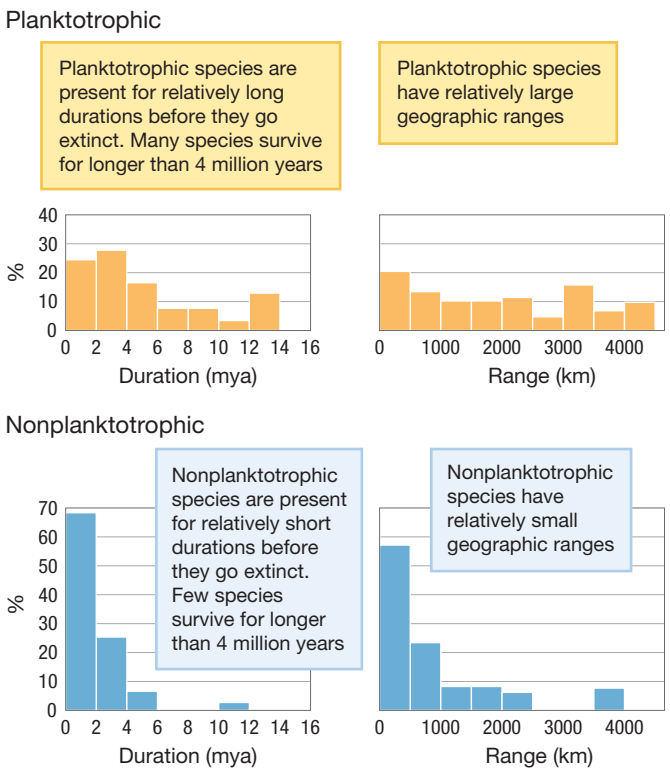


FIGURE 15.35 Geographic range and survival. Time is shown on the *x* axis, and a statistical measure called a log-odds ratio is shown on the *y* axis. A log-odds ratio of zero means no association between geographic range and probability of extinction, whereas a positive log-odds ratio indicates that species with broad geographic ranges show reduced extinction rates. Most values are above zero, so in general, larger geographic range is associated with lower rates of extinction. This effect is weaker during periods of mass extinction (blue bars). Thinner lines are 95% confidence intervals on estimated odds ratios. Adapted from Payne and Finnegan (2007).

adults at a relatively slow rate. Because they are small for a long period of time and live in the open water, planktotrophic larvae are often dispersed long distances, leading to a broad geographic range for species with such larvae compared to that of species with nonplanktotrophic larvae. When Jablonski examined the larval development patterns, extinction rates were half as high in planktotrophic species (**Figure 15.36**) (Jablonski and Lutz 1983).

FIGURE 15.36 Larval development and survival rates. The relationship between larval development mode (planktotrophic versus nonplanktotrophic), species' geographic range, and extinction in gastropods of the Late Cretaceous period. Adapted from Jablonski and Lutz (1983).



15.5 Rates of Evolutionary Change and Evolutionary Trends

In this last section of the chapter, we will discuss both rates of evolutionary change and evolutionary trends. These topics will allow us to connect our discussion of speciation in the previous chapter with our discussion of extinction in the current chapter and will help us to bridge the gap between microevolutionary and macroevolutionary approaches to the study of evolution.

Rates and Patterns of Evolutionary Change

Here we address questions about *rates* and *patterns* of evolutionary change. To put our discussion of these topics into context, we need to recognize that while paleontologists continue to provide us with a better and better picture of the fossil record, much of the fossil record remains unexplored. Think about it like this: Start at the surface of Earth, and imagine a giant forest made of rock that goes down (rather than up) in space. This forest descends for miles and miles all over the planet, and it is extraordinarily difficult to navigate. Fossil life in this forest is buried in hard-to-reach places, and it exists in a very fragile form, with older and older life being especially

rare and fragile. To make our search even more challenging (and frustrating), parts of the forest are gone forever as the result of a process known as **subduction**. As two tectonic plates collide, subduction occurs when one tectonic plate slides under the other and is carried down toward Earth's mantle, where any fossils are destroyed by the high temperature and pressure.

As we search and discover, catalog life, and generate new hypotheses, we realize that our inverted forest, spanning Earth's circumference and going miles and miles down, is a vast area, and much of it remains dark and hidden. This poses some difficult challenges, especially as we aim to infer the rates and patterns of change that have occurred over evolutionary time. In earlier chapters, we have seen cases where paleontologists could trace a lineage back through evolutionary time and obtain a fairly detailed record of the changes that have occurred in that lineage, including the birth and death of new species. But in most instances, we get a more fragmentary view of evolutionary change. We see snapshots at various points in time, with little information about what has occurred between these snapshots.

Fossil snapshots pose an interesting problem. Suppose that we find evidence for one particular species in the fossil record from 5 million years ago. And suppose that the fossil record from 4 million years ago shows two new species, both of which appear to be descended from that prior species. We may not have much in the way of fossil evidence from the interval spanning 5 million to 4 million years ago, either because we have not had the opportunity to investigate this stratum or because we have done some investigation but found few fossils. In either case, we might say that there is a gap in the fossil record.

With respect to rate and pattern of change, at least two interpretations of this sequence are possible. One possibility is that the fossil record may be hiding a series of small-scale changes that lead from the original species to its two descendant species. Another possibility is that very little change may have occurred during most of the period 5 million to 4 million years ago, and the changes that led to the diversification of species we uncovered may have happened quite rapidly, perhaps so rapidly that we would not expect them to be captured in the fossil record.

Presumably, if we had extensive fossil information from the period 5 million to 4 million years ago in our hypothetical case, we could distinguish between these two possibilities. The problem for evolutionary biologists is that these gaps in the fossil record are common, and they will likely remain so for the foreseeable future (recall our forest analogy). As a result, in the early 1970s, two conceptual schools developed around how to interpret the sorts of data on rates and patterns of evolutionary change when such gaps exist. One of these schools of thought is called phyletic gradualism, and the other is known as punctuated equilibrium (Eldredge and Gould 1972; Gould 1985, 2002).

The **phyletic gradualism model** of evolutionary change can be traced to Darwin and his argument that the adaptations that arise within a population are the result of a slow, gradual process, where any variant that provides the slightest net benefit slowly increases in frequency. Perhaps more critically, on many occasions, Darwin argued that this very same slow, gradual process eventually led to the formation of new species. The theory of phyletic gradualism

hypothesizes that new species arise from a gradual transformation of an ancestral species through slow, constant change. A classic example of phyletic gradualism is the case of equine evolution that we discussed in Chapter 5, in which slow, gradual changes in skull and limb morphology led to new equine species over evolutionary time.

New forms that appear in the fossil record may arise either through branching speciation events—a process known as **cladogenesis**—or through gradual modification of form over evolutionary time without branching speciation, a process known as **anagenesis**. Because paleontologists sample from the fossil record, when the lineage being studied has changed enough via anagenesis—through the slow, gradual accumulation of adaptive and nonadaptive changes—it is considered a new species. The earlier forms no longer occur in the fossil record, and it appears as if this earlier species has gone extinct. We call this phenomenon **pseudoextinction** (Smith et al. 2001), because the lineage has not actually died out; rather, its members have changed so much that they are now reclassified as a new species (**Figure 15.37**).

Eldredge and Gould's **punctuated equilibrium model** provides an alternative to phyletic gradualism. Eldredge and Gould propose that major evolutionary changes do not occur through a slow, gradual process. Instead, while *some* minor degree of change is always occurring within lineages, stasis—the *absence of change*—is the rule during the vast majority of a lineage's history. When evolutionary change does occur in lineages, it is not only rapid but also typically results in branching speciation; that is, cladogenesis. Thus, periods of rapid morphological change coincide with bursts of rapid branching speciation (**Figure 15.38**).

As an extreme example of a period of rapid evolutionary change, consider the **Cambrian explosion**. Fossil evidence from the Cambrian period, approximately 543 million to 490 million years ago, shows a huge spike not just in the number of marine species but also in the number of genera, families, and other taxonomic units, as well as in an exquisite array of new multicellular creatures with new

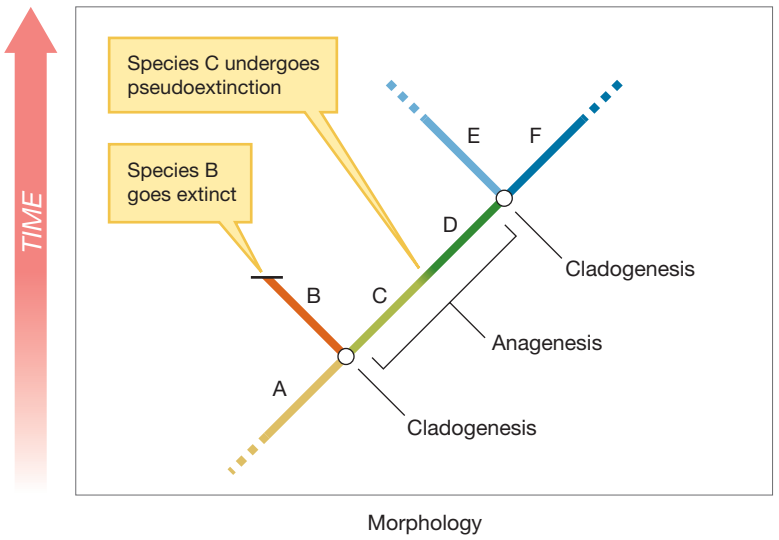


FIGURE 15.37 Cladogenesis, anagenesis, and pseudoextinction. This diagram illustrates the evolutionary fate of a lineage over geological time. Labels A–F indicate species as classified by paleontologists using morphological characters from the fossil record. In this diagram, species A undergoes cladogenesis, giving rise to species B and C by a process of branching speciation. The lineage that includes species B goes truly extinct, whereas the lineage that includes species C changes gradually and by the process of anagenesis is eventually classified as species D. We say that species C has undergone pseudoextinction. Species D then undergoes another round of branching speciation, resulting in species E and F.

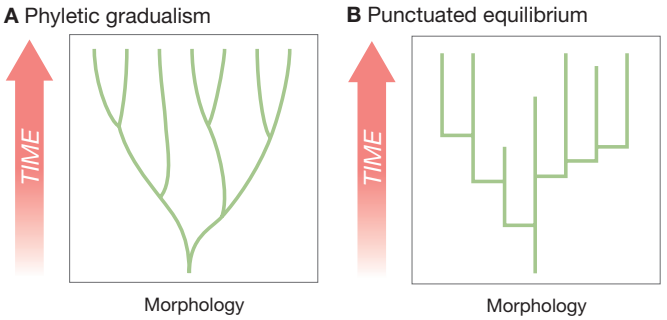


FIGURE 15.38 A schematic of evolutionary trees predicted by punctuated equilibrium and phyletic gradualism. **(A)** Phyletic gradualist models hypothesize that change is slow, gradual, and constant. **(B)** According to punctuated equilibrium theory, stasis—represented by long, vertical lines—is the status quo. When change occurs in lineages, that change is rapid and associated with branching speciation. Adapted from Benton and Pearson (2001).

body forms and shapes. Indeed, most of the animal groups that have ever lived appeared in the fossil record for the first time during the early Cambrian period (Conway Morris 1998, 2006; Smith and Harper 2013) (**Figure 15.39**).

Much of the evidence for the Cambrian explosion comes from an extraordinary bed of fossils known as the **Burgess Shale** (in British Columbia, Canada), which, for a complicated set of geological reasons, contains samples from soft-bodied species that elsewhere tend to fossilize poorly if at all. Active debate continues about the causes of the Cambrian explosion (Smith and Harper 2013). Our point here is not that the Cambrian explosion is best explained by punctuated equilibrium models—for example, molecular evidence suggests that many animal groups may have arisen long before the Cambrian but failed to leave fossil remains until this period (Erwin et al. 2011). The point is simply that the fossil record does show times in which evolutionary change seems to be rapid, such as during the early Cambrian period, and other times when this is not the case.

The theoretical underpinnings of punctuated equilibrium are tied to Mayr's peripheral isolate model of allopatric speciation (Chapter 14) (Eldredge and Gould 1972; Gould and Eldredge 1993). If speciation occurs most often in small peripheral populations, large-scale evolutionary changes likely arise out of rapid punctuated bursts of change. But why? Imagine a large mainland population of animals surrounded by an archipelago of small islands and that these islands are colonized by individuals from the mainland. (The argument applies equally well to other forms of geographic isolation.) As we have learned, when small, genetically isolated subpopulations are adjusting to local conditions, natural selection combined with genetic drift may lead to rapid evolutionary change. If such change is occurring on many small islands, all populated from some larger mainland, the rate of branching speciation may be rapid.

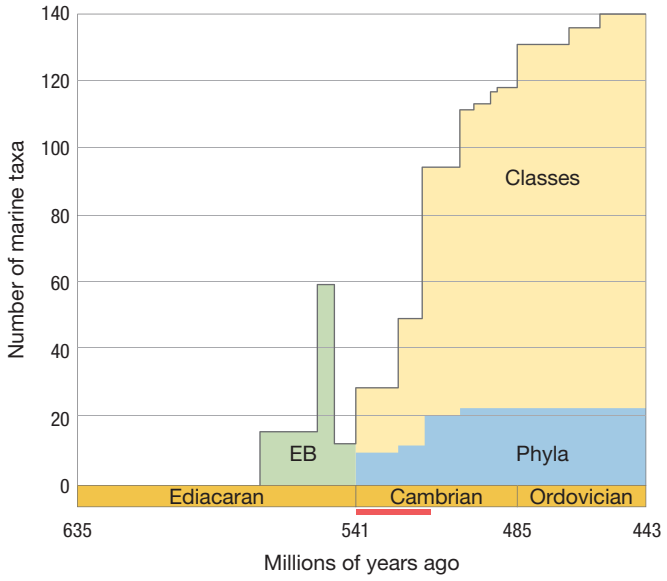


FIGURE 15.39 The Cambrian explosion. Fossil evidence from approximately 543 million to 490 million years ago shows a huge spike in the number of marine genera, families, and other taxonomic units. These include many multicellular creatures with new body forms and shapes. The Ediacaran biota (EB), described earlier in this chapter, are indicated in green. Few if any of these lineages survived into the Cambrian. The red underline denotes the period of the Cambrian explosion. From Smith and Harper (2013).

But there is more to it than that. If populations on islands diverge rapidly, then sometimes one of the new species on an island will migrate back to the mainland and coexist with its ancestral species. If we sample the fossil record, we might see the following: Initially, we will see our ancestral mainland population. If change occurs rapidly on the surrounding islands, we are unlikely to catch that speciation in the fossil record. When we next look back at the mainland, we may find our ancestral species and one of its descendant species that seems to have appeared from nowhere. But, of course, it didn't appear from "nowhere." Rather, the descendant species migrated from small, isolated populations that are unlikely to be sampled from the fossil record.

An example of the patterns of change expected under the punctuated equilibrium model was documented by Alan Cheetham in his work on speciation and evolutionary change in aquatic invertebrates called bryozoans (Cheetham 1986). Using the fossil record from the past 20 million years, Cheetham tracked speciation patterns in one genus of bryozoans (*Metrarabdotos*) by measuring change in 46 morphological characters—including the size and shape of various cells in a colony—in fossil bryozoans from the entire geographic range of genus *Metrarabdotos* (**Figure 15.40**).

The pattern of speciation uncovered by Cheetham resembles the pattern hypothesized by punctuated equilibrium theory. Most bryozoan species showed little change for long stretches of time (represented by the long vertical lines). Then in a punctuated burst of change, speciation occurred (represented by the horizontal lines). The question of what, if anything, caused rapid speciation to occur in many different lineages of bryozoans at about the same time—6 million to 8 million years ago—remains unresolved, although there is some suggestion that pulses of speciation in this group might be tied to the rise and fall of oceanic boundaries over time (Jackson and Cheetham 1999).

What are we to make of this work on punctuated equilibrium, phyletic gradualism, and rates of evolutionary change? One review of nearly 60 studies found evidence for patterns of punctuated equilibrium in some lineages and of phyletic gradualism in others. This review also found numerous studies in which phyletic gradualism best explained change for some period of evolutionary time in a given lineage, while change in other periods of time, for the same lineage, were better described by punctuated equilibrium (Erwin and Anstey 1995).

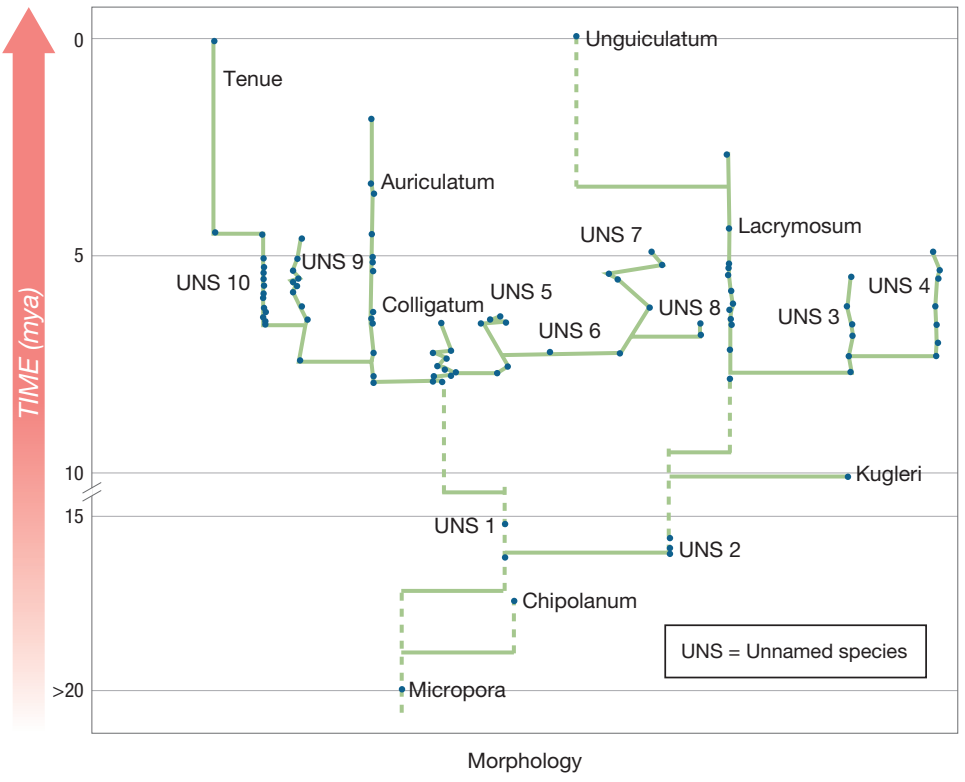


FIGURE 15.40 Punctuated equilibrium in bryozoans. A phylogeny of colonial bryozoan species from the genus *Metrarabdotos*. Time runs along the *y* axis, and morphological difference is depicted on the *x* axis. Stasis is shown in long vertical lines, while punctuated speciation is depicted in horizontal lines. Adapted from Cheetham (1986).

Evolutionary biologists want more than numeric estimates of the frequency of punctuated equilibrium, of phyletic gradualism, or of some model. What we seek is to understand whether the predictions from one of these theories are better supported by the data. Is punctuated change, for example, found more often in island archipelagoes or in environments that change often and dramatically? As of today, we can't answer these questions, but research on this topic continues.

KEYCONCEPT QUESTION

15.4 If geologist Charles Lyell (Chapter 2) had lived to see the debate between the proponents of phyletic gradualism and the proponents of punctuated equilibrium, whose side do you think he would have taken? Why?

Evolutionary Trends

Looking over macroevolutionary timescales, evolutionary biologists sometimes note *trends*—patterns of directional change over time—in clades (Gregory 2008). For example, what is called **Cope's rule** (named after paleontologist Edward Cope) asserts that species in mammalian clades tend to increase in body size over evolutionary time (Hone and Benton 2005). As another example, a number of researchers have argued in favor of a general increase in organismal complexity—measured in any number of ways—over the history of life (McShea 1998; see also Chapter 12).

Many, if not all, trends may hold only along certain branches of the tree of life or only during limited periods of time. For example, Cope originally stated his rule as the observation that mammalian lineages tended to increase in body size during the Cenozoic era. Studies of Cope's rule have since been extended to other groups and other time periods. Though there is still debate on the matter, some bird taxa appeared to follow Cope's rule throughout the Jurassic and Cretaceous periods (Hone and Benton 2005; Butler and Goswami 2008). But Cope's rule—like most other evolutionary trends—is not universal. During the Cretaceous, groups such as bivalves and gastropod mollusks did not change according to the predictions of Cope's rule (Jablonski 1997).

Directional changes of this sort can derive from a number of different evolutionary processes. One important distinction is that between a *passive trend* and an *active trend*. In the absence of any trend, we might expect a character state—body size for example—to spread out randomly in both directions as a clade diversifies, increasing in some lineages and decreasing in others (**Figure 15.41A**).

When the direction of diversification is limited by some kind of constraint on evolution—for example, when the ancestor was already the minimal viable size—variations in body size again will spread out throughout the clade, but only in the unconstrained direction (**Figure 15.41B**). The result is a trend in the sense that we see an increase in the mean body size within the clade, but we call this a *passive trend* because, away from the boundary, evolution is as likely to lead to a decrease in body size as to an increase in body size (Stanley 1973). In other cases, we may

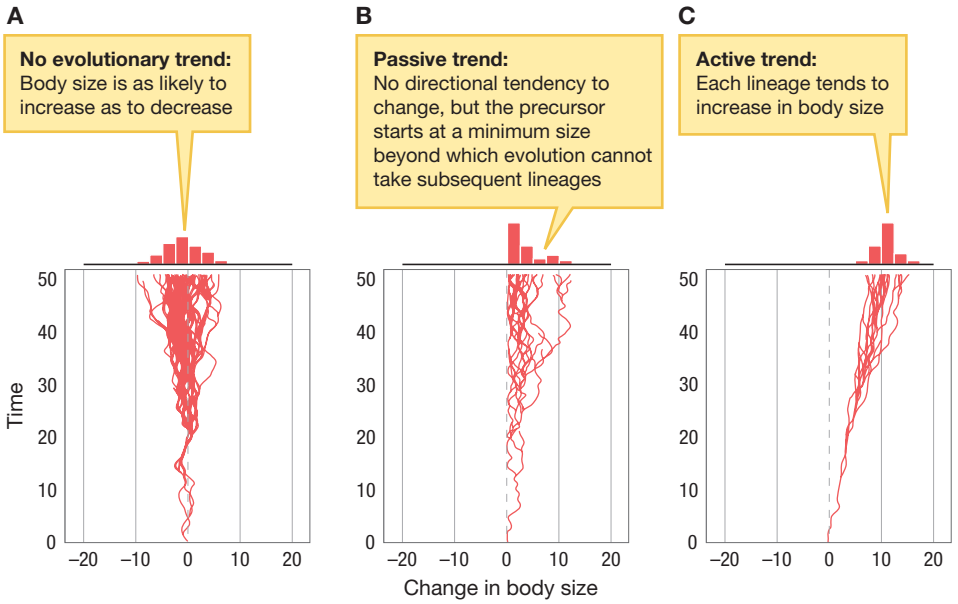


FIGURE 15.41 Passive versus active trends. These graphs provide a conceptual way to think of change in a clade over time. The *y* axis represents time, and the *x* axis represents a character state that we are interested in—body size, in this example. In each, a species starts at some point in character space, and over time, evolutionary changes and speciation events lead to increasing diversity in the character states represented within the clade. **(A)** No evolutionary trend. Different lineages diffuse out in both directions from the original ancestor. **(B)** No directional tendency to the evolution of any individual lineage, but our precursor species starts at a boundary—a minimum value—beyond which a lineage cannot go. As a result, all derived lineages have body sizes at least as large, and often larger, than that of the ancestor. This generates a *passive trend* in which the mean size within the clade increases over time, even though at any point away from the boundary, the evolutionary process is equally likely to lead to an increase or a decrease in size. **(C)** An *active trend*, in which each lineage tends to increase in the character state. Adapted from McShea (1994, 1998).

see a tendency for the entire distribution of body sizes within the clade to increase. Such an *active trend* results in an increase in mean body size even without relying on a boundary, as **Figure 15.41C** illustrates.

Ideally, evolutionary biologists would be able to link an active trend of this type to the underlying selective conditions that generate it. Kingsolver and Pfennig did precisely this in a large-scale analysis of body size evolution in plants, invertebrates, and vertebrates (Kingsolver and Pfennig 2004). They aggregated results from 42 studies of selection on 854 traits in 39 species, and they found that, on average, selection favored traits associated with increased body size. The direction of selection of other traits was evenly distributed between increasing or decreasing their magnitude (**Figure 15.42**). Other work analyzing body size in 30,000 species of ray-finned fishes has found that selection for increased body size is associated with bursts of speciation. Together, these sorts of studies fashion a bridge between microevolutionary studies of local selective conditions and macroevolutionary studies of long-term trends across taxa (Rabosky et al. 2013).

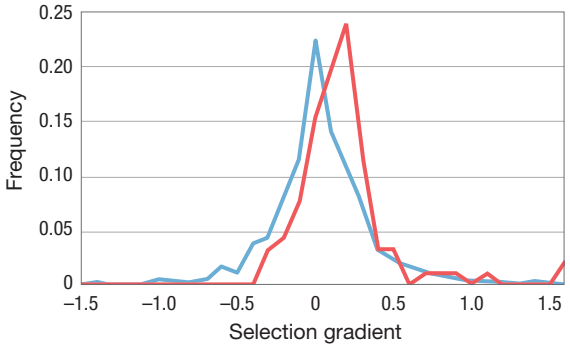


FIGURE 15.42 Selection for increased size. Selection gradients—a measure of the strength and direction of selection—for 854 traits from 39 species. Selection on increased body size tended to be positive (red line), whereas selection on other traits averaged zero (blue line). These results suggest a selective mechanism behind Cope's rule as an active trend. Adapted from Hone and Benton (2005).

Active trends can arise from two distinct processes (**Figure 15.43**). The most straightforward is a process by which the distribution of trait values (for example, size) in a clade shifts because the trait values within each subclade shift in parallel. Alternatively, the average trait value may increase because of **species selection**; that is, the result of speciation and extinction rates that vary according to the value of the trait in question. For example, if species with larger body size are more likely to speciate and/or less likely to go extinct, species selection can result in a shift of the distribution of trait values across species (**Figure 15.44**).

The distinction between parallel evolution within subclades and species selection is similar to the distinction we made in Chapter 2 between transformational and variational processes of evolution—except here we are applying these concepts at the level of species. If each subclade goes through a parallel process of evolutionary change, we have a transformational process at the species level. If instead species vary in some trait, and species with certain trait values are more likely to speciate (reproduce) or go extinct (die), we have a variational process at the level of species, in which species are *sorted* according to their trait values.

Consider the evolutionary trend manifested in the increase in morphological complexity of the Crustacea from the Cambrian period to the present. Focusing on a trait that has been well preserved in the fossil record—the morphological structure of the limb—researchers have documented that over the past 500 million years, there has been a trend toward increasing differentiation of limbs, with

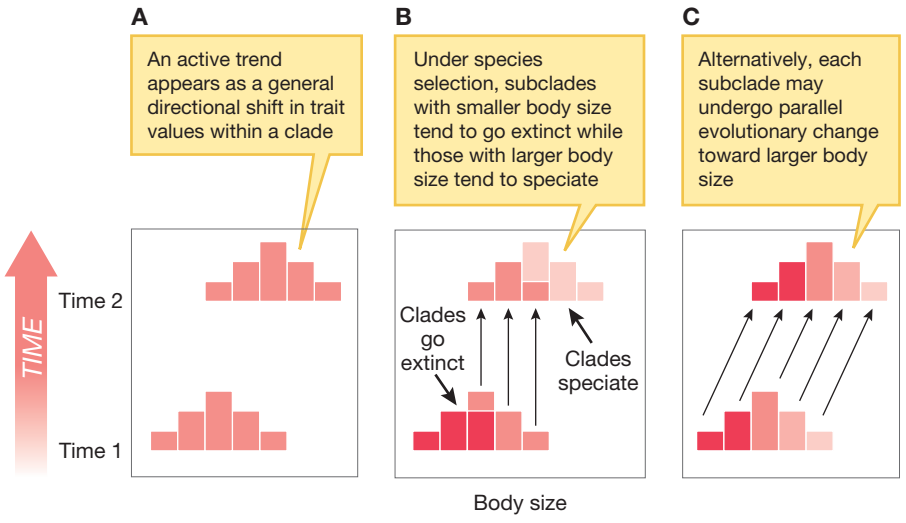


FIGURE 15.43 An active trend can arise from either of two evolutionary processes. **(A)** An active trend—for example, an increase in body size—results in an across-the-board change in the value of the trait of interest; here the entire distribution shifts to the right. **(B)** Species selection occurs when the trait value influences extinction or speciation probabilities. Here, smaller subclades tend to go extinct, larger subclades tend to speciate, and so new subclades tend to be larger in body size. **(C)** Alternatively, an active trend can occur when each subclade undergoes a parallel evolutionary change. Here, that change is toward larger body size. Adapted from Adamowicz et al. (2008).

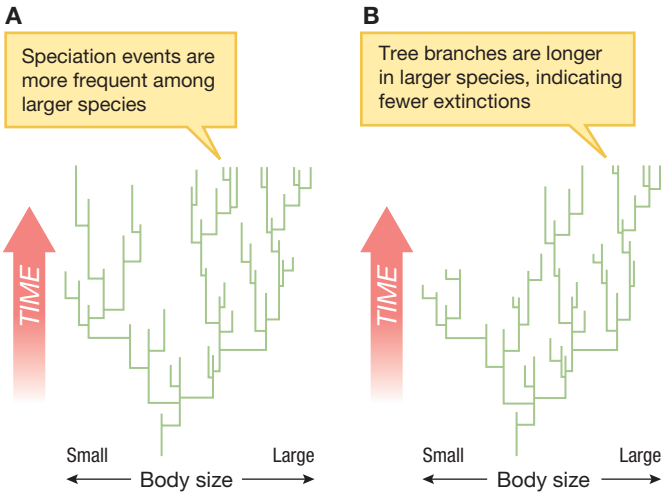


FIGURE 15.44 Species selection can result either from differential rates of extinction or speciation. **(A)** Species selection by differential speciation results in an active trend. Here, larger species speciate at a higher rate. **(B)** Species selection by differential extinction also results in an active trend. Here, larger species go extinct at a lower rate: We can infer this from the fact that branches of the phylogenetic tree are shorter in smaller species. Although the distinction between differential speciation and differential extinction is conceptually useful, both processes can occur simultaneously. Adapted from Gregory (2008).

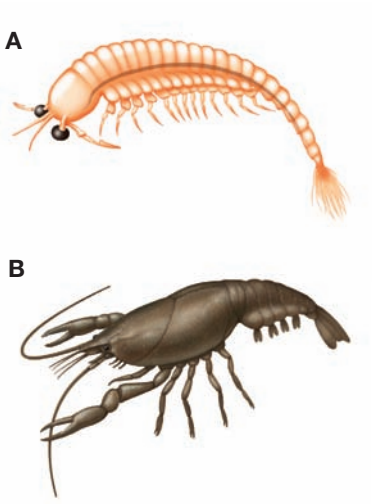


FIGURE 15.45 Comparing complexity of limb structure. This brachiopod **(A)** has minimal complexity in the structure of its leg segments, relative to the crayfish **(B)**.

different morphology on different body segments (Figure 15.45). But why? Is this an example of a passive trend, leading some but not all taxa away from a boundary (namely, having only one limb type)? Is it an active trend due to parallel evolution toward increasing limb complexity in each of many taxa? Or is it an active trend driven by some kind of species selection?

To resolve this question, Sarah Adamowicz and her colleagues compiled data on 66 crustacean orders from extant organisms and from the fossil record (Adamowicz et al. 2008). For each order, they computed an overall indicator of limb morphological complexity known as the Brillouin index, and they established the time at which the order was present in the fossil record. Plotting the Brillouin index as a function of time (Figure 15.46) and testing for statistical significance, they found that complexity has increased significantly from 500 million years ago to the present. The dearth of minimally complex orders at the present time suggests that this pattern is not simply the result of a passive trend.

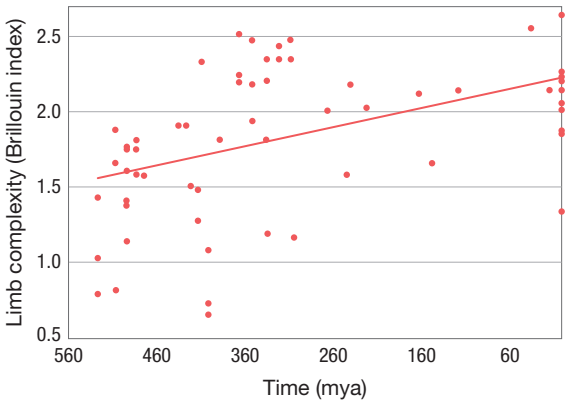


FIGURE 15.46 A trend toward increasing limb complexity in the crustaceans. The complexity of limb morphology in observed fossils increases as we move from 500 million years ago to the present. Note the dearth of low values for present-day and recent fossil species. Adapted from Adamowicz et al. (2008).

But is this increase in complexity due to the differential success of more complex orders, or is it due to a trend toward the development of increasing complexity within individual orders, or to both? To check for parallel evolutionary change within multiple subclades, Adamowicz and her colleagues selected 12 paired comparisons between fossil orders and their closest relatives, chosen so as to make each comparison phylogenetically independent from the others (recall our Chapter 5 discussion of methods to do this). They found that in 10 to 11 of these cases, the present-day orders exhibited greater complexity than that of the fossil

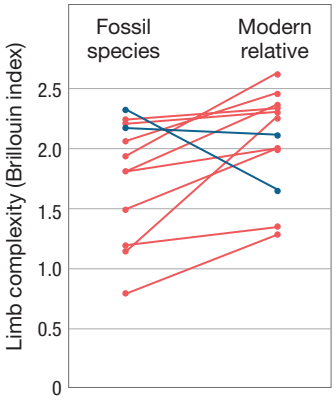


FIGURE 15.47 Increasing limb complexity using phylogenetically independent data points. Paired comparisons (chosen for phylogenetic independence) of fossil crustacean taxa with closest current relatives shows increasing complexity in 10–11 of 12 comparisons. This reveals that there has been parallel evolutionary change in the various subclades. Adapted from Adamowicz et al. (2008).

orders to which they were related (**Figure 15.47**). This indicates an active trend, with complexity increasing in parallel within each individual order.

Adamowicz and her team also found evidence for species selection, with both differential rates of speciation and differential rates of extinction: Newly originated taxa exhibited higher-than-average limb diversity, suggesting a correlation between limb diversity and speciation rate. In addition, they found that extinction events were correlated with low limb diversity. Taken together, these observations suggest that species selection has also contributed to the pattern of increasing limb diversity in the Crustacea.

Because of both the effect of extinction on the tree of life and the alarming rate at which species are going extinct at the hands of modern humans, it is critical that we understand the dynamics of extinction. In this chapter, we have examined background extinction, mass extinction, and evolutionary trends and how evolutionary biologists study both background extinction and mass extinction for clues to their causes and for information on which to build a conceptual framework for understanding extinction.

SUMMARY

1. A species is said to be extinct when all members of that species have died out and left no living representatives. If all species in a genus are extinct, then that genus is extinct. Most species that have ever lived have gone extinct.
2. When estimating extinction dates from the fossil record, evolutionary biologists must be aware of both backward smearing and forward smearing effects.
3. Rates of extinction vary over time. Extinction rates sometimes spike far above normal, or above what are sometimes called background levels. These spikes in extinction rate are called mass extinctions.
4. Many causes for background extinction have been studied, among them predation, competition, and disease. Both direct and indirect effects of predation, competition, and disease may lead to background extinction.
5. Mass extinctions affect many species over a broad geographic range. At least five (and perhaps as many as eight) such mass extinctions have occurred over 600 million years—at the end of the Ordovician, in the Late Devonian, at the end of the Permian, at the end of the Triassic, and at the Cretaceous–Paleogene (K–Pg) boundary.
6. Mass extinction not only leads to fewer species and genera but also decreases diversity with respect to morphology, behavior, the number of different types of niches inhabited by organisms, and developmental patterns.
7. The best studied and most famous of the mass extinctions is the K–Pg mass extinction. A large asteroid that collided with Earth approximately 65 million years ago likely initiated this mass extinction.
8. The late Permian extinction occurred approximately 250 million years ago, and 80% to 96% of all marine species went extinct. This mass extinction may have been triggered by a series of huge volcanic eruptions in Siberia that occurred about 251 million years ago.
9. Two different models—phyletic gradualism and punctuated equilibrium—have been proposed to explain rates of evolutionary change.
10. Across macroevolutionary timescales, evolutionary biologists sometimes find trends—patterns of directional change over time. Some of these trends are passive and some are active. Active trends may arise when the distribution of trait values in a clade shifts because the trait values within each subclade shift in parallel. Alternatively, the average trait value may increase because of species selection.

KEY TERMS

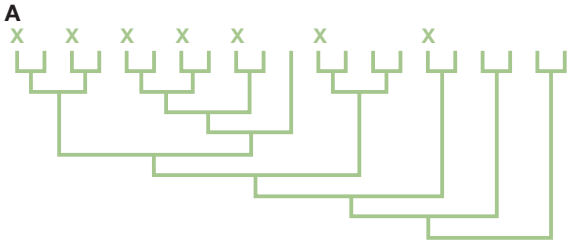
anagenesis (p. 557)
background extinction (p. 533)
Burgess Shale (p. 558)
Cambrian explosion (p. 557)
carbonization (p. 529)
cladogenesis (p. 557)
Cope's rule (p. 560)
endemic (p. 534)
evolutionary radiation (p. 535)
fossil (p. 529)
fossil record (p. 529)
half-life (p. 532)
K–Pg mass extinction (p. 545)
law of superposition (p. 532)
mass extinctions (p. 533)
paleomagnetic dating (p. 533)
phyletic gradualism model (p. 556)
pseudoextinction (p. 557
punctuated equilibrium model (p. 557)
radiocarbon dating (p. 532)
radiopotassium dating (p. 532)
Signor–Lipps effect (p. 533)
species selection (p. 562)
subduction (p. 556)

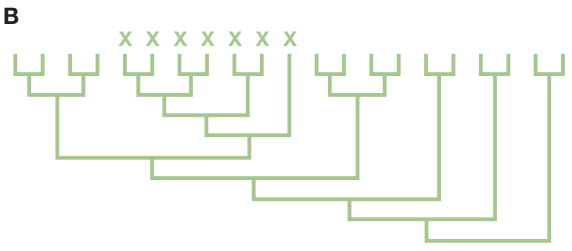
REVIEW QUESTIONS

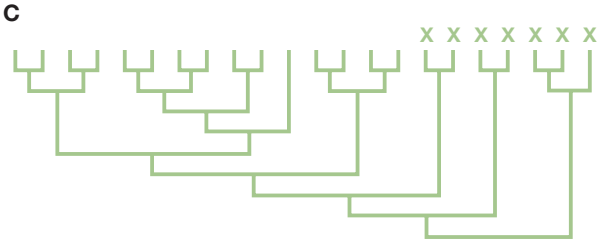
1. Why do we expect extinction rates to be much higher in species that are endemic to islands than in species found more broadly distributed?
2. Why is finding good fossil evidence a combination of skill and luck?
3. What is paleomagnetic dating?
4. What are backward and forward smearing?
5. What are some of the factors associated with background extinction?
6. How did an anomaly in iridium levels provide strong support for the asteroid-impact hypothesis for the K–Pg mass extinction?
7. Why is the Permian mass extinction sometimes referred to as the greatest extinction event ever?
8. What are phyletic gradualism and punctuated equilibrium?
9. What is an evolutionary trend?
10. Describe two different processes that can result in an active evolutionary trend.

KEY CONCEPT APPLICATION QUESTIONS

11. The figure below (adapted from Erwin [2008]) illustrates a number of possible extinction scenarios (extinction events are indicated by an X). Which of these would be the most desirable from the standpoint of conserving phylogenetic diversity? Which might be the least desirable?







12. Let's return to the quote from David Jablonski cited in this chapter: "To the conservation biologist, there is little positive to be said about extinction. From an evolutionary perspective, however, extinction is a double-edged sword. By definition, extinction terminates lineages and thus removes unique genetic variation and adaptation. But over geological time scales, it can reshape the evolutionary landscape in more creative ways, via the differential survivorship of lineages and the evolutionary opportunities afforded by the demise of dominant groups and the postextinction sorting of survivors." What do you suppose Jablonski meant by "reshaping the evolutionary landscape in creative ways?"
13. Why do you think that the study of the Pleistocene megafauna extinction was just the sort of work that would bring about collaborations between evolutionary biologists and researchers in archaeology, anthropology, and even sociology?
14. Imagine a clade of birds with an unornamented common ancestor. Over evolutionary time, the bird species in this clade become more highly ornamented on average. Explain the difference between an active trend and a passive trend using this clade as an example.
15. Leigh van Valen created a plot of species ages as a way to see whether the probability of extinction changes with species age (see Figure 15.34). His plot takes the shape it does because species have an approximately constant probability of extinction regardless of their age. Suppose that instead species all lived for approximately 10 million years and then went extinct with certainty. Sketch what van Valen's plot would have looked like in this case.

SUGGESTED READINGS

Alvarez, W. 1998. *T. Rex and the Crater of Doom*. Vintage, New York. A popular science book on the K–T mass extinction.

Benton, M. 2003. *When Life Nearly Died: The Greatest Mass Extinction of All Time*. Thames & Hudson, London. A nice general discussion of the Permian extinction.

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Harnik, P. G., C. Simpson, and J. L. Payne. 2012. Long-term differences in extinction risk among the seven forms of rarity. *Proceedings of the Royal Society B: Biological Sciences* 279: 4969–4976. An examination of the ways that geographic range and species rarity is associated with extinction rate in the marine fossil record.

PART IV

Evolutionary Interactions

Thomson's gazelles (*Eudorcas thomsonii*) sparring at the Masai Mara Reserve in Kenya.

16

Sex and Sexual Selection

◀ Tail feathers of the Raggiana bird of paradise (*Paradisaea raggiana*).

The New Zealand mud snail, *Potamopyrgus antipodarum*, looks fairly ordinary. It is small—about 4 to 7 millimeters—very common, and it serves as host to dozens of different parasites that lay eggs between the snail's body and its shell (**Figure 16.1**). But for the evolutionary biologist, there is something quite extraordinary about these little snails, and it is that different snails in the same lake reproduce in dramatically different ways.

In Lake Alexandrina, New Zealand, and in many other New Zealand lakes and streams, populations of *P. antipodarum* have two very different kinds of females. Some *P. antipodarum* females reproduce sexually, mating with and being fertilized by males in the population. Other females reproduce asexually: These females produce unfertilized eggs that mature into the next generation of females. In these asexual lineages, each offspring is a clone of its parent.

Among multicellular eukaryotic organisms, the vast majority of species reproduce only sexually. All species of birds and mammals, for example, reproduce sexually. Other species, such as aphids, reproduce sexually some

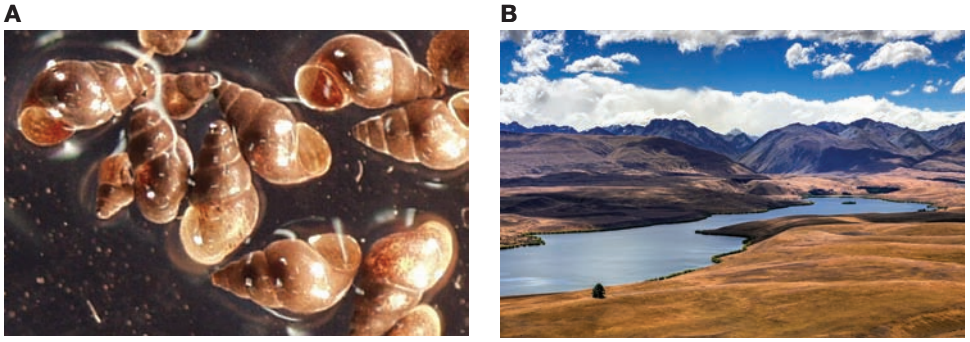


FIGURE 16.1 Asexual and sexual reproduction in snails. The evolution of sexual and asexual reproduction has been studied in the New Zealand mud snail, *Potamopyrgus antipodarum*. **(A)** *P. antipodarum.* **(B)** Lake Alexandrina, South Island, New Zealand, one of the study sites for work on *P. antipodarum.*

of the time and asexually at other times. Other species reproduce only asexually. But it is unusual to discover an animal such as *P. antipodarum* in which some lineages reproduce only sexually (obligate sexual reproduction), while other lineages reproduce only asexually (obligate asexual reproduction).

How can both asexually and sexually reproducing females coexist in the same population? After all, asexual females produce genetic clones of themselves, whereas sexually reproducing females produce offspring that contain genomes that are a mixture of their own and their mate's genes. And as we will see in subsequent sections, all else being equal, asexual lineages should multiply at twice the rate of sexual lineages. So why haven't asexually reproducing lineages of *P. antipodarum* replaced the sexual lineages? Why do we still see sexual reproduction at all in the New Zealand populations of *P. antipodarum?* Beginning in the late 1980s, Curt Lively and his colleagues set out to answer these questions (Lively 1987).

Lively tested three hypotheses regarding what might favor sexual reproduction (**Figure 16.2**). He tested the *environmental unpredictability hypothesis* by examining whether sexual reproduction was seen at higher frequencies in rapidly changing and unpredictable environments (Lively 1987). The basic idea here is that because of recombination, sexual reproduction generates a great deal of genetic variation, and this variation may allow sexual lineages to adapt to unpredictable environments faster than do asexual lineages. Prior work suggested that lakes have changed less over time than streams in New Zealand, so Lively could compare the frequency of sexual reproduction in lakes versus streams and test the prediction that sexual reproduction occurs more frequently in streams, the more unpredictable of the two environments.

The lakes and streams that Lively studied also differed in another fundamental way: Lakes had a greater number of distinct ecological niches—habitats with particular, well-defined resources, competitors, predators, parasites, and pathogens—that could be occupied by snails. Evolutionary theory predicts that sexual reproduction should be favored over asexual reproduction in environments with more niches because the huge number of genotypes generated by sexual reproduction may include genotypes that are able to colonize niches unavailable to individuals from asexual lineages. This idea is referred to as the *multiple niche hypothesis*. In the case of the New Zealand populations of *P. antipodarum*, the multiple niche hypothesis predicts that sexual reproduction

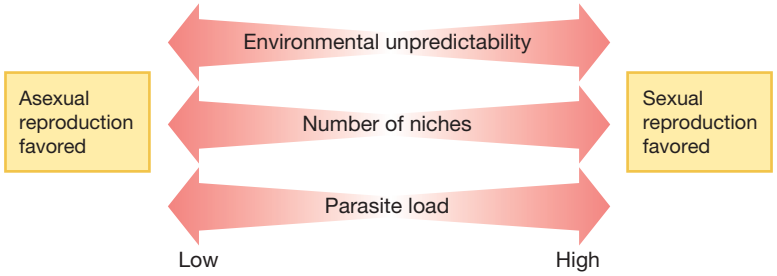


FIGURE 16.2 Three models of how environmental factors favor asexual or sexual reproduction. The environmental unpredictability model suggests that sexual reproduction is favored in unpredictable environments, but not in predictable environments. The multiple niche model suggests that sexual reproduction is favored when there are a large number of different niches available in the environment, but not when there are only a few. The Red Queen hypothesis suggests that sexual reproduction is favored when parasite load is high, but not when parasite load is low.

should be more common in lakes than in streams: the exact opposite prediction from that of the environmental unpredictability hypothesis.

Lively also tested a third model, the **Red Queen hypothesis**, named after the Red Queen in Lewis Carroll's *Through the Looking-Glass*. We will discuss this hypothesis in more depth later in the chapter, but for now, suffice it to say that this hypothesis predicts that the frequency of sexual reproduction will be related to the level of parasitic infection. In particular, the Red Queen hypothesis predicts that *if* parasites infect an asexual lineage, the parasites are likely to be very successful after a relatively small number of generations because in each generation their host's genome remains largely unchanged as a result of asexual reproduction. But in sexual lineages, though offspring still resemble their parents, they are not genetic clones of them. Over generations, the genetic variation produced by sexual reproduction creates a moving target for parasites. Even when parasites can successfully infect sexual lineages, the genetic variation produced by sexual reproduction—including the occasional production of new parasite-resistant genotypes—may favor sexual reproduction when parasites are abundant. Sexual reproduction may not enable the organisms to outrun the parasites totally, but it will at least enable the hosts to keep pace with the parasites rather than being totally overwhelmed by the parasites, as are organisms in asexual lineages.

Lively sampled snails from dozens of different lakes and streams across New Zealand, assayed parasitic infections in these snails, and used the frequency of males in each sample to measure the prevalence of sexual reproduction. He found that sexual reproduction was more common (1) in lakes than in streams, as predicted by the multiple niche hypothesis, and (2) in populations that had high parasite loads, as predicted by the Red Queen hypothesis (**Figure 16.3**). To distinguish between the multiple niche and the Red Queen hypotheses, Lively analyzed the data using statistical tools that allowed him to address the following question: If we control for differences in the frequency of parasites, do lakes and streams still differ in the frequency of sexually reproducing snails? The answer was "no": He found that if the effect of parasites was statistically removed from the analysis, no difference in the frequency of sexual versus asexual reproduction existed between lake and stream populations, weakening support for the multiple niche hypothesis. Next, Lively asked whether there was still a positive correlation between the frequency of parasites and the frequency of sexual reproduction when he controlled for whether the data came from streams or lakes. This time, the answer was "yes," sexual reproduction was more common when the frequency of parasites was high, both in lakes and in streams. The Red Queen model for the evolution of sex best explained the frequency of sexual reproduction in populations of *P. antipodarum.*

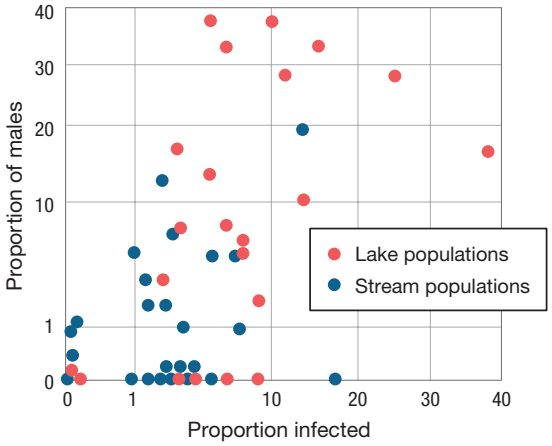


FIGURE 16.3 Parasites and sexual reproduction. *P. antipodarum* are infected with many different parasites. On the horizontal axis is the proportion of individuals infected with *Microphallus* or *Stegodexamene* parasites. The proportion of males in a population—a measure of the prevalence of sexual reproduction in that population—is shown on the vertical axis. Both axes are displayed on a transformed scale. Red circles represent data from lakes, and blue circles represent data from streams. Adapted from Lively (1987).

The literature on the evolution of sexual and asexual reproduction includes other such elegant tests of models. In this chapter, we will examine both asexual and sexual reproduction and discuss the costs and benefits of each type of reproduction. We will also discuss the role of environmental unpredictability and variation and how they can affect the evolution of asexual versus sexual reproduction. We move on to consider how sexual reproduction results in sexual selection, which occurs when individuals of one sex compete for mating opportunities with individuals

of the other sex, and/or members of one sex select mates from among those of the opposite sex. We will try to answer the following questions in this chapter:

- What are asexual and sexual reproduction, and how do we know that sexual reproduction is occurring or has occurred in a lineage?
- What can we infer about sexual and asexual reproduction from their phylogenetic distributions?
- What are the costs and benefits of sexual reproduction?
- What is sexual selection?
- What are conflicts of interest between the sexes, and how do they affect sexual selection?

16.1 Asexual and Sexual Reproduction

To understand the evolution of sexual and asexual reproduction, we will begin by defining these terms. We will then briefly look at the modes of asexual and sexual reproduction.

Asexual Reproduction

In multicellular eukaryotes, **asexual reproduction** is typically defined as the production of offspring from unfertilized gametes (Schurko et al. 2009). There are two forms of asexual reproduction: apomixis and automixis. In both cases, reproduction involves a single, female parent (we do not consider selfing, in which hermaphrodites self-fertilize, to be asexual reproduction). In **apomixis**, unfertilized gametes are produced by mitosis-like cell division, producing daughter cells with an unreduced number of chromosomes. These daughter cells are genetically identical to those of the mother. In plants, apomixis is sometimes referred to as apogamy. **Automixis** involves the production of haploid gametes via meiosis, but diploidy is usually restored by the fusion of haploid nuclei from the same meiosis (some biologists consider this a form of sexual reproduction). Offspring from automictic asexual reproduction are genetically different from their parent and their siblings, but much less genetic variation is generated than in sexual reproduction.

Sexual Reproduction

In a broad sense, **sexual reproduction** involves the joining together of genetic material from two parents to produce an offspring that has genes from each parent (Barton and Charlesworth 1998). More specifically, the process of sexual reproduction is characterized by amphimixis**,** which involves alternating phases of meiosis and gamete fusion (syngamy) (Kondrashov 1993). There are three steps in the process of amphimixis (**Figure 16.4**):

1. Recombination: the crossover between homologous chromosomes, which produces new chromosomal variants.
2. Gamete production: the production of haploid gametes by diploid individuals via reductive meiotic division.

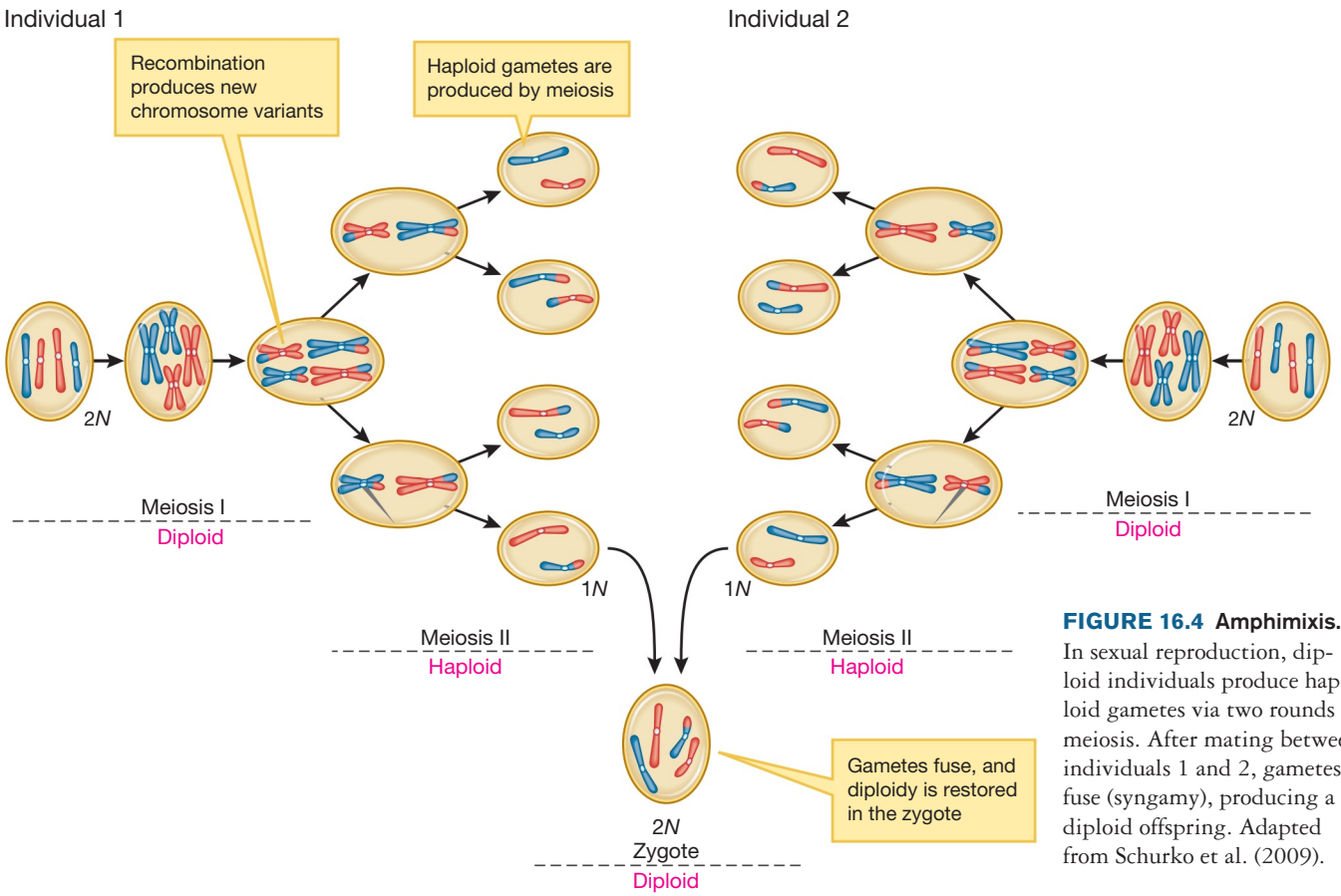


FIGURE 16.4 Amphimixis. In sexual reproduction, diploid individuals produce haploid gametes via two rounds of meiosis. After mating between individuals 1 and 2, gametes fuse (syngamy), producing a diploid offspring. Adapted from Schurko et al. (2009).

3. Gamete fusion: gametic exchange between individuals, in which haploid gametes fuse to produce a diploid offspring.

As we will see, the vast majority of multicellular eukaryotes reproduce only sexually, and virtually all eukaryotes reproduce sexually either at some point in their life cycle or periodically across generations. Mechanisms of genetic exchange among bacteria, such as transduction, conjugation, and transformation, are sometimes also referred to as "sex" (Redfield 2001; Franklin 2007; Michod et al. 2008; Vos 2009). But these mechanisms do not satisfy the definition of sexual reproduction we have presented above (Barton and Charlesworth 1998; Otto and Lenormand 2002).

Distinguishing between Sexual and Asexual Reproduction

Until recently, researchers used natural history to classify a species as reproducing sexually or asexually; that is, evolutionary biologists would search for direct and indirect physical clues of sexual reproduction. The most obvious of these clues would be to observe two individuals mating. In addition, courtship behavior implies sexual reproduction, as do sexual organs. And as all individuals in asexual species are females, the mere presence of males in a population strongly suggests sexual reproduction. While this natural history approach works fairly well for obligately sexual organisms that reproduce sexually every generation, it may be trickier to compile evidence for sex in some facultatively sexual organisms, who

may only have sex rarely or only under certain unobserved conditions. Fortunately, other approaches, such as looking at gene trees and at the genes involved in sex, can also be used to detect sexual reproduction.

One approach involves looking at the genome. The molecular machinery and cellular processes associated with sexual reproduction—recombination, gamete production, and gamete fusion—are complex and involve many genes operating simultaneously. In the nematode, *Caenorhabditis elegans*, more than 1400 such genes have been identified (Reinke et al. 2000). We can use the presence or absence of such genes and their homologs to infer whether reproduction is sexual or asexual in other taxa (Normark et al. 2003; Neiman et al. 2005, 2009; Schurko et al. 2009; Riley and Corradi 2013).

We can also compare phylogenetic trees to help us understand whether a species reproduces primarily sexually or asexually, as Paczesniak and colleagues (2013) did for the freshwater snails we discussed earlier. To see how, recall that mitochondrial DNA (mtDNA) is inherited only through females, but nuclear DNA is inherited through both parents. This means that in asexual species—which contain only females—phylogenetic trees based on mtDNA and nuclear DNA should be fairly congruent; that is, they should be similar to one another. But a comparison of phylogenetic trees based on mtDNA and nuclear DNA genes is predicted to be less congruent in sexual species (although this comparison can be skewed by population bottlenecks and population expansions). We can then use the degree of phylogenetic incongruity between nuclear DNA–based and mtDNA-based trees to infer mode of reproduction (**Figure 16.5**).

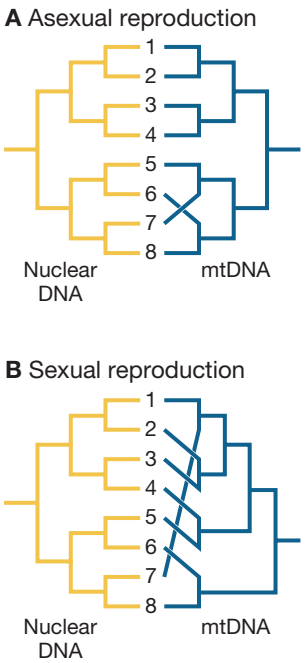


FIGURE 16.5 Using phylogenetic incongruence to infer sexual and asexual reproduction. Four hypothetical phylogenetic trees of eight species, based on either nuclear DNA or mtDNA, are shown. **(A)** When we compare the two trees in an asexual species, we expect them to be quite similar. In this example, only the resolution of the 5,6 and 7,8 subclades has changed. **(B)** When we compare two trees in a sexual species, we expect a greater degree of incongruence between the nuclear DNA tree and the mtDNA tree. In this case, the structures of the two trees differ in numerous ways.

A Phylogenetic Overview of Sexual and Asexual Reproduction

In a moment, we will examine the relative costs and benefits of sexual versus asexual reproduction, but before we do so, let's survey the rather striking phylogenetic distribution of these forms of reproduction in eukaryotes. In eukaryotes, very few species reproduce only asexually. Among vertebrates, for example, of the more than 42,000 species recognized, only 22 species of fish, 23 species of amphibians, and 29 species of reptiles reproduce exclusively by asexual reproduction (Vrijenhoek et al. 1989) (**Figure 16.6**).

Tallying the numbers of asexual versus sexual species tells only part of the story. Evidence also suggests that asexual taxa are short-lived compared to sexual taxa. Although there is much debate as to how to calculate "short-lived" in absolute time, in general, the consensus is that asexual species go extinct more quickly than sexual species (Law and Crespi 2002; Neiman et al. 2009). What is perhaps more critical is that at the level of genus or higher, there are almost no taxa entirely composed of species that only reproduce asexually (although there are a few possible exceptions to this rule, including the bdelloid rotifers, tiny freshwater invertebrates). This translates into a "twiggy" phylogenetic distribution for species that reproduce only asexually. Generally speaking, asexual species are rare and short-lived, and hence they tend to be tiny twigs on phylogenetic trees (**Figure 16.7**). Indeed, to date, the evidence suggests that all species of eukaryotes that reproduce only asexually are derived from an ancestral sexual species, and genes associated with meiosis have been uncovered in all major eukaryotic radiations,

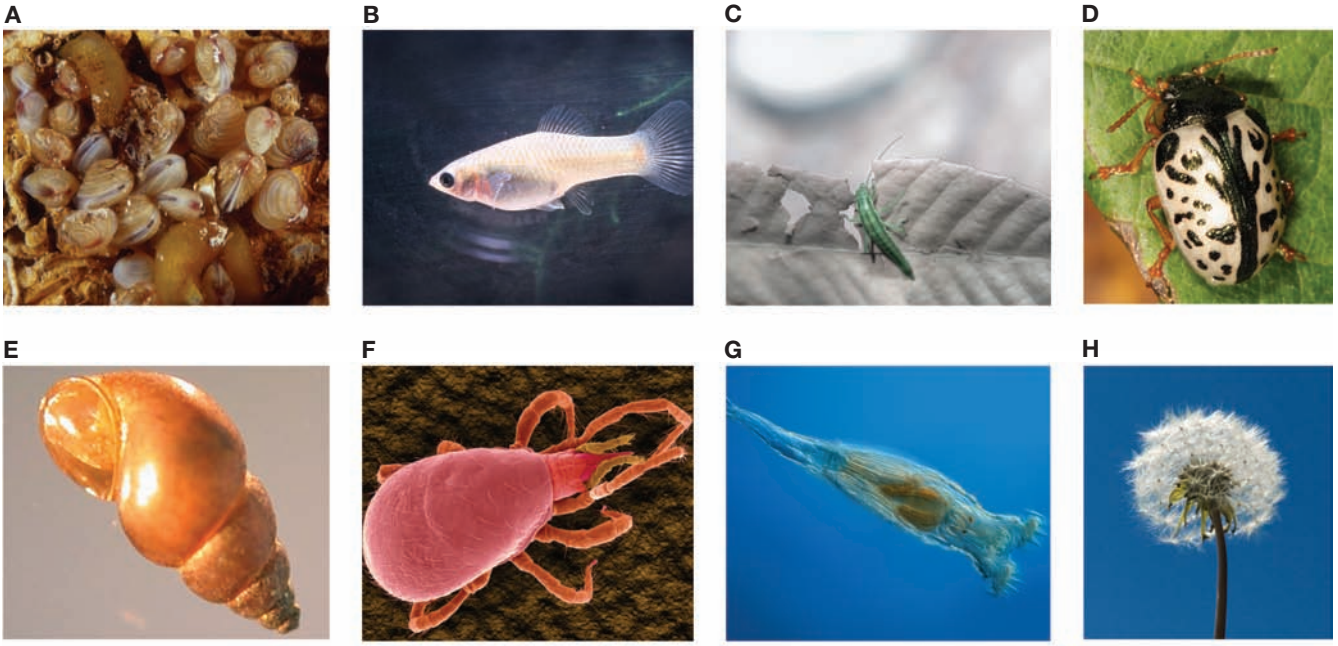


FIGURE 16.6 Examples of asexual species from a variety of taxa. Although asexual species are rare among eukaryotes, they can be found in a number of taxa as shown here. **(A)** *Lasaea australis*, marine clam. **(B)** *Poecilia formosa*, Amazon molly. **(C)** *Timema douglasi*, stick insect. **(D)** *Calligrapha suturella*, leaf beetle. **(E)** *Potamopyrgus antipodarum*, snail. **(F)** *Archegozetes longisetosus*, oribatid mite. **(G)** *Philodina roseola*, bdelloid rotifer. **(H)** *Taraxacum officinale*, dandelion.

strongly suggesting that sexual reproduction is the ancestral state in eukaryotes (Brawley and Johnson 1992; Ramesh et al. 2005; Kobiyama et al. 2007; Malik et al. 2008; Phadke and Zufall 2009; Fritz-Laylin et al. 2010; Lahr et al. 2011; Peacock et al. 2011; Vanstechelman et al. 2013; Goodenough and Heitman 2014).

KEYCONCEPT QUESTION

16.1 In what sense is it surprising that every eukaryote species that reproduces strictly asexually is derived from an ancestral sexual species?

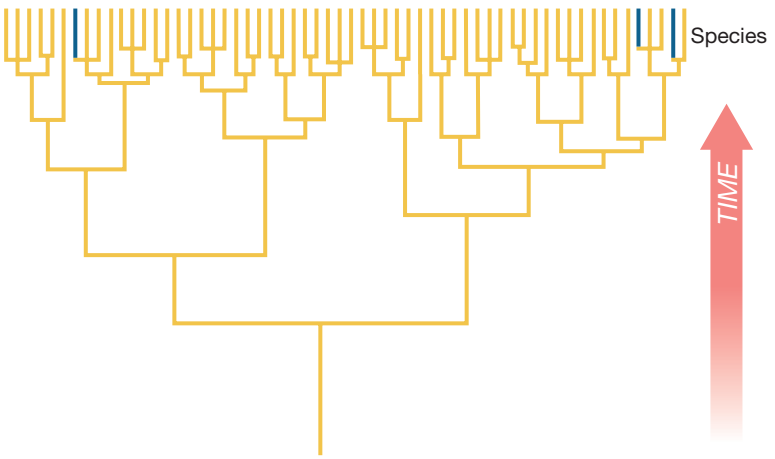


FIGURE 16.7 A hypothetical phylogenetic distribution of asexual species. This figure represents a typical animal phylogeny. In animals, asexual species (blue) compared to sexual species (gold) are rare, making up less than 0.1% of all animal species. Asexual lineages are also relatively short-lived on an evolutionary timescale. Adapted from Rice (2002).

16.2 The Costs of Sexual Reproduction

Our phylogenetic survey reveals that sexual reproduction is the norm among eukaryotes. This poses something of a challenge to evolutionary biologists, because sexual reproduction has a number of significant costs associated with it (Meirmans et al. 2012). The most obvious of these costs is that diploid sexual females produce haploid gametes containing only one of the two sets of chromosomes that they possess. A haploid gamete, should it successfully fuse with another haploid gamete, will produce a diploid offspring that contains only one set of its mother's chromosomes (and one set of its father's chromosomes). A diploid asexual female—all asexual individuals by convention are referred to as females—produces offspring that possess two sets of chromosomes from the mother. All else equal, the asexual female passes on twice as many copies of her genes as does the sexual female. Another way to say this is that, assuming no inbreeding, asexual females are twice as genetically related to their offspring as are sexual females (**Figure 16.8**).

In this section, we will discuss other costs to sexual reproduction, and in Section 16.3, we will describe some compensating benefits that may be responsible for natural selection favoring the evolution of sexual reproduction.

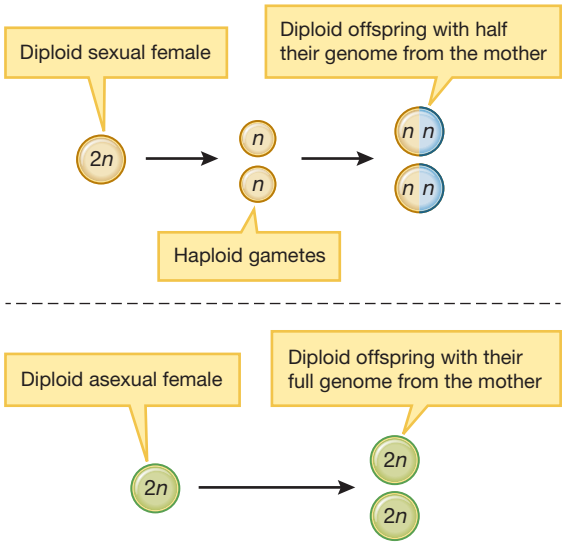


FIGURE 16.8 Sexual versus asexual diploid reproduction. As a result of meiosis, each gamete produced by a sexual female has only one set of the mother cell's chromosomes. Her diploid offspring also include a set of chromosomes from the father, here shaded in blue, so only one-half of the genome of the sexual diploid offspring comes from the mother. An asexual female passes two sets of chromosomes to each diploid offspring cell, so the entire genome of asexual diploid offspring comes from the mother. As such, diploid asexual females are twice as related to each of their offspring as are sexual females.

The Twofold Cost of Sex

In the 1970s, evolutionary biologist John Maynard Smith made the following argument: Consider a population that is made up of asexually reproducing females, as well as sexually reproducing males and females (Maynard Smith 1971, 1978). The number of asexuals in such a population should grow at twice the rate of the sexually reproducing individuals. The reason for this is, from a demographic perspective, a male's only function is to inseminate females; males never produce offspring directly. Asexual females avoid the "cost of producing males" by producing offspring that do not need to be inseminated in order to reproduce themselves; that is, by producing only females.

To see how the Maynard Smith model works, consider a population of sexually reproducing individuals, into which a small number of asexually reproducing females are introduced by mutation or migration. Let n = the number of asexually reproducing females in our population, and let N_m = the number of males and N_f = the number of sexually reproducing females in our population. For simplicity, let's assume an equal sex ratio in the sexually reproducing population, so that $N_m = N_f = N$. In generation t, let k be the number of offspring produced by a female, and assume that this number is not affected by whether the female reproduces sexually or asexually. Finally, let s = the probability that an offspring will survive and eventually breed. Maynard Smith calculated the number of adults in generation $t + 1$ (Maynard Smith 1971, 1978) as follows:

	Adults in t Generation	Offspring	Adults in $t + 1$ Generation
Asexually Reproducing Females	n	kn	skn
Sexually Reproducing Adults	$2N$	kN	skN

Now let's see what has happened to the proportion of asexual females in our population. At generation t, the proportion of asexual females—that is, the number of asexual females divided by the total number of individuals—was $n/(2N + n)$. In the next generation, this proportion is now

$$\frac{skn}{skN + skn} = \frac{sk(n)}{sk(N + n)} = \frac{n}{N + n}$$

The proportion of asexually reproducing females has gone from $n/(2N + n)$ to $n/(N + n)$. When n is small compared to N—that is, when a population is composed mostly of sexually reproducing individuals and only a few asexual females, n will be small compared to N—the proportion of asexual females will approximately double each generation, from approximately $n/(2N)$ to approximately n/N. Maynard Smith called this the **twofold cost of sex**. As n gets larger, the proportion of asexual females still increases each generation, but not at so fast a rate. In **Figure 16.9**, we show how to conceptualize the twofold cost of sex when we begin with one asexual population and one sexual population, rather than one population with both asexual and sexual individuals.

The twofold cost of sex is a consequence, not of sex itself, but rather of **anisogamy**, which is the production of two different kinds of gametes that are usually called sperm and eggs (Bell 1982). Imagine that the growth of a lineage is constrained by the amount of resources that parents can invest in the biomass of their gametes, and thus in the biomass of their offspring. In the case of sex with anisogamy, a female produces large gametes, which when fertilized have sufficient biomass to develop into an adults. For example, in **Figure 16.10A**, a female can produce two large gametes. In anisogamous sexual reproduction, males do not invest resources in offspring biomass. Rather, they produce millions to billions of tiny sperm, of which only a few will pass on genes—and essentially no other biomass—to the next generation. In Figure 16.10A, two parents produce two zygotes. So far as the growth rate of the *lineage* is concerned, the male reproductive effort is wasted on sperm, most of which do not ever fertilize eggs.

In the case of asexual reproduction, all individuals in the population are female, and thus all reproductive effort is invested in large gametes and thus in offspring biomass. In **Figure 16.10B**, each of two females produces two offspring. Two parents produce four zygotes, and thus this asexual lineage grows at twice the rate of the sexual lineage in Figure 16.10A. This is the twofold cost of sex that we have described earlier.

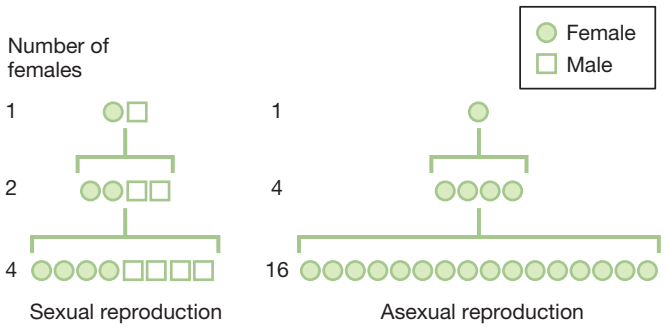


FIGURE 16.9 The twofold cost of sex. If sexual and asexual females each produce four offspring (four females in an asexual population, two males and two females in a sexual population), the population size increases twice as fast in asexual versus sexual populations.

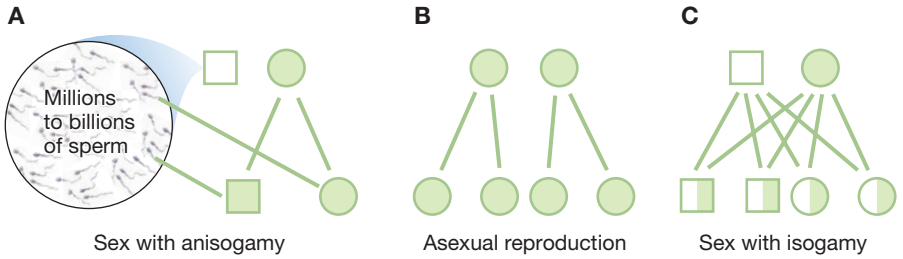
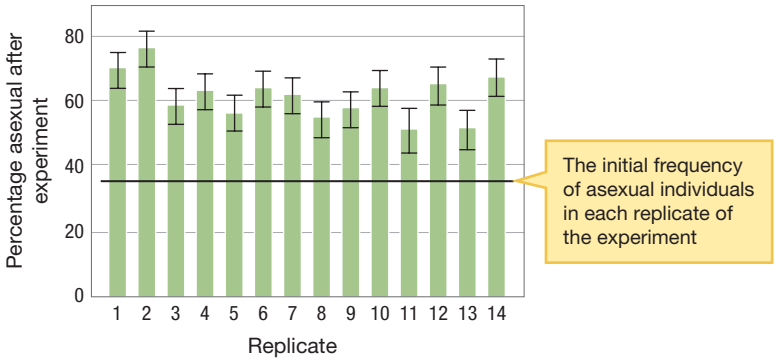


FIGURE 16.10 The twofold cost of sex arises only in anisogamy. **(A)** With anisogamous sexual parents, only the female invests in offspring biomass; the male invests in sperm that mostly go to waste so far as the growth rate of the lineage is concerned. **(B)** In asexual reproduction, all parents are female and invest entirely in offspring production. **(C)** With isogamous sexual parents, investment again goes to biomass rather than "wasted" sperm, and the lineage is able to grow at the same rate as an asexual lineage. Males are represented by squares; females by circles. Each offspring in panel C is colored white and green to indicate investment from both parents. (In isogamous mating systems, biologists often refer to "mating types" rather than males and females.)

In the case of sex with **isogamy**—when individuals in a population produce one type of gamete—each parent produces mid-sized gametes that, when they fuse, are together the size of the large gametes produced by anisogamous females. In **Figure 16.10C**, each parent can produce four mid-sized gametes. These eight gametes fuse to produce four zygotes. These two sexual isogamous parents produce as many offspring as two asexual parents—they do not pay the twofold cost of sex. Thus, the twofold cost of sex arises because under anisogamy, males invest in sperm—most of which are wasted—rather than in biomass that goes to the offspring. For this reason, the twofold cost of sex is sometimes called the "cost of males."

The twofold cost of sex is supported empirically as well as theoretically. For example, Curt Lively and his colleagues tested the prediction that the proportion of asexual females should increase in mixed populations of asexual and sexual individuals of *Potamopyrgus antipodarum*, the snail species that we discussed at the opening of this chapter. They created 14 replicate experimental populations, each composed of 120 sexual snails and 65 asexual snails—that is, each tank in their experiment was made up of 35% asexual individuals—from Lake Alexandrina in New Zealand. One year later, after two or three snail generations, the frequency of asexual individuals had increased dramatically in each and every experimental population—on average, the frequency of asexual individuals rose from 35% to 62% in a single year (Jokela et al. 1997) (**Figure 16.11**).

FIGURE 16.11 Competition experiment with asexual versus sexual snails. Each of 14 experimental replicates began with 35% asexual and 65% sexual individuals. The percentage of asexually reproducing snails significantly increased in this 1-year experiment. Adapted from Jokela et al. (1997).



Sex Can Break Up Favorable Gene Combinations

As we will discuss in more detail soon, there are many benefits to the genetic variation that is created as a result of the outcrossing and recombination during sexual reproduction (Felsenstein 1974, 1988; Felsenstein and Yokoyama 1976). But recombination also has its costs, in that it can potentially break up associations between gene combinations that have been favored by natural selection. When we speak of a favorable gene combination, we mean that an allele at one locus is favored when it occurs in the presence of a specific allele at another locus, but not otherwise. For example, imagine a two-locus combination, in which genotypes *AB* and *ab* are favorable haplotypes. Recombination breaks up these good combinations and produces disfavored *aB* and *Ab* haplotypes.

Other Costs of Sex

Compared to asexual reproduction, sexual reproduction has other costs as well. These costs include the following:

1. The search for potential mates requires time and energy. For example, John Byers and his team tracked individually marked pronghorns (*Antilocapra americana*). They then compared the energy used by pronghorns actively searching for mates and those not actively searching for mates. Over a 2-week period, the energy expended by members of these groups differed by approximately the energy used by an average pronghorn in half a day (Byers et al. 2005).
2. Courting potential mates takes time and energy. These costs have been examined in many frog species in which males form choruses to court females and sing for hours each evening—sometimes for weeks at a stretch—to attract females (Wells and Schwartz 2007). The cost of mating is not limited to males. In dumpling squid (*Euprymna tasmanica*), for example, matings last 3 hours. After such copulations, the swimming endurance of both males and females is cut in half, dramatically affecting their ability to forage and avoid predators (Franklin et al. 2012).
3. When individuals are searching for and courting potential mates, they are often less vigilant for predators in the environment. For example, experimental manipulations have found that individual *Littorina plena* snails that are part of "mating pairs" are more prone to being attacked and captured by predators than are other individual snails, and that snails respond to predation threat by decreasing mating in the presence of predators (Koch et al. 2007).
4. During the process of sexual reproduction, individuals may become infected with parasites from mates or potential mates. Parasitic infection may occur during courtship, copulation, and/or as gametes travel through an individual's reproductive tract (Lockhart et al. 1996; Knell and Webberley 2004, Ashby and Gupta 2013). Indeed, an entire class of *sexually transmitted diseases* (STDs), including those from viruses, bacteria, protozoa, fungi, and arthropods, has been the subject of investigation in both the medical sciences and evolutionary biology.

KEYCONCEPT QUESTION

16.2 Can you think of another cost of sexual reproduction associated with courting potential mates?

16.3 The Benefits of Sexual Reproduction

Despite all of the costs, sexual reproduction is the norm across the eukaryotes. How is this possible? The answer must involve the benefits associated with sex—so what are they?

Almost all of the hypotheses addressing the advantages of sexual over asexual reproduction are grounded in one of two ideas: (1) sexual reproduction purges deleterious mutations, and (2) sexual reproduction generates genetic variation, some of which is responsible for traits favored by natural selection. We will look at these hypotheses in turn, but note first that they are not mutually exclusive.

Sex Purges Deleterious Mutations

Offspring produced through sexual reproduction are genetically different from their parents. One consequence of this is that when a deleterious mutation arises in a sexual population, individuals with this mutation can produce offspring without it. This is not the case with asexual reproduction, in which whole genomes are passed on from parent to offspring. As a result, deleterious mutations can't be purged as readily in asexual species as in sexual species.

The irreversible buildup of deleterious mutations in asexual populations was first discussed by population geneticist Herman Muller and has come to be known as **Muller's ratchet** (Muller 1932, 1964), because a ratchet turns in only one direction and locks there until it turns in that direction again. Muller's basic idea amounts to this: Imagine a population of asexual organisms in which deleterious mutations occur, but back mutations—mutations from deleterious to wild type—do not occur. Let the smallest number of deleterious mutations present in any individual's genome be some number j. For the sake of illustration, let's say that j is 1. Eventually, a new deleterious mutation or set of mutations will emerge in each and every genome in which $j = 1$. When that happens, j increases to 2. The "ratchet" has clicked one turn, so that the minimal number of deleterious mutations per genome in our asexual population is now 2. Or it may be that any initial individuals with $j = 1$ die or fail to reproduce, leaving no descendants. Again, j will now be 2 (or more). Without the recombination that occurs in sexual reproduction, j can only increase; it can never decrease (**Figure 16.12**).

Note that looking at Muller's ratchet is not the same as looking at the fixation of deleterious alleles. The ratchet can turn without any particular deleterious mutation becoming fixed (**Figure 16.13A**). Similarly, a deleterious mutation can be fixed without causing the ratchet to turn (**Figure 16.13B**).

Muller noticed that one function of recombination is that it can reverse the ratchet effect we have been discussing. By recombining different segments of the chromosome, a region with few mutations from one parent can be combined with a region with few mutations from another parent to generate a new genome with fewer deleterious mutations than were present in any single individual parent (**Figure 16.14**). But recombination cannot in and of itself reverse the fixation of deleterious mutations; once a particular deleterious mutation is fixed in the population, recombination cannot undo this fact.

The ability to reverse Muller's ratchet and purge deleterious mutations is a beneficial consequence of sexual reproduction. But is there empirical evidence for this theoretical result? One way to answer this question is to directly

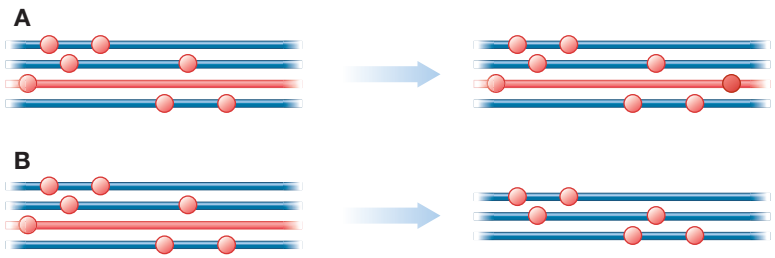


FIGURE 16.12 Muller's ratchet. **(A)** In the population on the left, most of the genomes (lines) have two deleterious mutations (red circles), but the highlighted genome (red line) has only a single deleterious mutation. When a new deleterious mutation (shown in dark red) arises in that genome, all genomes in the population have at least two deleterious mutations, and Muller's ratchet has turned. **(B)** Muller's ratchet can also turn if the genome or genomes with the fewest deleterious mutations fail to leave any descendants in the next generation. Here, the ratchet turns because the genome indicated by the red line fails to produce offspring, and thus all genomes in the subsequent generation have two deleterious mutations.

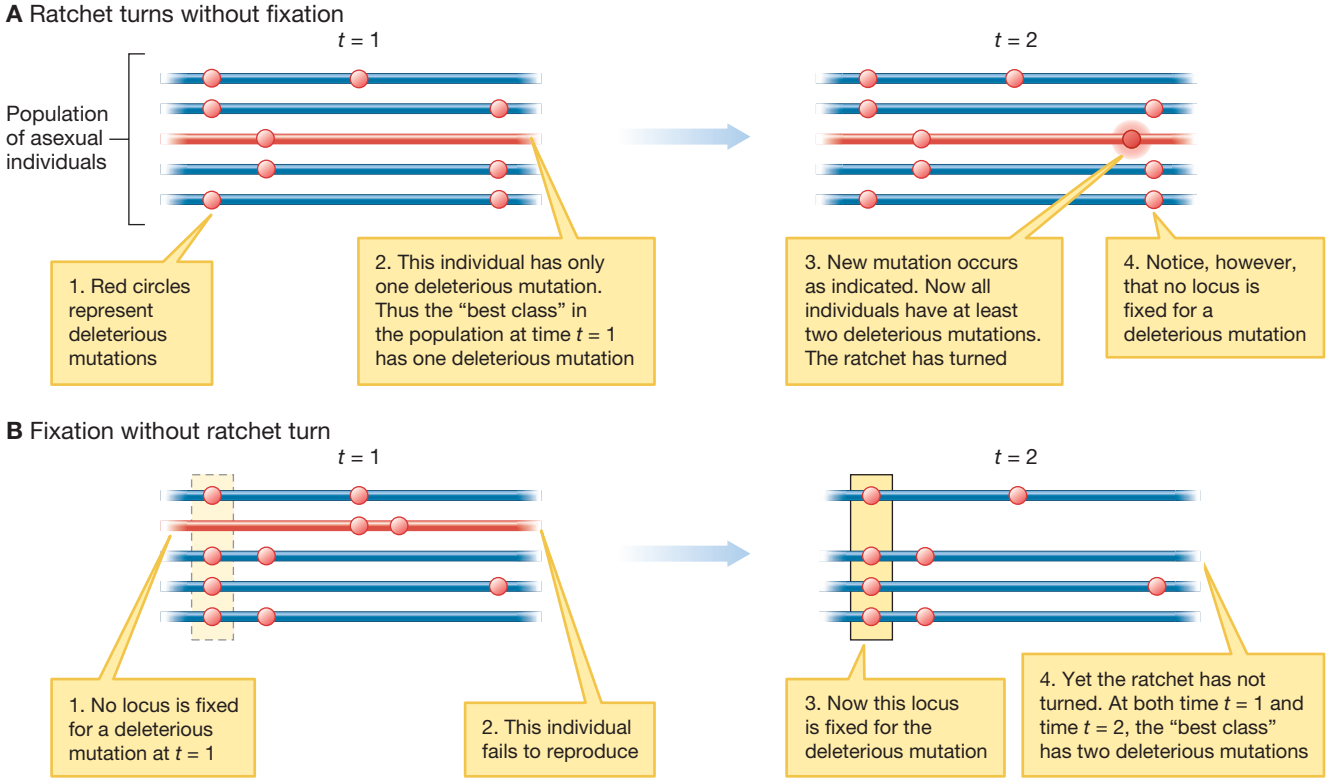


FIGURE 16.13 Muller's ratchet versus fixation of deleterious mutation. Muller's ratchet and the fixation of deleterious mutations are two processes by which deleterious mutations accumulate in an asexual population. In each image, the lines indicate chromosomal segments, and the red circles represent the positions of deleterious mutations. **(A)** Muller's ratchet turns without fixation of any particular deleterious mutation. At time $t = 1$, the "best class" in the population features one deleterious mutation only. All other individuals have two. At $t = 2$, this individual's offspring picks up a novel deleterious mutation. Now all individuals in the population have at least two deleterious mutations. Muller's ratchet has turned. Notice that fixation has not occurred; no locus (at $t = 1$ or at $t = 2$) is fixed for the deleterious mutation. **(B)** A deleterious mutation is fixed without a turn of Muller's ratchet. At $t = 1$, no locus is fixed for a deleterious mutation. But the sole individual without the deleterious mutation at the indicated locus fails to reproduce and leaves no offspring at $t = 2$. Now the indicated mutation has become fixed. Notice that the ratchet has not turned; both at $t = 1$ and $t = 2$, the "best class" in the population has two deleterious mutations.

compare sexual populations with asexual populations. Maurine Neiman and her colleagues asked this question by comparing sexual and asexual populations of the snail *P. antipodarum*, which we discussed at the beginning of the chapter. They found that recently derived asexual populations of this species had accumulated more mutations (Neiman et al. 2010). Similar work using sexual and asexual lineages of a species of water flea, *Daphnia pulex*, reveals that deleterious mutations accumulate at 4 times the rate in asexual versus sexual lineages (Paland and Lynch 2006).

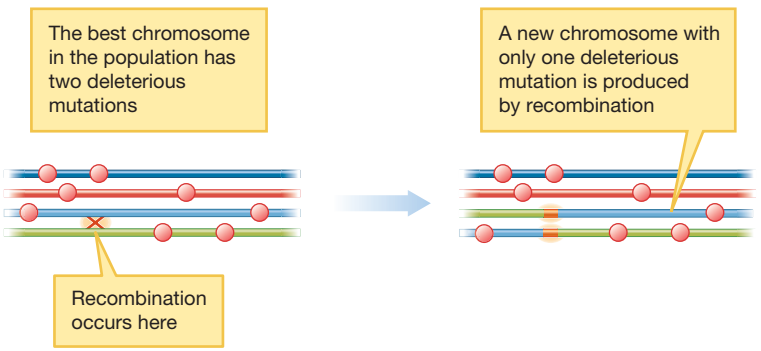


FIGURE 16.14 Recombination reverses Muller's ratchet. Prior to recombination, every chromosome in the population has at least two deleterious mutations, indicated by the red circles. Recombination then occurs as indicated by the orange crossover point. This creates two new chromosomal variants, one with only a single deleterious mutation, and one with three deleterious mutations. Recombination has driven the ratchet backward.

Comparable sexual and asexual populations can be hard to come by, but there is another way to test the theory using species that are entirely sexual. The key to this approach is that the Y chromosome has no homolog, it does not undergo recombination, and thus it functions much as if it were in an asexually reproducing species. By comparing the Y chromosome to the other chromosomes in a sexual species, we can get another view on how recombination affects the accumulation of deleterious mutations.

Comparisons of the Y chromosome to other chromosomes show that the Y chromosome is not only significantly smaller, with few functional genes, but also that this chromosome has "degenerated" and accumulated nonfunctional, likely deleterious genes at a faster rate than other chromosomes (Rice 1994, 2002; Lahn et al. 2001; Tilford et al. 2001; Wilson and Makova 2009; Sayres et al. 2014). These results are consistent with mutation accumulation models of sex, although other processes may also contribute to the degeneration of the Y chromosome.

Alexey Kondrashov has expanded Muller's ratchet model to consider how *epistasis*—interactions between the effects of alleles at different loci—influences the accumulation or purging of deleterious mutations. He considered the case in which the effects of mutations are synergistic, in the sense that two mutations that occur together have a stronger detrimental effect than the summed effect of each mutation alone (Kondrashov 1982, 1988, 2001). Kondrashov's model found that such synergistic epistasis strongly favors recombination, and hence sexual reproduction over asexual reproduction. Indeed, Kondrashov found that under synergistic epistasis, when the deleterious mutation rate per diploid genome per generation is greater than 1, sexual reproduction is favored over asexual reproduction.

The evidence available to date—primarily obtained from work on *Escherichia coli* and *Saccharomyces cerevisiae*—suggests that such synergistic epistasis among mutations occurs, but whether it is prevalent enough to explain the maintenance of sex remains unclear (Elena and Lenski 1997; He et al. 2010; Dettman et al. 2012). As evolutionary geneticists and molecular biologists gather more data on mutation rates and on the extent of synergistic epistasis among mutations, we will be better able to test this idea (Kondrashov 1988; Kondrashov and Kondrashov 2010).

Sex Accelerates Adaptive Evolution: The Fisher–Muller Hypothesis

One advantage to sexual reproduction is that recombination allows natural selection to operate at a quicker rate than is possible in asexual species. This idea was first proposed by R. A. Fisher (1930) and later discussed by Herman Muller (1932), each of whom made the following argument: Compare two large populations, one sexual population and one asexual population. Imagine that a beneficial mutation, *A*, arises and increases in both of our populations. Now suppose that a second beneficial mutation, *B*, arises. In an asexual population, *AB* individuals can only come about if the *B* mutation occurs in an individual with *A*. But, in sexual populations, recombination can bring the two beneficial mutations together even when the *B* mutation does not arise in an individual that already has *A*: an *AB* individual can be the product of a mating between one individual with *A* and

one with *B*. If a third beneficial mutation, *C*, now arises, we can use the same argument for how the frequency of *ABC* individuals can increase more quickly in sexual populations (**Figure 16.15**). The Fisher–Muller hypothesis, then, predicts that sexual reproduction will accelerate the speed at which evolution operates. In our example, we get high frequencies of *ABC* individuals more quickly in large sexual populations than in asexual populations. Notice that the key thing that sex is doing in this model is breaking down linkage disequilibrium (Felsenstein 1988). When beneficial mutations *A*, *B*, and *C* arise in different individuals, there is initially linkage disequilibrium among these loci; that is, the presence of *A* guarantees the absence of *B* and *C*, and so on. Sex makes it possible to have *A* and *B* or *A* and *C*, and so on, in the same individual, breaking down this nonrandom association.

Experimental evidence comparing fitness in asexual versus sexual populations of yeast and green algae (*Chlamydomonas reinhardtii*) supports the prediction of the Fisher–Muller hypothesis (Zeyl and Bell 1997; Greig et al. 1998; Colegrave 2002; Goddard et al. 2005). In one of these experiments, Austin Burt and his colleagues experimentally created asexual lines of yeast by deleting two genes (*SPO11* and *SPO13*) associated with meiosis and recombination in yeast cells from a line of yeast that has a typical sexual phase during its reproductive cycle (Goddard et al. 2005). They replaced one of these genes with a neutral marker—a marker gene that did not otherwise affect function in the yeast—allowing them to distinguish asexual lineages. These manipulations enabled them to compare populations of yeast that differed only with respect to whether reproduction was sexual or asexual.

Burt and his colleagues compared fitness in asexual and sexual lineages of yeast by measuring growth rates. Lineages were raised in one of two types of environments: (1) a "benign"

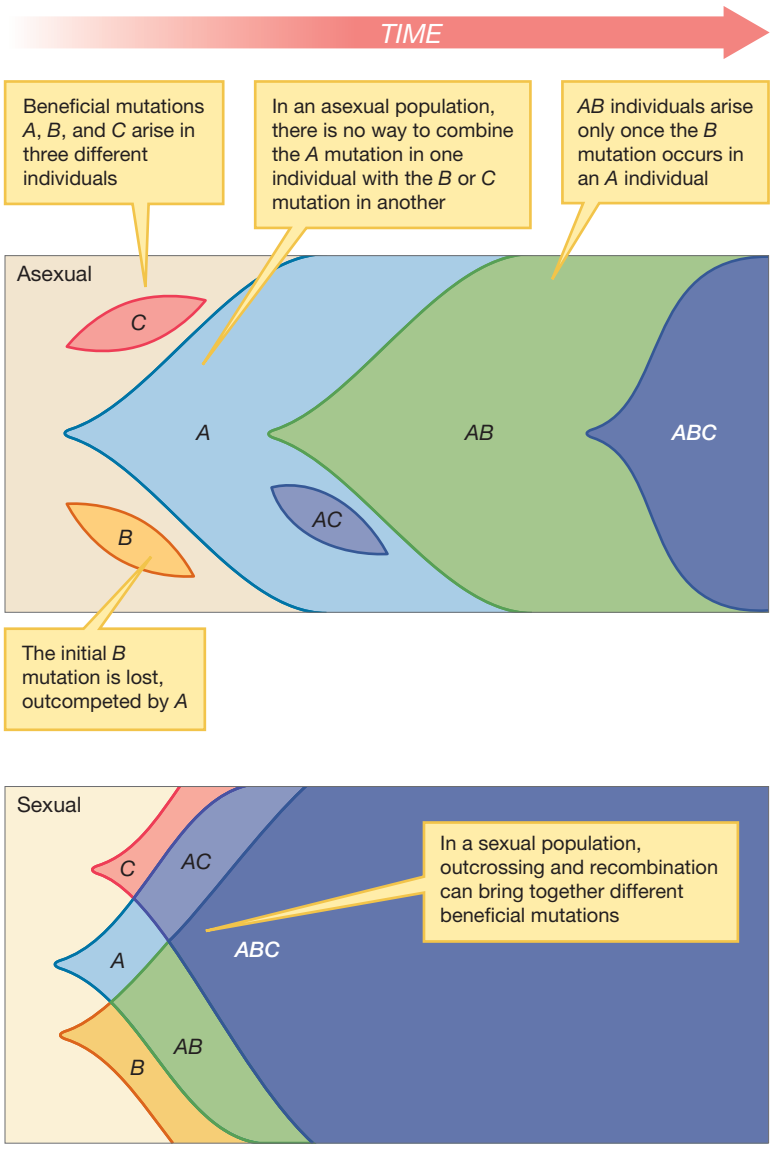


FIGURE 16.15 The Fisher–Muller hypothesis. A beneficial mutation, *A*, arises and increases in both large sexual and large asexual populations. If a second beneficial mutation, *B*, arises at a different locus, *AB* individuals emerge more quickly in a sexual population than in an asexual population. The same holds true for a third beneficial mutation *C*. This occurs because, in an asexual population, *AB* and *ABC* individuals can only arise if the *B* mutation occurs in an individual with *A*, and the *C* mutation then occurs in *AB* individuals (or if the *C* mutation occurs in an individual with *A*, and the *B* mutation then occurs in *AC* individuals). But, in sexual populations, recombination brings together beneficial mutations from separate lineages. Adapted from Crow and Kimura (1965) and Maynard Smith (1988), after Muller (1932).

environment, in which there were relatively high levels of glucose sugar that the yeast could use as a resource and a temperature that facilitated yeast growth, or (2) a "harsh" environment, in which glucose was more limited and the temperature was above optimal for yeast growth. In the benign environment, in which selection for favorable mutations was presumably weak because resources were relatively plentiful, there was no significant difference in the fitness of asexual and sexual populations when fitness was measured by growth rate. But, in the harsh environment, in which selection was more intense because of a relative dearth of resources, sexual lineages had significantly higher growth rates than asexual lineages (**Figure 16.16**). Although the precise mutations involved, and the order in which they occurred, could not be measured in this experiment, these findings are consistent with the Fisher–Muller hypothesis for the accelerated rates of natural selection in sexual populations.

Sex and the Red Queen

Let's again return to our discussion of sexual and asexual lineages of the New Zealand snail, *Potamopyrgus antipodarum.* We noted that Lively's work supported the Red Queen hypothesis for the evolution of sexual reproduction. Here, we explore the Red Queen hypothesis and the evolution of sex in more detail.

The key to understanding how the Red Queen hypothesis operates is that natural selection is constantly favoring hosts that can better defend themselves against parasites. Selection, of course, also favors parasites that can overcome a host's defenses. In this sense, the parasite–host relationship is an arms race (Chapter 18). But parasites have a built-in advantage in this arms race because, in almost all cases, their generation times are orders of magnitude shorter than those of their hosts. As a result, natural selection can drive more rapid evolutionary change in

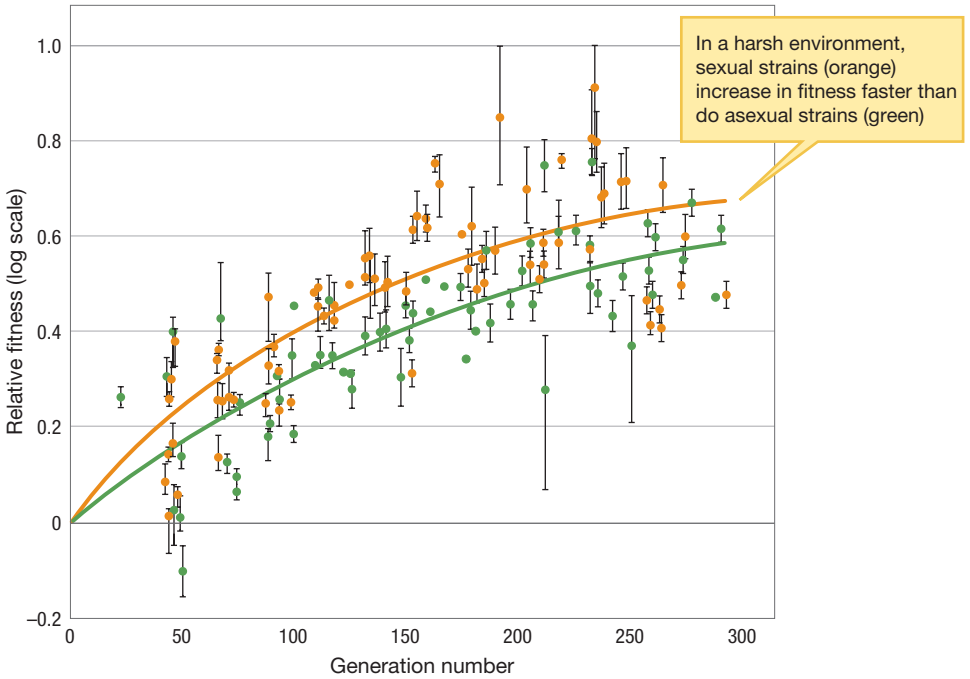


FIGURE 16.16 Relative fitness in asexual versus sexual populations. The relative fitness of asexual and sexual lineages of yeast was measured in a benign environment and a harsh environment by measuring population growth rates. In the benign environment (not shown), there was no difference in the fitness of asexual and sexual populations, but in the harsh environment, a significant difference was observed. Adapted from Goddard et al. (2005).

pathogens than in their hosts. For example, the generation time of many bacteria is on the order of an hour, whereas the generation time of their human host is on the order of two decades. One generation of natural selection in humans corresponds to more than 100,000 generations in a bacterial pathogen. Can hosts overcome this inherent disadvantage and, if so, how?

The Red Queen hypothesis proposes that sex provides hosts with a way around this disadvantage and predicts that host lineages that reproduce sexually will outcompete host lineages that reproduce asexually. When a new asexual lineage of host emerges, it may initially be resistant to parasites in its environment, and it may quickly grow to much higher frequencies in the overall population. But this increase in frequency is transient for asexual hosts because, as they become prevalent, natural selection quickly favors adaptations in parasites to overcome the defenses of their now-prevalent asexual hosts. Because asexual individuals in a lineage are genetically identical, when effective adaptations to circumvent a host's defense system evolve in parasites, the parasites will be particularly effective against asexual hosts.

If hosts reproduce sexually, the genetic variability generated by recombination makes it much more difficult for a parasite to home in on vulnerabilities and breach the host's defenses. As in the Fisher–Muller hypothesis, sex breaks down linkage disequilibrium; here, the advantage to the host is that with reduced linkage disequilibrium, it is more difficult for the pathogen to track the host genotype. This argument has been dubbed the Red Queen hypothesis for sexual reproduction because the continual generation of new genotypes by sexually reproducing species makes them akin to the Red Queen in Lewis Carroll's stories, who has to keep moving just to stay in place (Bell 1982). Similarly, sexual lineages must keep producing new genotypes, and keep breaking down linkage disequilibrium, to "keep up" with adaptations in parasites. The Red Queen hypothesis predicts:

1. Oscillations in the relative frequency of asexual lineages when parasites are present: An asexual clone may be resistant at first, and it may increase in frequency in the population relative to sexual lineages. But this relative advantage will dissipate as an asexual lineage becomes more common.
2. Time lags: Suppose an asexual host evolves an effective defense against parasites. Initially, this lineage will increase in frequency. But, as it becomes common in the host population, natural selection will act strongly on the parasite population to favor parasite variants that can evade the defenses of this now-common host strain. Because of these dynamics, we expect a short time lag between the emergence of an effective host defense and the evolution of pathogen traits that can counter that defense (**Figure 16.17**).
3. A correlation between parasite load and sexual reproduction: Sexual reproduction will increase in frequency relative to asexual reproduction when the level of parasitism in an environment is high (Jaenike 1978; Hamilton 1980; Hamilton et al. 1990; Salathe et al. 2008; King et al. 2011; Soper et al. 2014; Vergara et al. 2013).

At the beginning of this chapter, we presented evidence for the third of these predictions in the snail *P. antipodarum*, but there is also evidence in this system

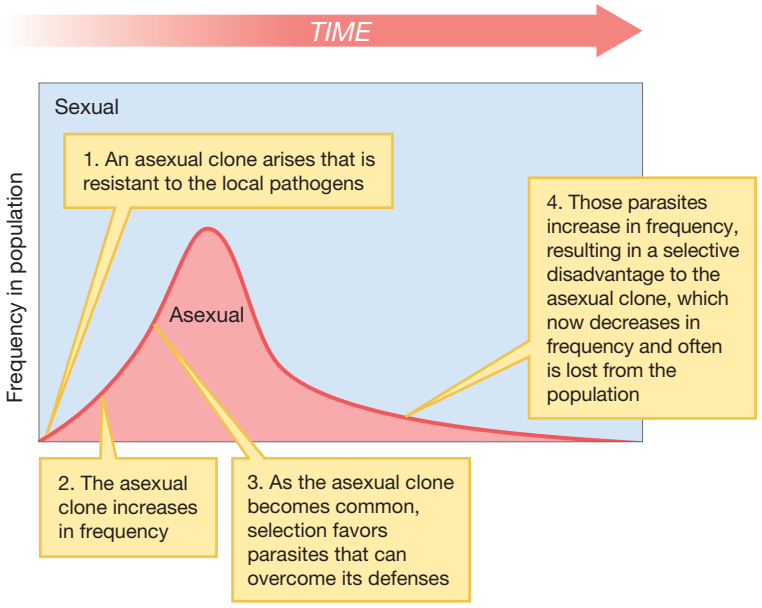


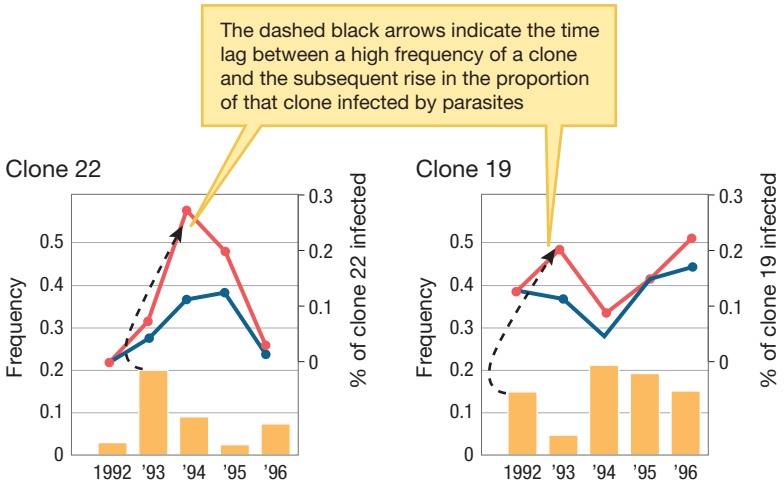
FIGURE 16.17 The Red Queen hypothesis. When a single parasite-resistant asexual clone arises within a sexual population, it increases in frequency quickly and reduces the relative proportion of the sexual genotypes in the population. As this clone increases in frequency, natural selection favors parasites that can infect this clone, which prevents its further increase. Adapted from Jokela et al. (2009).

that supports the first and second predictions. When Lively and his colleagues collected snail samples from lakes in New Zealand for four consecutive years, they found that the frequency of different asexual clones varied across years. As a clonal lineage reached higher frequencies, the proportion of individuals of that clone that were infected by parasites increased (prediction 1). A time lag was also observed. When the frequency of a clonal lineage increased in one year, the proportion of that lineage infected by parasites tended to increase in the subsequent year (prediction 2). This fieldwork was supplemented by laboratory experiments in which Lively and his colleague Mark Dybdahl raised uninfected snails from different asexual clonal lineages and then experimentally exposed them to parasites from the wild. Lively and Dybdahl then recorded the proportion of individuals in each clonal lineage that became infected. As predicted by the Red Queen hypothesis, the clonal lineages that were most common in nature had the highest proportion of infected individuals, while those clonal lineages that were rare in nature had a lower proportion of individuals that were infected (**Figure 16.18**) (Dybdahl and Lively 1998).

FIGURE 16.18 Parasite infection and frequency of asexual clones in nature. The frequency of two common asexual clones—clones 22 and 19—were plotted (gold bars) against the proportions of those clones infected by *Microphallus* parasites (blue) and by all parasite species present (red). Adapted from Dybdahl and Lively (1998).

Sex, Environmental Unpredictability, and Variation among Offspring

Environments vary in both time and space. Organisms in the same location may experience very different conditions—temperature, humidity, predators, parasites, and so forth—across time. What's more, *during* a specific time interval, different patches in the same area may have different conditions. Both spatial and temporal variability are often unpredictable.



This environmental variability and unpredictability can lead to changes in which traits are favored by natural selection and may have important consequences for the evolution of sex (Robson et al. 1999; Otto 2009). If environments are unpredictable, then natural selection may favor individuals that reproduce sexually. This is because sexually reproducing individuals produce a diverse set of genotypes among their offspring as a result of both crossing-over and the fact that offspring are made up of parts of the genomes of two different parents. This is akin to "bet hedging," in which individuals attempt to maximize the chance

that some of their lottery tickets (offspring) are winners (survive to reproduce). Reproducing sexually in such environments provides two different kinds of immediate benefits to individuals (Kondrashov 1993):

1. It increases the chance that at least one of an individual's offspring will be a good match to the environment in which the offspring find themselves (Williams and Mitton 1973; Ghiselin 1974; Williams 1975).
2. Because individuals with similar genotypes are likely to be competitors for the same resources, the genetic variability that exists among sexually produced offspring creates the opportunity for these offspring to specialize in different niches in an environment: Sexual reproduction reduces competition between siblings (Maynard Smith 1978; Bell 1982; Price and Waser 1982).

One way that evolutionary biologists have examined the role of environmental variability on the evolution of sexual reproduction is by examining cyclical parthenogens who *usually* reproduce asexually but *occasionally* reproduce sexually.

One way we can understand how sexual reproduction may be linked to environmental variability is by examining the conditions under which cyclical parthenogens reproduce sexually versus asexually. In particular, what factors cause diploid females to shift from asexual reproduction to sexual reproduction (Walsh 2013)?

In the water flea *Daphnia magna*, a known cyclical parthenogen, females respond to cues that environmental change is occurring or about to occur, and these cues trigger a shift from asexual to sexual reproduction (Herbert 1974; Herbert and Crease 1980). Introduction of a new predator—which represents a substantial environmental change—triggers sexual reproduction in some *D. magna* populations (Pijanowska and Stolpe 1996; Slusarczyk 1999). A decrease in the quality of food may also trigger sexual reproduction, as do cues that a temporary pond may be drying up (sexually produced eggs are very resistant to drying up) (Carvalho and Hughes 1983; Koch et al. 2009). All of these findings suggest that sexual reproduction in cyclically parthenogenic *D. magna* is favored as an adaptation to environmental variability and unpredictability. Recent quantitative trait loci (QTLs) work in *D. magna* has even begun to shed light on the molecular genetic underpinnings of the shift from asexual to sexual reproduction (Roulin et al. 2013).

16.4 Sexual Reproduction Leads to Sexual Selection

Once sexual reproduction is established and once anisogamy—the production of different-sized gametes by males and females—evolves (**Box 16.1**), selection begins to favor different traits in males and females.

Selection Operates Differently on Males and Females

Competition between members of one sex for mating access to the other sex is often much stronger among males than among females. This is, in part, due to a fundamental difference between the sexes. By definition, females produce fewer, but larger, gametes than males. Compared to sperm, each egg is extremely

BOX 16.1 The Evolution of Different-Sized Gametes: Anisogamy

Here, we examine when natural selection should favor anisogamy, the production of different-sized gametes; namely, small sperm and large eggs. Our model is based on work by Geoff Parker and his colleagues (Parker et al. 1972; Bulmer and Parker 2002) and on discussions of Parker's model by Maynard Smith (1978) and Randerson and Hurst (2001).

We begin by imagining an ancestral marine organism that sheds its gametes into the water, and these gametes then fuse with gametes from other parents to produce offspring. Imagine a population of individuals in which a wide range of gamete sizes are produced, and suppose that (1) there is a trade-off between the size of gametes and the number of gametes, so that the larger the gamete, the smaller the number of gametes an individual can produce; (2) the larger the size of a gamete, the less mobile it is; and (3) the probability that a zygote survives increases with its size, where the size of the zygote is a function of the sizes of the fusing gametes (Parker et al. 1972; Bulmer and Parker 2002).

Because those ancestral individuals that produce very small proto-sperm can produce many such gametes, most zygotes come from the fusion of two very small proto-sperm. But these zygotes have low survival rates compared to those of zygotes that were formed by the fusion of large gametes (proto-eggs). Clearly, selection favors proto-sperm that fuse with proto-eggs. All else being equal, proto-eggs should be favored to fuse with other proto-eggs, but all else is not equal. Proto-eggs, because of their large size, are relatively rare. As such, selection may favor proto-eggs that differentially fuse with proto-sperm, producing disruptive selection for proto-eggs and proto-sperm. Intermediate-sized gametes begin to decrease in frequency. Researchers have predicted that intermediate-sized gametes will decrease to a frequency of zero—leaving just proto-eggs and proto-sperm—when the relationship between zygote size and zygote fitness is as shown in **Figure 16.19** (Parker et al. 1972; Bulmer and Parker 2002).

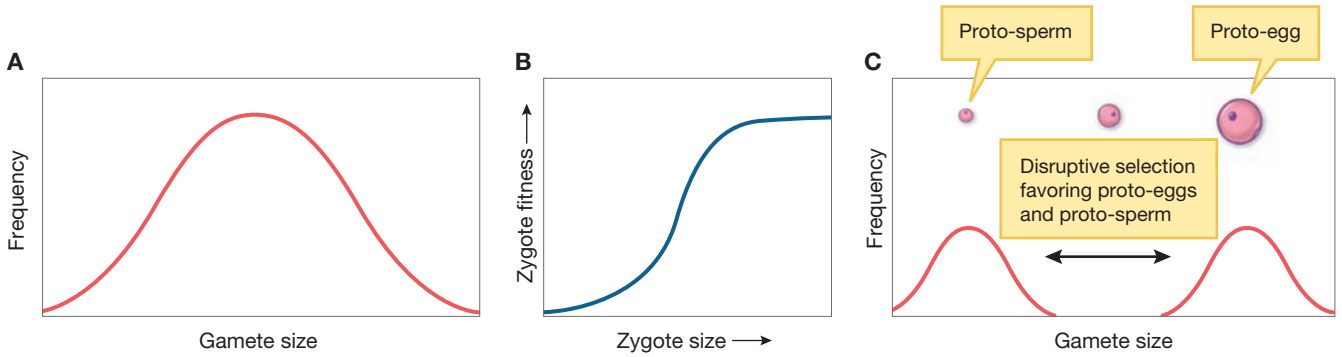


FIGURE 16.19 Anisogamy and disruptive selection. Mathematical models have found that when a population begins with a normal distribution of gamete sizes **(A)**, and the relationship between zygote size and zygote fitness takes the S shape seen in **(B)**, the population will be subject to disruptive selection, and proto-eggs and proto-sperm will be favored **(C)**.

valuable because of both its size and its relative scarcity. Each sperm, in contrast, requires much less energy to produce, and sperm are usually produced in very large quantities.

This means that male reproductive success is limited by the comparatively few eggs that are available to fertilize (Trivers 1985). By this logic, evolutionary biologists have hypothesized the following:

1. Because eggs are the limiting resource, males should compete for access to mating opportunities with females. But it is more than just eggs being a limiting resource that drives competition among males. The huge number of sperm produced by males means that males chosen by multiple females may have extraordinarily high reproductive success, again creating intense competition between males. Other males may have very few mating

opportunities. This leads to a great deal of variation between males in their reproductive success. The situation is different for females. Because of the relatively high costs related to egg production and the relative scarcity of eggs, variation in female reproductive success should be fairly low. This is especially likely in species in which females have internal gestation and devote resources to a developing embryo. In such instances, females cannot become pregnant again until after they give birth, further reducing the variance between females in reproductive success. Indeed, evolutionary biologists observe much greater variation in the reproductive success of males than in that of females (Bateman 1948) (**Figure 16.20**).

2. Because eggs are expensive and scarce, females should be the choosier sex. Theory predicts that females will tend to be more discriminating about which males have access to their gametes because females stand to lose more than males by making bad choices of mates. There are at least two reasons for this: (1) eggs are larger and more energetically expensive than sperm and so they have a higher replacement value, and (2) in species with internal gestation, females are usually the only sex to devote energy to offspring *before* they are born, and so females are under strong selection to choose good mates to secure the investment they have made.

KEYCONCEPT QUESTION

16.3 Figure 16.20 shows the distribution of reproductive success for male and female bitterling fish. As noted in the figure caption, the mean reproductive success is the same for males and females because each offspring has one male and one female parent. However, the median reproductive success need not be the same. On the basis of the figure, which sex has the higher median reproductive success? Do you think this pattern is typical for other vertebrate species?

The different ways that selection operates on males and females and the consequent differences in male and female physiology, anatomy, and behavior have been noted since the origin of evolutionary biology. Darwin, for example, noticed and was puzzled by extravagant traits such as the beautiful plumage of many male

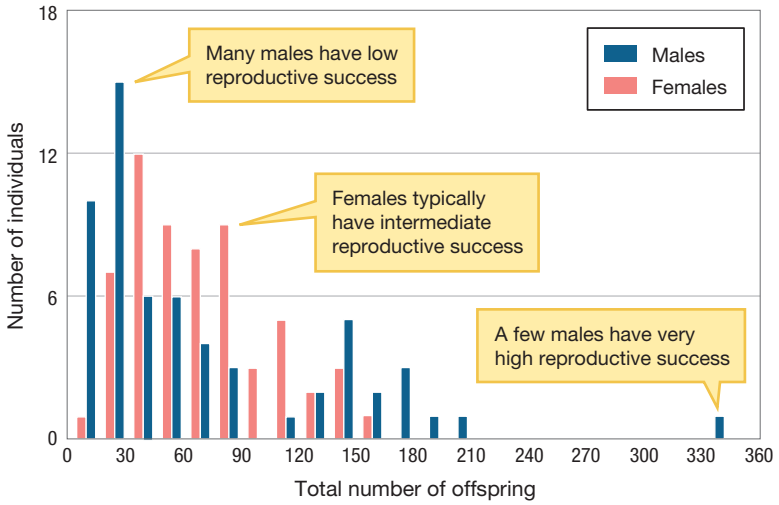


FIGURE 16.20 Male and female reproductive success. Lifetime reproductive success for male and female bitterling fish (*Rhodeus amarus*). Reproductive success in the fish follows a pattern seen in many animals: Males have a greater variance in reproductive success, with many males leaving no offspring and a few having a large number of offspring. Females have a smaller variance in reproductive success. Because each offspring has one male and one female parent, the mean reproductive success of each sex is equal. Adapted from Reichard et al. (2009).

birds, the melodic songs of many species across the animal kingdom, and the giant horns found on males in many mammals and insects. How, he wondered, could such extravagant, presumably costly traits ever be favored by natural selection? In *The Descent of Man and Selection in Relation to Sex*, Darwin proposed that such traits evolved via **sexual selection**—selection for traits that increase mating success rather than survival. In Darwin's words, sexual selection "depends on the advantage which certain individuals have over other individuals of the same sex and species *in exclusive relation to reproduction*" (Darwin 1871). When sexual selection acts differently on males and females in the same population, a *sexual dimorphism* may arise in the traits in question (**Figure 16.21**).

Following Darwin's lead, evolutionary biologists often divide sexual selection into (1) **intersexual selection**, in which individuals of one sex, usually females, select among individuals of the other sex as mates, and (2) **intrasexual selection**, in which members of one sex, most often males, compete with each other for mating access to the other sex. Mutual mate choice occurs when both sexes are selective in their choice of partners (Bergstrom and Real 2000).

From the time of Darwin until about the 1970s, much of the research on sexual selection focused on male–male competition and intrasexual competition, rather than on mate choice and intersexual selection (Andersson 1994; Andersson and Simmons 2006; Clutton-Brock and McAuliffe 2009). This bias may have resulted from the fact that male–male competition is relatively easy to observe in nature, and because some early, prominent evolutionary biologists had dismissed mate choice as unimportant, thus directing research toward male–male competition (Huxley 1938). But the research focus has shifted, and now the majority of studies done on sexual selection examine intersexual selection, typically but not exclusively involving female choice of males.

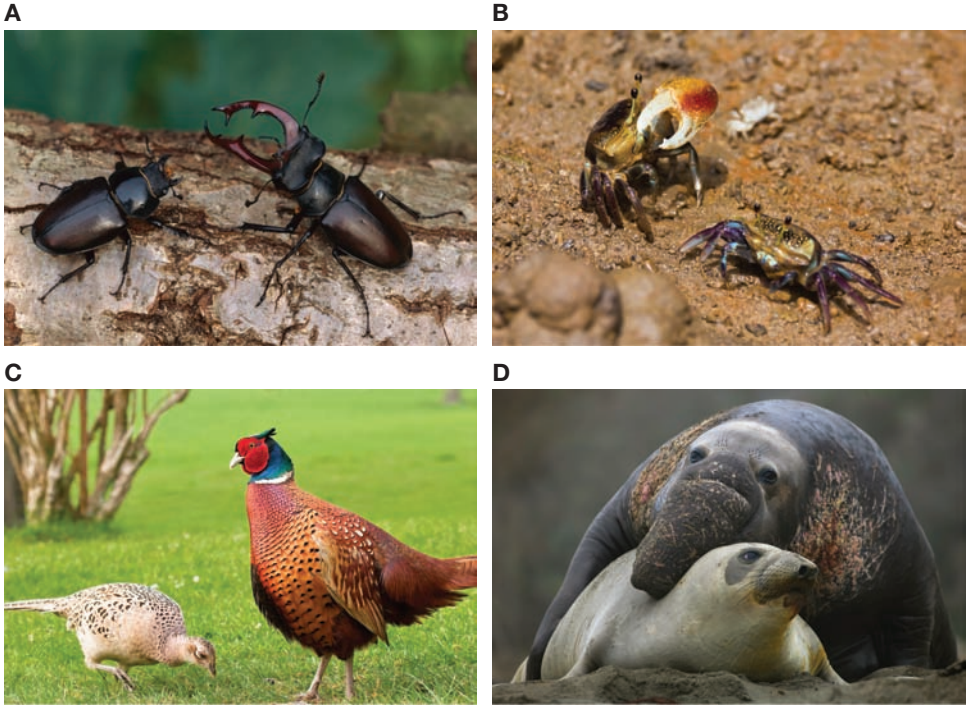


FIGURE 16.21 Sexual selection leads to sexual dimorphism. When sexual selection acts differently on males and females in the same population, it leads to sexual dimorphism, a difference in how the same trait manifests in males and females. Examples of animals that display sexual dimorphism are **(A)** stag beetles (*Lucanus cervus*), male on right, **(B)** fiddler crabs (*Uca forcipata*), male on left, **(C)** common pheasansts (*Phasianus colchicus*), male on right, and **(D)** Northern elephant seals (*Mirounga angustirostris*), male on top.

KEYCONCEPT QUESTION

16.4 Imagine a group of males that are engaged in a series of fights. Suppose that a female watches these fights and prefers winners as mates. How does this example blur the distinction between intrasexual selection and intersexual selection?

16.5 Intersexual Selection

We will begin our discussion of sexual selection by considering intersexual selection, focusing on male ornaments and female preferences for them. We will examine four evolutionary models of female mate choice: the *direct benefits*, *good genes*, *Fisherian runaway selection,* and *sensory bias* models (Kuijper et al. 2012). We will start by outlining the logic of the models, and then we will look at case studies. Our focus is primarily on case studies in which the evolution of a sexually selected trait is best explained by just one of our four models, but it is important to recognize that in many species, the evolution of sexually selected traits might best be explained by a combination of two or more models.

Direct Benefits

In the direct benefits model of sexual selection, selection favors females who have a genetic predisposition to choose mates that provide them with resources—above and beyond sperm—that increase their fecundity and/or survival (Kirkpatrick and Ryan 1991; Price et al. 1993; Andersson 1994; Møller and Jennions 2001). For example, Randy Thornhill has found that female hanging-flies, *Hylobittacus apicalis*, prefer males that bring them "nuptial gifts" of large prey items during courtship. Such gifts increase the amount of resources available to the female to expend on growth and future reproductive effort (**Figure 16.22**) (Thornhill 1976). In the subsection that follows, we will examine the direct benefits model when the benefit provided by males is protection from predators.

Direct Benefits and Safety from Predators

In many species of amphibians, insects, and crustaceans, males and females perform premating rituals that include *amplexus*, in which males and females are physically joined together, often with one individual on the back of the other. This form of mate guarding involves males defending the female from other males and positioning themselves so that they are present when females become receptive to copulation.

Amplexus is seen in crustaceans from the genus *Hyalella* (Cothran 2008). In *Hyalella* amphipods, males carry females using large, clawlike appendages called gnathopods. Such appendages are found in both sexes, but they are larger and more muscular in males. Females mate more often with larger males who have larger gnathopods, and behavioral work has shown that this is not a function of male–male competition (Strong 1973). Moreover, females are not forced to mate by larger males. Rather, the distribution of matings is the result of female preference for mates with larger gnathopods.

Why should females prefer mates with larger gnathopods? What benefits, if any, do females obtain as the result of such a preference? Rickey Cothran hypothesized that females may be safer from predators while in amplexus with larger males, and

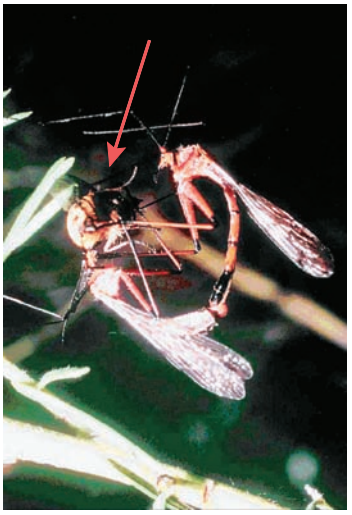


FIGURE 16.22 Direct benefits to hangingfly females. Male hangingflies present females with food items that the females eat during courtship or mating. Females prefer males that provide larger prey items. The red arrow points to a prey item captured by a male hangingfly and presented to the female during courtship.

he set out to address this question using *Hyalella* populations around the University of Oklahoma. Cothran collected a sample of *Hyalella* and brought them into the laboratory. He then randomly selected one male and one female and placed the pair in a large jar filled with lake water. Once a male and female were in amplexus, Cothran added a predator—a larval dragonfly—and noted whether the predator attacked the *Hyalella* pair, whether such attacks were successful, and, if they were, which individual was taken by the predator (Cothran 2008).

When Cothran analyzed the data on attack rate and predator success, he found that the size of a *female* in amplexus did not affect the probability that a predator attacked. But a female's probability of survival increased dramatically as a function of her partner's size. Females that mated with larger males were less likely to be eaten by predators (**Figure 16.23**). In the *Hyalella* system, then, one reason that selection favors females who mate with larger males is that females receive direct benefits—in the form of safety from predators—as a result of their choices.

Good Genes and Costly Signals

In many species, males do not provide females with any sort of direct resource such as food and shelter. How does female mate choice evolve in these species, given that the female receives nothing but sperm from her mate? In such species, we often observe that males display elaborate ornaments, and females exhibit a preference for these ornaments. Why? The evolutionary biologist R. A. Fisher addressed these questions a century ago:

> The first step to a solution lies in the fact that the success of an animal in the struggle for existence is not measured only by the number of offspring which it produces and rears, but also by the probable success of those offspring. So that in selecting a mate from a number of different competitors, it is important to select that one which is most likely to produce successful children. (Fisher 1915, p. 185)

Fisher's insight was that even if females receive only sperm, not all sperm will be equally good at producing successful children. Selection will favor females who choose mates that possess "good genes"; that is, genes that code for some suite of favorable traits (Fisher 1915; Kodric-Brown and Brown 1984; Andersson 1994; Kokko et al. 2003; Mays and Hill 2004). This sounds straightforward enough, but how can females assess whether males have good genes?

Wouldn't selection favor males who have traits that indicate that they possess appropriately good genes, even if they don't? The answer is "yes." This, in turn,

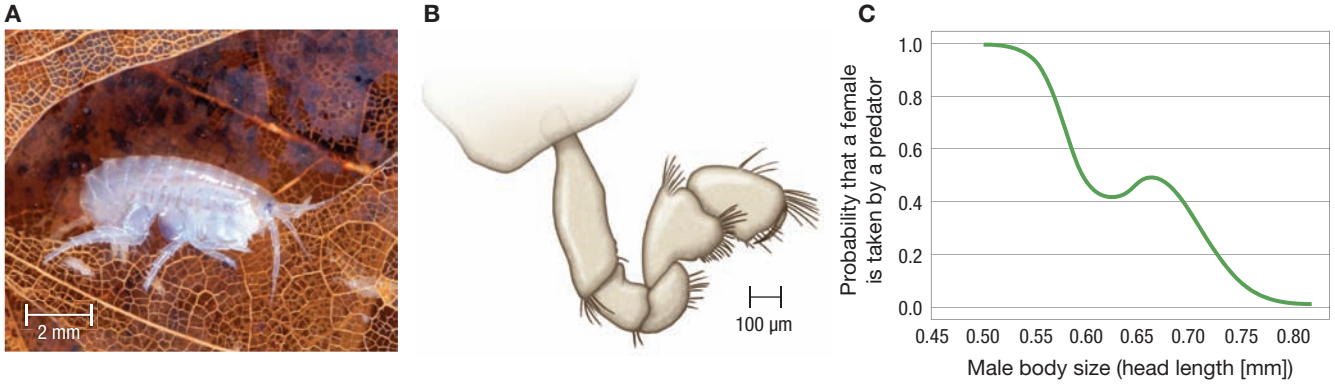


FIGURE 16.23 ***Hyalella*** **females receive a direct benefit in the form of safety.** **(A)** The amphipod *Hyalella azteca*. **(B)** The gnathopod of *H. azteca*. **(C)** The probability that a female is captured by a predator during mating decreases with the size of the male she chooses as a mate. The curve shown here has been estimated using a statistical procedure known as cubic spline estimation. Panel B adapted from Gonzalez and Watling (2002). Panel C adapted from Cothran (2008).

selects for females who can avoid being fooled. As a result, evolutionary biologists have hypothesized that only traits that are accurate and honest indicators of male genetic quality should be used by females when choosing mates.

To see how ornaments can serve as honest indicators of quality, we will explore the process of intersexual selection in peafowl (Gadagkar 2003). During the breeding season, male peafowl—referred to as peacocks and characterized by their dramatic and elaborate tails (often called trains)—set up and defend their own areas within small arenas called leks that contain no apparent resources such as food or shelter. Females then come to these leks and select mates from among the males present.

Females often visit many leks, and they prefer leks that contain the most males (Alatalo et al. 1992; Hoglund and Alatalo 1995; Kokko et al. 1998). At the lek, females carefully assess the displaying males. As demonstrated by recent work with eye-tracking software, the females pay particular attention to the males' tails (Yorzinksi et al. 2013) and choose those males that have elaborate, colorful tails with beautiful "eyespots." Early work had shown that females preferred males with longer, more elaborate tails. Indeed, experimental reduction of the number of eyespots on a tail reduced a male's attractiveness to females (Petrie et al. 1991). But why do females prefer males with ornate tails?

Because elaborate tails are costly to produce and maintain, Marion Petrie and her colleagues hypothesized that tail length and ornamentation are signals of male genetic quality. To test their hypothesis, Petrie and her team ran a series of mating experiments in natural enclosures that they built and placed in a field in Whipsnade Park, England (Petrie 1994). They put eight males in the experimental pens (one male per pen). They randomly selected eight sets of females, four females per set, and placed one set of females in the pen of each of the eight males. After mating, a total of 349 offspring hatched, and the researchers took measurements on the chicks at numerous points during their development. Petrie's team found a positive correlation between the weight of the chick (a sign of health) and both the tail length of the chick's father and the number of eyespots on the tail of its father. These results suggested that females who chose males with longer, more elaborate tails were indeed choosing males with good genes. The researchers next released the chicks that had been born during the experiment into the field at Whipsnade Park, and they checked survival rates of these individuals over the course of 2 years. They found a positive correlation between survival of the released birds and the size of the eyespots on an individual's father (**Figure 16.24**), in part because the chicks from fathers with large

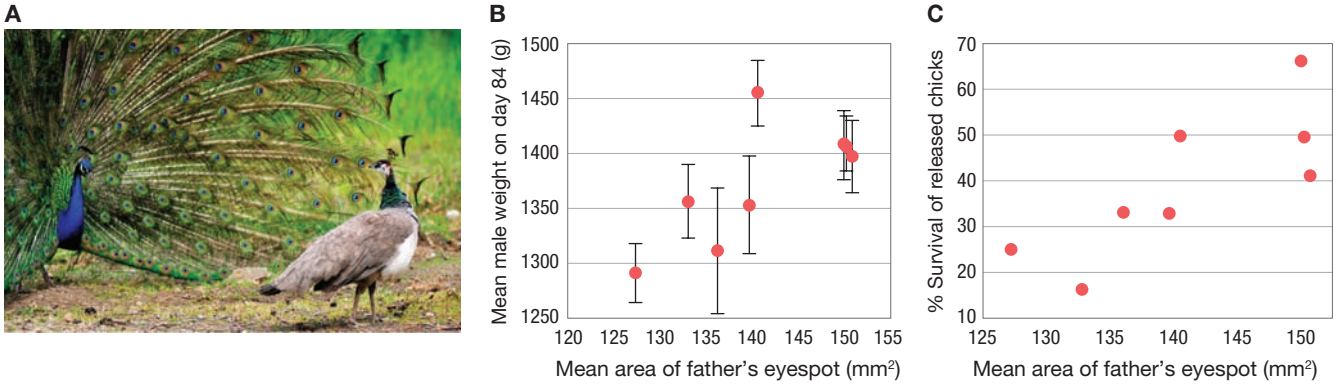


FIGURE 16.24 Good genes, offspring health, and survival. **(A)** Male (left) and female (right) peafowl. Males with more elaborate trains had **(B)** healthier chicks, as measured by weight at day 84 after birth (data for sons shown here), and **(C)** offspring with higher survival rates, after 2 years. Panels B and C adapted from Petrie (1994).

eyespots were particularly good at fending off infectious diseases (Hale et al. 2009). This provides even stronger evidence that females were selecting males with good genes as their mates, and that the females were using length and elaborateness of the peacock's tail as indicators of male quality when making such choices.

The peacock's use of a costly ornament as an honest signal is an example of a more general conceptual principle in evolutionary biology. In the early 1970s, natural historian Amotz Zahavi struggled to understand the peacock's tail and the many other extravagant displays that we observe in nature. It was a problem that had puzzled researchers since Darwin. Why do male birds of many species, the peacock included, display spectacular plumage? Why do baby birds beg so loudly? Why do gazelles jump up and down when they see a lion? Zahavi proposed that these and many other costly ornaments and elaborate behaviors function as signals (Zahavi 1977), just as the peacock's tail appears to be a signal used by prospective mates. Zahavi named his hypothesis the **handicap principle**, and he suggested that there is something about costly behaviors or physical features that makes for inherently reliable signals. Let's delve a bit more deeply into Zahavi's idea.

Applied to the peacock, Zahavi's handicap argument goes something like this: Females choose mates from a pool of suitors. Because the females cannot judge a male's genetic quality directly, they instead attend to signals that the male provides: Males advertise their quality with a long flamboyant tail. This advertisement is a handicap in the sense that it is energetically costly to produce and maintain, and it may reduce the male's ability to maneuver as well. Critically for Zahavi's hypothesis, the cost of producing flamboyant tails varies among males. A weak and sickly male likely cannot afford to divert energetic resources from basic metabolism to the production of ornaments. Moreover, an unhealthy bird would have a hard time flying if he were also hindered by an extravagant tail. By contrast, a strong and healthy male can afford the additional costs of producing an ostentatious tail, and he may be able to fly reasonably well even when hampered by a such a tail.

Because only high-quality males can afford lavish tails, females prefer mates with this characteristic. High-quality males, for their part, produce the flamboyant tails to ensure that they are chosen as mates. Low-quality males cannot afford to do so, and so they will produce less extravagant tails.

Thus, among peacocks, long tails with numerous ornate eyespots appear to be honest signals of male quality that are used by females to choose their mates. This is the basic idea behind the use of the costly signals in a sexual selection context. Of course, the costly signal need not involve extended tail feathers: bright colors, a large rack of antlers, an elaborate song, a captured prey item offered as a gift, or any number of other expensive ornaments or displays could serve equally well. Nor, for that matter, must the males be the signaling sex. In some cases, females may use costly signals to advertise their own qualities to male suitors.

A number of authors, most notably Alan Grafen and John Maynard Smith, have used mathematical models to demonstrate that a costly signaling mechanism can indeed allow the evolution and maintenance of honest communication, including honest communication in mate choice and in nestling begging (Grafen 1990; Maynard Smith 1991). Although the mathematics involved get rather complicated, we can capture a good fraction of the intuition behind the models with a simple graphical illustration.

Figure 16.25 shows the cost of producing a tail of a given length for peacock males of three different underlying genetic qualities: low, medium, and high. Producing an extravagant tail always costs more than producing a shorter tail, but producing an extravagant tail is comparatively less expensive for high-quality individuals. On the same graph, the fitness benefits that result from improved mating success are indicated as the black curve. The more extravagant the tail, the greater the mating success. In this graph, a male's fitness is maximized when the size of his tail maximizes the difference between the fitness benefits and the fitness costs. On the graph, low-quality, medium-quality, and high-quality males, respectively, produce tails of low, medium, or extreme size. Thus, each type of male maximizes its fitness with a different degree of tail elaboration: large flamboyant tails for high-quality males, small drab tails for low-quality males, and intermediate tails for medium-quality males. Zahavi's predictions are met. Tail elaboration as a signal will be (1) *honest*, in that the higher the male's quality, the more flamboyant his tail, and (2) *costly*, in that all males produce tails that impose significant fitness costs.

Fisherian Sexual Selection

In the good genes model of sexual selection, females prefer ornaments because they indicate other beneficial attributes, usually genetic quality. In the **Fisher process** of sexual selection, some females express a preference for an ornament, and simply because of this preference, selection favors both the male ornament and further female preference for it. In 1915, R. A. Fisher first proposed a verbal model of how a trait favored under the good genes model could be exaggerated by this mechanism (Fisher 1915, 1958). Subsequent researchers have developed mathematical models of this process even on arbitrary and uninformative male traits (Lande 1981; Kirkpatrick 1982; Kuijper et al. 2012) and proposed that this provides a sort of null model for the evolution of extravagant traits (Prum 2010).

We can illustrate the Fisher process by thinking about how it might drive the evolution of brightly colored males in a population of birds. Imagine a population in which a female preference for bright color somehow arises, and that some, but not all, of the females have an arbitrary, heritable preference for brightly colored males. Next, suppose that some males are more colorful than others, and that coloration is also a heritable trait. Females with the preference for bright colors will tend to mate with males that express the bright color trait, whereas females without this preference will mate at random with respect to male coloration. As a consequence, the offspring of choosy females and colorful males will tend to carry both the alleles for color preference and for bright coloration. Over time, sexual selection builds up a genetic association between color preference and bright coloration; that is, linkage disequilibrium arises between the loci associated with female

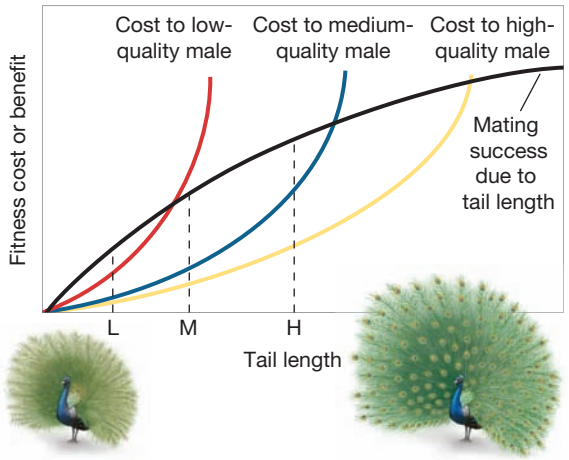


FIGURE 16.25 Costly signaling of male quality. Longer and more elaborate tails cost more to make and maintain, but they cost relatively less for higher-quality males. Females prefer longer tails, and thus mating success increases as a function of tail length. Low-, medium-, and high-quality males maximize fitness (as indicated by the black curve) by producing tails of the lengths L, M, and H, respectively. These optimal signals are both honest—higher-quality males produce longer tails—and costly, in that all males expend a substantial fitness cost on tail production. Adapted from Lachmann et al. 2001.

preference and with male phenotype. Linkage disequilibrium of this sort does not require that the loci in question are all located on a single chromosome.

Once linkage disequilibrium arises, sexual selection by females on alleles for male coloration has the side effect of also selecting on the alleles for female preference that now are statistically associated with the male coloration alleles. As a result, both the colorful male phenotype and the female preference increase in frequency and intensity in the population.

Note that once the female preference is present in the population, it is self-reinforcing. Females benefit from mating with colorful males because by doing so, they are more likely to produce sons who are colorful—and thus preferred by females—as well. This is sometimes known as the *sexy son mechanism* for female choice (Weatherhead and Robertson 1979).

Under most conditions, the Fisher process will cause the male trait and female preference to settle at intermediate values determined by a balance of natural selection and sexual selection. However, if the genetic correlation between trait and preference is extremely high, this system can "run away" in a positive feedback loop, like a snowball rolling down a snowy mountain and accelerating as it becomes larger and larger (Lande 1981; Kirkpatrick 1982). This is known as **runaway sexual selection**. Across generations, runaway sexual selection may produce increasingly exaggerated male traits—for example, extremely bright male coloration—coupled with strong and pervasive female preferences for this trait. There is a caveat: If female choice is costly, the Fisher process will not operate on its own, and the male trait will evolve to that favored by natural selection (Pomiankowski 1987).

The Fisher process model has been notoriously difficult to distinguish from the good genes model. Conceptually, the two models differ because in the good genes model, the ornament covaries with some other aspect of quality, whereas in the Fisher process model, the alleles for the ornament are valuable to the female in and of themselves (Kuijper et al. 2012). But actually distinguishing the two models is not easy. One problem is that the Fisher process inevitably operates on top of any good genes mechanism of sexual selection. Whenever and for whatever reason a heritable female preference arises for a heritable male trait, the Fisher process serves to strengthen this preference (Mead and Arnold 2004).

A second problem is that a key prediction of the Fisher process model—linkage disequilibrium between female preference and male trait—is also predicted by the good genes model (Lande 1980; O'Donald 1980; Pomiankowski 1988; Houde 1997). This means that evidence of a genetic correlation between female preference and male trait—a correlation that has been demonstrated in guppies, sticklebacks (**Figure 16.26**), stalk-eyed flies, and field crickets—is not sufficient, in and of itself, to distinguish between the good genes and Fisher process models (Houde and Endler 1990; Bakker 1993; Wilkinson and Reillo 1994; Gray and Cade 1999).

A third problem is that neither the good genes model nor the Fisher process predict a positive correlation between male ornamentation and male viability. Even in the good genes model, high-quality males may invest so much in the ornament that their survival drops below that of lower-quality individuals (Kokko 2001; Kokko et al. 2002). Some authors consider the Fisherian model and the good genes model to be manifestations of the same underlying process (Kokko 2001; Kokko et al 2002). Their basic argument is that beneficial alleles are "good

genes" whether they serve to improve mating success or to increase other components of fitness. Thus, they would argue, even the alleles for a Fisherian ornament are "good genes" in the presence of female preference for the trait.

The Sensory Bias Hypothesis

The last of the evolutionary models of female mate choice we consider is known as the **sensory bias model** (also called the sensory exploitation model or preexisting bias model) (West-Eberhard 1979, 1981; Endler and McLellan 1988; Ryan 1990). This model addresses the *origin* (but not the maintenance) of female preference. The sensory bias model hypothesizes that females initially prefer a certain male trait—let's call it M—but not because of any mating benefit, direct or indirect, that is associated with that male trait. Instead, the sensory bias hypothesis proposes that female nervous systems respond to trait M either because it is associated with some benefit outside of mate choice or simply as an artifact of how the stimulus excites their nervous systems. Males with trait M are then tapping into a preexisting sensory bias by females for trait M.

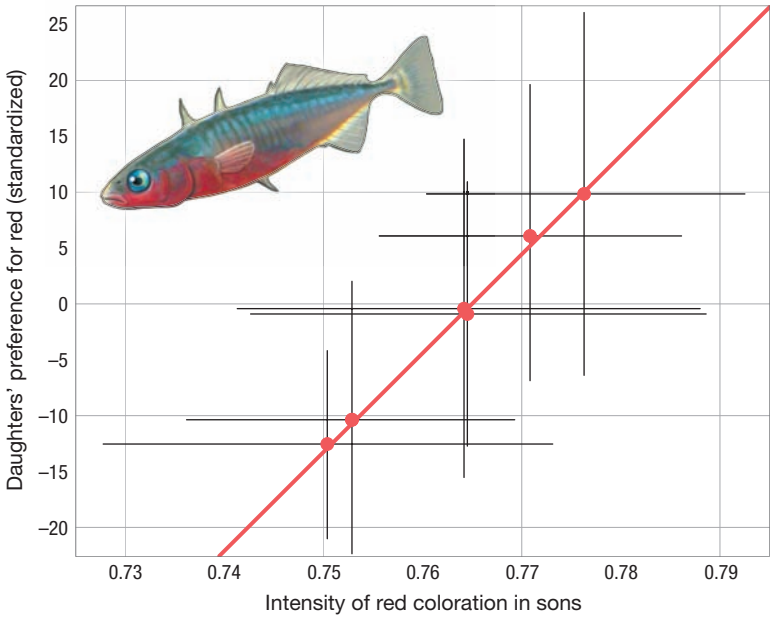


FIGURE 16.26 Genetic correlation in male trait and female preference. A genetic correlation between red color in males and preference for red color in females was found in the sons and daughters of six male stickleback fish. The horizontal lines show the standard deviation in red color among sons from a given father, and the vertical lines show the standard deviation in preference for red color among daughters from a given father. A line of best fit is shown in red. Adapted from Bakker (1993).

Suppose that red berries are the most nutritious food source available to a fruit-eating species of birds. Selection will favor individuals who are best able to search out and consume red berries, for example. That is, natural selection will fine-tune the neurobiology of the birds so that they are acutely aware of the color red and will home in on red things in their environment (Kirkpatrick and Ryan 1991).

Suppose that after selection has favored a nervous system that is especially adept at picking up red-colored objects in the environment, red feathers arise as a consequence of a random mutation in some individuals in the population. Red-feathered males may be chosen as mates because the female's nervous system is already designed to respond preferentially to red objects. Males with red feathers, then, are exploiting the preexisting neurobiologically based preferences of females—preferences that evolved as a result of selection for other functions.

The sensory bias model is unique among models of mate choice in that it leads to a clear, very simple prediction regarding the phylogenetic history of male traits and female preferences. When we look at a phylogeny that includes information on both female preference and the male trait preferred by females across closely related species, the female preference trait should predate the appearance of the male trait. In our example of red berries, the preference for red should be in place *before* red feathers evolve.

One of the earliest studies of sensory bias involved two closely related species of frogs, *Physalaemus pustulosus* and *Physalaemus coloradorum* (Ryan et al. 1990). Males of both species use advertisement calls to attract females. Males in both *P. pustulosus* and *P. coloradorum* species begin this advertisement with what is referred to as a

high-frequency "whine." But *P. pustulosus* males add a low-frequency "chuck" sound to the end of their call (**Figure 16.27**). When *P. pustulosus* females choose between *P. pustulosus* males who produce a chuck and those who do not produce a chuck, they prefer to mate with the former. Ryan and his colleagues hypothesized that the female preference for chucks was the result of a sensory bias in favor of such low-frequency sounds, which are detected by the amphibian papilla section of the inner ear.

Phylogenetic and behavioral evidence support the contention that the female preference for chucks in *P. pustulosus* is due to sensory bias. Recall that the chuck part of the call is absent in the call of *P. coloradorum* males. Indeed, when Michael Ryan and his colleagues used molecular and morphological data to reconstruct the evolutionary history of the genus *Physalaemus*, they inferred that the common ancestor of *P. coloradorum* and *P. pustulosus* did not use a chuck call (**Figure 16.28**). Yet, when computer audio technology is used to add a chuck call to the end of pre-recorded *P. coloradorum* male calls, *P. coloradorum* females show a preference for calls that include a chuck—as soon as chucks appear in a *P. coloradorum* population, females prefer males who produce such calls. These studies suggest the auditory circuitry in *Physalaemus* frogs is built in such a way as to produce a preference for a certain class of low-frequency calls like chucks. The *P. coloradorum* studies provide evidence that the preference for chucks predated the actual production of chucks in this species, in accordance with the sensory bias hypothesis.

16.6 Intrasexual Selection and Sexual Conflict

Although intrasexual selection—competition between members of one sex for mating access to the other sex—can occur in either sex, it is more common among males for reasons we discussed earlier in the chapter. Male–male competition can take many forms. Males may fight among themselves: For example, male stag beetles (*Lucanus cervus*) use their "horns" to fight, and male red deer (*Cervus elaphus*) battle each other with their antlers. The winners of such contests mate more often than the losers.

In this section, we will explore the various ways—sometimes obvious, other times more subtle—in which males compete with each other for access to females and the evolutionary consequences of such competition.

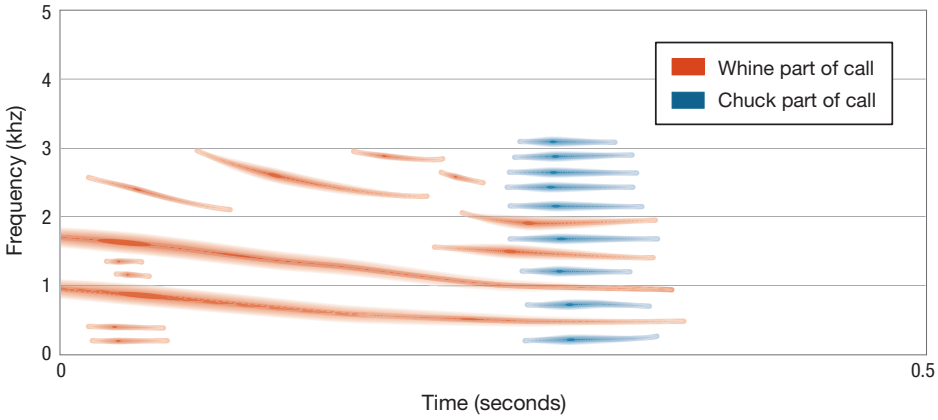


FIGURE 16.27 A whine and chuck call from the frog *Physalaemus pustulosus*. Other *Physalaemus* species produce the whine without the chuck. Adapted from the Ryan Lab (2011).

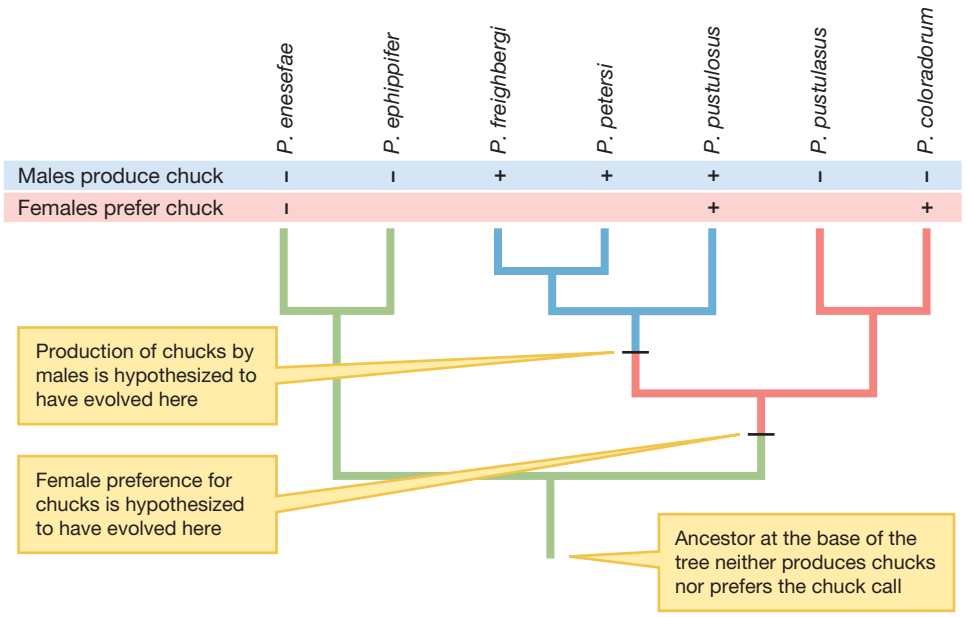


FIGURE 16.28 Preference for chuck calls arose before production of the calls themselves. The chuck call (or a similar "squawk") is thought to have evolved in the common ancestor of *P. pustulosus*, *P. petersi*, and *P. freighbergi*. Males of other species do not produce the chuck. Female preference for the chuck call has been observed in *P. pustulosus* and *P. coloradorum*, but was not found in *P. enesefae*. As such, it is hypothesized that the preference predated the chuck call itself. Adapted from Ryan and Rand (2003).

Male–Male Competition by Cuckoldry in Bluegill Reproductive Morphs

Male–male competition can occur in more subtle ways than direct fights between individuals. In many species, a single population may contain numerous different male reproductive "morphs" that have distinct combinations of physiological, endocrinological, and behavioral traits. These morphs compete with each other for access to mating opportunities in indirect and often complicated ways (Gross and Charnov 1980; Gross 1985). For example, in bluegill sunfish (*Lepomis macrochirus*), three male morphs—known as parental, sneaker, and satellite morphs—coexist within a number of populations (Gross 1982; Neff et al. 2003).

Parental bluegill males are light-bodied in color, but they have dark yellow-orange breasts. They build nests, and they are highly territorial, chasing off any other males that come near their territory. They also invest substantial amounts of energy in caring for their offspring by fanning the eggs to oxygenate them and defending the nest against predators during nesting (Coleman et al. 1985).

Sneaker bluegill males are smaller and less aggressive than parental males, and they do not hold territories. They camouflage themselves in hiding places near a parental male's territory, and when they see a parental male and female spawning, they quickly swim toward the pair, shed their own sperm, and swim away, all within about 10 seconds (Gross 1982). This strategy can be highly effective. Using molecular paternity analysis, researchers have found that, depending on their relative numbers in a population, sneaker males fertilized up to 58.7% of all bluegill eggs laid in Lake Opinicon, Canada (Philipp and Gross 1994).

A third male reproductive morph, called the satellite morph, is also found in some bluegill populations. Satellite males look like females, and they often swim between a spawning pair that contains a parental male and a female. If the parental

male attempts to spawn with both the female and the satellite male that is posing as a female, the satellite male will release his own sperm (**Figure 16.29**).

The continued coexistence of parental, sneaker, and satellite males demonstrates how males compete for mating opportunities in complex ways.

Male–Male Competition by Sperm Competition in Bluegill Reproductive Morphs

Sexual selection acts not only on behavior and external morphological traits but also on traits that affect a sperm's ability to reach and ultimately fertilize an egg. In such cases, competition occurs *after* a female has mated with more than one male. If sperm from more than one male is present, sperm may compete with one another over access to fertilizable eggs. When such sperm competition exists, selection can operate directly on various attributes of sperm, such as sperm size and shape. Sperm competition is one form of what is known as **postcopulatory sexual selection** (Eberhard 2009).

To see this in more detail, let's return to the case of the bluegill male reproductive morphs. Because the three different male morphs exhibit very different reproductive behaviors, Bryan Neff and his colleagues reasoned that there might also be differences in sperm production and sperm quality across morphs (Neff et al. 2003). In particular, they hypothesized that because of their "hit-and-run" mating strategy, sneaker males might invest most heavily in sperm *production*. The results of their investigation are consistent with this prediction. Although parental males are larger than sneakers and have testes that are larger, when Neff and his colleagues examined the ratio of testes size to body size—that is, the relative investment in testes—sneaker males had the highest ratio, followed by satellites and then parental males.

The relative investment in testes size is an indirect measure of sperm production. A more direct measure would be the number of sperm produced per ejaculate. Given that the sperm produced by sneakers are always competing with parental sperm, but parental sperm are not always competing directly with sneaker sperm, a high density of sperm per ejaculate should be more strongly favored in sneaker males. When Neff and his team looked directly at the density of sperm per ejaculate, they found that sneakers indeed produced more sperm per ejaculate.

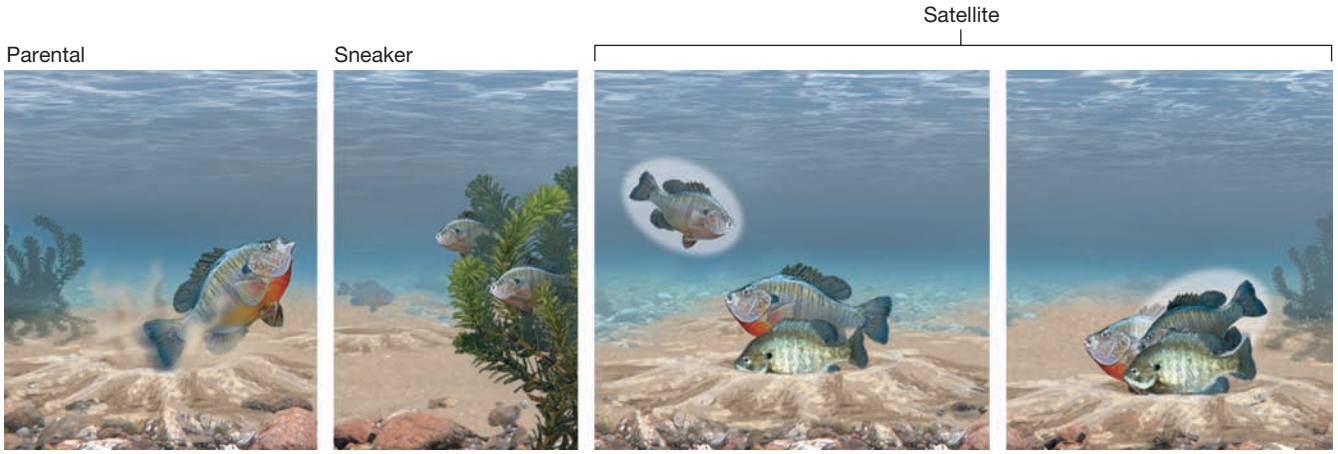


FIGURE 16.29 Parental, sneaker, and satellite males. Bluegill morphs, from left to right: a bluegill parental male preparing a nest; sneaker males hiding behind plants awaiting a chance to sweep quickly into a parental nest; a satellite male (outlined in white oval) about to swim over a nest containing a parental male and a female; and a satellite male swimming between a parental male and a female. Adapted from Gross (1982).

Nonetheless, sneakers do pay costs for investing so heavily in sperm production. First, their sperm survive shorter periods of time than parental sperm. Second, when Neff and his colleagues removed sperm from both sneakers and parentals, and then released the same number of parental and sneaker sperm over eggs, parental sperm were more likely to fertilize eggs than were sneaker sperm. Sneakers invest in producing many short-lived, lower-quality sperm, while parentals invest in producing fewer but higher-quality sperm (**Figure 16.30**).

Sexual Conflict

As we have seen throughout this chapter, selection operates differently on males and females with respect to mating behavior. When these differences are sufficiently strong, **sexual conflict** may result: traits that evolve in one sex may be detrimental to individuals of the other sex. As an extreme example of this type of sexually antagonistic coevolution, consider the case of the yellow dung fly, *Scathophaga stercoraria*. Male dung flies fight for access to females, and these fights are so intense at times that females are killed in the melee (Parker 1979).

At a more general level, sexual conflict between males and females will emerge over the type of mating system in place. For example, in a bird known as the dunnock (*Prunella modularis*), some males and females are monogamous, while others mate with multiple partners. Underlying much of the variation in mating systems, including that of the dunnock, is the fact that the fitness of males and females is affected in different ways by the mating system.

In general, a conflict of interest between the sexes exists with respect to what constitutes the optimal breeding system (Arnqvist and Rowe 2005; Rowe and Day 2006; Fricke et al. 2009a; King et al. 2013). For a male, *potential* reproductive success of the most successful individuals will often be lowest when they have shared access to a single female (polyandry), and the reproductive success of the most successful individuals will increase as follows: sole access to a single female (monogamy), joint access to two females (polygynandry), and access to numerous females (polygyny).

Potential reproductive success in the most successful females increases in precisely the opposite direction to male reproductive success, with polyandrous and polygynandrous females having the highest reproductive success. In dunnocks, females appear to be winning this battle of the sexes over breeding system (at least for now): One study found that over the course of 10 years, 75% of females and 68% of males were involved in either polyandrous or polygynandrous mating groups (Davies 1992) (**Figure 16.31**). It is difficult to know exactly why females currently are winning this sexual conflict, but it may be because early in the breeding season, females compete with each other to establish territories, and such female territories are chosen independently of the position of males. Males may then attempt to overlay their own territories on as many female territories as possible. This pattern of dispersal and territoriality may allow females a degree of control over the mating system because female territories are already established when males try to establish their territories.

Competition over which mating system should be in place is not the only way that conflicts of interest

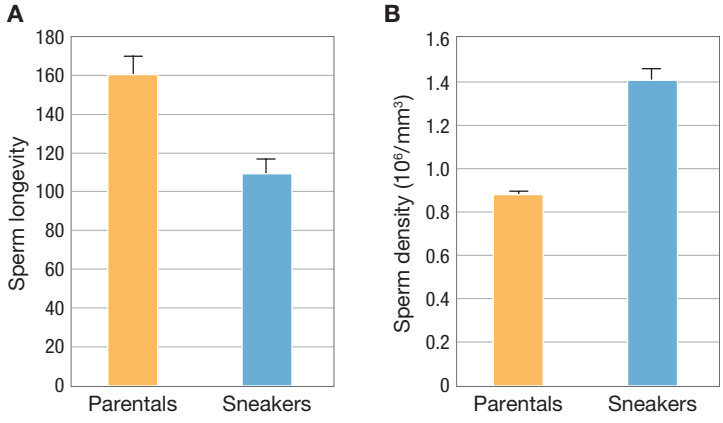


FIGURE 16.30 Sperm production in bluegill morphs (parental and sneaker). Sperm longevity **(A)** and ejaculate sperm density **(B)** in parental and sneaker morphs. Adapted from Neff et al. (2003).

can manifest themselves in sexual selection. Conflicts of interest may also arise when a trait, expressed in only one sex, has positive effects on individuals of that sex but negative effects on members of the opposite sex. An example of this sort of antagonistic relationship is seen in the evolution of proteins in the seminal fluid of *Drosophila* (fruit fly) males (Chapman et al. 1995, 2003; Chapman 2001).

The seminal fluid of *Drosophila* males contains at least 80 different proteins produced primarily in what are called accessory glands. The genes that code for proteins produced in the accessory gland proteins are all found on autosomes but they are only expressed in males, and they evolve at relatively quick rates compared to other autosomal genes (Swanson et al. 2001). The accessory gland proteins have many different functions. They facilitate egg production and laying in females, decrease a female's receptivity to other males, and form part of what is called a mating plug—a gelatinous mass that temporarily blocks a female's reproductive tract so that she cannot successfully mate with other males (Chapman 2001; Fricke et al. 2009b; Wigby et al. 2009; Gioti et al. 2012).

The benefits to males of such accessory gland proteins are obvious, but what, if any, are the benefits to females? It might seem that the increased egg production and laying associated with the accessory gland proteins transferred in seminal fluid would be beneficial to females as well. This would be the case if eggs were cost-free to produce. But, of course, they aren't. And when a female increases her egg production as a result of contact with the accessory gland proteins transferred to her by one male, this may reduce her chances of surviving and producing offspring with other males in the future. Tracey Chapman and her colleagues hypothesized that this might impose a mating cost on female fruit flies, and they designed an experiment to test the idea (Chapman et al. 1995). They used four experimental, transgenic lines of fruit flies. Males in these lines differed from one another as follows:

- Treatment 1: Males produced no sperm or accessory gland proteins. These males behaved normally and mated with females.
- Treatment 2: Males produced no sperm or accessory gland proteins. These males behaved normally, except that they had their genitalia experimentally altered so that they could not mate with females.
- Treatment 3: Males produced seminal fluid and accessory gland proteins, but not sperm. These males behaved normally and mated with females.
- Treatment 4: Males produced seminal fluid and accessory gland proteins, but not sperm. These males behaved normally, except that they had their genitalia experimentally altered so that they could not mate with females.

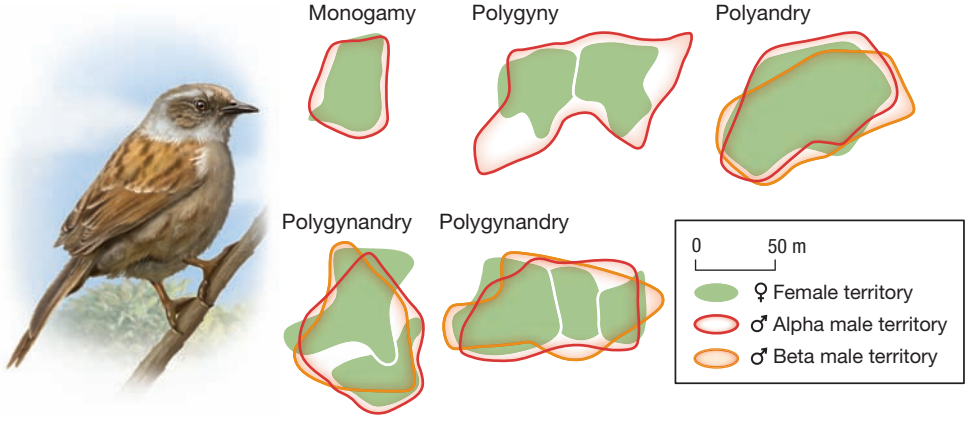


FIGURE 16.31 Territories and mating systems in dunnocks. Female territories are shown in green, territories of alpha (dominant) males are depicted by solid red lines, and territories of beta (subordinant) males are shown by solid orange lines. In a single dunnock population, mating systems ranging from monogamy to polygyny, polyandry, and polygynandry can be found. Adapted from Davies (1992).

This combination of treatments allowed Chapman's team to isolate the possible negative effects of male accessory gland proteins on females, while holding constant other attributes of sperm, as well as male behavior outside of courtship behavior. *If* there was a cost to females associated with accessory gland proteins transferred by males during mating, then that cost should show up in treatment 3 but not in the other treatments, because it was only in treatment 3 that accessory gland proteins were produced by males who mated with females. When Chapman and her team looked at survival probabilities of females across the four treatments, they found that treatment 3 females died earlier than females from other treatments, suggesting a significant cost to females from exposure to male accessory gland proteins (**Figure 16.32**). Accessory gland proteins benefit males, but they are detrimental to females: the signature of sexual conflict.

In this chapter, we began by examining the evolution of sexual reproduction. Sexual reproduction is the predominant reproductive mode of eukaryotes, while asexual reproduction in eukaryotes is relatively rare and has a very twiggy phylogenetic distribution. Yet, sexual reproduction has many costs associated with it, not the least of which is that parents and offspring are no longer genetically identical (as they are in asexual reproduction). The compensating benefits associated with sexual reproduction have helped evolutionary biologists understand the origins and maintenance of this form of reproduction. Sexual reproduction results in two (or more) sexes, whose interests are rarely identical, leading to sexual selection and sexual conflict. We concluded the chapter by examining the underpinnings of sexual selection theory, a topic that has fascinated biologists since Darwin first discussed it. In the next chapter, we will move on to the evolution of sociality, including the evolutionary relationship between cooperation and conflict, as well as the role of communication in promoting social interaction.

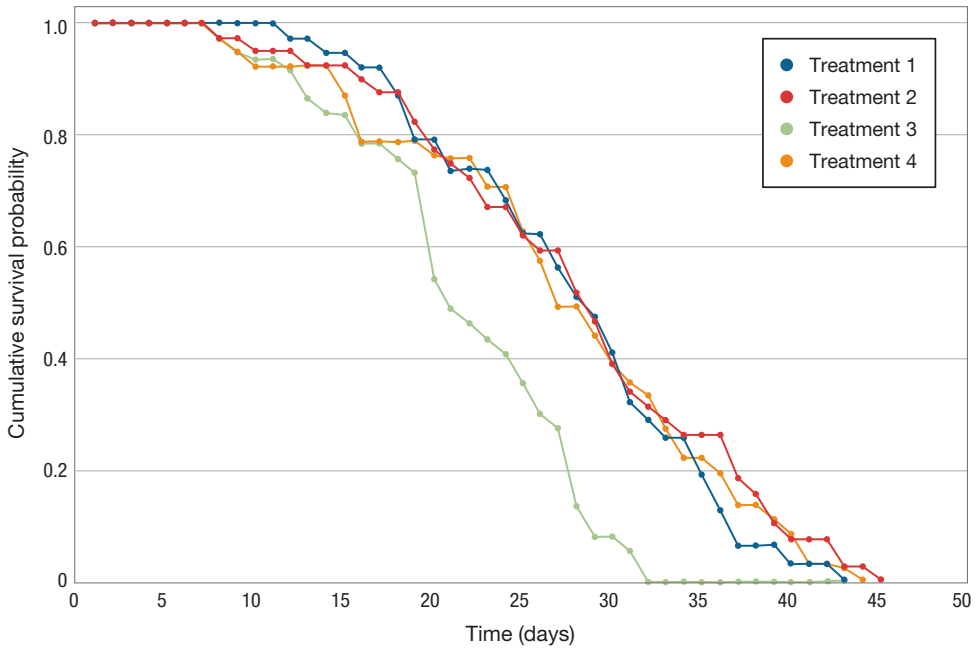


FIGURE 16.32 Cost to females of accessory gland proteins in male seminal fluid. Female survival when exposed to accessory gland proteins in male seminal fluids. Treatment 1: Males with no sperm or accessory gland proteins; these males behaved normally and mated with females. Treatment 2: Males similar to males in treatment 1, except that they had their genitalia experimentally altered so that they could not mate with females. Treatment 3: Males produced seminal fluid and accessory gland proteins, but not sperm; males behaved normally and mated with females. Treatment 4: Males similar to males in treatment 3, except that they had their genitalia experimentally altered so that they could not mate with females. Adapted from Chapman et al. (1995).

SUMMARY

1. Asexual reproduction involves the production of offspring from unfertilized gametes. Sexual reproduction involves the joining together of genetic material from two parents to produce progeny that have genes from each parent. Researchers can determine whether a species reproduces sexually or asexually by observation, but also by molecular genetics and phylogenetic comparisons.
2. In eukaryotes, species that reproduce only asexually are rare and short-lived on an evolutionary timescale. Their phylogenetic distribution has a "twiggy" appearance.
3. Despite its ubiquity, sexual reproduction has many costs associated with it, including the "twofold cost of sex" and the cost of breaking up of favorable gene combinations.
4. The advantages of sexual reproduction can be divided into two general categories: (1) sexual reproduction is more efficient at purging deleterious mutations from a genome than asexual reproduction, and (2) sexual reproduction leads to the production of more variable offspring through the processes of recombination and gametic fusion.
5. In *The Descent of Man and Selection in Relation to Sex,* Darwin defined sexual selection as selection that "depends on the advantage which certain individuals have over other individuals of the same sex and species in exclusive relation to reproduction."
6. Sexual reproduction leads to sexual selection, which can occur via (1) intersexual selection, wherein individuals of one sex choose individuals of the other sex as mates, and/or (2) intrasexual selection, in which members of one sex compete for mating access to the other sex.
7. The four main evolutionary models of female mate choice are the direct benefits model, good genes model, Fisherian runaway selection model, and sensory bias model.
8. When selection operates differently on males and females, sexual conflict, where the traits that evolve in one sex are detrimental to individuals of the other sex, may occur.

KEY TERMS

anisogamy (p. 577)
apomixis (p. 572)
asexual reproduction (p. 572)
automixis (p. 572)
Fisher process (p. 595)
handicap principle (p. 594)
intersexual selection (p. 590)
intrasexual selection (p. 590)
isogamy (p. 578)
Muller's ratchet (p. 580)
postcopulatory sexual selection (p. 600)
Red Queen hypothesis (p. 571)
runaway sexual selection (p. 596)
sensory bias model (p. 597)
sexual conflict (p. 601)
sexual reproduction (p. 572)
sexual selection (p. 590)
twofold cost of sex (p. 577)

REVIEW QUESTIONS

1. What are the three steps associated with sexual reproduction?
2. How can phylogenetic techniques distinguish between sexually and asexually reproducing lineages?
3. What are isogamy and anisogamy?
4. What are some of the costs of sexual reproduction?
5. What are the two main benefits of sexual reproduction?
6. What are the two basic types of sexual selection?
7. What are the four main models of female mate choice?
8. What is the handicap principle?
9. What is postcopulatory sexual selection?
10. What do evolutionary biologists mean by sexual conflict?

KEY CONCEPT APPLICATION QUESTIONS

11. In 2010, NASA researchers found a new, shrimplike creature living 600 feet below the ice sheets of the Antarctic. We know almost nothing about this new species—including whether it reproduces sexually or asexually. Why might the deep waters under the ice sheets of the Antarctic be the sort of environment especially likely to house asexual creatures?
12. Why would you predict that females in species with internal fertilization and long periods of gestation would be choosier in selecting their mates than females in other species?
13. What would you expect to happen to the variance in male reproductive success if females copied the mate choice of other females?
14. How might ectoparasites—external parasites—play a role in the direct benefits model of female choice?
15. On the diagrams below, bars represent chromosomes and circles represent deleterious mutations. Add to the diagrams to illustrate (a) the turning of Muller's ratchet and (b) how sex can reverse the ratchet.

Illustrate Muller's ratchet turning

Illustrate how sex can reverse the ratchet

16. Suppose you hypothesize that the antlers of male deer are costly signals of fighting ability, directed toward other males. List three testable predictions this hypothesis makes?

SUGGESTED READINGS

King, E. D. A., P. B. Banks, and R. C. Brooks. 2013. Sexual conflict in mammals: Consequences for mating systems and life history. *Mammal Review* 43: 47–58. A review of the causes and consequences of sexual conflict in mammals.

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17

The Evolution of Sociality

◀ An adult emperor penguin (*Aptenodytes forsteri*) amidst a crèche of chicks in Antarctica.

Slime mold cells are a social lot. In Chapter 12, we described how slime mold cells join together to form mobile slugs. The slugs are ensembles of individual cells that unite to form a sort of pseudo-multicellular creature. Slime mold slugs produce reproductive structures called fruiting bodies, and within each fruiting body, some of the cells make up a stalk that holds up a capsule full of reproductive spores, while others are the reproductive spores (**Figure 17.1**).

Evolutionary biologists have found that slime mold cells that are large and well nourished generally become spore cells, and those that are less well nourished become stalk cells (Kessin 2001; Bonner 2003). But this does not tell us how selection could ever favor a mechanism that leads a once free-living cell to forfeit an opportunity for reproduction and instead become part of the nonreproducing stalk. To understand that, we need two more pieces of information: (1) more stalk cells result in taller stalks, which allow greater dispersal distances for spores and reduce competition with other slugs; and (2) slime mold slugs are composed of cells that are close genetic relatives. By increasing the reproductive success of

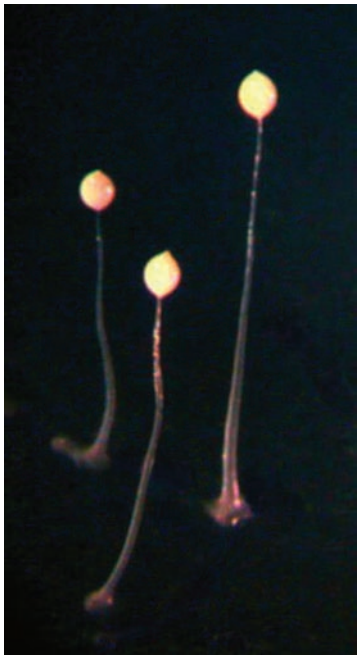


FIGURE 17.1 Fruiting bodies of the slime mold, *Dictyostelium discoideum.* The fruiting body is composed of a stalk and a capsule on the top of the stalk that holds reproductive spores.

their close genetic relatives—the spores in the capsule—stalk cells may increase the number of copies of their own genes that make it into the next generation, albeit indirectly through their genetic kin.

Slime molds also teach us another lesson about sociality. Under certain conditions, not all individual cells that form a slug are close genetic relatives. In those situations, when individuals do not benefit their genetic kin by adopting the role of stalk cell, selection will favor cheating behavior, with cheaters suppressing the tendency of other cells to differentiate into spores and increasing their own probabilities of ending up as spores rather than stalk cells. Such cheater mutants have been found in natural populations and have also been created by knockout gene experiments (Ennis et al. 2000; Santorelli et al. 2013).

In this chapter, we will examine the important effect that genetic relatedness can have on social behaviors such as cooperation in organisms ranging from microbes to vertebrates. But our goals are broader than this, as we wish to provide an overview of important topics in the area of social behavior. By social behavior, we mean the interactions that organisms have with others—most often, their conspecifics. When we look at behavior in a social context, the actions taken by one individual affect not only its own fitness but also the fitness of those around it.

In particular, we will address the following questions in this chapter:

- What are the evolutionary processes leading to cooperation?
- How do evolutionary processes lead to conflict?
- How has signaling behavior—involved in both cooperation and conflict—evolved?

17.1 Cooperation

We begin with two definitions. **Cooperation** is usually defined as an outcome of some interaction in which two individuals each receive a net benefit from their joint actions. A **cooperator** is an individual that acts in a way to allow cooperation potentially to occur. Each cooperator may pay an immediate cost for its action, but the overall effect on its fitness, *if* cooperation is achieved, is positive. Yet even when everyone benefits, it is not obvious why natural selection would favor cooperative behavior. The reason is that *free riding*—receiving benefits from others but not paying the costs of generating benefits for others—may be possible, and may be more beneficial than cooperation to an individual free rider. To understand when natural selection favors cooperation, we need to understand how natural selection has solved the following two related problems:

1. The problem of **altruism**. Why would natural selection favor an individual who performs an altruistic action: one that has the immediate consequence of reducing its own fitness while increasing the fitness of another?
2. The **free-rider** problem. In many cases, groups of individuals cooperate, each investing time, energy, and other resources in activities that benefit everyone in the group. Why are individuals selected to do so when they could instead *free ride* on the efforts of others, receiving the public benefits while shirking their own duties?

Somehow, natural selection must have solved these problems, because as we will see in this section, cooperation and altruism are widespread in the natural world. To understand how, we will explore three evolutionary "paths" to cooperation: (1) kinship, (2) reciprocity, and (3) group selection.

KEYCONCEPT QUESTION

17.1 As we saw in the slime mold example, even microbes cooperate. But microbes don't have neurons, let alone brains: How can they cooperate with one another?

Path 1: Kinship and Cooperation

Most of us feel a special loyalty to our familial kin. "Blood is thicker than water, is it not? If cousins are not friends, who can be?" asks Anthony Trollope in *The Belton Estate* (1866). While among humans this sentiment may partly be a learned cultural convention, there are strong evolutionary reasons why we might expect to see cooperation and altruism among close relatives of any species. The basic reason is that genetic relatives share alleles that they have inherited from common ancestors—parents, grandparents, and so on. Here, we will explore how this genetic relatedness relates to cooperation and altruism.

Common Ancestry and Shared Alleles

Alleles that are shared because of common ancestry are referred to as *identical by descent*. For example, you and your sibling share some of the same copies of alleles that you both inherited from common ancestors—in this case, your mother and father. In a similar way, you and your cousins are genetic kin because you share genes in common; in this case, your most recent common ancestors are your grandparents. In general, a *most recent common ancestor* is the most recent individual through which two (or more) organisms can trace gene copies that they share by descent. Full siblings share the same mother and father, cousins share some of the same grandparents, and so on.

Inclusive Fitness and Genetic Relatedness

There are two ways for an individual to increase the probability that copies of her alleles will reach the next generation. The straightforward way is to produce surviving offspring of her own. The less direct way is to act in a manner that increases the number of offspring produced by her genetic relatives, as these relatives share her genes with some probability, and they may pass copies of those genes on to *their* offspring. Thus, an individual can get copies of her genes into the next generation either by producing more offspring herself or by helping her kin in their reproductive endeavors. This observation forms the basis for the notion of **inclusive fitness**.

To formalize this intuition, British evolutionary biologist W. D. Hamilton (1936–2000) proposed that we broaden our definition of fitness (Hamilton 1963, 1964). He proposed that an individual's total fitness can be viewed as the sum of (1) its **direct fitness**, which is the number of viable offspring that it produces,

and (2) its **indirect fitness**, which is the incremental effect that the individual's behavior has on the (direct) fitness of its genetic relatives. The latter quantity reflects the fact that when an individual increases the number of its genetic kin that survive and reproduce, it is indirectly getting copies of some of its own genes into the next generation. Hamilton termed the sum of the two components the *inclusive fitness* of an individual (Hamilton 1963, 1964; Costa 2013).

KEYCONCEPT QUESTION

17.2 When calculating inclusive fitness, why is it important to distinguish between genetic kinship and kinship in the everyday sense of "family"?

Because genetic relatedness is one path to the evolution of cooperation, we would like to have a way of quantifying the relatedness between two individuals. The **coefficient of relatedness** between two individuals is defined as the probability that an allele in one individual has a copy that is identical by descent in the other individual.

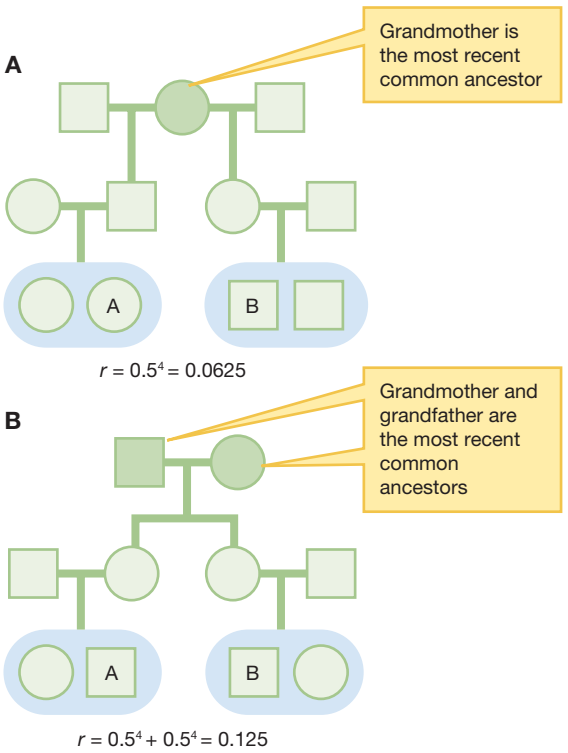


FIGURE 17.2 Pedigrees for calculating relatedness. Squares represent males; circles represent females; dark-shaded squares or circles indicate most recent common ancestors. **(A)** Individuals A and B have the same grandmother (shaded circle) but different grandfathers: Their grandmother is their sole most recent common ancestor. **(B)** Individuals A and B have the same maternal grandmother (shaded circle) and the same maternal grandfather (shaded square): Both maternal grandparents are the most recent common ancestors.

In diploid species, there is a straightforward algorithm for calculating this probability. To calculate the coefficient of relatedness (often denoted r) between two individuals "A" and "B," we follow these steps:

1. We locate the most recent common ancestor or ancestors of individuals A and B. This may be a single individual as in **Figure 17.2A** or it may be a mated pair as illustrated in **Figure 17.2B**.
2. For each most recent common ancestor, we calculate the probability that a given allele copy in that ancestor has been passed on to *both* individuals A and B. This computation is straightforward. In sexual diploid organisms, the process of meiotic segregation occurs once per generation. Thus, for any given allele copy in the parent, there is a 50% chance that this allele will be passed on to each offspring and a 50% chance that the homologous allele will be passed on instead. To compute the coefficient of relatedness r, we simply tally the number of meiotic divisions that occur along the paths from the common ancestor to individual A and from the common ancestor to individual B. Going through multiple generations is straightforward: Each generation reduces the probability of obtaining a particular allele that is identical by descent by one-half. According to the rules of probability, if there are two meiotic divisions, the probability is then $0.5 \times 0.5 = 0.25$. If there are four meiotic divisions, the probability is $0.5 \times 0.5 \times 0.5 \times 0.5 = 0.0625$. In general, if there are k meiotic

divisions separating individuals A and B, the probability that they share an allele through a single most recent common ancestor is 0.5^k. In Figure 17.2A, individuals A and B share a single ancestor—a grandmother—and are separated by four meiotic divisions (A's grandmother to A's father, A's father to A B's grandmother to B's mother, B's mother to B). Thus, the coefficient of relatedness r between individuals A and B is $0.5^4 = 0.0625$.

3. If individuals A and B have only one most recent common ancestor, the calculation is complete, and we have the coefficient of relatedness r for the individuals in question. If individuals A and B have two most recent common ancestors, they could share a given allele through either of those ancestors (but not both). Therefore, again following the rules of probability, we *add* the probability that individuals A and B share an allele through one of the most recent common ancestors to the probability that individuals A and B share an allele through the other most recent common ancestor. In Figure 17.2B, individuals A and B have two most recent common ancestors—their maternal grandparents. The chance they share an allele through one specific grandparent is $0.5^4 = 0.0625$, so the total chance they share an allele through either grandparent is $r = 0.5^4 + 0.5^4 = 0.125$.

In **Box 17.1**, we show how to calculate the coefficient of relatedness r for other sets of genetic relatives.

There is a straightforward way to use genetic relatedness as one variable in a model to predict whether an allele for helping one's relatives is favored by natural selection. As a rule of thumb, Hamilton showed that an allele X for helping a relative increases in frequency whenever

$$rb > c$$

where b is the benefit that the genetic relative receives from traits associated with allele X, r is the coefficient of relatedness, and c is the cost accrued to the individual expressing the trait (Lush 1948; Hamilton 1963, 1964; Grafen 1984). The equation is often called Hamilton's rule in Hamilton's honor. The logic behind the equation is as follows: Normally, natural selection favors a behavior if the benefit (b) exceeds the cost (c). In the case of Hamilton's rule, one individual pays the cost and a different individual—a relative—receives the benefit. So when comparing benefit and cost, we need to discount the benefit somewhat—by the relatedness r. If the discounted benefit is still greater than the cost, selection favors the action.

Hamilton's rule shows that the extent to which natural selection favors assisting family members depends on how closely related individuals are to one another and how high or low the associated costs and benefits turn out to be. When relatedness r is high, benefit b to the recipient is high, and cost c to the actor is low, then natural selection should strongly favor individuals who help their kin (Emlen 1995).

BOX 17.1 Calculating Genetic Relatedness

Let us work through a few more examples of calculating genetic relatedness. In **Figure 17.3A**, individuals A and B are half siblings, with the same mother but different fathers. To compute the coefficient of relatedness r between individuals A and B, we first must find the most recent common ancestor or ancestors. In this case, there is one most recent common ancestor: their mother. Second, we compute the probability that a given allele copy in the mother is passed to both offspring. The probability is 0.5 that the allele will be passed to individual A, and the probability is 0.5 that it will be passed to individual B, so the probability that it will be passed to *both* is $0.5 \times 0.5 = 0.25$. Because the mother is the sole most recent common ancestor, this is the total coefficient of relatedness r.

In **Figure 17.3B**, individuals A and B are full siblings, with the same mother and the same father. Thus, both parents are the most recent common ancestors. For each, we compute the probability that a given allele copy will be passed to both offspring. The calculation is as above. With probability $0.5 \times 0.5 = 0.25$, a given allele in the mother will be passed to both offspring, and by similar logic, with probability 0.25, a given allele in the father will be passed to both offspring. The total coefficient of relatedness will be the sum of these two paths: $0.25 + 0.25 = 0.5$.

In **Figure 17.3C**, individuals A and B have a single most recent common ancestor who is individual A's maternal grandmother and individual B's mother. The chance that a given allele copy in this ancestor reaches individual A is 0.25, because there is a 0.5 chance that it will reach individual A's mother, and if it does, there is an additional 0.5 chance that it will go on to reach individual A, for a net chance of 0.25. The chance that a given allele will reach individual B is 0.5, because only a single meiosis separates individual B from the common ancestor. Thus, the chance that the given allele copy will reach *both* individuals A and B is $0.25 \times 0.5 = 0.125$. The coefficient of relatedness between individuals A and B is therefore 0.125. (If B had been a full sibling to A's mother, the coefficient of relatedness between A and B would have instead been 0.25). Similar calculations allow us to compute the genetic relatedness between any pair of individuals with a known pedigree.

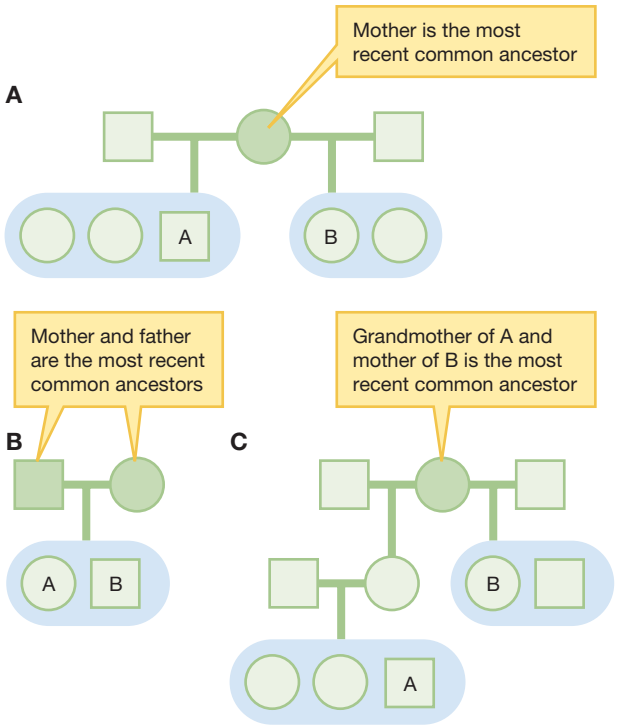


FIGURE 17.3 Example pedigrees for computing coefficients of relatedness. **(A)** Individuals A and B are half siblings. **(B)** Individuals A and B are full siblings. **(C)** A more complicated scenario, with individuals A and B coming from different generations.

KEYCONCEPT QUESTION

17.3 Pelicans have clutches of two offspring. Suppose among pelicans a new allele arises that causes a nestling to share its food with its nestmate if it is not particularly hungry. This gene imposes a fitness cost of 0.2 on those who carry it, while conferring a benefit of 0.5 on the sibling who receives the additional food. Will this gene increase in frequency if nestmates are always full siblings, sharing the same mother and father? What if nestlings are always half-siblings, sharing the same mother but different fathers?

Inclusive Fitness and Alarm Calls

Paul Sherman's work on alarm calls in Belding's ground squirrels (*Urocitellus beldingi*) was one of the first explicit tests of the power of inclusive fitness to explain altruism (Sherman 1977). Sherman marked squirrels in a population that he was studying in the Sierra Nevada mountains, and over the course of 3000-plus hours of observations, he and his colleagues observed many cases of a ground squirrel standing up on its hind legs and emitting a piercing alarm call when a predator was spotted. In response to that call, other squirrels in the area then headed for safety: Recipients of the call clearly benefited from the alarm caller's behavior. Sherman also had data that demonstrated that giving an alarm call increased the caller's chance of being attacked by the predator: Alarm calls were costly to the caller. Together, these data suggest that ground squirrel alarm calls are altruistic. But why give these calls? Why hasn't selection acted against emitting alarm calls?

Sherman noted certain patterns in alarm calling. When predators were spotted by ground squirrels, 65% of calls emitted came from adult females, even though they only made up 30% of the population, and the opposite pattern was found in adult males, who gave only 2% to 3% of the alarm calls but made up 20% of the population (**Figure 17.4**). It was this sex bias in alarm calls that provided Sherman with the critical piece of information he needed to answer the question of *how* natural selection could favor the evolution of alarm calling in ground squirrels. To see why, we need to know something about the demography and natural history of Belding's ground squirrels.

When male squirrels reach sexual maturity, they emigrate to new populations, where some eventually find mates. Female squirrels, however, spend their entire lives in their natal (birth) populations. This asymmetry in emigration patterns means that adult females tend to be surrounded by many of their genetic relatives: mothers, offspring, nieces, and aunts. In contrast, except for their own offspring, mature males are interacting with individuals to whom they are unrelated. What this means is that when a female gives an alarm call, it is likely to benefit a larger number of close relatives than when a male gives an alarm call.

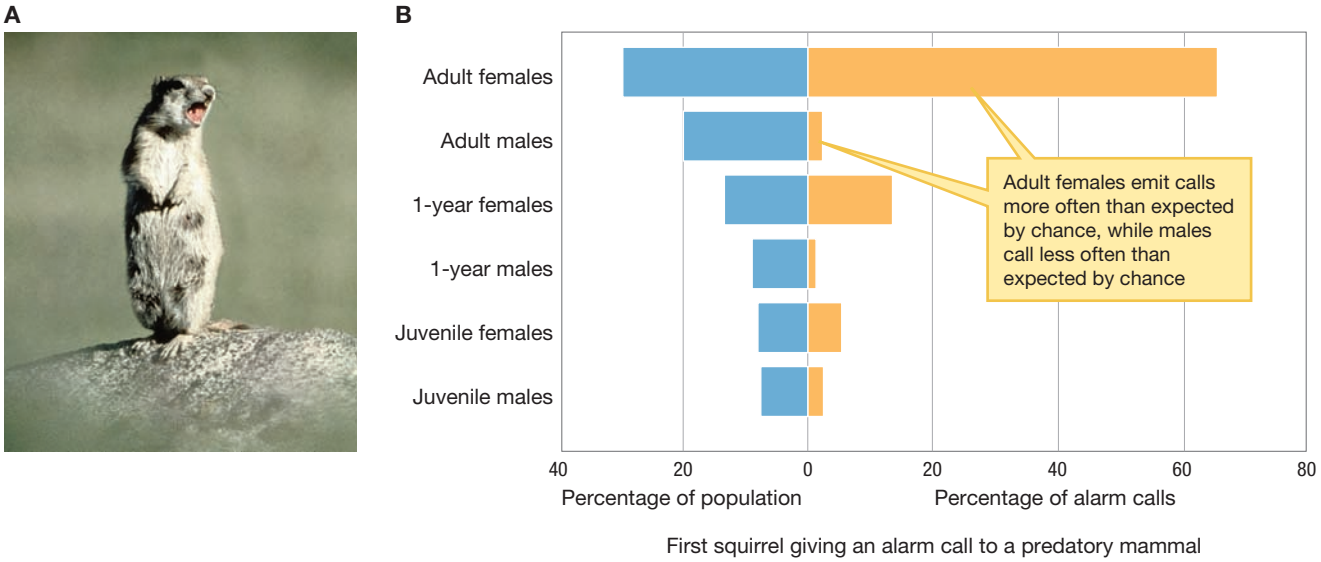


FIGURE 17.4 Ground squirrel alarm calls. **(A)** A female Belding's ground squirrel emitting an alarm call. **(B)** While adult females make up only 30% of the population, they are responsible for 65% of the alarm calls. Adult males make up 20% of the population but give only about 2% of alarm calls. Panel B adapted from Dugatkin (2013).

First squirrel giving an alarm call to a predatory mammal

Hamilton's rule is easily extended to a case like this where there are multiple beneficiaries. If relatives 1, 2, . . . , n are related by r_1, r_2, . . . , r_n and receive benefits b_1, b_2, . . . , b_n, an action with cost c will be favored by natural selection if the following inequality is met:

$$\sum_{i=1}^{n} r_i b_i > c$$

In words, this means an action with cost c will be favored by natural selection if the sum of the benefits, discounted by the coefficients of relatedness, exceeds the cost. Because a female ground squirrel tends to be around more relatives, the sum of benefits multiplied by relatedness (that is, the sum of $r \times b$ for all relatives) will be larger than it will be for males—and thus selection more likely will favor alarm calling in females than males.

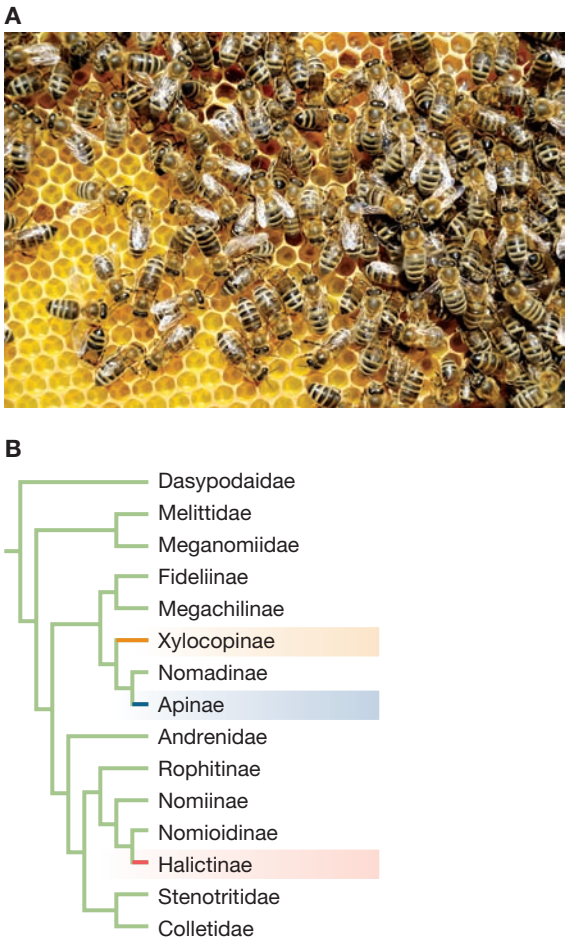


FIGURE 17.5 Eusocial insects. **(A)** Ants, wasps, and bees are eusocial, often living in large, complex societies such as the honeybee colony seen here. **(B)** A phylogeny of bees at the subfamily level. Eusociality is thought to have emerged five separate times among bees alone, once in the Xylocopinae (orange), once in the Apinae (blue), and three times in the Halictinae (red). Panel B adapted from Danforth (2007).

KEYCONCEPT QUESTION

17.4 Why would you expect individuals in some populations to be able to gauge their genetic relatedness to those around them? When would such behavior be favored by natural selection?

Inclusive Fitness and Eusociality

Many evolutionary biologists have argued that inclusive fitness theory has played a prominent role in understanding the major transition from solitary to group living (Chapter 12). In particular, inclusive fitness theory has been used to examine the evolution of eusocial behavior and group living. Although debate continues over how best to define **eusociality** (Batra 1966), it is most often defined as a social system with the following properties (Alexander et al. 1991):

1. Reproductive division of labor. Only a fraction of the population is breeding at a given time; others are infertile, often as a result of some form of direct reproductive suppression.
2. Cooperative rearing of young. Multiple individuals, beyond the immediate parents, work together to feed and care for the young.
3. Overlapping generations. Not only do the generations of a eusocial species overlap (unlike annual plants or many annual insect species), but the members of different generations also live together and work together in a single group.

Eusociality has evolved in termites, beetles, aphids, thrips, shrimps, and mammals such as the naked mole rat. But this extreme form of sociality is most often associated with ants, bees, and wasps. This group is part of the insect order Hymenoptera.

Phylogenetic analysis suggests that eusociality has evolved independently on at least nine separate occasions in hymenopterans (Hughes et al. 2008) (**Figure 17.5**).

We can use inclusive fitness theory to understand why we see eusociality evolving so often in ants, wasps, and bees. First, bee, ant, and wasp nests, which often contain hundreds or thousands of individuals, are composed primarily of genetic relatives. So, the altruistic acts associated with eusociality may benefit not just one but many, many genetic relatives. For example, when a worker bee defends the hive, she may save hundreds of genetic relatives—including, most importantly, the queen—by her act. But this logic applies to any colonial species in which relatives live close together, not just the Hymenoptera. While not all hymenopteran species are eusocial, many are. Why is Hymenoptera particularly prone to evolve eusociality?

The answer may lie, in part, in the unusual genetic architecture of the hymenopterans. Ants, bees, and wasps are **haplodiploid**; that is, all males are haploid and all females are diploid. Because of the genetics of haplodiploidy, when a queen in a colony mates with a single male, sister workers are related to one another on average by a coefficient of relatedness r of 0.75; that is, the probability that a given parental allele ends up in both sisters is 0.75. Here's why. The probability that the sisters share a given allele copy through their mother is 0.25 (as in the case of diploid species), but because all males are haploid, the probability that sisters share an allele copy through their father is 0.5. Adding these probabilities gives us our genetic relatedness value of 0.75 for full sisters (**Figure 17.6**).

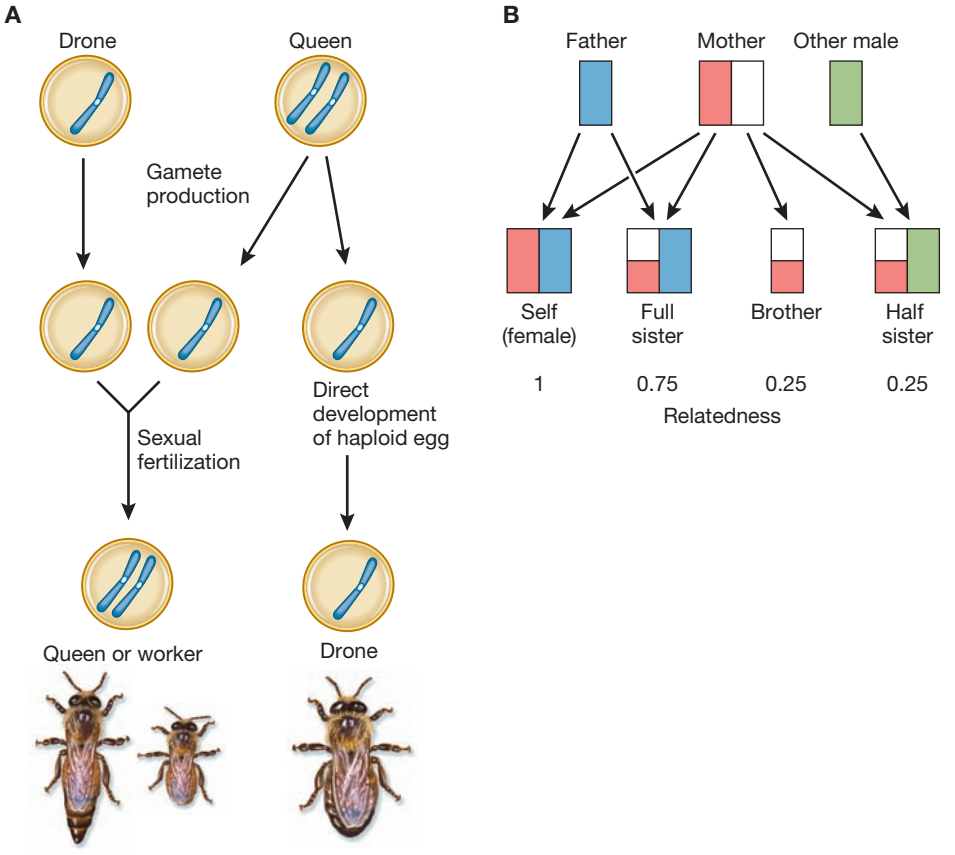


FIGURE 17.6 Relatedness in haplodiploids. **(A)** Haplodiploid genetics in bees. **(B)** Coefficients of relatedness among haplodiploids. Blue represents the paternally derived alleles; red represents maternally derived alleles in the focal female labeled "Self." Relatedness to self is always 1. Adapted from Queller (2003).

A genetic relatedness of 0.75 between sisters has the remarkable effect of making females more related to their sisters than to their own offspring. Think about it like this: The queen of the hive produces both females (workers) and males (drones). If the female workers produce their own offspring, they have a genetic relatedness of 0.5 to such offspring, but if they help their mother produce more workers—more sisters—they have a genetic relatedness of 0.75 to such new sisters. Females are not as closely related to their male sibs. A female worker and a male sibling share an r of 0.25, whereas r between siblings in a diploid species is 0.5. Because of the asymmetries in genetic relatedness, we predict that eusocial behaviors should be displayed by female workers but not by males (drones). And, indeed, that is what we see: Females tend to the brood at the hive, they defend the nest (even at the cost of their lives), and they do the foraging for the nest.

Eusociality in hymenopterans is not *completely* explained by the high genetic relatedness between workers that comes about via their haplodiploid genetics. After all, *all* hymenopteran species are haplodiploid, but only some hymenopteran species are eusocial. What's more, there are also examples of eusociality in diploid species such as naked mole rats and termites. So, haplodiploidy *alone* is neither necessary nor sufficient for the evolution of eusociality, but it does help partly to explain why eusociality is overrepresented in hymenopterans.

The unusual genetic architecture of hymenopterans also has a dramatic effect on sex ratios in these species. To see why, we need to return to Fisher's original argument for sex ratio evolution, which we explored in Chapter 1. Recall Fisher's conclusions: For most systems of genetic inheritance, natural selection will favor a 1:1 sex ratio, assuming that the cost of producing a male is the same as the cost of producing a female. If the costs differ, an analogous argument reveals that selection will favor parents who invest an equal *amount of resources* in offspring of each sex. As a result, parents will produce more of whichever sex is less expensive to produce.

This result can be extended yet further, to treat the curious genetics of haplodiploid species. An extension of Fisher's argument reveals that when relatedness varies by sex, as in haplodiploid species, natural selection will favor individuals who invest in kin of a given sex in proportion to their relatedness to kin of that sex.

As we have seen, haplodiploid mothers are equally related to their sons ($r = 0.5$) and their daughters ($r = 0.5$). Assuming an equal cost of producing male and female offspring, queens are expected to favor a 1:1 sex ratio. But because female workers, who care for offspring by providing food and protection to them, are 3 times as related to their sisters ($r = 0.75$) as to their brothers ($r = 0.25$), they will favor a 3:1 female:male sex ratio. In many social insect species, sex ratios are closer to 3:1 than 1:1, suggesting that workers do influence sex ratio (Trivers and Hare 1976; Nonacs 1986). In those species, inclusive fitness considerations affect not only social behavior but also the sex ratio of the population.

The idea that high genetic relatedness is important to the evolution of eusociality in at least some hymenopterans (especially in bees, but perhaps not in ants; Wilson and Holldobler 2005a,b) is supported by a comparative phylogenetic analysis. Genetic relatedness is highest in social insect groups when queens are *monandrous*; that is, when they have a single mate. When females are *polyandrous*—that is, when they mate with many males—the average genetic relatedness in groups goes down, as many individuals in the hive or nest do not share the same father. Given

the high degree of relatedness among bees in a hive, inclusive theory predicts that eusociality in bees will often be associated with a monandrous mating system.

To test the hypothesis, William Hughes and his colleagues took advantage of the fact that prior phylogenetic analyses had found that eusociality has independently evolved nine different times in hymenopterans: five times in bees, three times in wasps, and once in ants (Hughes et al. 2008; Ratnieks and Helantera 2009). When we look at these eusocial lineages today, we see both monandry and polyandry. But Hughes and his colleagues hypothesized that for eusociality to have taken hold in these groups to begin with, their evolutionary histories should indicate that the *ancestral* mating system was monandrous.

A phylogenetic analysis of eight of the nine lineages (267 different species; data were not available to test one lineage of bees) indicates that, as predicted by inclusive fitness theory, monandry was the ancestral state in *all* eusocial lineages examined (**Figure 17.7**). This suggests that eusocial species that are not currently monandrous (about one-third of all eusocial species) evolved from monandrous ancestors after eusociality was already in place. Why the evolution to polyandry occurred in some hymenopteran species has not been fully explained.

Path 2: Reciprocity

In 1971, Robert Trivers hypothesized that if individuals benefited from *exchanging* acts of altruism, then this sort of reciprocal exchange system—which Trivers called **reciprocal altruism**—might be favored by natural selection (Trivers 1971). If individual A pays some cost to help individual B, but the cost is recovered at some point in the future (when B helps A), then natural selection might favor behaviors that lead to this type of reciprocity. Reciprocal altruism might be especially likely to occur among individuals living in stable groups because they are likely to have ongoing interactions with the same set of partners.

The Prisoner's Dilemma

Trivers addressed the question of the evolution of reciprocity using a theoretical framework known as *game theory*. Game theory allows us to analyze decision making in a social context and is useful when dealing with strategic situations, in which the results of one participant's actions depend on the behaviors that other participants adopt. In particular, Trivers, with some help from W. D. Hamilton, suggested that the evolution of cooperation could best be understood by using a mathematical game called the prisoner's dilemma.

The prisoner's dilemma game, initially developed by Merrill Flood and Melvin Dresher of the RAND Corporation, is based on a scenario in which two criminal suspects are caught by the police. They are taken to two different rooms and interrogated separately. The police have enough circumstantial evidence to put each suspect in prison for 1 year, even without a confession from either. In an effort to get the two suspects to testify against one another, the police offer each suspect the following deal: "If you testify against the other guy, you'll walk away a free man and the other guy will go to prison for 5 years." The catch is that if *both* prisoners agree to testify, the police won't set them free, but instead each will be convicted on the grounds of the other's testimony, and each will have to serve 3 years in prison. The prisoners are aware of this catch.

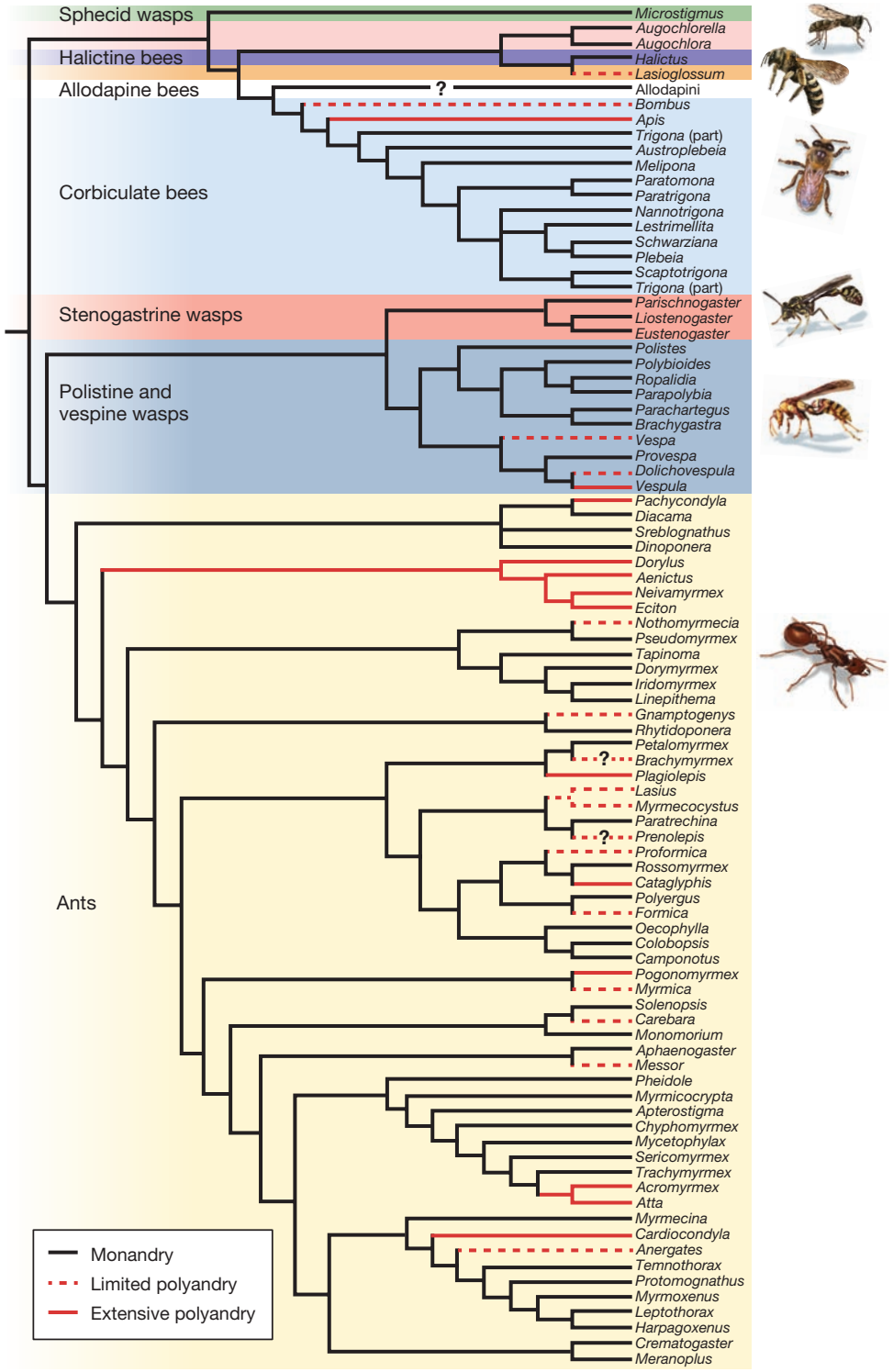


FIGURE 17.7 Phylogeny of ant, bee, and wasp species. This phylogeny is for ants, bees, and wasps for which data on female mating frequency are available. Each independent origin of eusociality is indicated by a different color for that clade. Clades exhibiting high polyandry (a queen and multiple male mates) are depicted by red branches, and completely monandrous (a queen and a single male mate) genera have black branches. Colored in this way, the phylogeny reveals that as Hughes and colleagues hypothesized, the common ancestor of each eusocial clade is monandrous. Data on mating frequency for Allodapine bees (in white) are not available. Adapted from Hughes et al. (2008).

BOX 17.2 Evolutionarily Stable Strategies

An evolutionarily stable strategy (ESS) is defined as "a strategy such that, if all the members of a population adopt it, no mutant strategy can invade" (Maynard Smith 1982). Here, "mutant" refers to a new strategy introduced into a population, and successful invasions depend on the relative fitness of established and mutant strategies. If the established strategy is evolutionarily stable, the payoff from the established strategy is greater than the payoff from the mutant strategy. To see this more formally, let's consider two strategies, I and J (for example, I might be to cooperate, while J might be not to cooperate). We will denote the expected payoff of strategy I against strategy J as $E(I,J)$, the payoff of J against I as $E(J,I)$, the payoff of I against I as $E(I,I)$, and the payoff of J against J as $E(J,J)$. Strategy I is an ESS if for every possible alternative strategy J, either

$$E(I,I) > E(J,I) \tag{17.1}$$

or

$$E(I,I) = E(J,I), \quad \text{but} \quad E(I,J) > E(J,J) \tag{17.2}$$

If the first condition (Equation 17.1) holds true, then I does better against other I's than J does. Thus, if everyone is playing I, no one can do better by unilaterally shifting to J. Thus, condition 1 ensures that strategy I is what is called a Nash equilibrium.

If strategy I is *a strict Nash equilibrium*—that is, if $E(I,I) > E(I,J)$ for all other strategies, J—we are done. I is an ESS. But if strategy I is a *weak Nash equilibrium*—that is, if there is at least one strategy J that does as well against I as I itself does—we need an additional condition to ensure that I can resist invasion by J. The second condition (Equation 17.2) provides this. If I is only a weak Nash equilibrium, it can still be an ESS as long as it does better than J when paired up against J; that is, when $E(I,J) > E(J,J)$.

What should the prisoners do? If both refuse to testify, each will serve only 1 year. But each has an incentive to testify against the other: not serving any time in prison. Notice that an individual serves a shorter sentence if he agrees to testify, *irrespective* of what the other suspect decides to do. In particular, if each player's strategy in this game is to testify, neither player can do better by changing what he alone is doing. In game theory, a pair of strategies in which neither player can benefit by unilaterally changing his strategy is known as a Nash equilibrium. In **Box 17.2**, we examine the related topic of an evolutionarily stable strategy (ESS).

In game theory, the prisoner's dilemma is the paradigmatic model of the altruism problem that we presented at the start of this chapter. Let's see why. In the prisoner's dilemma, each player has the opportunity to help the other player—by refusing to testify—but a player pays a cost for making that choice. If both players refuse to testify, each does better than if both testify. For this reason, the prisoner's dilemma is thought of as a model of cooperation, and the players' strategy of "refuse to testify" is labeled as "cooperate," while "agree to testify" is labeled as "defect" (notice that, when defined this way, to cooperate means to cooperate with one's codefendant, not with the authorities, and to defect is to no longer cooperate with one's codefendant).

Figure 17.8 depicts the payoffs—measured as years in prison—to each suspect as a function of what he decides to do and what the other suspect decides to do. If both suspects cooperate, they both receive a payoff of R (the reward for mutual cooperation; 1 year in jail), but if they both defect, each receives P (the punishment for mutual defection; 3 years in jail). If suspect 1 defects, but suspect 2 cooperates, the former receives a payoff of T (the temptation to defect; no time in jail), and the latter receives S (the sucker's payoff; 5 years in jail). If we order the payoffs in this matrix from high to low, we see that $T > R > P > S$. It is this series of inequalities—$T > R > P > S$—that defines our game as a prisoner's dilemma.

With the game laid out in this way, we can explore the strategic problem facing our two suspects: Suspect 1 will receive a higher payoff individually (serving fewer years

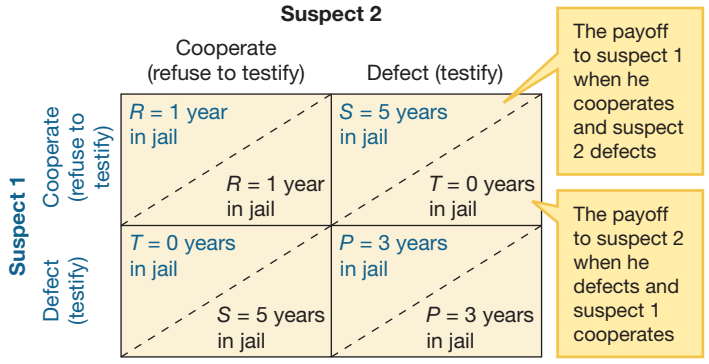


FIGURE 17.8 The prisoner's dilemma game. In this game, each player labeled suspect 1 or suspect 2 can either cooperate or defect. To cooperate is to refuse to testify; to defect is to testify. Each cell shows the payoff to suspect 1 (above the dashed diagonal line) and the payoff to suspect 2 (below the dashed diagonal line). For example, in the lower left cell, when suspect 1 defects and suspect 2 cooperates, the former gets no time in jail, while the latter gets 5 years in jail. For the matrix to qualify as a prisoner's dilemma game, it must be true that $T > R > P > S$, where T is "temptation to defect" payoff, R is "reward for mutual cooperation" payoff, P is "punishment for mutual defection" payoff, and S is "sucker's payoff." Technically, in order for the game to be a prisoner's dilemma, it must also be true that the payoff for mutual cooperation ($2R$) is greater than the sum of the payoffs received by two players in a cooperator–defector interaction; that is, $2R > T + S$. Adapted from Dugatkin (2009a).

in prison) if he defects, irrespective of what suspect 2 does. As such, suspect 1 should always defect, assuming that he prefers to minimize the length of his prison sentence. The same holds true individually for suspect 2, and he should also always defect. So, if both subjects want to minimize the lengths of their prison sentences, each should defect and agree to testify against his codefendant. The *dilemma* in the prisoner's dilemma is that while each suspect receives P (3 years in prison) when they testify against one another, both suspects would have received better payoffs (R, which is only 1 year in prison) if they had both refused to testify; that is, if they had cooperated with each other. The seemingly intractable problem that the prisoners face is that once taken to their separate interrogation rooms, each has no way to ensure that the other will cooperate if he does so himself—and in fact by the logic above, each has every reason to suspect the other will defect instead.

So, why would we ever see cooperative behavior in games that take the form of the prisoner's dilemma? In *one-shot games*—that is, in circumstances in which the game is played only once—the answer is that we do not expect to see cooperation. Defection is a Nash equilibrium (neither player can benefit by changing his strategy and cooperating), and it is the only Nash equilibrium.

But what if the game is played repeatedly? Then perhaps the logic of reciprocal altruism may lead to cooperative behavior. Indeed, it can, but only under certain conditions. The key insight is that each player can "demand" cooperation from the other, using the promise of future cooperation and the threat of future defection as carrot and stick. One notable strategy of this sort is known as tit for tat (TFT). In the tit-for-tat strategy, an individual cooperates on the initial encounter with a partner and subsequently copies the partner's previous move. This means that after the first move, TFT operates under an if–then rule: if the partner cooperated in the previous round, then cooperate now; if the partner defected in the previous round, then defect now. That is, TFT reciprocates both acts of cooperation and acts of defection. Playing repeatedly against a TFT player, one can defect now, but at the cost of being defected against in the next round. So, can this make cooperation advantageous?

To see, let's first suppose that players 1 and 2 know that they are going to play the prisoner's dilemma game with one another 10 times in a row. We might imagine that each would cooperate in the early rounds, so that the other would continue to cooperate throughout the series of games. But does this really work? Think about what each player should do on the 10th and final round of the game. In this final round, as in any single round of the prisoner's dilemma game, he will do strictly better by defecting. Moreover, there are no further rounds to worry about, so each player may as well defect on the final round. Now step back to the second-to-last round. Knowing that the other player is likely to defect on the final round, by the logic above there is no harm in defecting on the second-to-last round, because there is no cooperation to preserve. So, each should defect on the

second-to-last round as well. By the same logic, each should defect on the third-to-last round, the fourth-to-last round, and so on, all the way back to the first round of the game. Thus, in a repeated prisoner's dilemma where the players know there will be some *fixed number of interactions*, the only Nash equilibrium that remains is to defect throughout. Simply playing repeatedly does not necessarily solve the altruism problem.

But the altruism problem can be solved with a bit of uncertainty about how many times the game will be played. If neither player knows when the game will end, neither can apply the logic described earlier. There is no definitive "last round" in which defection is the obvious choice. Instead, at any present time, each player must cooperate now so as to ensure cooperation by the other player in the future.

Robert Axelrod and W. D. Hamilton used both analytical techniques and computer simulations to examine what sorts of behavioral strategies fared well in an iterated (repeated) prisoner's dilemma game (Axelrod and Hamilton 1981; Axelrod 1984). They found that while the strategy "always defect" is the only Nash equilibrium in the single-shot prisoner's dilemma, the tit-for-tat strategy was one Nash equilibrium in the iterated prisoner's dilemma that has an uncertain end point. This work established the basic theoretical foundation for reciprocal altruism.

Numerous studies have examined reciprocity in animals (Dugatkin 1997). Here, we examine one such study that addresses reciprocity in the context of predator mobbing by birds.

Reciprocity and Mobbing in Birds

Along with the altruism problem, we described a closely related problem known as the free-rider problem. The gist of the free-rider dilemma is that it may be hard to establish costly cooperation in groups because each individual has an incentive to "free ride" on the efforts of the others. The behavior of mobbing a predator provides a good example.

Mobbing behavior is an antipredatory tactic, in which one or more individuals approach, chase, and sometimes even attack a potential predator that may be much larger than individuals of the mobbing species. This sort of behavior is common among birds, where mobbing behavior often causes a potential predator to leave an area as a result of continual harassment (Sordahl 1990) (**Figure 17.9**).

Mobbing behavior can be costly, both in terms of the time and energy invested and because mobbing individuals are occasionally caught by the predator they are trying to mob (Sordahl 1990; Krama and Krams 2005). But once a predator is driven away, *all* of the prey individuals in that area benefit, not just those that were involved in mobbing. So, why do individual birds join a mobbing group? Why don't they simply let others take on the cost and risk? Indrikis Krams and his colleagues designed an experiment to examine whether reciprocity played a role in solving this problem with respect to the mobbing behavior of the pied flycatcher (*Ficedula hypoleuca*) (Krams et al. 2008). The researchers examined

FIGURE 17.9 Pied flycatchers mobbing an owl. Two pied flycatchers mob an owl predator in an attempt to force the owl to leave the vicinity. Evidence from some species suggests that this sort of antipredator behavior may involve reciprocity among the mobbers.

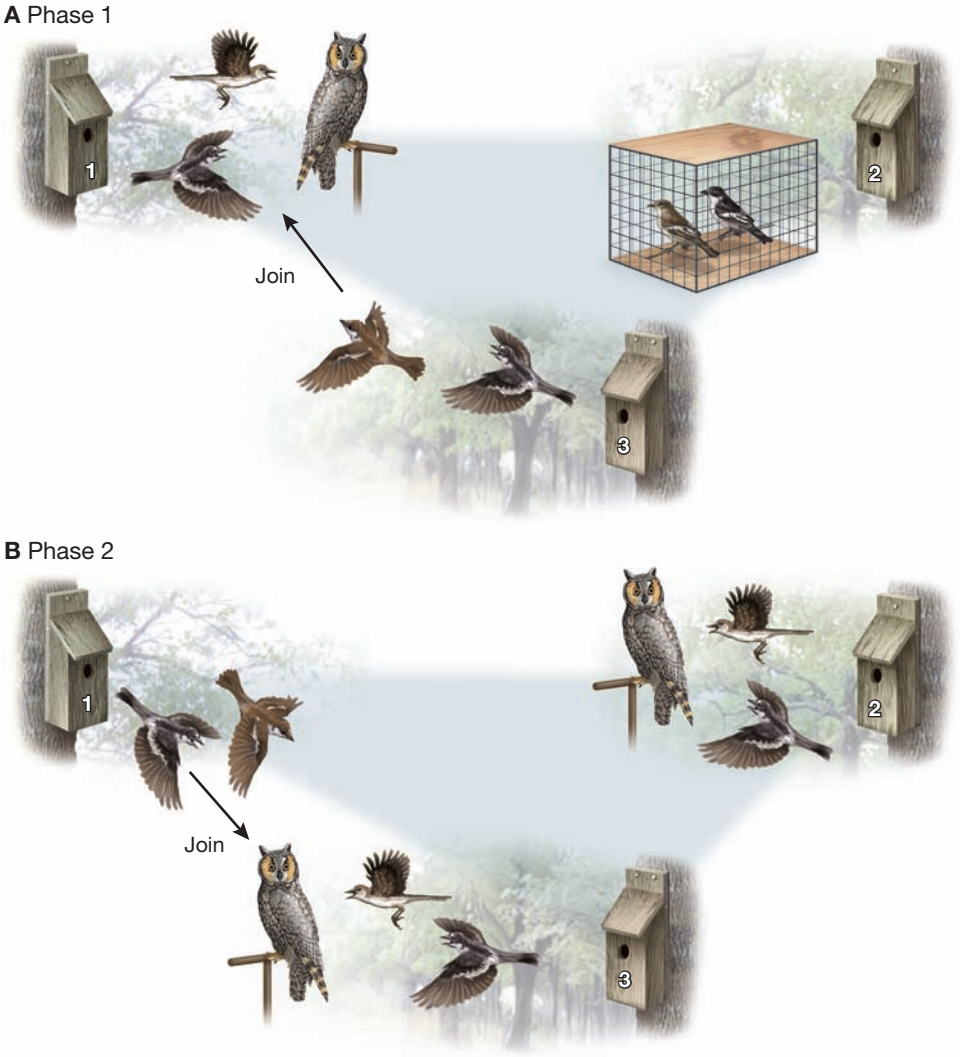


FIGURE 17.10 Reciprocal mobbing in pied flycatchers. Three nestboxes were placed on a triangular grid spaced roughly 50 meters apart. **(A)** Phase 1: A stuffed predator (owl) was placed near nestbox 1. Pied flycatchers from nestboxes 1 and 3 mobbed the predator at nestbox 1, but pied flycatchers from nestbox 2 could not join this mob. **(B)** Phase 2 (conducted 1 hour after phase 1): A stuffed predator was placed at nestboxes 2 and 3. Pied flycatchers from nestbox 1 joined the mob at nestbox 3, but not at nestbox 2. Adapted from Wheatcroft and Price (2008).

whether flycatchers were more willing to risk the danger associated with mobbing when they had partners who had helped them in the past. To test this, they set up three nestboxes that each housed a pair of flycatchers. They placed the nestboxes about 50 meters from one another, and birds in each nest could see all the other nestboxes.

To begin the experiment, a stuffed "model" predator was placed near nestbox 1 (**Figure 17.10**). Birds from nestbox 1 mobbed the predator, and they were joined by birds from nestbox 3. But the experimenters had placed the birds in nestbox 2 in a cage, so that they could not join the mobbing event at nestbox 1. As a consequence, the birds in nestbox 1 had the experience of being aided by those at nestbox 3, but not by those at nestbox 2. In two follow-up experiments in which a stuffed predator was placed at nestboxes 2 and 3, birds from nestbox 1 joined birds at nestbox 3 in mobbing a predator—they reciprocated the aid they had received—but they did not join a mob when a predator was placed near nestbox 2. Together, these experiments suggest that pied flycatchers exhibit reciprocal altruism when mobbing dangerous predators in their environment.

Path 3: Group Selection

A third evolutionary path to cooperation is via group selection. Ideas about group selection have a long history (D. S. Wilson 1980; Sober and D. S. Wilson 1998; D. S. Wilson and E. O. Wilson 2007; Nowak et al. 2010; Abbot et al. 2010; Boomsma et al. 2011). Although still quite controversial (Lehmann et al. 2007; Reeve and Holldobler 2007), *modern* group selection models—sometimes called trait-group selection models—of cooperation are conceptually straightforward. Before treating these, however, we will briefly review the history of group selection thinking and the critiques that brought earlier group selection approaches into disfavor.

Group Selection and "Good of the Species" Logic

From the time that Darwin and Wallace laid out their theory of evolution by natural selection through the 1960s, evolutionary biologists would sometimes attempt to explain certain aspects of animal behavior or physiology as adaptations that had arisen "for the good of the species" or "for the good of the population"; that is, adaptations that would minimize the chances that the species or population as a whole would go extinct. Wallace himself and, to a lesser extent, Darwin were proponents of such ideas (Ruse 1980). Nobel prize–winning ethologist Konrad Lorenz (1903–1989) used this type of argument to explain why animal fights are rarely fatal, despite the seemingly lethal armaments that many species carry (Lorenz 1966). The "good of the population" type of thinking perhaps reached its pinnacle in V. C. Wynne-Edwards's 1962 book *Animal Dispersion in Relation to Social Behavior* (Wynne-Edwards 1962, 1986, 1993). In his book, Wynne-Edwards presented a survey of traits that he believed to be adaptations that favored the survival of groups. Wynne-Edwards was particularly interested in the reproductive restraint that organisms appeared to display, and he viewed this as a group-level adaptation to avoid overexploiting their food supply and other resources. For example, individuals defend territories that are larger than they seem to need for survival and reproduction, with the consequence that the landscape is divided into fewer breeding territories. Some individuals are then unable to establish territories on which to breed, and thus fewer offspring are produced in the population. Wynne-Edwards attributed this to group selection (**Box 17.3**).

American evolutionary biologist George Williams (1926–2010) vigorously challenged this approach in an influential 1966 book entitled *Adaptation and Natural Selection* (Williams 1966). In his book, Williams noted that most of Wynne-Edwards' examples could also be explained by natural selection at the level of the individual, rather than at the level of the group. For example, Williams hypothesized that individuals might defend large territories as a hedge against unusually poor environmental conditions, not to keep the population growth rate down. Even more critically, Williams offered a decisive argument against naive use of the logic of group selection. He stressed the following thought experiment: Imagine a population of individuals showing altruistic restraint (in their acquisition of resources, severity of fighting, rate of reproduction, or any other purportedly group-level adaptation). Now imagine that a mutation arises that causes its bearer not to exercise such restraint. While this may be bad for the population in the long run, in the short run the mutant individual will take more resources, win more fights, or leave more offspring than the individuals who exhibited restraint. As a

BOX 17.3 The Tragedy of the Commons

In a famous 1968 essay, Garrett Hardin presented a metaphor for the overexploitation of natural resources, which he called "The Tragedy of the Commons" (Hardin 1968). Hardin describes the following pastoral fable:

> Picture a pasture open to all. It is to be expected that each herdsman will try to keep as many cattle as possible on the commons. Such an arrangement may work reasonably satisfactorily for centuries because tribal wars, poaching, and disease keep the numbers of both man and beast well below the carrying capacity of the land. Finally, however, comes the day of reckoning; that is, the day when the long-desired goal of social stability becomes a reality. At this point, the inherent logic of the commons remorselessly generates tragedy (Hardin 1968, p. 1244).

Hardin proceeds to explain why this leads to tragedy:

> As a rational being, each herdsman seeks to maximize his gain. Explicitly or implicitly, more or less consciously, he asks, "What is the utility to me of adding one more animal to my herd?" (Hardin 1968, p. 1244)

Hardin points out that adding one additional animal to his personal herd—grazed on communal land—offers both benefits and costs to the individual herdsman. The benefit accrues to the individual herdsman alone; he now has one more animal that he can use or sell. This brings him a net benefit of one animal.

The cost of adding one more animal to his personal herd comes in the form of the further overgrazing to the commons that is caused by the added animal. This cost is shared among all of the people who graze animals on the commons, and thus even if that cost is quite large, the part that the individual herdsman must pay is only a small fraction of one animal. Based on this logic, Hardin explains,

> The rational herdsman concludes that the only sensible course for him to pursue is to add another animal to his herd. And another; and another. . . . But this is the conclusion reached by each and every rational herdsman sharing a commons. Therein is the tragedy. Each man is locked into a system that compels him to increase his herd without limit—in a world that is limited. Ruin is the destination toward which all men rush, each pursuing his own best interest in a society that believes in the freedom of the commons. Freedom in a commons brings ruin to all. (Hardin 1968, p. 1244)

Hardin's tragedy of the commons is yet another form of the altruism problem or free-rider problem. In this case, the cooperative or altruistic thing to do would be to show restraint and limit one's own herd, but this creates group benefits at an individual cost. In the context of natural selection, we can then ask why natural selection would favor such moderation; indeed, this is precisely the question that led Wynne-Edwards to advocate the form of group selection thinking that he did.

result, the frequency of the mutation will increase over time within the population, as its bearers outcompete the more restrained wild type, and natural selection will eliminate restraint. Williams' point is that natural selection typically acts more strongly on individual-level traits than on group-level traits. For this reason, he argued that appeals to group-level selection should be an absolute last resort for evolutionary biologists.

Modern Approaches to Group Selection: Trait-Group Selection Models

Although Williams' arguments against group selection are sound, they do not entirely rule out the possibility of selection acting at the level of a group. Modern *trait-group models*, first developed by D. S. Wilson, address this, and they specify the precise circumstances in which selection can favor group-beneficial traits even when such traits impose individual-level costs (D.S. Wilson 1975; Cohen and Eshel 1976).

A *trait group* is defined as a set of individuals that affect one another's fitness. Many such trait groups make up a population. The essence of trait-group selection models is that natural selection is a hierarchical process that operates at two levels: within-group selection and between-group selection. In the context of cooperation, within-group selection acts *against* cooperators who pay some cost that others do not. Free riders—selfish individuals who do not cooperate—are

always favored by within-group selection because they receive any benefits that accrue through the actions of cooperators, but they pay none of the costs.

As opposed to within-group selection, between-group selection favors cooperation if groups with more cooperators outproduce other groups; for example, by producing more total offspring or being able to colonize new areas faster. Consider alarm calls, but now, unlike the case of the ground squirrels we discussed earlier, imagine that individuals do not live in groups with their relatives. Alarm callers pay a cost within groups, as they will be the most obvious target if a predator is alerted by such a call. But their sacrifice may benefit the group overall, as other individuals—including other alarm callers, as well as those that don't call—are able to evade predators because of the alarm call. Thus, groups with many alarm callers may outproduce groups with fewer alarm callers. For such group-level benefits to be manifest, groups must differ in the frequency of cooperators within them, and groups must be able to "export" the productivity associated with cooperation (for example, by having more total offspring, by moving more quickly to colonize newer areas, and so on).

Many evolutionary biologists argue that group selection models (including trait-group selection models) can be translated mathematically into "classic" models of natural selection; that is, they claim that group selection models simply partition the effect of a trait into within-group and between-group components, but that if the effects are summed over all groups making up a population, you get the same solution as a classic model would produce by tracking gene frequency in an entire population (Queller 1992; Lehmann et al. 2007; Reeve and Holldobler 2007). This is absolutely correct. We can always take a group selection model and translate the mathematics into a model of alternative alleles in which natural selection favors one allele over another in a given population. Despite the mathematical equivalence, however, group selection models do shed new light on behavior, as trait-group selection models focus attention on what is happening within and between groups, and this is not necessarily the case for more classic models (Dugatkin and Reeve 1994; Kerr and Godfrey-Smith 2002). Thus, under certain conditions, trait-group selection models may spur investigators to conduct experiments or pursue lines of research that would not have been obvious had they been using classic models. We now illustrate with an example of foraging behavior in ants.

Within-Group and Between-Group Selection in Ants

Cooperative colony foundation occurs in a number of species of ants in which cooperating cofoundresses are *not* closely related (Holldobler and Wilson 1990; Bernasconi and Strassmann 1999). This type of cooperative foundation has been especially well studied in the desert seed harvester ant *Messor pergandei*, a species in which nests are often initiated by two unrelated queens (cofounders). Cofounding queens in a nest assist in excavating their living quarters, and each produces approximately the same number of offspring.

Steve Rissing and his colleagues have found a positive correlation between the number of cooperating foundresses in a nest and the number of initial workers produced by that colony (Rissing and Pollock 1986, 1991). The number of workers produced by a nest is important for nest survival because *brood raiding* is common in this species. Brood-raiding ants attack nearby colonies and capture their larvae and pupae. The stolen brood is brought to the nests of the victorious ants. Colonies that lose their brood in such interactions die; such a fate often befalls colonies

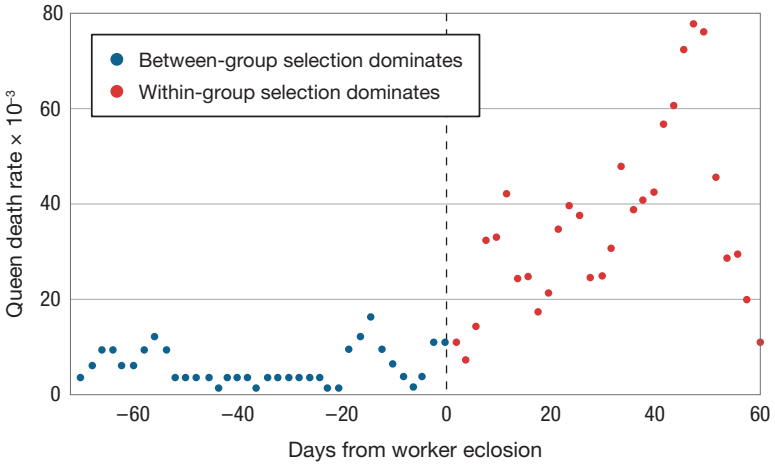


FIGURE 17.11 From cooperation to aggression. Cofounding queens in *Messor pergandei* are cooperative during worker production, with very little queen–queen aggression during this phase of colony development. But once workers emerge—known as *worker eclosion*, starting at day 0—aggression between queens escalates, as does the queen death rate. Adapted from Rissing and Pollock (1987).

that are just starting up. This competition between nests favors cooperation at the level of the group (Wheeler and Rissing 1975; Ryti and Case 1984). Nests with more cooperating foundresses—and thus with more workers—are more likely to win brood raids (Rissing and Pollock [1987], but see Pfennig [1995] for a critique of this work).

Until workers emerge, *M. pergandei* queens within a nest do not fight, and no dominance hierarchy exists (**Figure 17.11**). After workers emerge and the between-group benefits of having multiple foundresses are already set in place with the presence of brood raiders, all that remains is within-group selection, which always favors noncooperative behavior. It is at this juncture that queens within a nest often fight to the death.

One of the strongest cases for group selection comes from Rissing's work on another ant, *Acromyrmex versicolor* (Rissing et al. 1989). In this species, nests are often founded by multiple queens, there is no dominance hierarchy among queens, and all *A. versicolor* queens produce workers. As was the case for *M. pergandei*, brood raiding among starting nests is common, and the probability that a nest survives the brood-raiding period is a function of the number of workers it has produced.

In *A. versicolor*, a single queen in the nest takes on the role of forager for that entire nest (**Figure 17.12**). Foraging entails bringing vegetation back to the nest, where this resource is added to a "fungus garden" from which the ants feed. As a result of increased predation pressure outside of the nest, foraging is a dangerous activity for a queen. Yet, once a queen takes on the role of forager, she remains in that role.

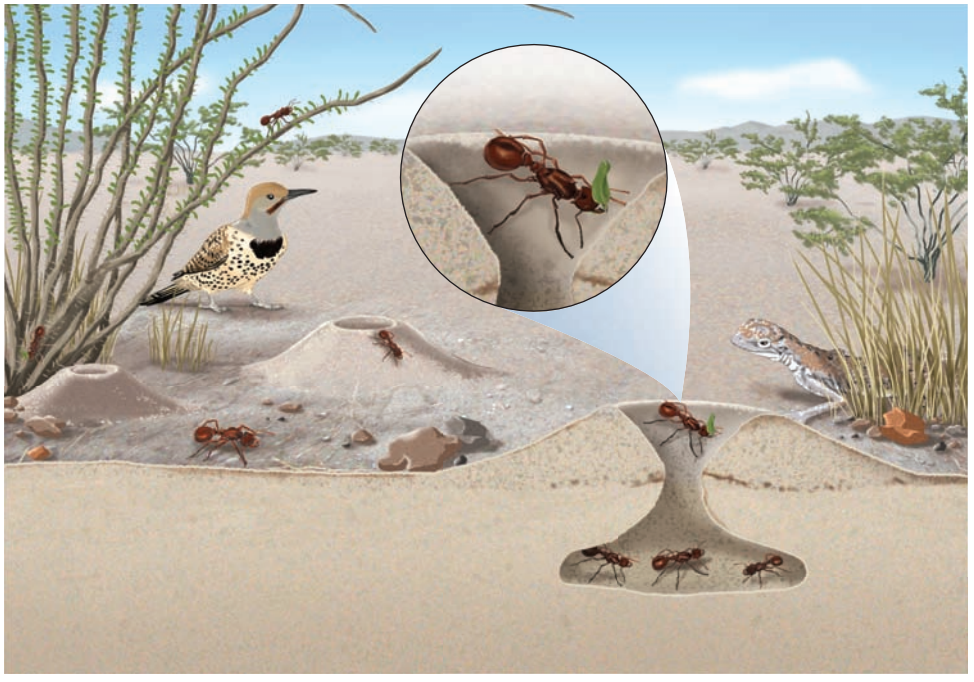


FIGURE 17.12 Cooperation by foraging queens. In the ant *Acromyrmex versicolor*, a single queen (shown in the blowup circle) acts as the sole forager for a nest. Such foraging is very dangerous, but all food collected is shared equally among the queens. Cofounding queens in *A. versicolor* are genetically unrelated. Adapted from Dugatkin (2009a).

TABLE 17.1

Cooperation among Forager and Nonforager *Acromyrmex versicolor* Queens Leads to Equal Reproduction by All the Queens

	Forager	Nonforager
Mean number of primary eggs	8.6	8.5
Mean primary egg length	0.52	0.54
Mean number of total eggs	20.37	18.94

Adapted from Rissing et al. (1989).

The queen that is the sole forager for her nest shares all the food she brings into her nest with her cofoundresses. This means that the forager assumes both the risks and the benefits of foraging, while the other queens in her nest reap the benefits without paying the costs (**Table 17.1**). Once again, however, cooperation within nests—in this case, on the part of the forager—appears to lead to more workers. The increase in workers in turn affects the probability that a given nest will be the one to survive the period of brood raiding, thus providing the between-group component necessary for cooperation to evolve (Rissing et al. 1989; Seger 1989).

Rather than separating these two ant examples into within-group and between-group selection, we could have analyzed the cooperation described in both examples in terms of the relative success of alternative alleles, and we would have come to the same conclusions we arrived at from the trait-group perspective. For example, in the case of *A. versicolor*, we could say that foraging by the foraging specialist is favored over not foraging because decreased survival rates associated with foraging are, on average, made up for by the increased expected survival of her reproductive brood owing to enhancement of the worker defense force. Here, we have averaged survival rates over all groups, rather than separating our example into what happens within and between groups. Both explanations are correct in that they are mathematically equivalent, but in both *M. pergandei* and *A. versicolor,* we see systems in which population biology and demographics match those postulated in trait-group models. The multiple nests, intense competition between nests, and multiple foundresses in these species make them ideal for an analysis at the within-group and between-group levels.

17.2 Conflict

Thus far, our discussion of the evolution of sociality has centered on cooperation. But prosocial behavior such as cooperation is only one type of social behavior that evolutionary biologists study. Indeed, much of the work on the evolution of animal interactions focuses not on prosocial behavior but instead on the behaviors associated with conflict. In a sense, conflict is easier to understand than cooperation. When resources are limited, sometimes they are worth contesting.

Conflict manifests itself in many ways in nature. The most obvious form is aggressive behavior, such as when two rams butt horns or when two male elephant seals fight for access to mating opportunities. But conflict can also occur in unexpected places, such as between genetic relatives, where we would generally

expect cooperation. Finally, conflict is not limited to conflict between individuals. Conflict, in the broadest sense, can occur at many levels, including among genes in the same genome. In this section, we will work through examples of each—conflict among nonkin, conflict within families, and conflict within genomes.

Conflict among Nonkin

In the previous chapter, we considered various types of sexual selection. We noted that intrasexual selection involves direct competition among members of the same sex—typically, although not always, males—for territory or access to members of the opposite sex. This sort of male–male competition is a major source of conflict in nature (**Figure 17.13**). Many of the conflicts are resolved by direct fights, which in turn have a strategic dimension. For example, when should an individual risk a fight, and when should it flee? As mentioned earlier in this chapter, evolutionary biologists can use game theory models as a tool for thinking about social interactions and their fitness consequences. For example, if an individual is willing to fight for a contested resource, the outcome will depend on whether its opponent opts to fight or simply to flee, and so we can model fighting behavior using game theory.

The hawk–dove game is a classic model of the evolution of aggression, and it was among the first applications of game theory in evolutionary biology (Maynard Smith and Price 1973; Maynard Smith 1982). John Maynard Smith and George Price wanted to understand why in contests among organisms with lethal armaments—sharp teeth, claws, horns, and so forth—one individual often backed down, thereby avoiding a fight that might lead to lethal injury. They used the hawk–dove game to understand why this happens.

The modern form of the hawk–dove game models two individuals contesting a single resource with a value v. They face off over the resource, and they can adopt one of two behavioral strategies when contesting the resource: Each can play the aggressive "hawk" strategy or the cautious "dove" strategy. If both select hawk, they end up in a damaging fight incurring total cost c. After fighting, each gets half of the resource (or, alternatively, we can think of the probability that a given individual gets the resource as 0.5). If one individual selects hawk and the other selects dove, the hawk gets the resource, while the dove retreats and gets nothing. If both select dove, they share the resource. We can write down the payoffs for this game as in **Figure 17.14**. We assume here that the cost of a fight c is greater than the benefit of the resource v.

Just as when we analyzed the prisoner's dilemma game, we are interested in finding the Nash equilibrium (or Nash equilibria, if there are more than one) for the hawk–dove game. Thus, we want to find a pair of strategies for player 1 and player 2 such that neither player can benefit from unilaterally changing his strategy. In the hawk–dove game, there are two such strategy pairs: If player 1 always plays hawk and player 2 always plays dove, neither player can benefit by switching his strategy alone. If player 1 switched to dove, he would have to share the resource with player 2 instead of getting it all for himself. If player 2 switched to hawk, he would end up in a costly fight against player 1,

FIGURE 17.13 Conflict. Two oryx lock horns in a struggle over access to mates.

who was also playing hawk. We see this kind of Nash equilibrium in some territorial interactions in nature: Often, a territory holder will be willing to fight to keep the territory (thereby playing a hawklike strategy), and an invader will flee immediately when challenged by the territory holder (thereby playing a dovelike strategy). The second Nash equilibrium, which is equivalent to the first, occurs when player 1 plays dove and player 2 plays hawk.

But what if the two individuals don't *know* who is player 1, and who is player 2; that is, what if there is not any salient cue, such as the status of territory owner or invader, that distinguishes the roles of the two players? Then it is impossible to play either of the Nash equilibria described earlier because players cannot condition their strategy on whether they are player 1 or player 2. In this case, no strategy by itself is a Nash equilibrium in the hawk–dove game. But there is a Nash equilibrium in which each player plays hawk some fraction of the time, with probability p, and plays dove the rest of the time, with probability $1 - p$ (or alternatively, a proportion p of the individuals play hawk always, and $1 - p$ play dove always). This type of equilibrium is called a *mixed Nash equilibrium.* **Box 17.4** shows how we calculate the mixed Nash equilibrium for the hawk–dove game.

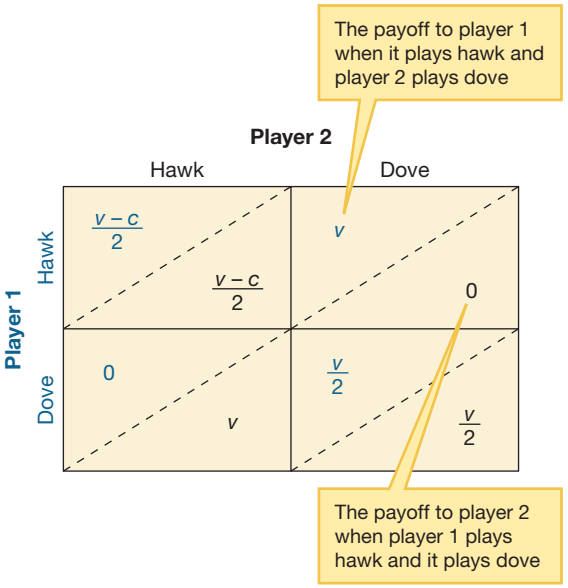


FIGURE 17.14 Payoffs for the hawk–dove game. The row indicates the strategy hawk or dove of player 1, and the column indicates the strategy of player 2. Each cell shows the payoffs to player 1 (above the diagonal line) and player 2 (below the diagonal line). For example, in the upper right box, player 1 plays hawk and player 2 plays dove. In that scenario, player 1 gets a payoff of v and player 2 gets a payoff of 0.

BOX 17.4 The Mixed Nash Equilibria for the Hawk–Dove Game

One equilibrium in the hawk–dove game is a *mixed Nash equilibrium*: There is a fraction p such that if everyone plays hawk with probability p and dove with probability $1 - p$, no one can benefit from unilaterally changing their strategy. Here, we will show how to find the value of p.

We can find the mixed Nash equilibrium by using a trick. It turns out that at the mixed Nash equilibrium, both strategies give the same payoff. Imagine that this wasn't the case. Then one strategy would provide a higher payoff than the other, and a player could shift to playing only the higher-paying strategy and unilaterally increase his payoff. But, by definition, at any Nash equilibrium, players *cannot* unilaterally increase their own payoff. So, we know that at a mixed equilibrium, the two strategies cannot give different payoffs.

To find a mixed Nash equilibrium, then, we look for a point where both strategies give the same payoff. Suppose that everyone else in the population is playing hawk with probability p and dove with probability $1 - p$. Then we calculate the payoff if an individual plays hawk: With probability p our individual plays against another hawk and gets payoff $(v - c)/2$, and with probability $1 - p$ he plays against a dove and gets payoff v. This gives an expected payoff of $p\,(v - c)/2 + (1 - p)v$. We can also calculate the payoff if an individual plays dove. In that case, he plays against a hawk with probability p and gets the payoff of 0, and he plays against a dove with probability $1 - p$ and gets the payoff $v/2$. This gives an expected payoff of $(1 - p)v/2$. At a mixed Nash equilibrium, the payoff from playing hawk must equal the payoff from playing dove. So, at the mixed Nash equilibrium, the following equation must hold:

$$\frac{p(v - c)}{2} + (1 - p)v = \frac{(1 - p)v}{2}$$

Solving this equation for p, we get

$$p = \frac{v}{c}$$

This is the mixed Nash equilibrium frequency of playing hawk. The frequency of playing dove is then $1 - p = 1 - v/c$. Notice that the lower the cost of fighting c and the higher the value of the contested resource v, the more often individuals will play hawk.

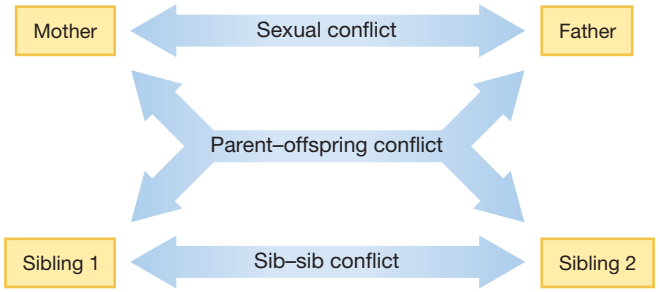


FIGURE 17.15 Conflict within families. Familial conflicts include sexual conflict between parents, parent–offspring conflict, and sib–sib conflict. All of these potential conflicts can influence parental investment in offspring. Adapted from Parker et al. (2002).

Conflict over Parental Investment

As we discussed earlier in this chapter, genetic relatedness plays a pivotal role in understanding the evolution of cooperation. Inclusive fitness theory can also be used to understand conflict within families.

A major source of familial conflict is *parental investment*: the resources—food, shelter, defense—that parents provide to their offspring. At first glance, it might seem that parental investment should be a straightforward matter: Natural selection favors the parents who leave the most surviving offspring, so where is the potential for conflict? But as we look more closely within an inclusive fitness framework, areas of potential conflict rise to the surface. Even within a family, individual interests vary, creating the potential for multiple conflicts (**Figure 17.15**). Parents face sexual conflict over issues such as who should provide how much parental care. Each parent is selected to hand off as much of the parental care as possible to the other. Siblings face **sib–sib conflict** over which sibling receives the most resources from the parents. Each is selected to try to obtain more than an even share of the total (Mock and Parker 1997). Parents and offspring face **parent–offspring conflict** over how parents allocate resources to their offspring. All else being equal, parents are selected to invest equally in all of their offspring. But individual offspring seek more for themselves, even at the expense of their siblings. In the subsection that follows, we will examine parent–offspring conflict.

Parent–Offspring Conflict

Because in diploid species parents and their offspring have a coefficient of relatedness r of 0.5, inclusive fitness theory predicts that parents should go to great lengths to help their offspring. And, indeed, they generally do just that. Hundreds of studies have shown that parents—mothers in particular—provide aid in many forms to their offspring.

Natural selection favors individuals who produce the most surviving offspring, and thus selection often favors parents who provide food, shelter, and other sorts of aid—collectively called *parental care*—to their offspring. Yet, there are limits to how much aid parents are selected to provide. These limits were first conceptualized by Robert Trivers in his parent–offspring conflict model (Trivers 1974). From the perspective of the parent, these decisions are affected by how much energy the parent has available to help current offspring and by how many offspring the parent is likely to have in the future.

In principle, a parent could use every bit of energy it has to provide one particular offspring with all the benefits at its disposal. But if such an effort kills the parent or severely hampers the parent from producing other offspring in the future, then natural selection may not favor such behavior, as it might not maximize the *total number of offspring* that the parent is able to produce over the course of his or her lifetime. So, there are limits on parental investment with respect to any given child.

Now, let's look at parental investment from an offspring's perspective. The offspring will receive some inclusive fitness benefits when its parent provides aid to both current and future siblings; if they are full siblings, these individuals are related to the offspring in question by $r = 0.5$. Yet, the individual offspring is more related to itself ($r = 1$) than to any of its siblings. As such, in terms of inclusive fitness, the

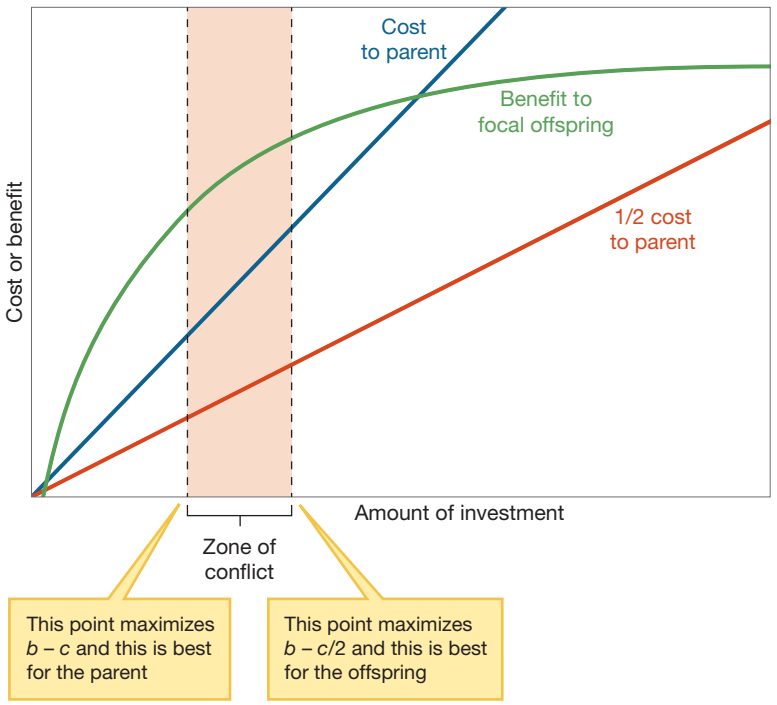


FIGURE 17.16 Parent–offspring conflict. A parent can either allocate resources to a "focal" offspring or redirect those resources to other current or future offspring. The *x* axis represents the amount of resources that the parent invests in the focal offspring, and the *y* axis represents fitness costs (*c*) to the parent (blue and orange lines) or benefits (*b*) to the offspring for that investment (green line). Benefits refer to increases in the fitness of the focal offspring, and costs are quantified in terms of decreases in fitness of other offspring. The more resources that a parent invests in the focal offspring, the greater the benefits to that offspring—albeit with decreasing returns—but the greater the costs as well. The parent is equally related to all of its offspring, and so it is selected to maximize the difference between benefit and cost. But the offspring is only half as related to its full siblings as it is to itself, and thus by the logic of inclusive fitness, it is selected to maximize the difference between benefit and cost divided by 2. As a result, parent and offspring prefer different amounts of resource allocation. This zone of conflict is shaded in the figure. To the left of this zone, parents and offspring alike benefit from increasing allocation to the offspring. To the right of this zone, parents and offspring alike benefit from decreasing allocation to the offspring.

offspring values the resources it receives from its parent more than the resources that its parent provides to its current or future siblings. The conflict between parent and offspring arises because although each offspring will value the resources it receives more than those dispensed to its siblings, all offspring are equally valuable to a parent. These different valuations set up a zone of conflict between how much an offspring would optimally receive from a parent and how much a parent would optimally provide to an offspring (the former always being greater than the latter). This zone is where parent–offspring conflict takes place (**Figure 17.16**).

KEYCONCEPT QUESTION

17.5 Why might you expect the zone of conflict between parents and offspring to decrease as a parent's age increases?

Parent–Offspring Conflict and Mating Systems in Primates

The degree of parent–offspring conflict predicted in any population depends on the mating system that exists in that population (Long 2005; Hain and Neff 2006). To see why, recall that in any parent–offspring conflict situation, natural selection favors offspring that balance (1) the inclusive fitness benefits associated with receiving continued parental assistance versus (2) the inclusive fitness benefits of curtailing the degree of parental assistance received and thereby leaving a parent with more resources to produce future offspring.

The degree of relatedness between current offspring and future offspring is not fixed, but rather it is a function of the mating system. In a strictly monogamous species, current offspring and future offspring will have an average genetic relatedness of $r = 0.5$ because they are likely to be full siblings (they have the same mother and the same father). But suppose the mating system is polyandrous, with

a female mating with many males. Then the genetic relatedness between current and future offspring will be somewhere between 0.5 (for full siblings) and 0.25 (for half siblings). Assume that the mother provides the majority of the parental care. Then compared to the case of monogamous mating systems, in polyandrous mating systems natural selection will favor an offspring who attempts to extract more in the way of parental assistance—the siblings from which it is effectively taking resources are not as closely related as they would be in a monogamous system. Thus, parent–offspring conflict should be more intense in polyandrous than in monogamous mating systems (Trivers 1974; Mock and Parker 1997).

Tristan Long tested the hypothesis that offspring will attempt to extract more resources from parents in polyandrous systems than in monogamous systems. He did this by asking whether there was evidence that fetuses grow faster in utero—taking more maternal resources—in polyandrous primate species.

Long used the method of independent contrasts (Chapter 5) to examine whether strong parent–offspring conflict was more likely in polyandrous or monogamous primate species. He began by using a phylogenetic tree for primates. From the tree, he was able to find 16 pairs of primates to use in his independent contrast analysis. Each pair was made up of species that had diverged from a recent common ancestor—one member of the pair was a monogamous species, and the other member of the pair was a polyandrous species. Long then compared already published data on fetal growth rates for each of the species in his pairwise comparison (Long 2005). He predicted that in polyandrous mating systems, a fetus would attempt to sequester more resources during development, and hence it would show faster rates of growth than would a fetus from a species that was monogamous. Long's analysis found just such a relationship.

Conflict within the Genome

In Chapter 6, we reviewed Mendel's law of segregation, which states that the two alleles at each locus segregate at meiosis so that each gamete receives one but not both alleles. We tend to think of this process as "fair," in the sense that each allele is equally likely to make it into a viable gamete. Thus, we tend to expect that, on average, half the gametes produced by a heterozygote at a given locus will contain one allele at that locus, and half the gametes will contain the other allele. But this is not always the case.

Segregation Distortion

If a particular allele could somehow distort the process of segregation in its own favor—if it could increase its representation to being more than half the gametes produced by an individual—that allele would be favored by natural selection, all else being equal. Indeed, some alleles can do that. Such alleles are known as **segregation distorters** (or **meiotic drive alleles**). When these alleles are present, we can speak of a genetic conflict of interest within individuals.

Segregation distortion has best been studied in fruit flies and mice, but it has also been found in many other species (Hartl 1972; Lyttle 1991; Hurst and Werren 2001), and its evolution has been modeled mathematically by evolutionary biologists (Dunn et al. 1958; Haig 2010). In *Drosophila melanogaster,* one of the best-studied cases of meiotic drive involves two linked loci (Hiraizumi and Crow 1957; Hiraizumi and Nakazima 1965; Hartl et al. 1967; Larracuente and Presgraves 2012). A segregation distorter locus houses either the active allele *Sd* or the inactive allele Sd^{+}, while a different responder locus houses what is known as the responder

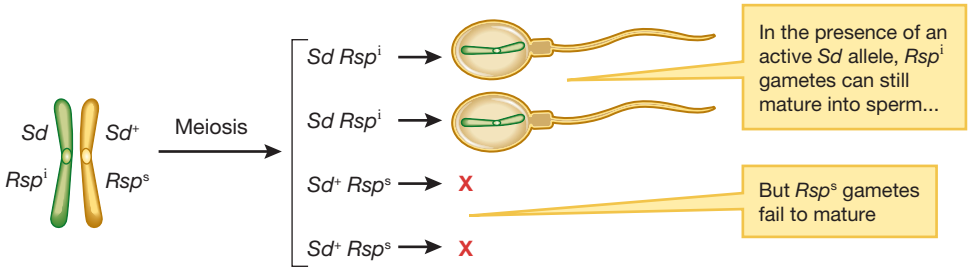


FIGURE 17.17 Meiotic drive in *Drosophila*. The segregation distorter system in *Drosophila* involves two linked loci, *Sd* (with alleles *Sd* or Sd^+) and *Rsp* (with alleles Rsp^i or Rsp^s). *Sd* and Rsp^i are often found together, as are Sd^+ and Rsp^s. In the presence of an *Sd* allele on either of the homologous chromosomes, sperm that have the Rsp^s allele break down: In a double heterozygote, 99% of the sperm are Rsp^i and, because of linkage, 99% are *Sd*. Adapted from Hurst and Werren (2001).

gene (*Rsp*), which is either Rsp^i (response insensitive) or Rsp^s (response sensitive). The loci are in linkage disequilibrium, in that *Sd* and Rsp^i are typically found together, as are Sd^+ and Rsp^s. In the presence of the active *Sd* allele, sperm that have the Rsp^s allele break down: 99% of surviving sperm in such individuals are Rsp^i and, because of genetic linkage, 99% of these sperm are *Sd* (Merrill et al. 1999) (**Figure 17.17**). In this extreme example, rather than observing an allele in half the gametes produced by heterozygotes, we see it in virtually all of the gametes.

This raises a question: If segregation distortion is so strongly favored by selection, why do we see segregation distorters such as *Sd* at intermediate frequencies in populations? The answer is that many segregation distorters probably go to fixation very quickly, and we do not see them because the disadvantaged allele is quickly lost. The ones that we do see are special cases in which the segregation advantage to a segregation distorter is balanced by a severe fitness cost paid by the distorter when it is found in homozygotes; that is, in individuals with two copies of the "driving" allele (Hartl 1972). For example, in the *t*-allele meiotic drive system in mice, individuals that are homozygous for the driving t^+ allele have greatly reduced survival and fertility. This can lead to an evolutionary equilibrium in which the driving t^+ allele remains in the population but is unable to reach high frequency (Dunn and Bennett 1967).

Parent-of-Origin Conflict and Genomic Imprinting

For reasons we will delve into more in a moment, in sexually reproducing species, selection may act differently on males and females with respect to how resources should be allocated to offspring. Behavioral ecologists have used models and experiments to explore the differences between males and females in how they allocate time and energy to feeding offspring, guarding offspring, and a suite of other parental duties (Parker et al. 2002; Houston et al. 2005, 2013; Johnstone and Hinde 2006). But this conflict of interest between males and females about how to allocate resources to offspring begins to play out long before offspring are born. To understand how this works, we begin this subsection with a discussion of a phenomenon known as *genomic imprinting*.

In mammals, every cell, besides sperm and eggs, has two copies of each chromosome: one copy inherited from the mother and one copy from the father. For the vast majority of genes in mammals, it makes no difference whether they are located on the maternally derived or paternally derived chromosome. These genes are expressed and function exactly the same regardless of parent of origin. But, starting in the late 1970s, genetic

engineering experiments with mice suggested that not all genes operated like this. In these experiments, researchers began with a fertilized egg that had been emptied of male- and female-derived chromosomes. In one treatment of this experiment, one set of paternal and one set of maternal chromosomes were injected back in the cell (as would be the case for a normal cell in mice); in another treatment, two sets of maternal chromosomes were injected into the emptied cell; and in the third treatment, two sets of paternal chromosomes were injected into the emptied cell.

If the parent of origin (male or female) did not affect gene expression and function, then eggs in all treatments should survive and develop equally well—both had two full sets of chromosomes—but researchers found that only cells with both a maternal and a paternal set of chromosomes survived at all, suggesting that chromosomes differ in some important way based on which parent they have been inherited from (**Figure 17.18**) (McGrath and Solter 1984; Surani et al. 1984). Soon after, researchers figured out *why* one chromosome from each parent was essential: Specific genes were only

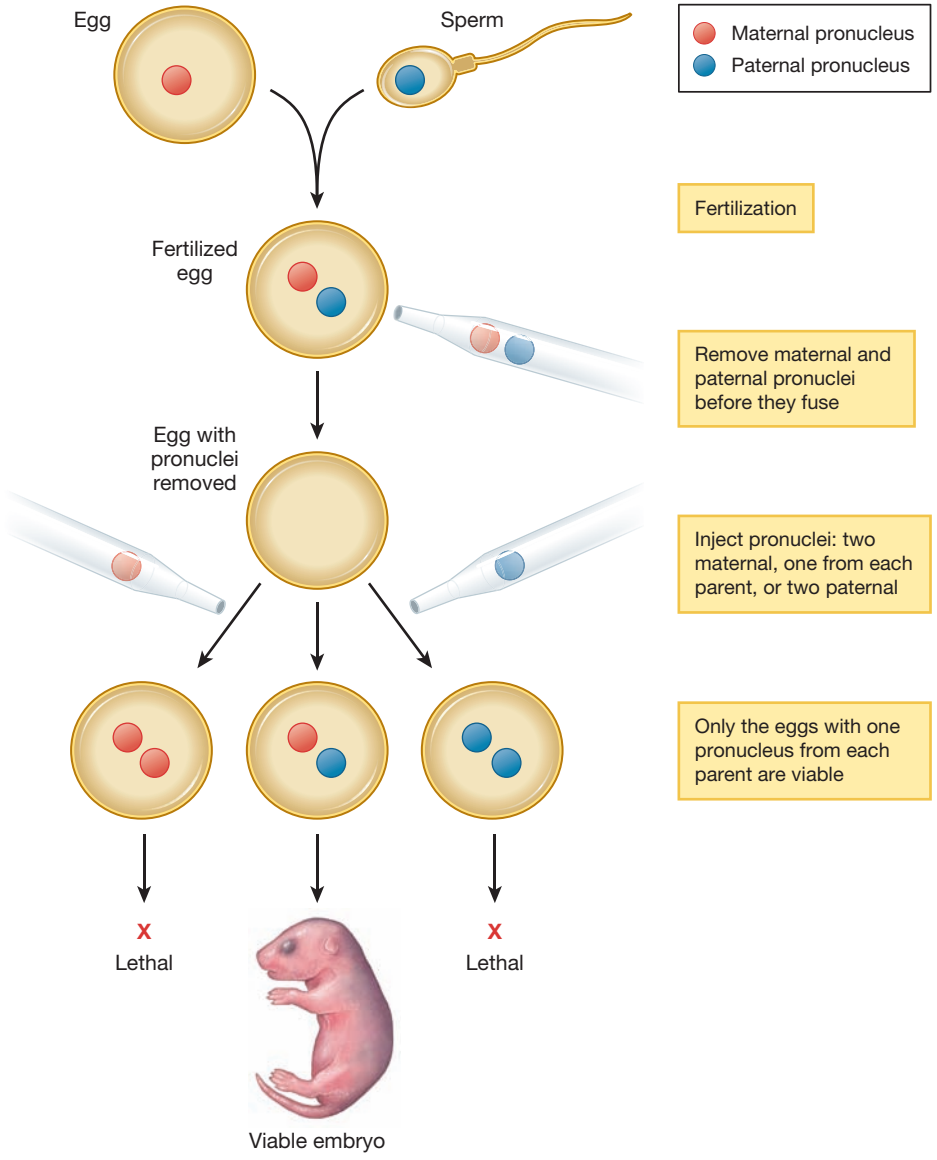


FIGURE 17.18 Parent-of-origin of chromosomes affects zygote viability. A series of genetic engineering experiments in mice began with egg cells immediately after fertilization, before the pronuclei—the nuclei of the sperm and egg—had broken down and the chromosomes fused to form the new nucleus. Both the maternal and paternal pronuclei were removed and replaced with either (1) two maternal pronuclei from other cells, (2) one maternal pronucleus and one paternal pronucleus, or (3) two paternal pronuclei. Only the egg cells with both a maternal pronucleus and a paternal pronucleus produced a viable embryo. Adapted from Barlow and Bartolomei (2014).

expressed on maternally derived chromosomes or paternally derived chromosomes (Barlow et al. 1991; Bartolomei et al. 1991; DeChiara et al. 1991; Ferguson-Smith et al. 1991).

Exactly what causes genes to function differently based on the parent of origin is an area of ongoing research. One well-studied mechanism is a form of epigenetic marking: DNA methylation of sperm and oocytes. Certain sites on the DNA are methylated when an enzyme (a DNA-methyltransferase such as Dnmt3a or Dnmt3b) adds a methyl group (CH_3) to the fifth position of the ring of molecules that make up the nucleic acid cytosine (**Figure 17.19**). This leaves a methyl "mark" on the cytosine. Precisely how methyl marks affect gene function probably varies from gene to gene and is still largely unknown, though recent work suggests that in some instances, DNA methylation prevents transcription factors from binding and thereby prevents transcription, possibly leading to a "silencing" of that gene when it is marked.

The methyl mark stays on cytosine as cells divide during subsequent development of the individual, but numerous processes are in place to remove methylation marks so that they are reset each generation. DNA-methylation marking of sperm and oocytes based on parent of origin, and the subsequent "erasing" of that marking in the next generation, then, allows for the parent-of-origin effects (Li and Zhang 2014).

Today, researchers have identified a few hundred imprinted genes in mammals. For these loci, gene expression—and hence gene function—depends on whether the gene is located on the paternal or maternal chromosome. In some cases, a gene is expressed when located on the maternal chromosome but silenced when it is located on the paternal chromosome. We say that such genes are *paternally imprinted*. In other cases, a gene is expressed when located on the paternal chromosome but silenced when it is located on the maternal chromosome. We say such genes are *maternally imprinted* (Barlow and Bartolomei 2014).

Soon after imprinted genes were first discovered, evolutionary biologist David Haig proposed what came to be known as the "tug-of-war" model for the evolution of genomic imprinting (Haig and Westoby 1989; Moore and Haig 1991; Haig 2000; Wilkins and Haig 2003). Haig's theory centers on the allocation of resources to current versus future offspring. To see how this theory works, consider the case of a pregnant female mouse. Obviously, this female needs to provide resources for her developing embryos, but how much? The developing embryos are just one clutch of the many clutches of offspring she may have in her life. So, the answer to the allocation problem involves taking into account not just the needs of current offspring but those of future offspring as well. From the pregnant female's perspective, her genetic relatedness to all those offspring, current and future, is an r of 0.5 (the relatedness between mother and child).

Now consider the resource allocation problem from the perspective of the female's *current* mate. He too will sire many clutches, and he too will have a genetic relatedness of $r = 0.5$ to all his offspring, current and future. But this male, in all likelihood, will not be the sire of the same female's future clutches. So, from the male's perspective, his genetic relatedness to his current mate's *future* offspring is 0, whereas her genetic relatedness to those same future offspring is 0.5. This asymmetry between the male and female partners selects on the male to divert more resources to current offspring than is optimal from the female's perspective. Haig proposes that this results in selection for certain genes to be imprinted. In

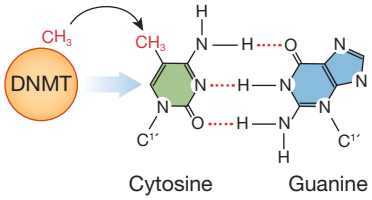


FIGURE 17.19 DNA methylation marks cells. During DNA methylation, the enzyme DNA-cytosine-5-methyltransferase (labeled DNMT) adds a methyl (CH_3) group to cytosine, creating a methyl "mark." DNA methylation does not impede cytosine's ability to bind with guanine. The black arrow indicates the addition of the CH_3 group shown in red; the blue arrow indicates where DNMT associates with cytosine. Adapted from Li and Zhang (2014).

particular, he hypothesizes that the function of most imprinted genes will revolve around such traits as "placental growth, suckling . . . appetite, nutrient metabolism and postnatal growth rate," with maternally imprinted (paternally expressed) genes functioning so as to obtain as much nourishment as possible for developing embryos above and beyond the maternal optimum and paternally imprinted (maternally expressed) genes acting to counter this effect.

A number of lines of evidence support the predictions of Haig's tug-of-war model of imprinting. In mammals, most known imprinted genes are indeed linked to resource allocation. And maternally imprinted genes (for example, *Igf2, Peg1, Peg3, Rasgrf1,* and *Dlk1*) are almost always associated with allocating resources in a way that leads to maximal growth rates for developing embryos, while paternally imprinted genes (for example, *Igf2r, Gnas, Cdkn1c, H19,* and *Grb10*) often counter this effect by slowing embryo growth rate (Barlow and Bartolomei 2014). For example, in mice, the insulin-like growth factor 2 gene (*Igf2*), linked to the production of growth hormones and to cell proliferation, is maternally imprinted, while the insulin-like growth factor 2 *receptor* gene (*Igf2r*), which "scavenges" the growth factor hormone and binds it, is paternally imprinted. The effects of these imprinted genes tend to cancel each other out, leading to the production of normal-sized offspring. When the maternally imprinted gene is experimentally knocked out, dwarf offspring are produced. When the paternally imprinted gene is knocked out, offspring are far larger than normal (Eggenschwiler et al. 1996) (**Figure 17.20**).

A further prediction of the tug-of-war model is that genomic imprinting should be selected most strongly in species where males can directly affect maternal allocation of resources to offspring. In mammals, imprinted genes have been found in placental species such as humans and mice and in marsupials such as opossums and wallabies. But in egg-laying mammals, where paternally expressed genes can less readily influence maternal resource allocation, no imprinted genes

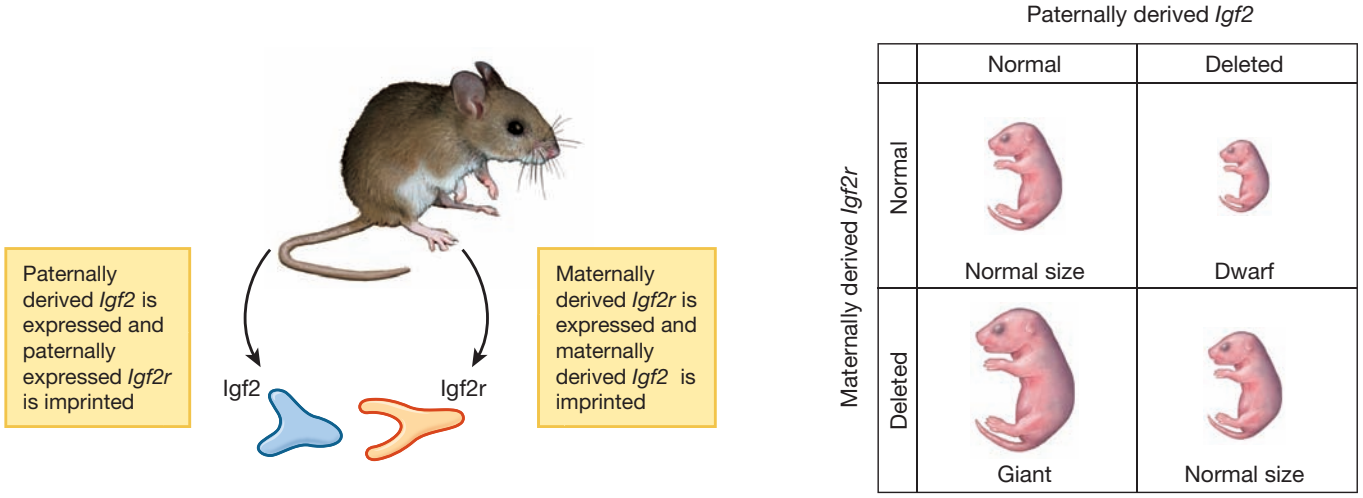


FIGURE 17.20 Genomic imprinting and *Igf2* in mice. In mice, the insulin-like growth factor 2 gene (*Igf2*) is maternally imprinted. This gene is associated with the production of growth hormones and accelerated cell proliferation. The insulin-like growth factor 2 receptor gene (*Igf2r*) slows growth by binding the growth factor hormone. This gene is paternally imprinted. When both genes are present, or both genes are absent, normal-sized offspring are produced. Dwarf offspring are produced when the maternally imprinted gene is experimentally knocked out, and giant offspring are produced when the paternally imprinted gene is knocked out.

have been uncovered (**Figure 17.21**). While other possible explanations cannot yet be ruled out, the distribution of imprinted genes across mammals is consistent with the predictions of the tug-of-war model.

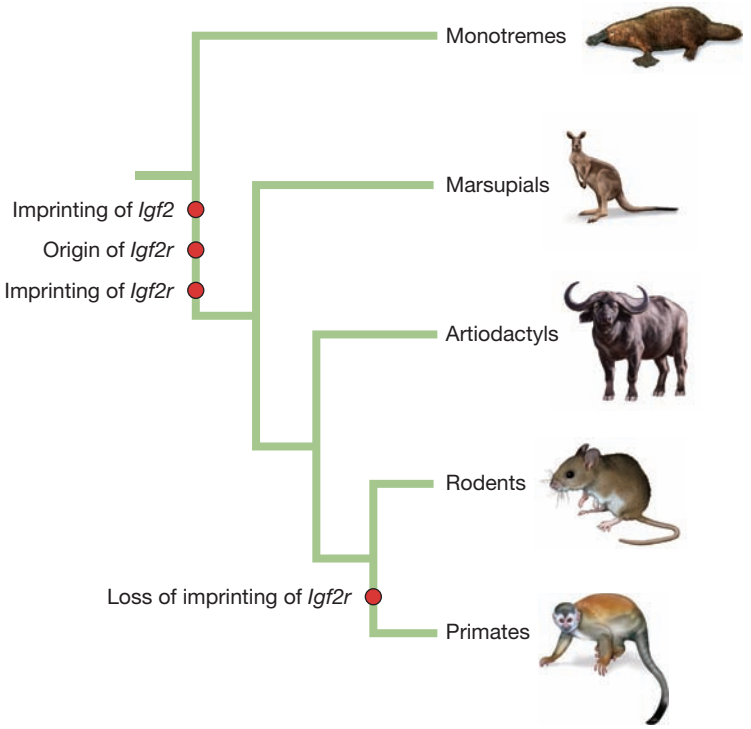


FIGURE 17.21 A phylogenetic history of *Igf2* imprinting in mammals. A partial phylogeny of mammals with the origin and loss of *Igf2* and *Igf2r* mapped onto the phylogeny. *Igf2* is not imprinted in monotremes but is imprinted in marsupials, rodents, artiodactyls, and primates. *Igf2r* is imprinted in marsupials, rodents, and artiodactyls but not in monotremes or primates. Adapted from Wilkins and Haig (2003).

17.3 Information and Communication

Regardless of whether social behavior involves cooperation, conflict, or the mating decisions we discussed in Chapter 16, information is likely being transferred. Signals are involved in virtually all social interactions. And so we need to understand the evolutionary processes associated with signaling.

As resources go, *information* is remarkably well suited for sharing. Compared to a nest or a heavy carcass, information is easy to transport from place to place. More important, unlike food or shelter or mates, one individual can share information with another, *without losing it himself.* There is perhaps no clearer way to express this than by an aphorism commonly attributed to George Bernard Shaw:

> If you have an apple and I have an apple and we exchange apples then you and I will still each have one apple. But if you have an idea and I have an idea and we exchange these ideas, each of us will have two ideas.

Of course, not all information can be shared without cost. If I tell you where an indivisible food resource is located, you may collect it at my expense. If I show you a safe hiding place, you can take it before I do. But when I give you information, I do not give up the information itself (**Figure 17.22**).

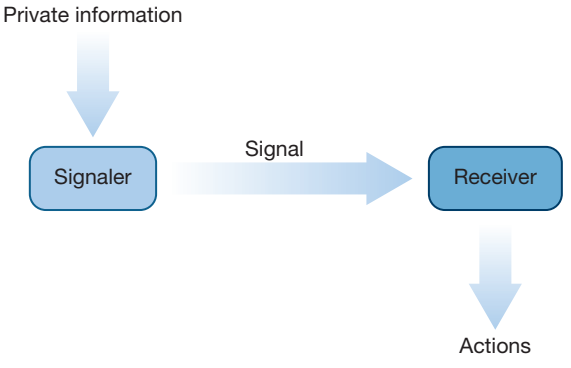


FIGURE 17.22 Signaling. A *signaler* with private information sends a signal to a *receiver*. The signal informs the receiver about the state of the world. The receiver may then act on the information.

Because of this unique property, information sharing is ubiquitous in nature. In many cases, it is relatively straightforward to understand how information sharing might evolve. If two individuals have entirely coincident interests, it is straightforward to see why both would benefit from communication. One striking example occurs between humans and a bird known as a honeyguide, *Indicator indicator* (**Figure 17.23**). This species has been documented to lead human hunters to bees' nests, where the hunters can use smoke and other techniques to extract the honey that would otherwise be inaccessible to the birds. In the process, the birds obtain some of the honey. Here, both sides benefit from honest communication. The humans are led to a food source, and the birds gain access to resources they could not otherwise have exploited (Isack and Reyer

FIGURE 17.23 The greater honeyguide *Indicator indicator*. The honeyguide forages on beehives disturbed by honey badgers (*Mellivora capensis*). People use the behavior of *I. indicator* to find beehives and gather honey.

1989). In this case, there is no incentive for birds to mislead humans about where the honey is located. Mathematical models reveal that signaling systems can readily evolve under such circumstances (Skyrms 2010).

Honest Signaling

Matters get substantially more complicated when, despite some commonality of interests, signalers have incentives to deceive. The key problem can be summarized very simply: Two individuals have access to different information. They could both gain if they could honestly share this information. But their interests do not coincide entirely, and so each has an incentive to deceive the other. How can honest communication be ensured? Evolutionary biologists have proposed a number of solutions to this puzzle. We will treat each of them in turn.

Mind Reading versus Manipulation

One possibility is that honest communication is *not* ensured. Rather, the signals and responses that we observe may result from an ongoing antagonistic coevolutionary process. In the *mind reading versus manipulation* view of communication proposed by Richard Dawkins and John Krebs, signaling arises when receivers attempt to gain an edge by closely observing the cues—not necessarily meant as signals—sent by another individual (Dawkins and Krebs 1978; Krebs and Dawkins 1984).

Krebs and Dawkins illustrate this idea with the example of a dog baring its teeth. If a dog is to bite a rival without severing its own lip in the process, it must pull its lip back prior to striking. This motion, however small, can tip off the rival that there will be an impending attack. By watching for such a cue, a rival can *mind read*, pushing an antagonistic interaction up to the point that an attack is imminent, and then fleeing before actual harm is done. Where mind reading aids in avoiding injury, it will be favored by natural selection.

But once a rival attends to the cues that the angry dog is sending, the dog has a "handle" by which to *manipulate* its rival's behavior. It can now influence its rival's behavior by altering the type or timing of the cues that it sends. For example, the angry dog can cause a rival to flee simply by baring its teeth, even when it does not actually intend to bite. Such a behavior will be selected when it confers an advantage in antagonistic interactions.

According to this view, signals emerge not as cooperative solutions to exchanging information, but rather through a process of antagonistic coevolution: Receivers attempt to obtain an edge by mind reading; signalers respond by sending cues to manipulate receiver behavior; receivers counter by adjusting their responses; and so forth.

Costly Signaling Theory

The mind reading versus manipulation view presents signals and responses as tactics in a coevolutionary arms race. If this view is correct, we would not expect the *same* signals to be maintained over long stretches of evolutionary time. To explain cases in which the same signals are maintained over evolutionary time, we would need some other explanation of how signaling evolves and is maintained. One such explanation arises from what is known as **costly signaling theory**. The basic

structure of costly signaling arguments is as follows: Suppose that signals are costly and that for one reason or another, dishonest signals cost more than honest signals. If telling the truth is cheap enough and telling a lie is costly enough, it may be worthwhile to communicate honestly and not to lie.

Without further exposition, it is not easy to see exactly how or why this might work. Fortunately, Zahavi's handicap principle, which we discussed in Chapter 16, provides one possible solution to how this sort of communication could evolve. Here, we use the handicap principle to try and understand the evolution of "signals of need."

SIGNALS OF NEED If you have ever located a bird's nest by listening to the begging nestlings within, you've heard a costly signal. The loud begging calls that nestlings make are thought to be costly signals of hunger or need. Consider the strategic problem that the mother bird faces when she returns with a morsel of food. Arriving at the nest, she finds herself faced with an array of gaping beaks. Which of them should she feed? Natural selection will favor efficient allocation of the food among her offspring, and so a mother bird would benefit from knowing precisely how much food each nestling truly needs.

But will the nestlings be willing to signal their true hunger levels? Here, we have another example of parent–offspring conflict. The parent would prefer to feed the hungriest chick, but each offspring would like to receive the food itself. As a result, nestlings may exaggerate the signals they emit regarding their levels of hunger, unless some mechanism prevents such deception.

Costly signals can provide a way out of this dilemma. Suppose that nestlings must signal their hunger by squawking loudly—the louder a chick squawks, the hungrier the mother infers it to be. And suppose that squawking in this way is not without its risks. Among other things, begging calls may attract predators to the nest.

Under these conditions, the nestlings may end up honestly revealing their hunger levels. If a nestling is satiated, the risk of predation will outweigh any potential gain from begging. By contrast, if a nestling is starving, then the predation risk may be outweighed by the need for food. As a result, the hungry chicks will beg, the satiated ones will stay silent, and the mother will receive honest information about each offspring's condition. Because the begging signal is costly in terms of predation, it ends up being honest as well (**Figure 17.24**).

The costly signaling explanation of begging makes at least three empirical predictions. If begging calls are costly signals, we would expect that (1) parents will deliver more food in response to stronger begging, (2) begging intensity will reflect the hunger level of nestlings, and (3) begging will be costly (Searcy and Nowicki 2005). Each of these predictions has been tested extensively. Here, we briefly consider studies that test each of the predictions above.

To determine whether parents heed begging calls and deliver more food in response to more intense begging, Katie Price recorded the begging calls of yellow-headed blackbird (*Xanthocephalus xanthocephalus*) nestlings (Price 1998). She then divided a set of blackbird nests into two groups. For each of the nests in the treatment group, she played back the begging

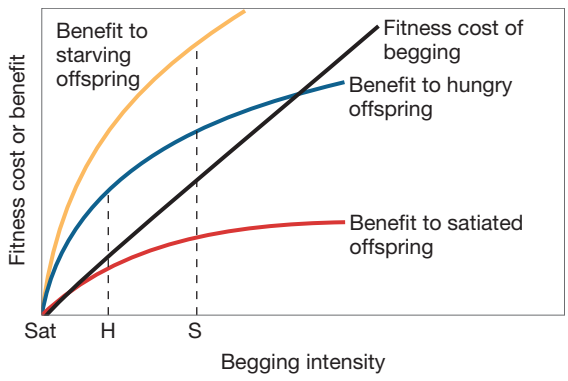


FIGURE 17.24 Costly signaling of need. Noisy begging carries a risk of attracting predators to the nest, and thus higher levels of begging impose higher fitness costs (black line). Begging also induces the parents to feed the offspring. This creates substantial benefits for starving offspring, intermediate benefits for hungry offspring, and minimal benefits for satiated offspring. Satiated, hungry, and starving offspring therefore optimize fitness by begging at levels that maximize the difference between their benefit and cost: Sat, H, and S, respectively.

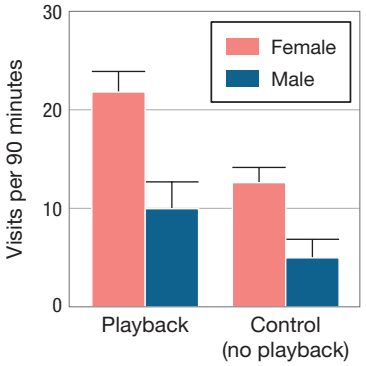


FIGURE 17.25 Parents respond to begging calls. Both male and female parents brought more food to treatment nests where begging calls were played back from hidden speakers than to control nests with no playback. Adapted from Price (1998).

calls from concealed speakers near the nest. For each nest in the control group, a concealed speaker was placed nearby and turned on, but no begging calls were broadcast. Price then compared the rate at which parents brought food to the treatment nests to the rate at which parents brought food to the control nests. She found that the rate at which parents brought food to the treatment group was significantly greater than the rate at which they brought food to the control group. Both male and female parents approximately doubled their rate of provisioning in response to calls played back from the hidden speakers (**Figure 17.25**). The added provisioning translated into weight gain for the nestings. Price also found that nestlings in the treatment nests gained significantly more weight than those in the control nests.

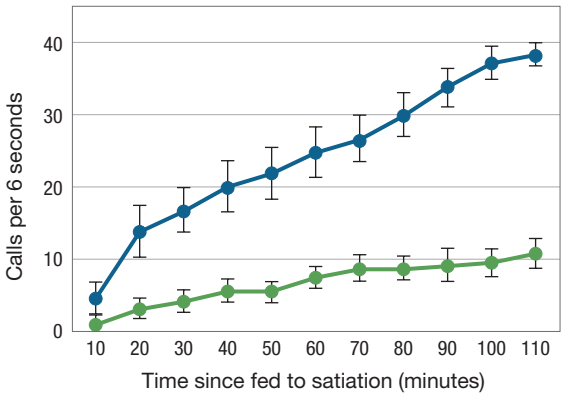
To establish that begging accurately reflects hunger levels, Rebecca Kilner and her colleagues fed a group of reed warbler nestlings (*Acrocephalus scirpaceus*) until they were satiated; then, they measured the begging rate as they withheld food over the subsequent 110 minutes (Kilner et al. 1999). They found that for both 3- to 4-day-old chicks and 6- to 7-day-old chicks, begging rate increased with the time since last feeding and thus presumably with hunger (**Figure 17.26**). From these results, Kilner and her colleagues concluded that begging intensity is an honest signal of hunger in reed warblers.

To explore whether begging calls are costly, researchers have measured two different potential costs associated with begging: the metabolic cost of the begging and the predation risk associated with the begging. Results from the metabolic cost studies suggest that begging only slightly raises metabolic rate above baseline levels. Given that nestlings are not begging continually, this minor increase during a small fraction of time confers minimal metabolic cost (Searcy and Nowicki 2005). But the predation costs associated with begging behavior appear to be more substantial.

To estimate those costs, Juan-Diego Ibanez-Alamo and his colleagues examined begging behavior in the common blackbird (*Turdus merula*) (Ibanez-Alamo et al. 2012). They created three experimental groups. In one group, an additional blackbird chick was added to a nest. In a second group, one of the blackbird chicks was removed from a nest. In a third group, a great spotted cuckoo chick (*Clamator glandarious*) was added to a nest of blackbird chicks. Great spotted cuckoos are nest parasites, meaning that females lay their eggs in the nests of other species. *Clamator,* the genus name of the great spotted cuckoo, translates to "crier," and work by other researchers had shown that chicks in nest parasite species begged at much higher rates than those of blackbird chicks (Davies et al. 1998; Dearborn 1999; Soler et al. 1999). Ibanez-Alamo hypothesized that nests with the added cuckoo chick would suffer higher rates of predation than nests in either of the other groups. What they found was that cuckoo chicks begged at higher rates, and nests with such chicks were attacked sooner and more often than nests in the other experimental groups, suggesting a real cost to begging behavior (**Figure 17.27**). Other work, using a different experimental design, has found similar costs to begging (Haskell 1994; Leech and Leonard 1997).

From this set of studies and others, researchers have amassed considerable evidence consistent with the hypothesis that begging behavior is costly. In addition to the begging example

FIGURE 17.26 Begging intensity reflects hunger. Begging rate of 6- to 7-day-old nestlings (blue) and 3- to 4-day-old nestlings (green) as a function of time since feeding. Adapted from Kilner et al. (1999).



considered here, costly signaling theory has been applied in many other domains as well—from threat displays to antipredator signals. While not all of these cases have been tested as rigorously as has the begging case, costly signaling is an important explanation for how honest signaling can evolve. The brilliance of Zahavi's solution was that he took two major puzzles in evolutionary biology—"Why are signals honest despite conflicting interests?" and "Why are signals extravagant despite selection for efficiency?"—and recognized that these puzzles, when coupled, resolve one another. Signals are honest because they are extravagant (in the right way); signals are extravagant because such extravagance may be required to ensure honesty.

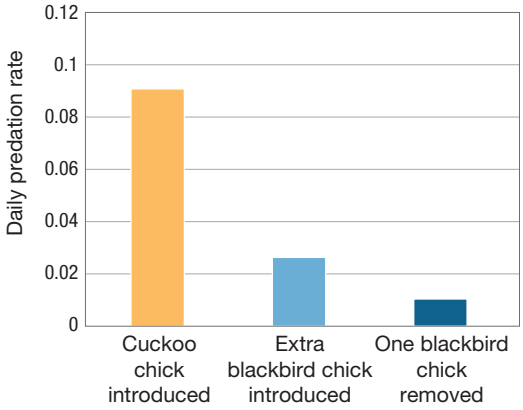


FIGURE 17.27 Begging intensity and predation risk. In experimentally manipulated blackbird nests, predation rates were highest on nests that had a great spotted cuckoo chick added. The cuckoo chicks begged more often than blackbirds, leading to increased predation. Adapted from Ibanez-Alamo et al. (2012).

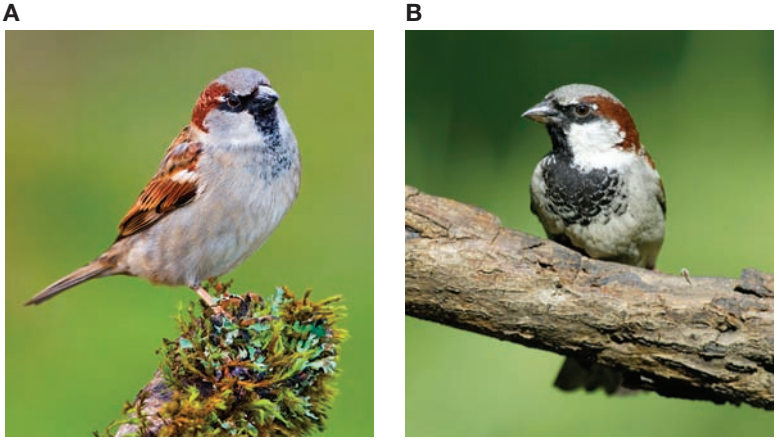
Conventional Signals

Although costly signaling may be important in explaining many examples of honesty, it cannot be the only mechanism that serves this purpose. The words that you are reading now do not have the sort of production costs associated with them that make begging calls honest. Moreover, costly signaling can be an extremely wasteful way of communicating. Indeed, in some cases, costly signaling can be so costly that both signaler and signal receiver end up worse off than if they had not communicated in the first place (Bergstrom and Lachmann 1997).

To see how signals can be honest without extravagant cost, we turn to the house sparrow (*Passer domesticus*). Members of this species, like many other sparrow species, use subtle variations in plumage coloration to signal fighting ability and social dominance. A house sparrow's fighting ability is indicated by the size of its black throat patch (**Figure 17.28**). The larger the throat patch, the less likely a bird is to be challenged and the more likely it is to win in a fight if it is challenged.

The sparrow's throat badge is inexpensive to produce, as it entails only a small color change in a small number of feathers. Signals of this type are known as **conventional signals**; that is, their meaning is established by a convention, rather than intrinsically connected with their structure. But what keeps conventional signaling systems honest? Why, for example, aren't sparrows who are poor fighters adorned with deceptively large throat patches? The answer appears to be social enforcement: If they are discovered, birds that have exaggerated their condition with a large throat badge, but are poor fighters, tend to be attacked by more dominant sparrows (Rohwer 1977).

FIGURE 17.28 Badges of status. House sparrows signal fighting ability by means of black throat badges. (**A**) This bird has a small badge, indicating low fighting ability. (**B**) This bird has a large badge, indicating high fighting ability.



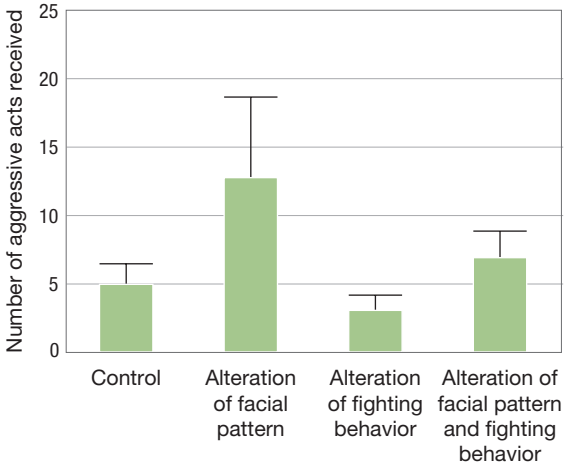
Elizabeth Tibbetts has demonstrated that paper wasps (*Polistes dominulus*) use a similar type of conventional signal to communicate their fighting abilities, and that these signals are kept honest by social punishment (Tibbetts and Dale 2004; Tibbetts and Lindsay 2008; Tibbetts and Izzo 2010). *Polistes dominulus* wasps have variable black facial patterns. In an initial study, Tibbetts and Lindsay demonstrated that the "brokenness" (fragmentation) of the black facial patterning signals dominance in this wasp species (**Figure 17.29**). Brokenness could

FIGURE 17.29 Conventional signaling of fighting ability by paper wasps. Moving from left to right, we see increasing "brokenness" of the black patterning on the face (from zero black facial spots to two black facial spots). This increased brokenness is a conventional signal associated with increased fighting ability in paper wasps.

be assessed by noting the number of black facial spots on the wasps: zero spots was correlated with low fighting ability and low dominance; two spots were correlated with high fighting ability and high dominance. The researchers manipulated facial patterns of individual wasps, adding spots with paint, and they found that wasps preferred to contest food resources with other wasps that had fewer black facial spots (which signaled lower quality and therefore lower fighting ability).

In a follow-up study, Tibbets and Izzo explored why these signals were honest. They wanted to know why wasps of low fighting ability didn't fake dominance by adopting broken facial patterns. They hypothesized that wasps could recognize when signals were not honest; that is, when the wasp's facial pattern indicated dominance and high fighting ability while the wasp actually had low fighting ability. To test this, they manipulated either the wasp's facial pattern using paint, the wasp's dominance behavior by applying an artificial hormone (which increased aggressive behavior), both, or neither. They found an increased incidence of aggressive behavior toward wasps whose facial patterns indicated dominance (and high fighting ability) but whose behavior did not (**Figure 17.30**). From their results, the authors argue that wasps detect dishonest signals as mismatches between markings and behavior. Wasps impose a social cost to such dishonest signals, attacking those individuals with facial markings that falsely indicate dominance and high fighting ability.

FIGURE 17.30 Aggression toward wasps with exaggerated facial patterns. Wasps with facial patterns that falsely indicated high fighting ability (dishonest signals) suffered higher levels of aggression from other wasps than did wasps in any of the other experimental treatments. Adapted from Tibbetts and Izzo (2010).



While the logic of conventional signals—such as those displayed by sparrows and paper wasps—seems at first glance quite different from that of costly signals—such as those displayed by begging birds—we can apply the same sort of cost–benefit framework to understand why conventional signals are honest. In doing so, we learn something important about how signal cost relates to signal honesty as illustrated by **Figure 17.31** (compare this to the corresponding figure in the sexual selection chapter, Figure 16.25). In Figure 17.31, signalers pay no cost unless they overstate their quality. If they do overstate their quality, they will face social punishment, and thus they will pay substantial costs. Here, each individual does its best to signal its true fighting ability, so the signals will be honest (Lachmann et al. 2001).

Conventional signals are honest, but they are not costly. *Deviations* from these signals—namely, exaggerations of fighting ability—would be costly, however, and it is this cost of deviation that keeps signals honest. Thus, we see that it is not the cost of

the signal per se, but rather the cost of shifting to a dishonest signal, that keeps signaling honest in each example we've treated.

One question remains: Why do some communication systems rely on costly signals, while others use conventional signals? Why do chicks produce expensive alarm calls to signal their hunger, while wasps can use inexpensive conventional signals to indicate their fighting ability? The difference between the two cases is that in the begging chick case, the signal receiver—the mother—cannot readily assess the honesty of the message. Was the chick that was begging the loudest actually the one that needed food the most? The answer to that question is difficult for the parent bird to ascertain. In the case of the wasps, the signal receiver can directly probe the accuracy of the signal by instigating a fight. Thus, we might expect that conventional signals can be used when communicating about verifiable traits, whereas costly signals will be required otherwise.

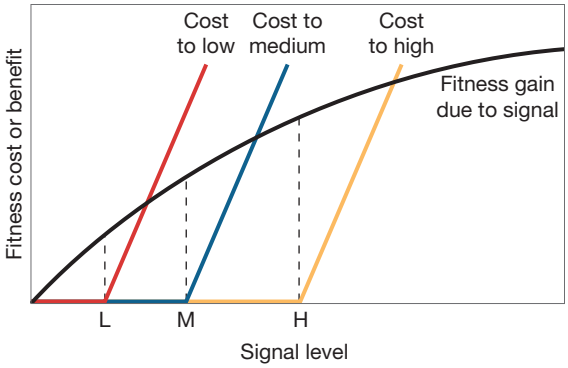


FIGURE 17.31 Conventional signals of fighting ability. Individuals pay no cost of signaling if they do not overstate their fighting ability. If they do overstate their fighting ability, they are attacked and punished by other members of the group, generating the cost curves shown for individuals of low, medium, and high fighting ability, respectively. Because individuals that signal high fighting ability have privileged access to food and other resources, there are fitness benefits that come from higher signal levels (black curve). Individuals with low, medium, and high fighting ability maximize fitness by signals at levels L, M, and H, respectively. These optimal signals are honest—better fighters signal at higher levels—but note that they are not costly. Each individual chooses a signal such that the cost is 0. Compare this to Figure 16.25, in which signals are costly.

In this chapter and the preceding one, we have focused on the evolution of behavior. We have examined sexual selection, including intrasexual selection and intersexual selection, and the evolution of cooperation, conflict, and signaling behavior. Throughout, we cast our evolutionary questions within a conceptual and theoretical framework and then examined empirical studies on both the costs and benefits of the behavior in question and the phylogenetic history of the subject matter. In the next chapter, we address conceptual, theoretical, and empirical questions related to coevolution, in which changes to traits in one species cause changes to traits in other species, which feed back to affect traits in the first species, and so on, back and forth.

SUMMARY

1. We can study the evolution of social behavior using many of the same tools we use to study the evolution of other traits.
2. Social behavior involves interactions that organisms have with others—most often, their conspecifics. In these interactions, the actions taken by one individual affect not only its own fitness but also the fitnesses of those around it.
3. Cooperation occurs when two or more individuals each receive a net benefit from their joint actions, even though individuals may pay a cost for interacting cooperatively.
4. At least three different paths can lead to the evolution of cooperation: (1) kinship, (2) reciprocity, and (3) group selection. All three paths are susceptible to cheaters—those who receive the benefits of cooperation but do not pay the costs.
5. Evolutionary theory predicts that cooperation and altruism should be common among close relatives because relatives are likely to share common genes that they have inherited from common ancestors—parents, grandparents, and so on. This idea has been formalized in inclusive fitness theory.
6. A second path to cooperation is via reciprocal altruism in which individuals benefit from exchanging acts of altruism. One formal model for reciprocity is called the repeated prisoner's dilemma game.
7. A third path to cooperation may be via group selection, although this is a matter of heated debate among evolutionary biologists. The core concept underlying modern group selection models is that natural selection operates at two levels: within-group selection and between-group selection.

8. Conflict can occur between unrelated individuals, including parents, and, under certain conditions, between genetically related individuals. Evolutionary biologists have developed and tested models predicting when and where such conflict should occur.
9. Segregation distorters, alleles that bias the process of Mendelian segregation in their own favor, exemplify the evolutionary conflict that can occur within genomes.
10. Signals of one sort or another are involved in virtually all social interactions, whether they revolve around cooperation or conflict.
11. In some cases, it is straightforward to understand how information sharing might evolve. If two individuals have entirely coincident interests, it is easy to see why both would benefit from communication.
12. Despite some commonality of interests, signalers often have incentives to deceive. Evolutionary biologists have developed and tested many models of communication that address the incentive-to-cheat problem.
13. Costly signaling theory suggests that if signals are costly and if, for one reason or another, dishonest signals cost more than honest signals, it may be worthwhile to communicate honestly and not to lie.
14. Conventional signals—that is, signals with meanings established by a convention, rather than signals with meanings that are intrinsically connected with their structure—can be honest if those who violate conventions are punished.

KEY TERMS

altruism (p. 608)
coefficient of relatedness (p. 610)
conventional signals (p. 641)
cooperation (p. 608)
cooperator (p. 608)
costly signaling theory (p. 638)
direct fitness (p. 609)
eusociality (p. 614)
free rider (p. 608)
haplodiploid (p. 615)
inclusive fitness (p. 609)
indirect fitness (p. 610)
meiotic drive alleles (p. 632)
parent–offspring conflict (p. 630)
reciprocal altruism (p. 617)
segregation distorters (p. 632)
sib-sib conflict (p. 630)

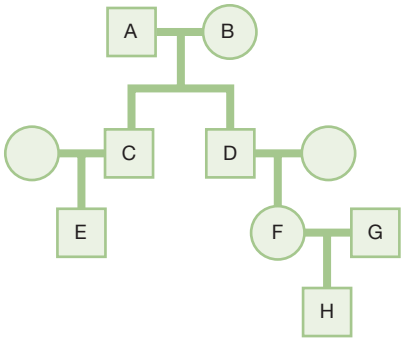
REVIEW QUESTIONS

1. What is the free-rider problem?
2. Name three paths that evolutionary biologists propose lead to cooperation.
3. In inclusive fitness theory, what is meant by *most common recent ancestor* and *coefficient of relatedness?*
4. What three requirements must be met for a species to be considered eusocial?
5. What is the tit-for-tat strategy?
6. What is the zone of conflict for parent–offspring resource allocation?
7. What are segregation distorters?
8. What is genomic imprinting?
9. Why is information an especially easy resource for organisms to share?
10. What is costly signaling theory, and how does it relate to honest signaling?

KEY CONCEPT APPLICATION QUESTIONS

11. With respect to parent–offspring conflict in mammals, why might we expect offspring to have more leveraging power in utero than after birth?
12. You are playing five rounds of the prisoner's dilemma game against an opponent you've never met before and will never see again. Both of you know that the game will go only five rounds.
    a. What is your optimal strategy in the fifth round of the game?
    b. What is your opponent's Nash equilibrium strategy (in every round)?
    c. If your opponent indeed plays that Nash equilibrium strategy, what is your optimal strategy in every round of the game?

13. In the pedigree illustrated below, A and B have offspring C and D, who are full siblings. Then, C and D have offspring E and F, who are cousins. Assume that all individuals on the pedigree are unrelated unless otherwise indicated on the pedigree.



    a. Compute the coefficient of relatedness between E and D.
    b. Compute the coefficient of relatedness between A and G.
    c. Compute the coefficient of relatedness between B and H.
    d. Suppose C can help D at a cost of 0.05. How great must the benefit to D be in order for natural selection to favor this helping behavior?

14. In general, coefficients of relatedness are the same in both directions: A mother is as related to her daughter as the daughter is to her mother, for example. In haplodiploids, this is not always the case. Provide an example to illustrate.

15. In the iterated prisoner's dilemma model that we considered, each player was able to perfectly ascertain what the other player did on the previous round. In this case, the tit-for-tat strategy proved very effective. Now imagine that players were occasionally mistaken about what their opponent had done on the previous round. What would happen when one tit-for-tat player faced another tit-for-tat player?

16. You hypothesize that the antlers of male deer are costly signals of fighting ability that are directed toward other males. What three testable predictions does this hypothesis make?

17. In a 1974 review paper on social evolution, Richard Alexander minimized the importance of parent–offspring conflict by making the following argument: Imagine a "rotten kid" allele that drives selfish behavior on the part of an offspring toward its parents. This rotten kid allele may be beneficial to the offspring while it is young, but any benefits that an individual receives from being selfish as a juvenile will be countered by the cost of having selfish offspring of its own. Critique Alexander's argument.

18. In Box 17.3, we considered Garrett Hardin's tragedy of the commons. How does Hardin's logic apply to the problem of antibiotic resistance?

SUGGESTED READINGS

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18

Coevolution

All over the planet, lichens grow on rocks and trees. Everything about lichens—the way they look, the way they reproduce, the way they respond to environmental change—would make the casual observer think that they are well-integrated multicellular organisms. And they are, but not in the usual sense: Every lichen is made up of two different species (Brodo et al. 2001).

There are thousands of different kinds of lichens, each of which is composed of one fungal species and one species of either photosynthetic algae or cyanobacteria. In the case of fungal–algal lichens, fungal cells typically surround the algal cells to form the body, or thallus, of a lichen. Each species in a lichen derives benefits from the other. The fungi use sugars produced by photosynthesis in the algae. The algae benefit from the fungi's ability to retain water, and they also use some of the resources that fungal cells extract from soil. The algae and fungi in a lichen live in a mutualistic relationship—each benefits the other. The codependency between algae and fungi is so complete that, for most lichens, neither the fungal nor the algal species can survive in the absence of its partner. As a result, the fungi and

◀ Bees visit a water lily at Mole National Park, Ghana.

algae have evolved to disperse together. One remarkable form of reproduction in lichens occurs through the spread of *diaspores*, which contain both algal and fungal cells.

When species interact in a deep and integrated fashion, as fungi and algae do in lichen, natural selection acting on one species may cause selection to operate in new ways on the other species. Evolutionary biologists say that coevolution occurs when changes to heritable traits in species 1 drive changes to heritable traits in species 2, which in turn feed back to affect heritable traits in species 1, and so on, back and forth. When the interaction of the two species increases the fitness of both species, this is called a mutualism.

In the case of fungi and algae, we can examine how the two species can coevolve in lichen. Above and beyond their remarkable natural history, lichens are an excellent model system for formulating and testing hypotheses about coevolution, particularly molecular genetic and phylogenetic questions regarding coevolution. This is because: (1) biologists have the tools to make molecular genetic comparisons among many species involved in lichen formation, and (2) many species of fungi that are part of a lichen have sister species that are not in a lichen association, which allows us to use the comparative method to address coevolutionary questions. For example, evolutionary biologists have hypothesized that the transition to, and the maintenance of, a mutualistic relationship like that seen in lichens must be complex and require many changes to the genomes of both species involved. We can then ask: Is there evidence for such changes to the genomes of algae and fungi that associate to form lichens?

To answer that question, François Lutzoni and Marc Pagel used the comparative method and examined the rate of nucleotide substitution in free-living versus mutualistic fungi (Lutzoni and Pagel 1997). They compared 1550 ribosomal nucleotide sites in 16 species of mutualistic fungi (primarily in lichens, but some in liverworts) and 13 species of free-living fungi that are closely related to the mutualistic lichens species. Lutzoni and Pagel found that there was a faster rate of molecular evolution in the mutualistic fungi. Specifically, the rates of nucleotide substitution were much higher in fungal species involved in mutualistic relationships with algae and liverworts than the rates in the closely related, free-living fungal species. Moreover, the researchers found evidence consistent with the hypothesis that the transition to mutualism was responsible for accelerating the rate of molecular evolution. They discovered that the increased rate of nucleotide substitution occurred only during and after the transition to the mutualistic relationship, not before. Finally, they also found that the increased rate of nucleotide substitution in mutualistic species was not constrained to one specific area of the genome, but rather it was widespread across many sections of the genome (**Figure 18.1**). Not only are the fungal and algal species that are in a lichen association coevolving, but also the process of coevolution has quickened the pace of evolutionary change throughout the genome of at least one of the partners in this mutualistic relationship. Subsequent work suggests that rates of substitution may be especially high when lichens move to new niches with new moisture requirements—where new selective conditions are at play (Lumbsch et al. 2008).

At a very general level, the long-term evolutionary dynamics of coevolution can lead to (1) mutualistic interactions, where each species benefits the other species (Boucher 1985; Bronstein 1994; Connor 1995; Thompson

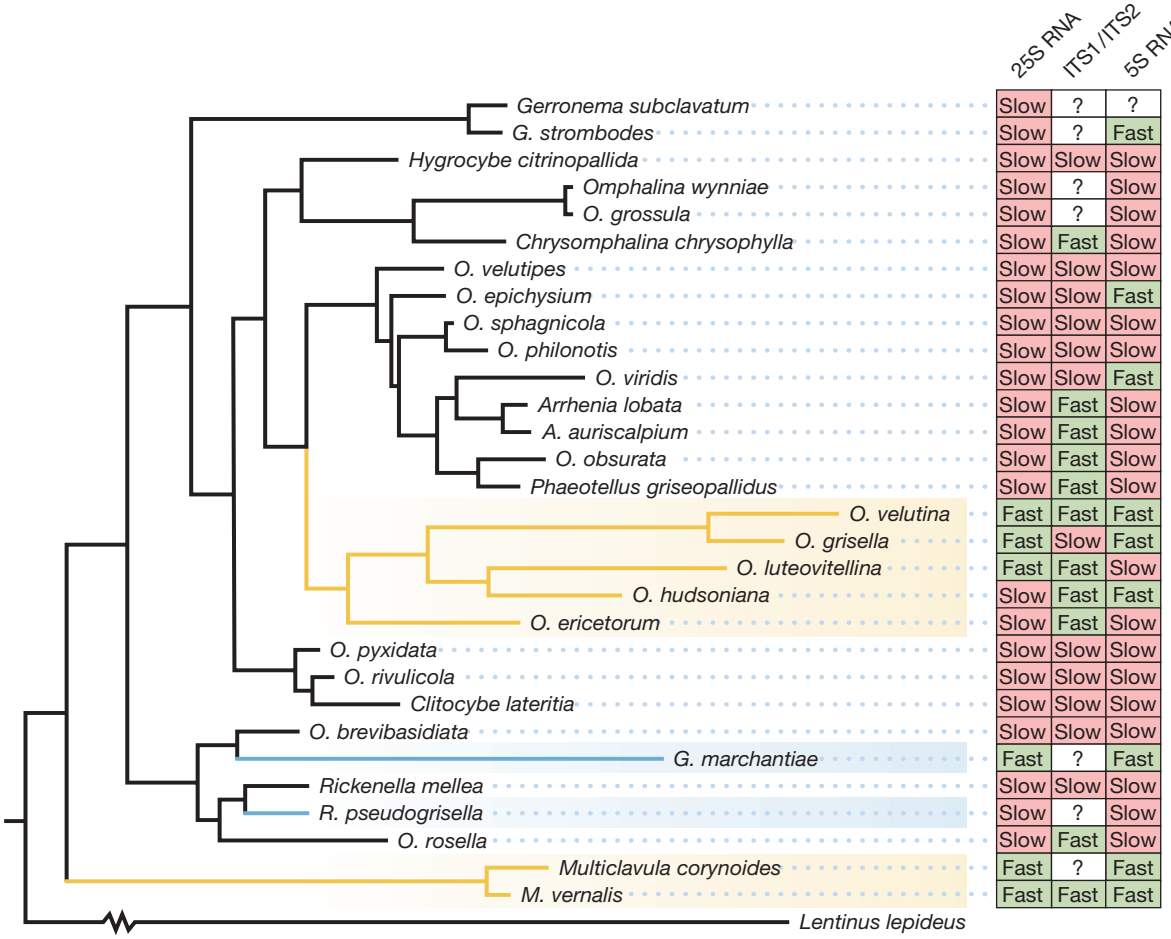

FIGURE 18.1 Rates of evolutionary change in mutualistic and free-living fungal species. A phylogenetic tree of the fungal genus *Omphalina* and related species reveals a more rapid pace of molecular evolution in the species involved in mutualistic associations. On the phylogenetic tree, clades associated with lichen-forming algae are indicated in yellow; species involved in mutualistic associations with liverworts are shown in blue. In the columns on the right, the rate of molecular evolution for each species is assessed in three different ribosomal RNA regions: the 25S RNA region, the regions around the spacers ITS1/ITS2, and the 5S RNA region. The columns on the right indicate slow (in red) and fast (in green) rates of nucleotide substitution in the fungi. An unknown rate of molecular evolution is indicated by a question mark. Fast evolutionary change tends to be associated with a mutualistic lifestyle. Adapted from Lutzoni and Pagel (1997).

2005), and (2) **antagonistic coevolution**, in which each species decreases the fitness of the other species. While we will often consider these separately throughout the chapter, keep in mind that a species may be involved in antagonistic coevolution with one partner and mutualistic coevolution with another partner. For example, *Photorhabdus luminescens*, a bacteria that is pathogenic to many insects (an antagonistic relationship), forms a mutualistic relationship with certain nematode species (Somvanshi et al. 2012).

A classic example of antagonistic coevolution is the relationship between predators and their prey, where selection for antipredator traits in prey—faster escape time, camouflage ability, and so on—favors traits in predators that produce better success at catching these prey, which selects for new antipredator behavior in

the prey, and on and on. Antagonistic coevolution produces an "evolutionary arms race" between predator and prey that may go on indefinitely, producing a wide array of both mechanisms by which prey can protect themselves against predators and systems by which predators can find and capture prey. We will discuss such evolutionary arms races in depth later in this chapter.

So far, we have been presenting a particular coevolutionary relationship as involving only two species; that is, what is often called pairwise coevolution. Pairwise coevolution is so common that sometimes the word *coevolution* is used as a synonym for it. In many instances, though, coevolution will involve more than two species. When this is the case, changes in any of the species involved may cause changes in some or all of the other species, which then set in motion the feedback loop we described in pairwise evolution. The more species involved, the more difficult it is to tie a specific change in one species to specific changes in one or more of the other species. This is referred to as **diffuse coevolution**. For example, imagine a simple case of diffuse coevolution in which a species of hawk preys upon a species of rabbit and a species of mouse. Changes in habitat preferences of the mouse species may impose new selection pressures on the hawk population, leading to new traits being favored in the hawk species. Resultant changes in the physiology of the hawks may set in motion further selection not only on the mouse population, as in pairwise coevolution, but also on the rabbit population. Moreover, the new strength of selection may not be the same on the rabbits as it is on the mice.

Diffuse coevolution is not limited to antagonistic interactions. It also plays an important role in mutualistic coevolution. For example, panic grass, *Dichanthelium lanuginosum*, can live in the geothermally heated soils of Yellowstone National Park, where soil temperature often exceeds 130°F. To survive in this scorching environment, the plant requires the presence of a fungus, *Curvularia protuberata*. For the fungus to survive in the soil, it in turn requires that a virus, called the *Curvularia* thermal tolerance virus (CThTV), be present in the environment (Marquez et al. 2007). Changes in any of these species feed back and affect selection on the others (**Figure 18.2**).

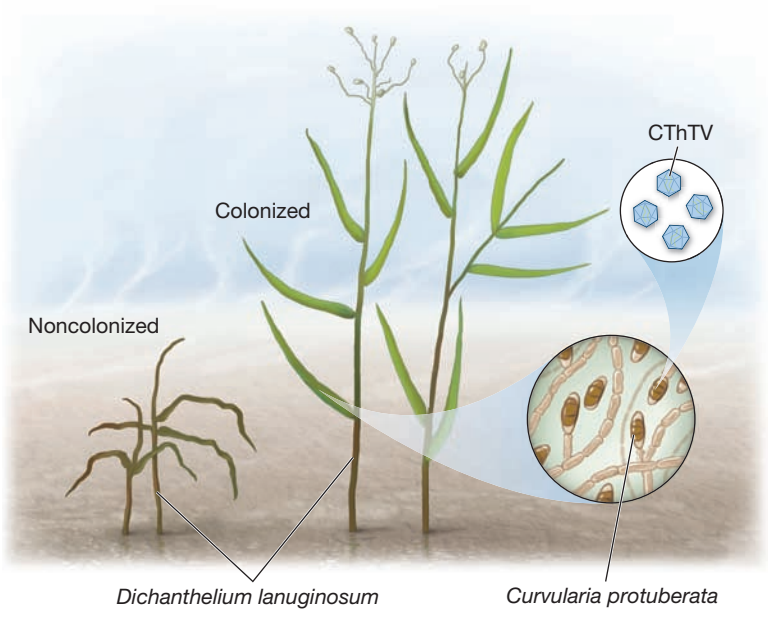

FIGURE 18.2 A diffuse three-way mutualism. *Dichanthelium lanuginosum* lives in the hot soils of Yellowstone National Park. To survive, the plant needs the fungus *Curvularia protuberata*. For the fungus to survive, the *Curvularia* thermal tolerance virus (CThTV) must be present. The plant on the left has not been colonized by *Curvularia protuberata* or CThTV, while the plant on the right has been colonized by *Curvularia protuberata* and CThTV. Adapted from Roossinck (2011).

Diffuse coevolution may also involve a combination of antagonistic and mutualistic interactions (Vannette et al. 2013). For example, a mutualistic relationship exists between hummingbirds and the hummingbird-pollinated shrub *Mimulus aurantiacus*. Prior work on plant–pollinator mutualisms had found that microbes that live in floral nectaries may affect the strength of such plant–pollinator mutualisms. To examine the effect of such "third parties" on the mutualism between hummingbirds and *Mimulus*, Racheal Vannette and her team experimentally manipulated the presence of two microbes—a

bacterium and a fungus—known to inhabit the floral nectary of *Mimulus aurantiacus*. They found the bacterium *Gluconobacter* had detrimental effects on the seed set of the plant, the amount of nectar consumed by pollinators, and the pollination success in *M. aurantiacus*. None of these detrimental effects were found when the microbe in the floral nectary was the fungus. The bacterium and the fungus, though both inhabitants of floral nectaries, had dramatically different effects on the costs and benefits of the hummingbird–shrub mutualism.

In this chapter, we will examine the following questions:

- How can mutualistic interactions between members of different species increase the fitness of individuals in each species and result in coevolution?
- In what situations does antagonistic coevolution occur between interacting species, and what are the evolutionary consequences of antagonistic coevolution?
- How can natural selection result in mosaic coevolution, in which there are mutualistic interactions between members of two species in some communities but antagonistic interactions between members of the same two species in other communities?
- What is gene–culture coevolution?

Before we move on to these questions, it is important to recognize the general power of coevolution. In a sense, coevolution may be the most important process driving evolutionary change. What we mean here is this: Evolutionary biologists are interested in understanding the history and diversity of life. Natural selection is a primary driver of biological diversity, and the sources of selection on a population at any point in time will be both abiotic (temperature, humidity, acidity, and so on) and biotic (the results of other living creatures). Traits that are favored in response to some abiotic factor in the environment, however, do not necessarily feed back on the environment, causing the environment to change and create further selection. If thicker fur is favored in Arctic environments, that has no effect on the temperature there. But, as we have argued earlier, traits that are favored in response to biotic factors—that is, other species, be they symbionts, competitors, predators, prey, or parasites—feed back on these other species.

Coevolution, and the changes it produces, can have dramatic effects on diversity in ways that abiotic factors cannot. This change in diversity can be studied in real time in certain systems. For example, antagonistic coevolution between the blue-green algae *Synechococcus* and the RIM8 virus was studied for 6 months in a series of laboratory microcosms. In each microcosm, in just 6 months, researchers documented 4–13 new viral phenotypes and 4–11 newly evolved *Synechococcus* phenotypes that differed in viral resistance (Marston et al. 2012). Similar sorts of rapid change and diversification have been found in other experimental microcosms in which the process of coevolution can be monitored and measured in real time (Kashiwagi and Yomo 2011; Brockhurst and Koskella 2013).

Coevolution can lead to changes in diversity in other ways as well. When novel traits are favored in a species as a result of coevolution, such novel traits may open up completely new, potentially very large, niches to that species in a process called *escape-and-radiate coevolution* (Ehrlich and Raven 1964; Fordyce 2010). An extreme example of escape-and-radiate coevolution is evident in the evolutionary history of interactions between beetles and angiosperms (flowering plants). A molecular phylogeny, combined with evidence from the fossil record, has found that beetle diversity increased dramatically, via a series of adaptive radiations, as this clade evolved new ways of feeding on angiosperms. The increase in beetle diversity as a result of this escape-and-radiate coevolution is estimated at an astonishing 100,000 species (Farrell 1998).

18.1 Coevolution and Mutualism

In Chapter 17, we examined the evolution of intraspecific (that is, within-species) cooperation. The mutualisms that we discuss in this chapter are examples of interspecific cooperation. But why make a distinction between intraspecific and interspecific cooperation? Part of the answer is historical. Different sets of researchers, with different research questions, have studied intraspecific versus interspecific interactions, and many of them have developed their own set of terms. But there is also a conceptual reason to make such a distinction. When interactions are intraspecific, the interactants share the same gene pool, and natural selection operates on alternative alleles in that gene pool. In contrast, as we will see throughout the course of this chapter, when interactions are interspecific, and interactants do not share the same gene pool, evolutionary interactions are different from those in the intraspecific case. To see why, we will begin with a discussion of how mutualistic relationships originate.

The Origin of Mutualisms

When we study a specific mutualism, we are looking at a snapshot of one point in evolutionary time. But we can also ask how mutualisms evolved. The answer is that there is no one set path by which a mutualism originates and evolves. In some cases, mutualisms have evolved from initially neutral interactions between species, in which neither party initially affected the other's fitness. Some mutualisms have evolved from interactions in which one species benefited and the other species was initially unaffected. Yet other mutualisms have evolved from an initially parasitic relationship when the costs and benefits of that parasitic relationship changed and favored mutualism. And in yet other instances, the relationship between species has been mutualistic from the very start of their interaction.

In **Table 18.1**, we present a few of the numerous systems in which the evolution of mutualism has been studied. While this gives us a sense of the wide array of mutualisms that exist in nature, to understand fully the exquisite adaptations that result from the evolutionary dynamics of mutualisms and to comprehend better the complex, often indirect interactions between the parties in such mutualisms,

TABLE 18.1

Examples of Mutualisms

Example	Partner 1	Partner 2	Context
		SURVIVAL AND GROWTH	
Mitochondria	Eukaryotes	Bacteria	Cellular energy
Chloroplasts	Eukaryotes	Cyanobacteria	Photosynthesis
Marine reefs	Corals	Dinoflagellates	Photosynthesis
Lichens	Fungi	Green algae/cyanobacteria	Nutrition
Mycorrhizae	Plants	Fungi	Plant nutrition
Rhizobia	Plants	Bacteria	Nitrogen fixation in soil
Gut symbionts	Animals	Bacteria	Digestion in animals
Gut symbionts	Termites	Protozoa, bacteria	Ability to digest cellulose
Fungus gardens	Ants	Fungi	Agriculture by ants
Chemosymbiosis	Bacteria	Invertebrates	Colonization of deep sea vents
		REPRODUCTION	
Pollination	Plants	Animals	Sexual reproduction in plants
Seed dispersal	Plants	Animals	Sexual reproduction in plants

Adapted from Thompson (2010).

we need to delve more deeply into some well-studied systems. We will begin with a mutualism between ants and fungus.

Ant–Fungus Mutualisms

Approximately 50 million years ago, ants began cultivating their own food by entering into mutualisms with certain species of fungi and tending "fungal gardens" (**Figure 18.3**) (Mueller and Rabeling 2008). These mutualisms continue to this day, and ants are one of the few taxa on the planet that grow their own food. The ants promote the growth of the fungi, while eating some of the vegetative mycelium—threadlike hyphae that absorb nutrients from the soil and break down plant material—produced by their fungal partners.

Cameron Currie and his colleagues have found a fascinating adaptation in attine ants such as the leaf-cutter ant *Acromyrmex octospinosus* and other fungal gardening ant species such as *Cyphomyrmex costatus* and *Apterostigma pilosum*. Remarkably, these ants not only provide a safe haven for fungi to grow but also protect the fungi from disease (Currie et al. 1999a,b; Cafaro and Currie 2005; Mangone and Currie 2007;

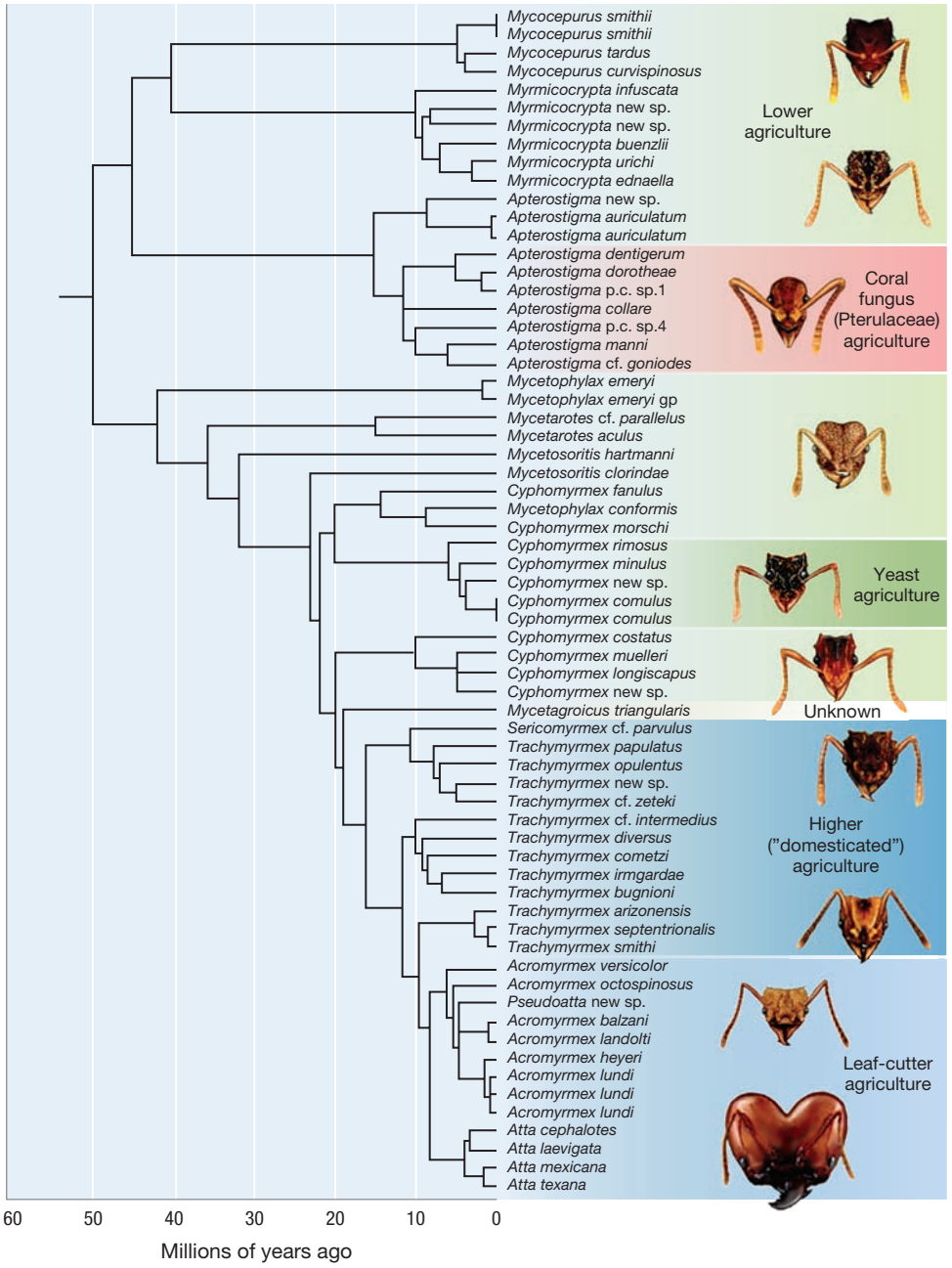

FIGURE 18.3 A phylogeny of attine, fungus-growing ants. The phylogenetic history of the five known ant agricultural systems: lower agriculture, coral fungus agriculture, yeast agriculture, higher agriculture, and leaf-cutter agriculture. Adapted from Schultz and Brady (2008).

Clardy et al. 2009; Cafaro et al. 2011). Researchers who study ants that tend fungal food gardens have long known of a whitish-gray crust found on and around many species of these ants (but not in species who do not tend gardens) (**Figure 18.4**). Currie and his team discovered that this whitish material is a mass of bacteria—primarily *Pseudonocardia* and *Streptomyces* bacteria.

Pseudonocardia and *Streptomyces* bacteria produce numerous antibiotic substances. Knowing this, Currie and his colleagues hypothesized that ants use the antibiotics produced by these bacteria to kill parasites that grow in their fungal gardens, thereby protecting their fungal food supply. A number of lines of evidence support this hypothesis: (1) all 20 species of the fungus-growing ants that

Currie and his team examined had *Streptomyces* bacteria associated with them; (2) these bacteria prevent the growth of certain parasitic fungi on the ants' fungal crop; (3) the antibiotics produced by these bacteria inhibit only *certain* parasitic diseases that directly threaten the fungal crop; (4) ants transmit the bacteria across generations, with parents—primarily mothers—passing the bacteria on to offspring; and (5) some species of fungus-growing ants—but no other ant species—have specialized indentations known as fovea all over their bodies. The bacteria live in these fovea, and the fovea appear to be supplied with nutrients of some type by exocrine glands (Currie et al. 2006).

One thing that makes the use of antibiotics by the ants in this mutualism so remarkable is that the antibiotics are specifically targeted toward pathogens that are dangerous to the fungal food crop growing in the ants' garden. When Currie and his colleagues tested the antibiotics produced by the bacteria that made up the white crust on the ant, they found that these antibiotics were potent only against the parasitic *Escovopsis* fungus—a serious threat to the ants' fungal garden. Other parasitic fungal species (those not a danger to fungus-growing ants) were unaffected by the antibiotics produced by *Streptomyces*, suggesting that selection has favored the use of the *Streptomyces* bacteria by the ants. These kinds of protective mutualisms are not found among ants alone. Recent work suggests that termite species that grow fungus also use antibiotics to protect their fungal gardens (Visser et al. 2012).

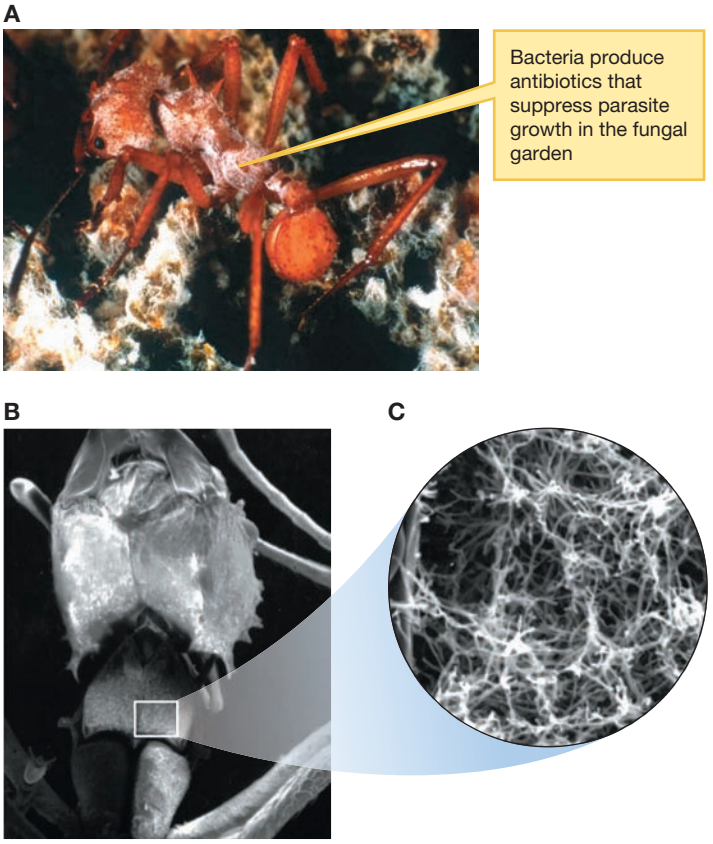

FIGURE 18.4 Leaf-cutter ants protect their fungal garden. **(A)** A worker of the leaf-cutter ant (*Acromyrmex octospinosus*) tending a fungal garden. The thick whitish-gray coating on the worker is composed of bacteria that produce the antibiotics that suppress the growth of parasites in the fungal garden. **(B)** Scanning electron micrograph of a worker, showing the location of the bacteria. **(C)** Detail of the micrograph in panel B.

Other work by Currie and his team has uncovered even more subtle components to the ant–fungus mutualism. In addition to directly using the antibiotics produced by *Streptomyces* to protect their fungal gardens, the ants meticulously groom these gardens and physically remove fungus from their garden that has been infected with *Escovopsis* (Mangone and Currie 2007). Ants pick up parasitic fungal *Escovopsis* spores and hyphae and place them in areas of their body called infrabuccal pockets. Inside these pockets, the spores and hyphae are killed by the antibiotics that are also present in the infrabuccal pockets. The ants then take the dead spores and hyphae and deposit them in a separate pile away from the fungal garden (Little et al. 2003, 2006).

Because many leaf-cutter ants grow their own food via fungal gardens, and because the fungal gardens break down lots of plant material that then becomes available to the ants, Currie and his team predicted that compared to species that do not grow their own food, leaf-cutter genomes would show evidence for the loss of genes associated with nutrient acquisition and normal digestion. When a team of researchers sequenced the entire genome of one leaf-cutter species (*Atta cephalotes*) that relies on fungal gardens, they discovered a number of lines of evidence supporting this prediction. For example, they found extensive reduction in the production of enzymes

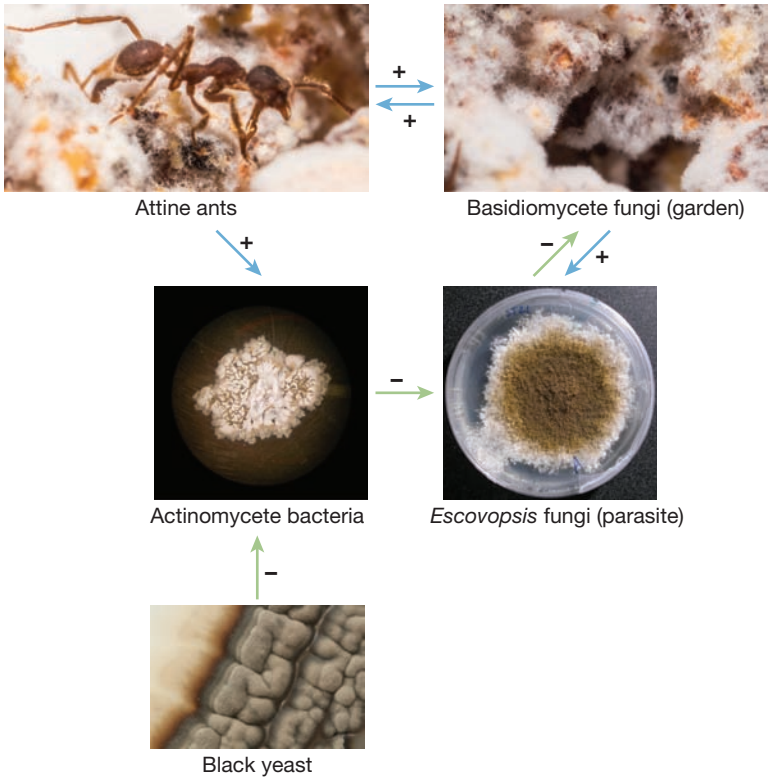

FIGURE 18.5 Five species are linked in a web of mutualistic interactions. Arrows indicate positive (+) or negative (–) direct effects of one species on another. There is an even more extensive web of indirect effects, such as the harmful effect that black yeast have on ants by feeding on the actinomycete bacteria (*Pseudonocardia* and *Streptomyces*) that help the ants exclude the *Escovopsis* parasite from their basidiomycete fungal gardens. Adapted from Little and Currie (2008).

(serine proteases) typically used during digestion. Currie and his team also found evidence for the loss of genes involved in producing the amino acid arginine, leading to a new, but as of yet untested, hypothesis that leaf-cutter ants obtain this amino acid directly from the fungi in their garden (Suen et al. 2011).

As complex as this multiorganism symbiosis seems, recent studies have also revealed another player, a black yeast that grows upon the ants' cuticle where the *Pseudonocardia* and *Streptomyces* bacteria are found (Little and Currie 2007). These yeast appear to feed on the bacteria themselves, rather than on the ants or the fungal garden. The consequences propagate on to the garden, though: Black yeast inhibits bacterial growth, which reduces antibiotic production, which in turn allows the *Escovopsis* parasite to grow, with a negative effect on the productivity of the fungal garden (Little and Currie 2008) (**Figure 18.5**).

The relationship between ants and their fungal gardens demonstrates that mutualistic relationships involve not only pairs of species, but also whole ecological communities joined together by complex and subtle interactions that generate a network of positive and negative relationships.

Ants and Butterflies: Mutualism with Communication

Natural selection should favor communication between individuals from different species that are involved in a mutualistic relationship if such communication increases the fitness of the individuals in each species. To examine the role of communication in mutualistic relationships, Naomi Pierce and her colleagues studied a mutualistic relationship between the imperial blue butterfly (*Jalmenus evagoras*) and the ant *Iridomyrmex anceps* (Pierce et al. 2002) (**Figure 18.6**). Both parties benefit substantially from this mutualism. The butterfly larvae and pupae secrete a sugary nectar composed of sucrose and fructose that nourishes the ants; the ants protect the larvae and pupae from predators such as wasps. Pierce and her colleagues have found that butterfly larvae have reduced survival rates when ants are experimentally removed. While ants can survive in the absence of the nectar that they consume from larvae and pupae, under normal conditions they obtain a significant portion of their nutrients from their butterfly larvae partners (Pierce et al. 1987; Fiedler and Maschwitz 1988).

Though the net effect of the mutualism is positive for both species, this ant–butterfly mutualism involves costly investment by both parties. Butterfly larvae that are not tended by ants develop into much larger pupae than butterfly larvae that are tended (Pierce et al. 1987). Why? Larvae that are not tended by ants reduce the amount of nectar they secrete and use the nutrients normally provided

to ants for their own development. Because size in both male and female butterflies is related to reproductive success, pupating early leads to lower reproductive success for the butterflies, and hence it represents a significant investment in the mutualistic relationship (Elgar and Pierce 1988; Hill and Pierce 1989; Hughes et al. 2000) (**Figure 18.7**). There is probably also a cost to ants for protecting butterfly larvae. Ants involved in a mutualistic relationship with butterflies probably have an increased risk of detection by their own predators and parasitoids and bear metabolic costs that are associated with defense of the butterfly larvae (Pierce et al. 1987).

FIGURE 18.6 Butterfly–ant mutualism. Butterflies and ants in a mutualistic relationship. In the mutualism between the butterfly *Jalmenus evagoras* shown here and the ant *Iridomyrmex anceps*, butterfly larvae are less likely to survive in the absence of ants, and ants receive some of their food from the nectar produced by the butterfly larvae.

Given that ants and butterflies are tied together in a mutualism that is costly to maintain, researchers hypothesized that communication between the two species would be favored by natural selection. They decided to test whether such communication was indeed taking place. Because ants are almost deaf when it comes to airborne sounds but are quite sensitive to vibrational signals traveling through solid substrates, Travasso and Pierce (2000) focused on vibrational communications. They noticed that when ants were in the vicinity, the buttefly larvae more vigorously produced vibrational signals by rubbing their stridulatory organs together. This observation s*uggests* that the larvae use these signals as a way to communicate with their ant guardians.

In a follow-up experiment, Travasso and Pierce, "muted" one of a pair of butterfly pupae by applying nail polish to its stridulatory organs and allowing the other member of the pair to stridulate normally. Then, using a preference testing device that included two bridges on which the ants could move about, Travasso and Pierce tested which butterfly pupae the ants were more attracted to (**Figure 18.8**). They found that ants demonstrated a clear preference for associating with the pupae that could and did produce vibrations, providing evidence that vibrational communication plays a role in this ant–butterfly mutualistic relationship.

Although Travasso and Pierce did not directly measure whether the butterfly pupae stridulate more when their ant partners are present, it appears that the fitness benefits accrued by both parties in the ant–butterfly mutualism are valuable enough that natural selection has favored a form of vibrational communication between these mutualistic partners.

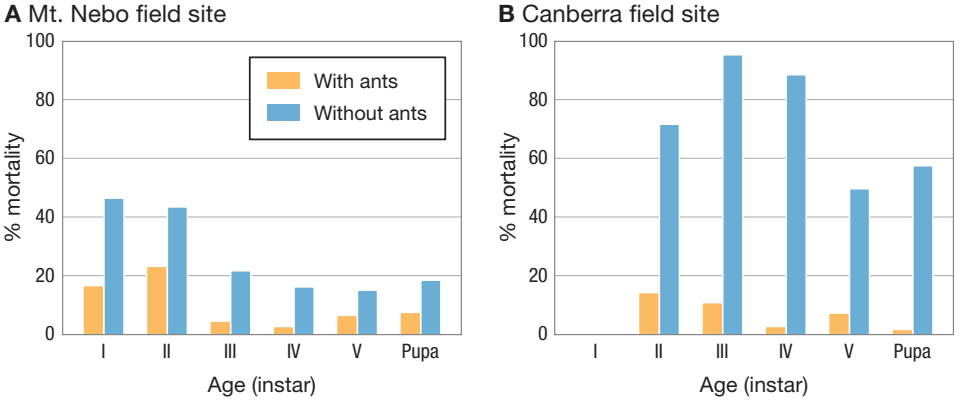

FIGURE 18.7 Butterflies benefit from their ant partners. The probability of mortality of *Jalmenus evagoras* larvae and pupae when faced by predation was much lower (and thus survival was much higher) when ants were present than when they were experimentally excluded at two Australian field sites: **(A)** Mt. Nebo site and **(B)** Canberra site. Adapted from Pierce et al. (1987).

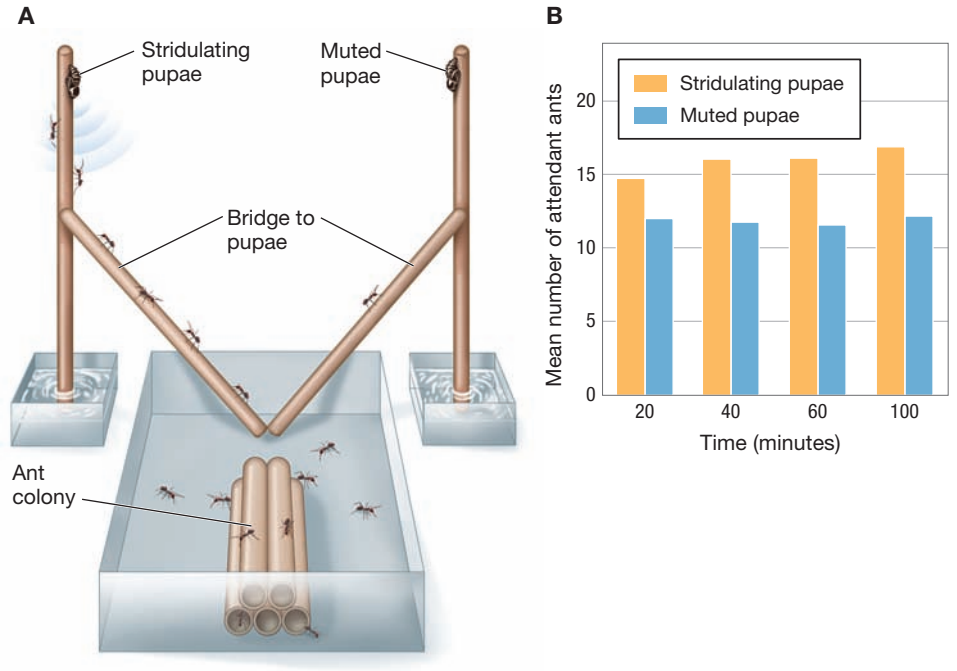

FIGURE 18.8 Communication between mutualists. **(A)** The apparatus used in preference tests. Ants from a colony could choose to move along either bridge. One of the pupae at the top was "muted." **(B)** Stridulating attracts ants. Stridulating *J. evagoras* pupae attracted more ants than *J. evagoras* pupae that had been experimentally muted. Differences between treatments were significant at all time intervals (20, 40, 60, and 100 minutes). Adapted from Travasso and Pierce (2000).

KEYCONCEPT QUESTION

18.1 Many species of ants and acacia trees have evolved a mutualistic relationship, in which the ants protect the acacia from mammalian and insect predators, and the acacia trees provide food for the ants. The acacias provide a carbohydrate-rich liquid (via extrafloral nectary glands) that the ants eat. Acacias also have Beltian bodies that produce proteins which the ants consume. How might you design an experiment to test the hypothesis that if the protection that the ants provide becomes less necessary, natural selection will favor acacia trees that produce fewer resources for ants?

FIGURE 18.9 Soybean–rhizobial bacterium mutualism. Soybean legumes (*Glycine max*) are involved in a mutualistic relationship with rhizobial bacteria (*Bradyrhizobium japonicum*). Pictured here is a soybean with nodules containing *B. japonicum*.

Mutualism and the Response to Cheaters

As we discussed in Chapter 17, where there is cooperation, there are often corresponding incentives for one or more of the parties involved to cheat, as cheaters can reap the benefits of cooperation without having to pay the associated costs. How do interspecific mutualists handle this "cheater problem"? Does cheating occur, and equally important, does one partner in a mutualism respond when the other cheats? To address this question, Toby Kiers and her colleagues examined the mutualism between a soybean legume, *Glycine max*, and a rhizobial bacterium, *Bradyrhizobium japonicum*, a soil bacterium that forms nodules on the roots of the soybean plant (Kiers et al. 2003) (**Figure 18.9**). In a process known as nitrogen fixation, *B. japonicum* converts inorganic N_2 in the root nodules of the plant into an organic form of nitrogen, providing a critical resource that the plant uses for growth and synthesis. On the flip side of this mutualism, the soybean plant provides

carbohydrates and other energetic resources to *B. japonicum*, which the bacteria use for their growth and maintenance.

What would happen if one party in this mutualism cheated? For example, nitrogen fixation is costly for *B. japonicum*, as the resources used to fix nitrogen could instead be used by the rhizobial bacterium for its own growth and reproduction. What if *B. japonicum* cheated in such a manner? Could soybean plants respond to reduced nitrogen fixation by *B. japonicum* in a way that would reduce such cheating (Denison 2000; West et al. 2002a,b; Kiers et al. 2006; Kiers and Denison 2008)?

To address this question, Kiers and her team experimentally forced *B. japonicum* to "cheat"—that is, to not fix nitrogen in the root nodules of their hosts—by creating a nitrogen-free atmosphere in one treatment condition of their study. The nitrogen-free atmosphere per se did not reduce the growth rate of *B. japonicum*, which can survive without fixing nitrogen, but such an atmosphere created an indirect cost for the plant—the absence of accessible nitrogen produced by *B. japonicum* (Layzell et al. 1979). Did soybean plants in the nitrogen-free treatment respond and "punish" *B. japonicum* for failing to fix nitrogen?

Even though the nitrogen-free atmosphere does not itself affect growth rates in *B. japonicum*, Kiers and her team found that *B. japonicum* populations grew to much larger numbers in plant nodules in an experimental treatment in which nitrogen was present compared with *B. japonicum* population numbers in a treatment in which the atmosphere was nitrogen-free. This was the case even in a "split root" treatment in which, by a clever experimental protocol involving precise control of atmospheric conditions in growth chambers, a single plant had some nodules subjected to a normal nitrogen atmosphere and some nodules subjected to a nitrogen-free atmosphere.

One interpretation of these results is that the soybean plant punished cheating by the bacteria, leading to decreased *B. japonicum* growth in the nitrogen-free treatment. How did the soybean plant do so? The mechanism appears to be curtailing the O_2 available to *B. japonicum* by changing the permeability of the nodule membrane, which in turn reduces *B. japonicum's* growth rate. The split-nodule control treatment condition also demonstrated it isn't just that plants with *B. japonicum* that fail to fix nitrogen have lower levels of O_2 themselves, and hence have less to put into nodules. Rather, the results indicated that plants differentially allocated O_2 to nodules with nitrogen-fixing *B. japonicum* over nodules containing experimentally created *B. japonicum* cheaters. These results suggest that when one party of a mutualism cheats, the other party responds in kind. This ability to punish cheating has the effect of stabilizing the mutualism by reducing cheating. Subsequent work in plant–fungal mutualisms suggests that other mechanisms also exist for stabilizing these mutualisms: Plants provide more carbohydrates to those fungal species that provide them with the best resources, while fungal partners increase the amount of nutrients they provide to plants whose roots provide them the most carbohydrates (Kiers et al. 2011).

Mutualism and Cospeciation

When the benefits of mutualism to both species are high and the mutualistic relationship has been in place over long periods of evolutionary time, the link between mutualists may result in **cospeciation**, in which speciation in one partner in a coevolutionary relationship leads to speciation in the other (de Vienne et al.

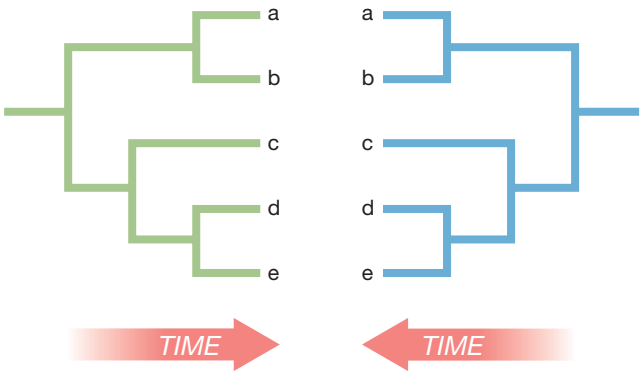

FIGURE 18.10 Cospeciation. Cospeciation in two clades (shown in green and blue). In this case of perfect cospeciation, the left and right phylogenies are mirror images of one another. Adapted from de Vienne et al. (2013).

A

B

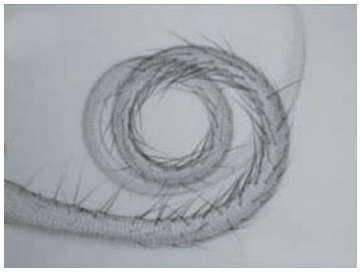

FIGURE 18.11 Pollination of *Glochidion* tree flowers by *Epicephala* moths. **(A)** Pollen on the proboscis of a female moth is shown at the tip of the red arrow. **(B)** One section of the proboscis of a female *Epicephala* moth. The hairlike projections (sensilla) in the females of pollinating *Epicephala* have likely evolved as a specialized trait associated with pollination. Panel B from Kawakita and Kato (2006).

2013) (**Figure 18.10**). But how might a simultaneous breakdown of gene flow *within each* of two mutualistic species occur and allow for cospeciation? Geographic separation provides one possible mechanism (Chapter 14). If some physical barrier separates communities that contain both mutualistic species, then a pair of species involved in a mutualistic relationship in community 1 could evolve independently of a pair in community 2, leading to cospeciation in allopatry.

As an example of cospeciation, we will examine the mutualism that exists between *Glochidion* trees and the *Epicephala* moths that pollinate them (Kawakita et al. 2004; Hembry et al. 2013). The *Glochidion* trees and *Epicephala* moth study system (which we label as the G–E system) is an **obligate mutualism**, in which each partner can only survive and reproduce successfully in the presence of the other (Thompson 1994, 2005).

In the G–E system, a female moth transports pollen between *Glochidion* tree flowers on a tubelike mouthpart called a proboscis. A comparison of the proboscis of male and female moths shows that female moths possess specialized hairlike projections called sensilla that play a role in pollination. Females lay their eggs in the flower's style, and their larvae feed on developing seeds, destroying a small portion of the seeds in the process (**Figure 18.11**). The larvae rely on these seeds as their food source, and the trees rely on the moths for pollination (Kawakita et al. 2004; Kawakita and Kato 2006).

The G–E system is made up of 300 species of *Glochidion* distributed across Asia, Australia, and Polynesia. The exact number of *Epicephala* moth species associated with these trees is unknown, but evidence suggests that the number is likely large, with some *Epicephala* species specializing in pollinating a single *Glochidion* species (Kato et al. 2003). To examine cospeciation in the G–E system, Kawakita and colleagues used molecular phylogenetic analysis of nuclear ribosomal DNA to investigate relationships among 18 species of *Glochidion* and their respective *Epicephala* pollinators. They then compared patterns of speciation across these mutualistic species (Kawakita et al. 2004).

After reconstructing the phylogenetic history for each partner in the G–E mutualism, the researchers used two different statistical approaches to see whether speciation in *Glochidion* trees was associated with speciation in their moth pollinators (Ronquist 1995). Their results indicate that although speciation patterns in trees and their moths were not identical, they were very similar, with somewhere between 6 and 10 cospeciation events (**Figure 18.12**). The reciprocal reliance in the G–E system, wherein each species cannot survive in the absence of the other, has led to significant cospeciation, and hence an increase in diversity in both *Glochidion* trees and *Epicephala* moths.

18.2 Antagonistic Coevolution

Antagonistic coevolution occurs when each of two species has a negative effect on the other. Here, we will examine the two most common forms of this type of coevolution: (1) between predator and prey, and (2) between parasite and host.

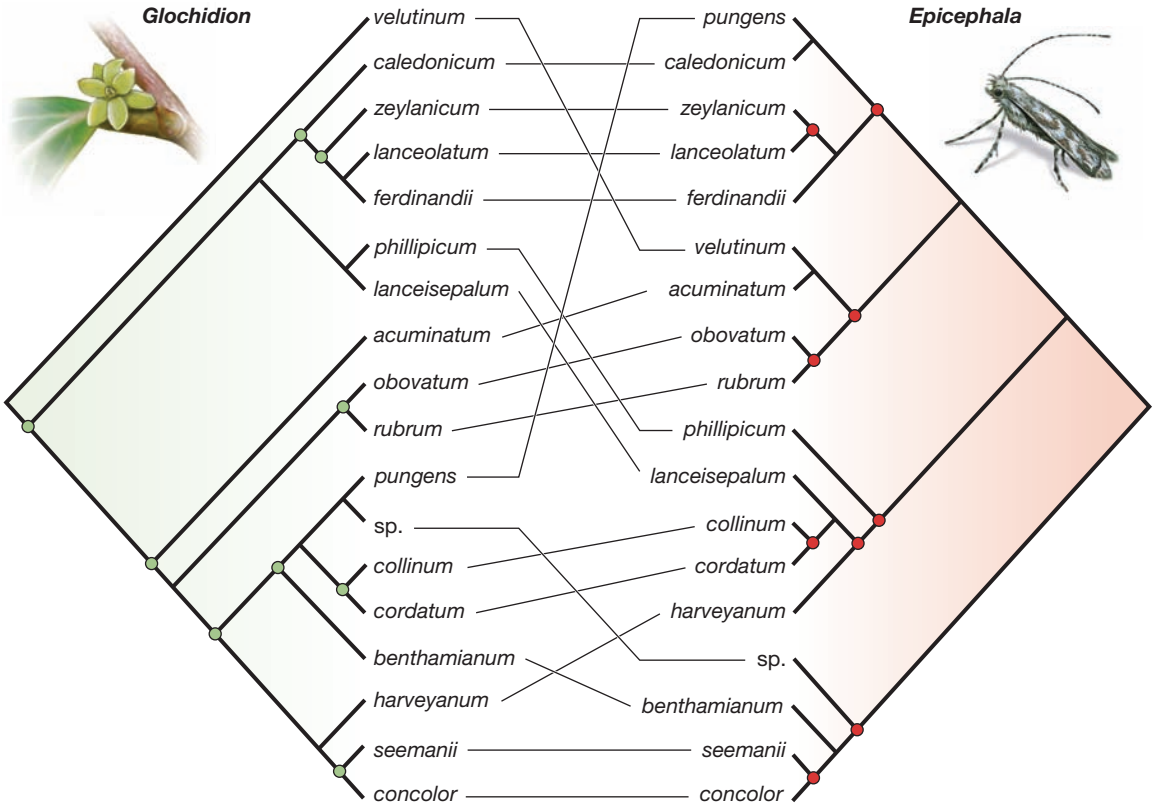

FIGURE 18.12 Cospeciation in *Glochidion* and *Epicephala*. Phylogenetic trees for *Glochidion* (left) and *Epicephala* (right) showing tree–moth associations. Species of *Glochidion* are also designated by their species names ("sp." indicates an unnamed species). The *Epicephala* moths in this study were all undescribed species, so each is indicated here by the species name of its host tree. Lines connect moth species and tree species that are associated with them. Nodes associated with cospeciation are indicated with colored circles. Adapted from Kawakita et al. (2004).

Predator–Prey Coevolution

Consider a simple predator–prey system in which a predator feeds on only one species of prey, and this species of prey is preyed on by only this one predator. All else equal, selection will favor any trait in the prey that increases its chances of escaping predation. If such a trait evolves in prey, this immediately intensifies selection on predators for traits that increase their probability of capturing and consuming their now better-adapted-to-escape prey. Such a trait in predators will then favor any trait in prey that allows them to escape their now better-adapted-to-kill predators, and so on (Vermeij 1993). This coevolutionary dynamic is known as an evolutionary arms race.

To understand such evolutionary arms races better, let's examine predator–prey interactions between the predatory whelk *Sinistrofulgur* and its bivalve prey *Mercenaria* (Dietl 2003a,b). In this system, the fossil record is detailed enough that it is possible to record both successful and unsuccessful attempts at predation over very long periods of evolutionary time. During an attack, a whelk "mounts" its prey, and it uses its shell lip to chip away at the bivalve shell. When it is successful, it kills the prey, but even when it is unsuccessful, the telltale chips and cracks from a failed predation attempt are preserved in the fossil record. The cost of predation can also be documented in the fossil record, as the whelk occasionally breaks its

own shell while trying to open its prey, and such damage and subsequent repair can be seen when examining whelk shells (**Figure 18.13**).

Evidence for an evolutionary arms race can be seen in the fossil record of the *Sinistrofulgur–Mercenaria* system. Over evolutionary time, selection has favored an increased shell size and shell thickness in *Mercenaria* prey, which would reduce its probability of being eaten by *Sinistrofulgur*. As *Mercenaria* evolved a thicker shell, selection then favored any trait in *Sinistrofulgur* that allowed it to kill its thicker-shelled *Mercenaria* prey. The fossil record shows that over the same time period that *Mercenaria* were evolving a thicker shell, *Sinistrofulgur* predators were also increasing in size. Larger *Sinistrofulgur* predators would have been able to penetrate the shells of their *Mercenaria* prey more easily (and would have been safer from their own predators). This would also produce selection for increased size in *Mercenaria* prey, and back and forth in an evolutionary arms race with *Sinistrofulgur* with respect to size.

Is it possible that, rather than a predator–prey arms race, natural selection acted on size, outside the context of predator–prey interaction, and independently in each species? Could this explain the increase in size in both *Sinistrofulgur* and *Mercenaria*? In principle this is possible, but the fossil record also shows that over evolutionary time, *Sinistrofulgur* predators changed the typical position they assumed during an attack in such a way as to increase the probability of successfully killing their *Mercenaria* prey. The positional change recorded in the predator is consistent with the hypothesis that the adaptive change was in response to adaptations in its prey rather than selection acting independently on predator and prey.

Evolutionary arms races may even leave their marks long after one of the species involved is extinct. Take the case of the pronghorn antelope, a modern-day species in which individuals can run at speeds approaching 60 miles an hour—much faster than any of the predators they face today. Why can pronghorns run that fast? John Byers has hypothesized that the pronghorn's speed today is a result of a predator–prey arms race that ended more than 10,000 years ago (Byers 1997). For millions of years, pronghorns shared their environment with speedy predators such as the

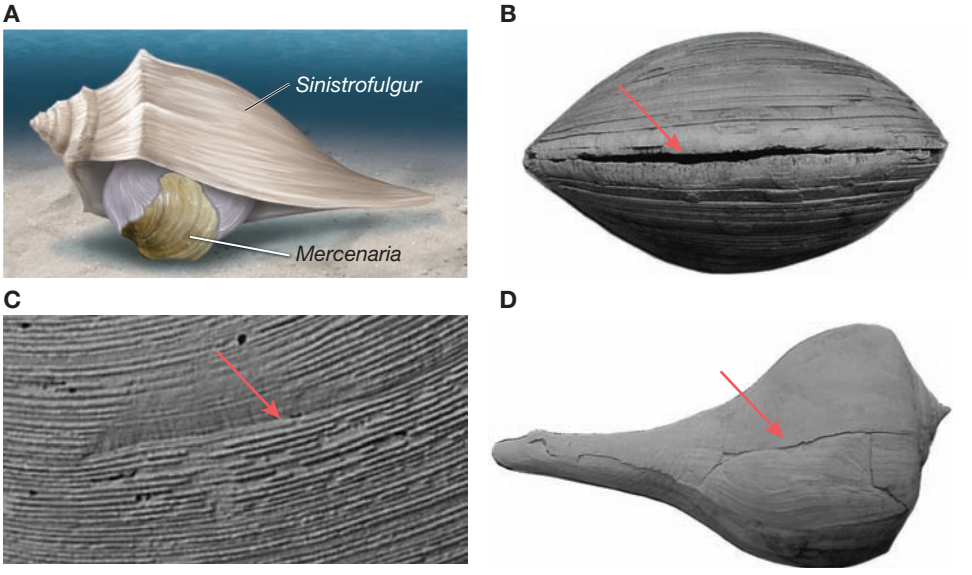

FIGURE 18.13 Predator–prey interactions and coevolution. **(A)** A predatory whelk *Sinistrofulgur* mounts its bivalve prey *Mercenaria* and chips away at its shell. Panel A adapted from Dietl (2003a). **(B)** Evidence of a successful attack on *Mercenaria* (red arrow). **(C)** Evidence of an unsuccessful attack on *Mercenaria*. The *Mercenaria* shell is worn down (red arrow) but not cracked by *Sinistrofulgur* shell chipping. **(D)** A whelk occasionally breaks its own shell while trying to open its prey. Damage is indicated by the red arrow. Panels B–D from Dietl (2003b).

North American cheetah (*Miracinonyx*) and the North American lion (*Panthera leo atrox*), both of which went extinct about 10,000 years ago. While this hypothesis remains to be tested, it suggests that evolutionary arms races can have lasting consequences even after one of the participating species is gone.

Host–Parasite Coevolution and Cospeciation

Earlier in this chapter, we discussed how mutualistic interactions can result in cospeciation and a resulting increase in species diversity. Parasites and hosts may also cospeciate, as initially suggested more than 100 years ago by Kellogg and later by Fahrenholz, both of whom hypothesized that phylogenies of parasites and hosts often change in parallel (Kellogg 1896; Fahrenholz 1909; Klassen 1992).

When populations of a host species become geographically isolated from one another, this will often produce geographic isolation among the parasite populations. As the host populations diverge, selection acts in new ways, not only on individuals in the host populations but also on the parasite populations they carry. If divergence in the host species is great enough, and host speciation occurs, this could lead to speciation in the parasite as well (Moran and Baumann 1994; Wade 2007).

Dale Clayton and his colleagues examined the role of parasite–host coevolution and cospeciation in ectoparasitic feather lice (*Columbicola*) that complete their life cycle on their pigeon and dove hosts, feeding on the bird's abdominal feathers (Clayton and Johnson 2003; Clayton et al. 2004; Johnson et al. 2009, 2011). Using nuclear and mitochondrial DNA sequences, these researchers constructed phylogenies of both lice and their hosts, and then they compared these phylogenies to test whether cospeciation had occurred. Their analysis uncovered eight cospeciation events (**Figure 18.14**).

What drove cospeciation in this system? One clue came when Clayton and his team found that the lice species that had larger individuals tended to live on larger species of pigeons and doves. The researchers hypothesized that there were benefits to lice if they stayed on size-matched species: small lice with small host species and large lice with large host species. If this was correct, then when speciation occurred in birds, their lice were constrained to remain on their hosts because of the benefits of size matching. The result was a tight linkage between host and parasite, leading to cospeciation events. But what exactly were the benefits that lice received for size matching with their hosts?

Clayton and his team experimentally examined whether body size matching allowed lice to remain attached to their hosts more efficiently. Lice were placed on feathers from either a host species or a nonhost species, and these feathers were attached to a fan to test the ability of the lice to remain on their hosts. Results indicate that size matching did not improve the ability of lice to attach to the feathers. Other work also found that body size matching did not affect the feeding ability of lice. Body size matching did, however, have a significant effect on the ability of lice to escape the defensive preening behavior of their host species. Compared to the case of lice on their natural hosts, when lice were experimentally placed on nonhost species that differed in size from their host species, they were unable to evade preening (self-cleaning) and they were eaten by

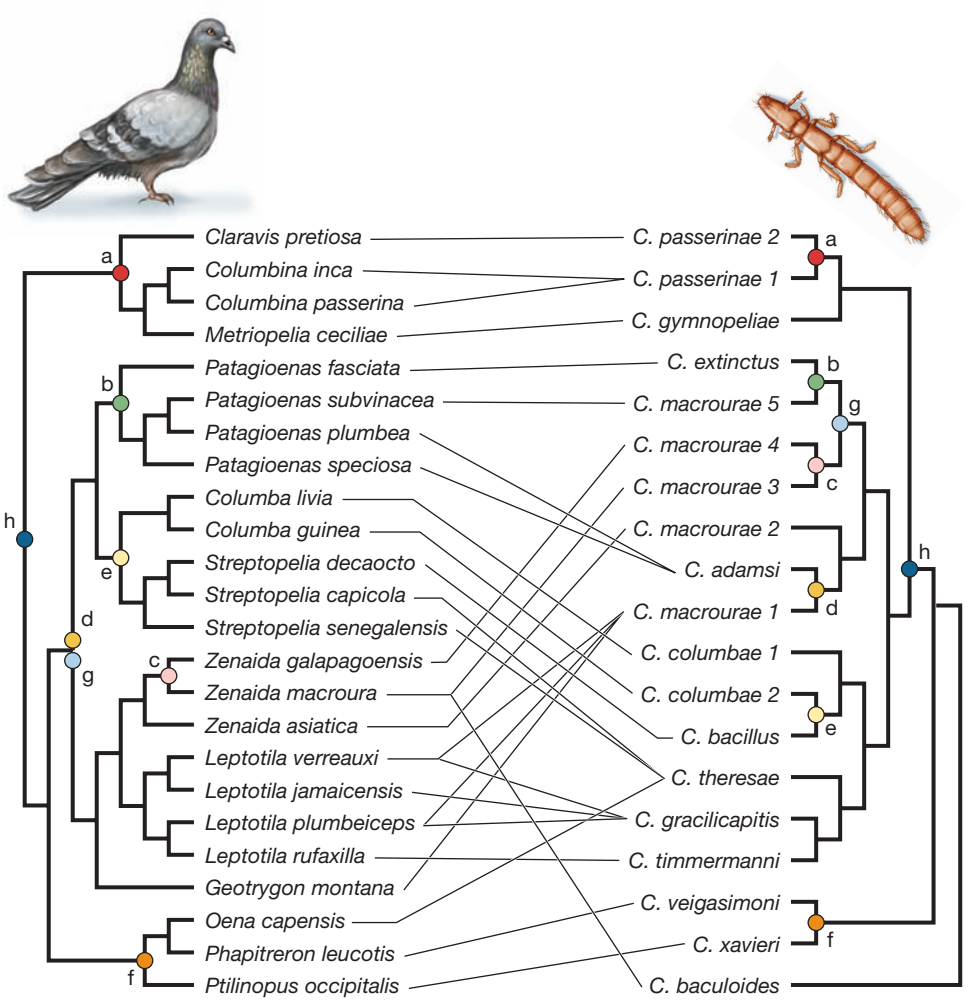

FIGURE 18.14 Parasite–host cospeciation. Phylogenies of pigeons and doves and their lice (genus *Columbicola*). Lines connect host–parasite associations. Cospeciation events are color coded: Matching colors and letters on each side indicate cospeciation events. Adapted from Clayton et al. (2003).

birds at high rates; as such, lice could not establish populations on hosts that differed in size from their normal host (**Figure 18.15**).

The process of cospeciation between birds and their parasitic lice in this example appears to unfold as follows: After a speciation event occurs in a bird group, lice are constrained to remain on their host species because they often fare poorly when switching hosts. Such switches might involve living on a new host that is a different size than their original host, which could potentially make the lice susceptible to significant predation by the new host. The lice are constrained to remain on their original bird host, and when natural selection acts on the bird host, its lice also experience selection operating in new ways. This can lead to new adaptations by the parasites as well, and cospeciation leading to an increase in species diversity in both parasite and host may occur.

Mimicry and Coevolution

In this subsection, we look at another way that coevolution may occur and result in increased species diversity. Recall the *Ensatina* salamanders we discussed as a classic example of a ring species in Chapter 14. Here, we return to these salamanders, but we will focus on one particular subspecies, *Ensatina eschscholtzii xanthoptica,* also known as the yellow-eyed salamander. This subspecies displays striking colors—an orange

ventral region and yellow eyes, neither of which are found in other *Ensatina* salamanders. Why have these dramatic colors evolved in *Ensatina eschscholtzii xanthoptica*? One hypothesis is that they are warning signals to predators that *E. e. xanthoptica* is unpalatable. Such **aposematic** (warning) **coloration** is common in salamanders and other animals—but *E. e. xanthoptica* is *not* unpalatable to predators. Instead, orange body color and yellow eyes in *E. e. xanthoptica* appear to be the result of coevolution. In the case of the yellow-eyed salamander, the second species in this story of coevolution is the California newt, *Taricha torosa*, which lives sympatrically with *E. e. xanthoptica* populations (**Figure 18.16**).

The California newt, in addition to possessing orange body coloration and yellow eyes, also produces a neurotoxin called tetrodotoxin in its skin. While this toxin is potent, predators whose attacks fail but who ingest a small dose of tetrodotoxin often survive and learn to avoid this potential prey type.

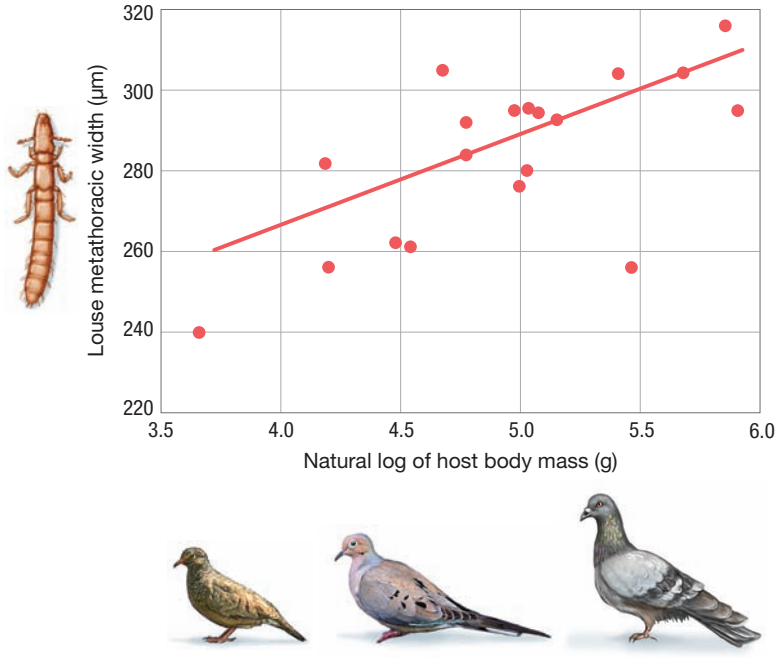

FIGURE 18.15 Body size in parasite and host. Parasite body size in relation to host body size across the associations shown in Figure 18.14. Adapted from Clayton et al. (2003).

More than 60 years ago, George Ledyard Stebbins (1906–2000) hypothesized that the orange body color and yellow eyes of the yellow-eyed salamander had been selected because these traits mimic the coloration of the California newt. Such mimicry would protect yellow-eyed salamanders from predators who might confuse it with the toxic California newt (Stebbins 1949). This sort of mimicry, in which one species is palatable and the other is not, is called **Batesian mimicry** (Bates 1862), and it is different from **Müllerian mimicry** (Müller 1879), in which multiple *unpalatable* species evolve similar phenotypes which reinforces warning signals that predators can pick up.

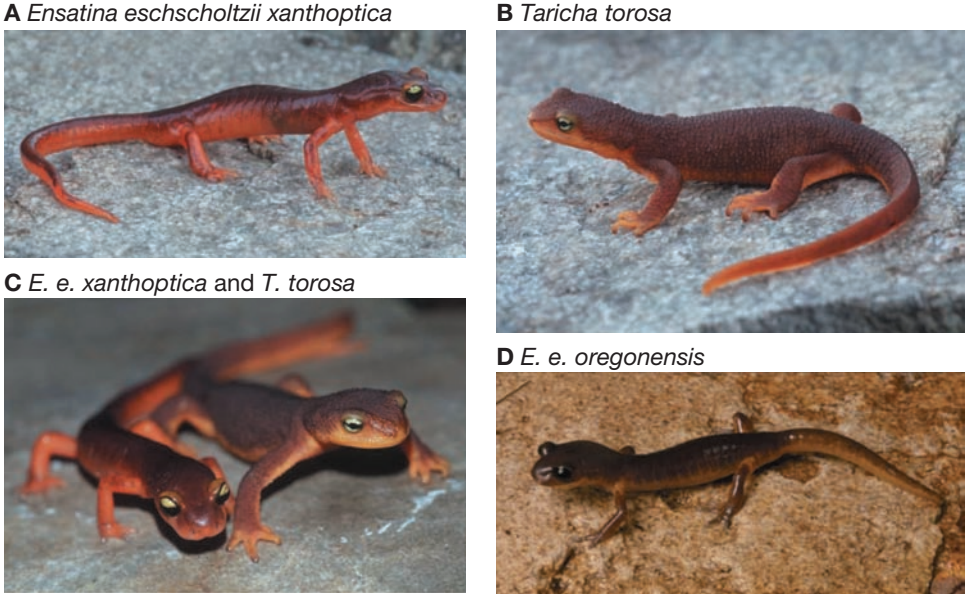

FIGURE 18.16 Mimicry and coevolution. (A) The nontoxic mimic, *Ensatina eschscholtzii xanthoptica*, **(B)** the toxic model, *Taricha torosa*, **(C)** the mimic (*E. e. xanthoptica*, on the left) and the toxic model (*T. torosa*, on the right) together, and **(D)** *E. e. oregonensis*, a non-toxic salamander species used as an experimental control because it lacks orange and yellow coloration.

KEYCONCEPT QUESTION

18.2 What sort of differences would you expect in coevolutionary dynamics between Batesian mimicry and Müllerian mimicry? For example, what differences might you expect with respect to antagonistic coevolution and mutualistic coevolution?

But how can we test whether the yellow-eyed salamander's brilliant colors are a result of coevolution via Batesian mimicry? Shawn Kuchta first attempted to answer this question by setting up an experiment in which he placed in the field clay salamander models that either looked like *E. e. xanthoptica* or were otherwise identical models that lacked the orange body color and yellow eyes of *E. e. xanthoptica*. His results showed that predators attacked the yellow-eyed salamander models significantly less often than models without the yellow-eyed salamander coloration (Kuchta 2005).

In a follow-up experiment, Kuchta and his colleagues examined rates of predation on yellow-eyed salamanders in a controlled laboratory setting in which a predator was provided with the opportunity to feed on live salamanders (Kuchta et al. 2008). Western scrub jays taken from the field were used as predators because they had experience with toxic California newts. But the Western scrub jays also had no experience with yellow-eyed salamanders, which are not usually found in the Western scrub jay habitat. The researchers presented a scrub jay with a California newt, and then they presented the jay with either a yellow-eyed salamander or an individual from the closely related subspecies *Ensatina eschscholtzii oregonensis*, which is morphologically similar to the yellow-eyed salamander but lacks orange and yellow coloration. Kuchta and his team found that after experience with the California newts—some of which the jays attacked—the jays took more time to approach yellow-eyed salamander than to approach *E. e. oregonensis* individuals.

From their encounters with the California newts, the scrub jays had learned to avoid creatures that had orange body color and yellow eyes and a newt-like body. As a result, jays were very hesitant to approach yellow-eyed salamanders, even though that species does not possess the neurotoxin found in the newts. In an encounter in the wild, such extra time could make the difference between survival or being eaten by a jay (**Figure 18.17**).

In this case, we have seen how traits in one species (the toxic California newt) influence the operation of selection on another (the nontoxic yellow-eyed salamander), but, unlike many of the other examples we have discussed, we do not know yet whether the yellow-eyed salamander has a reciprocal influence on the toxic California newt. There is, however, a testable prediction here: Because predators will occasionally eat yellow-eyed salamanders, they also will occasionally eat a *Taricha torosa* newt, and in so doing they select upon newts to signal their toxicity in a slightly different way. Future research could look for hints of such a change and continue to explore whether coevolution, in this case via mimicry, leads to increased diversity of antipredator traits.

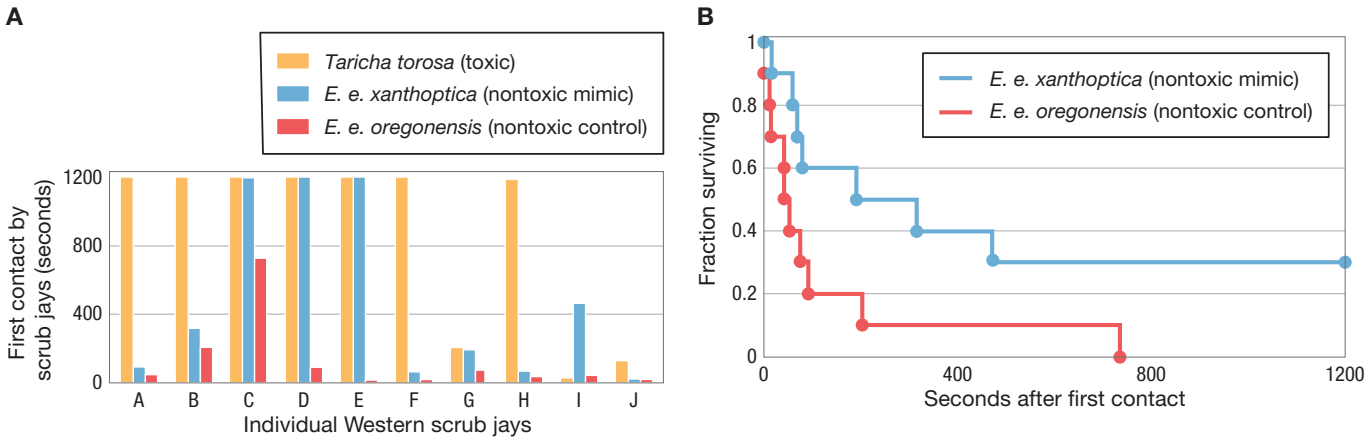

FIGURE 18.17 Survival from predation based on mimicry of toxic species. (A) Most Western scrub jays take longer to first contact a toxic salamander species and its non-toxic mimic than the non-toxic control. (B) The non-toxic mimic *E. e. xanthoptica* survives in the presence of a jay predator for longer than non-toxic control *E. e. oregonensis*. Adapted from Kuchta et al. (2008).

18.3 Mosaic Coevolution

Up to this point we have examined the dynamics of coevolution leading to mutualism or antagonistic coevolution between species. But it is important to understand that depending on the ecology and behavioral interactions, natural selection can result in mutualism between a pair of species in some communities, but antagonistic interactions between the *same* pair of species in other communities. This idea, which shows another way in which coevolution can lead to diversity, centers on geographic *variation* in coevolutionary outcomes and has been dubbed the theory of **mosaic coevolution** (Thompson 1982, 1994, 1999, 2009).

John Thompson and Bradley Cunningham studied mosaic coevolution in interactions between the herbaceous plant *Lithophragma parviflorum* (also known as the woodland star) and the moth *Greya politella* (Thompson and Cunningham 2002; Thompson and Fernandez 2006; Thompson et al. 2013). The moth lays its eggs into developing flowers of the woodland star by inserting its ovipositor down into the floral ovaries. But while inserting its eggs into numerous plants, the moth also pollinates the woodland star. Woodland star plants pay a cost for this pollination, because when moth larvae mature, they eat some of the woodland star's seeds. *Greya politella* is completely reliant on the woodland star as its sole host. But the woodland star plant is not always reliant on the moth as its sole pollinator. In some populations, the moth is indeed the *sole* pollinator of the woodland star. But in other populations, it is one of many species that act as pollinators for this plant, and many of these other pollinators do not produce larvae that eat the plant's seeds, and so they are less costly to the woodland star. This means that there is geographic variation in the costs and benefits of the plant–moth interactions. Thompson and Cunningham tested whether this geographic variation in costs and benefits was correlated with geographic variation in mutualism versus antagonistic coevolution (**Figure 18.18**). They hypothesized that the more reliant the plant was on this moth species as a pollinator, the more mutualistic the coevolutionary dynamics would be.

In four populations in which *G. politella* acted as the *sole* pollinator and the woodland star was the *sole* host for *G. politella*, Thompson and Cunningham found a mutualistic relationship between plants and moths. In these populations, the woodland star rarely aborted flower capsules that contained moth eggs (doing so

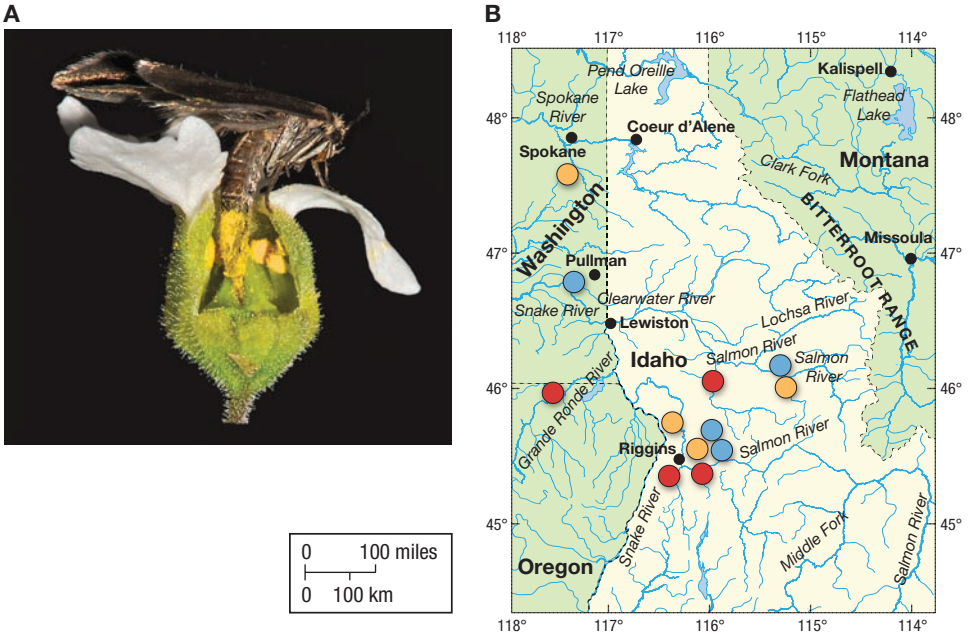

FIGURE 18.18 Mosaic coevolution in plants and moths. **(A)** A female *Greya politella* moth pollinates a woodland star (*Lithophragma* sp.). Researchers cut a small window in the flower to document pollination during oviposition. The moth's ovipositor here is covered in yellow pollen. From Thompson et al. (2013). **(B)** Mosaic coevolution must be studied across many sites. In their work on the interaction between the woodland star and its moth pollinator, Thompson and Cunningham sampled 12 sites. Note the geographic variation in what sort of evolution was occurring. Orange circle = mutualism, red circle = antagonistic coevolution, blue circle = commensal relationship (no effect of *Greya* on *Lithophragma*). Adapted from Thompson and Cunningham (2002).

would also kill the moth larvae) preferentially over capsules that had no moth eggs. But, depending on the costs and benefits to the plants and moths, mutualism need not be the outcome of coevolution in this system. In four other populations, in which the woodland star had numerous pollinators besides *G. politella*, the researchers found evidence of an antagonistic relationship between plants and *G. politella*. The plants selectively aborted flower capsules that contained moth eggs. In these populations, where alternative pollinators were present, the costs of having the moth pollinator outweighed the benefits, and selection favored an antagonistic, rather than a mutualistic, response from the plant. Coevolution was occurring in all cases, but the costs and benefits to the involved parties determined whether selection favored a mutualistic or antagonistic coevolutionary relationship. Just as important, with a solid understanding of the natural history of the species involved, evolutionary biologists can derive hypotheses about which populations will head down one coevolutionary path and which will head down the other.

KEYCONCEPT QUESTION

18.3 In the *G. politella*/woodland star example of mosaic coevolution, there are two species involved. How might yet other species in a community—their absence, presence, or behavior—affect mosaic coevolution of the two primary species a researcher may be studying?

18.4 Gene–Culture Coevolution

In addition to studying coevolution between species, over the past 30 years evolutionary biologists and anthropologists have begun examining **gene–culture coevolution**; that is, the coevolutionary dynamics between genetic and cultural traits, both within and between species. Throughout this book, we have examined how allele frequencies change over time. In that sense, we have already delved deeply into the "gene" part of gene–culture coevolution. Let's briefly examine how cultural evolution operates, and then we will move on to discuss gene–culture coevolution.

In Chapter 3, we noted that for natural selection to act on a trait, a mechanism for transmitting that trait across generations is required. Once Mendel's work on genetics was rediscovered in the early 1900s, it became clear that genes are one means of transmitting traits across generations. Culture provides another means, and recently evolutionary biologists have become interested in this phenomenon as well.

Cultural transmission is typically defined as the transfer of information from individual to individual through social learning. A slightly different way of saying this is that cultural transmission is a system of information transfer that affects an individual's phenotype via social learning (Bonner 1980; Cavalli-Sforza and Feldman 1981; Boyd and Richerson 1985).

Although cultural and genetic transmission each provide a means of passing traits down from one generation to another, there are a number of unique aspects of cultural transmission. When individuals learn from others—that is, when social learning occurs—information can be spread through a population very quickly. As a consequence, the behavior of a single individual can dramatically shift the behavior patterns of an entire group. For example, consider the case of Imo, a Japanese macaque monkey who lived on Koshima Island in the 1950s. After Imo learned the trick of washing the potatoes that researchers were providing, others in her group copied her, and potato-washing behavior spread quickly through Imo's group (Kawamura 1959; Kawai 1965). Cultural transmission can change the frequency of behavioral traits not only across generations, when younger individuals learn from older individuals, but also within a single generation, when individuals from the same cohort learn from one another (Boyd and Richerson 1985, 2004). When cultural transmission leads to changes in the frequency of traits within or between generations, we call this **cultural evolution**.

There are different types of cultural transmission. Vertical cultural transmission refers to the case in which information is transmitted between generations from parent(s) to offspring. Horizontal cultural transmission involves the transfer of information between individuals who are in the same age cohort. Oblique cultural transmission involves the transfer of information across generations when young receive information from adults that are not their parents.

For an interesting case study illustrating the importance of cultural evolution and social learning in animals, let's examine foraging behavior in rats. As scavengers, rats sample many new foods. Yet, scavenging can present a dilemma. A new food source may be an unexpected bounty for a rat. But new foods can also be dangerous. They may contain elements such as poisons that are inherently bad for rats. Or, because a rat doesn't know how a new food should smell, it is difficult to tell if a novel food is fresh and will serve as nourishment or spoiled and may make the rat sick.

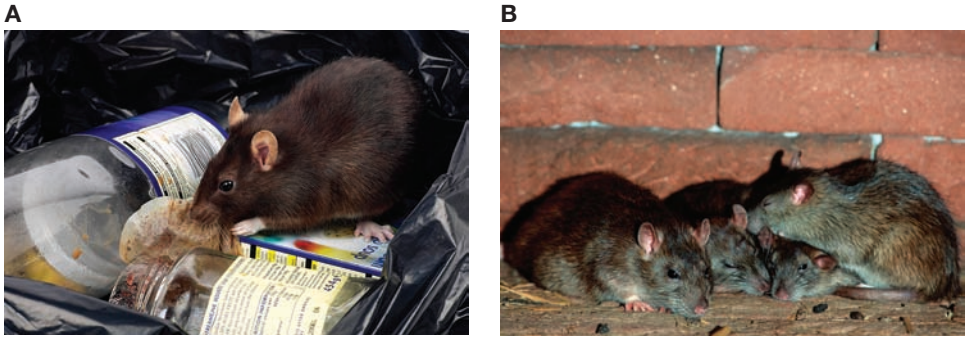

FIGURE 18.19 Scavenging and cultural transmission. **(A)** A scavenging rat often encounters new food items while foraging. **(B)** Smelling another rat provides olfactory cues about what it has eaten. If this affects a rat's behavior, transfer of information from one rat to another about safe foods is a form of cultural transmission.

Jeff Galef and his colleagues have studied the role of cultural transmission in the scavenging behavior of Norway rats (*Rattus norvegicus*) (Galef and Wigmore 1983; Galef and Laland 2005; Galef and Whiskin 2006). To test whether cultural transmission via social learning plays a role in rat foraging, the researchers examined whether observers could learn about a new food source simply by interacting with a demonstrator that had experienced such a new addition to its diet (**Figure 18.19**).

After two rats had been caged together for days, one rat was removed and taken to another experimental room, where it was given one of two novel diets: either rat chow flavored with cocoa or rat chow mixed with ground cinnamon. This "demonstrator" was then brought back to its home cage and allowed to interact with the other rat, known as the observer rat, for 15 minutes. The demonstrator rat was removed from the cage. For the next 2 days, the observer rat was given two food bowls, one with rat chow and cocoa, the other with rat chow and cinnamon. Although the observer rat had no direct experience with either of the novel food mixes and it had not *seen* the demonstrator rat eating these new food items, it was more likely to eat the food that the demonstrator rat ate, both when the demonstrator had eaten rat chow with cocoa and when it had eaten rat chow with cinnamon, strongly suggesting that Norway rat foraging behavior was affected by cultural transmission via olfactory cues (**Figure 18.20**).

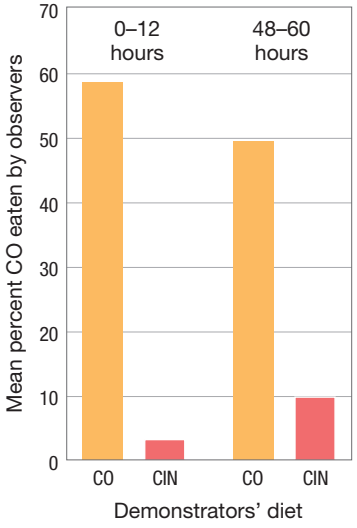

FIGURE 18.20 Cultural transmission across generations. Social learning and foraging in the Norway rat. Observer rats had a "tutor" (demonstrator) who was trained to eat rat chow containing either cocoa (CO) or cinnamon (CIN) flavoring. Rats with "cinnamon tutors" preferred cinnamon-flavored food (not shown here), and rats with "cocoa tutors" preferred cocoa-flavored food (shown here). Adapted from Galef and Wigmore (1983).

With the growing realization that cultural transmission affects many different types of behavior, evolutionary biologists have become interested in the ways in which genetic and cultural transmission can interact. Do genetic changes affect cultural evolution? Do changes in cultural evolution affect genetic evolution? Do they both affect each other? Researchers are actively looking into these questions, and the answers have promise for helping us to understand the coevolution of genetic and cultural transmission.

Gene–Culture Coevolution in Darwin's Finches

We begin with a discussion of gene–culture coevolution in Darwin's finches. The medium ground finch (*Geospiza fortis*) and the cactus finch (*G. scandens*) live on the Galápagos island of Daphne Major. These species are capable of interbreeding. Hybrid offspring produced from *G. fortis* by *G. scandens* matings do not appear to suffer a decrease in fitness compared to offspring from *G. fortis* parents or *G. scandens* parents (**Figure 18.21**). Nevertheless, interbreeding between *G. fortis* and *G. scandens* remains a rare event. Why is it that the two species seldom interbreed? Here, we will examine work that suggests a role for

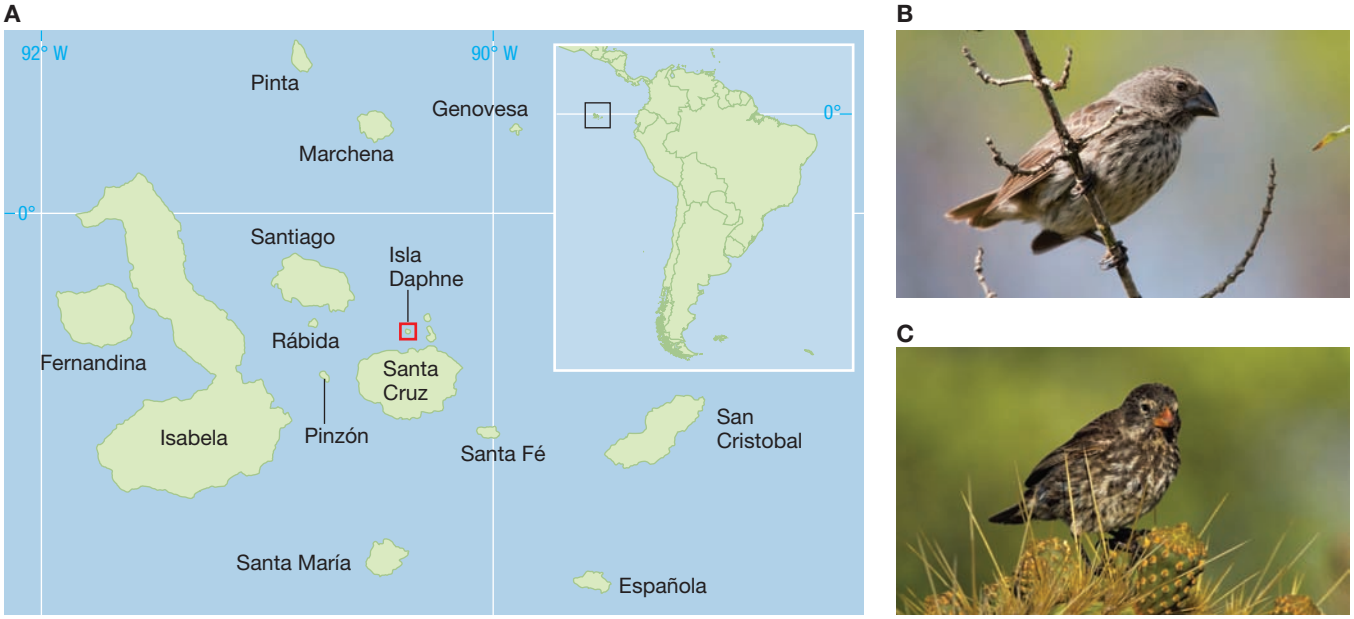

FIGURE 18.21 Cultural evolution in birdsong. **(A)** The Galápagos Islands, including Daphne Major Island (in red). **(B)** *Geospiza fortis*. **(C)** *Geospiza scandens*.

cultural transmission in inhibiting such matings (Nelson et al. 2001; Slabbekoorn and Smith 2002; Freeberg 2004; Lachlan and Servedio 2004).

When Peter and Rosemary Grant examined the songs of *G. fortis* and *G. scandens* during the birds' mating season, they found evidence of cultural transmission across generations (Grant and Grant 1996): Sons sing songs similar to their fathers' songs. To understand what was happening, the Grants compared the songs of sons, fathers, and grandfathers. One hypothesis for why father and son finches have very similar songs is that the song is a genetic trait passed from father to son; another hypothesis is that cultural transmission of the song from father to son takes place when the son hears and learns the song sung by his father. To test the two hypotheses, they compared the songs of sons to those of their paternal and maternal grandfathers. If song types are genetically controlled, we normally would expect the songs of the sons to be similar to the songs of both their paternal and maternal grandfathers, as the son inherits genes from both grandfathers. But if cultural transmission from father to son is the mechanism, then the songs of the sons should resemble those of their paternal grandfathers, not those of their maternal grandfathers, as it is the paternal grandfather who would have transmitted the song to the father, who in turn would have transmitted the song to the son (**Figure 18.22**). Comparison of the son's song to those of the maternal and paternal grandfathers shows that the son's song resembles the song of the paternal grandfather, not the song of the maternal grandfather—suggesting that birdsong is culturally transmitted.

KEYCONCEPT QUESTION

18.4 What other type of inheritance would have a male resemble his paternal grandfather but not his maternal grandfather?

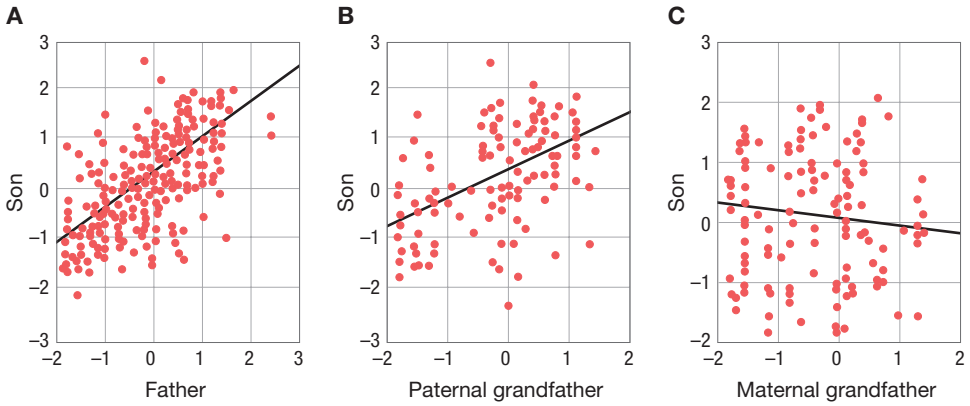

FIGURE 18.22 Finch songs across generations. Male finches' songs are positively correlated with those of their fathers **(A)** and those of their paternal grandfathers **(B)**, but not with those of their maternal grandfathers **(C)**. The significant positive correlation between the songs of sons and paternal grandfathers coupled with the absence of a positive correlation between the songs of sons and maternal grandfathers suggests that songs are culturally transmitted. The horizontal axis and vertical axis are in units that summarize many components of the songs. Adapted from Grant and Grant (1996).

In studying the birdsong of the two finch species, the Grants found that the songs varied significantly from one another: The birdsong of *G. scandens* has shorter components that are repeated more often than the components of the birdsong of *G. fortis* (Grant and Grant 1994, 1997). These differences in their songs—a culturally transmitted trait—have a dramatic impact on gene flow between ground finches and cactus finches. The researchers sampled 482 females and found that more than 95% of them mated only with males who sang the song typically produced by males of their own species. This suggests that cultural transmission plays a large role in why ground and cactus finches rarely mate, even though hybrid offspring suffer no fitness costs. The song sung by males—a culturally transmitted trait—provides females with a means to recognize individuals of their own species, which in turn leads to few between-species matings (the "gene" part of gene–culture coevolution). Further support for this interpretation comes from the fact that the Grants uncovered 11 cases in which the male of one species sang the song of another species; most of these males mated with females from the other species. In such cases of cross-species breeding, viable hybrid offspring were produced. Remove the normal pattern of cultural transmission, and the barrier to breeding across species disappears.

KEYCONCEPT QUESTION

18.5 How might the speed at which cultural evolution operates affect the rate of genetic evolution?

Gene–Culture Coevolution and Lactose Tolerance in Humans

While cultural transmission of information has been studied in many nonhuman species, there is little question that culture is more complex and more prominent in humans than in any other species. Virtually no human behavior is unaffected by what we see, hear, and read about others doing. Within psychology there is a whole subdiscipline—social psychology—devoted to this subject.

Many evolutionary anthropologists argue that gene–culture evolution is a powerful force driving human evolution. "Genes and culture resemble a symbiosis," note leaders in this field; they are "two inheritance systems occupying the same physical body" (Richerson et al. 2010, p. 8986). One of the most well-studied examples of gene–culture coevolution in our species is that of lactose tolerance in certain human populations. The story of lactose tolerance and gene–culture

evolution begins at the end of the last glacial age about 11,500 years ago. Once the environment became warmer and wetter, many human populations developed simple agriculture systems. Not long after that, humans began to domesticate animals (though wolves were likely domesticated long before this) as well as plants. In particular, cattle domestication is thought to have emerged on two separate occasions, once about 10,500 years ago, and again about 8500 years ago (Zhang et al. 2013). The many changes in domesticated animals and plants that have occurred over time are the result of artificial selection, but the process of domestication itself is an example of cultural evolution by humans.

When cattle domestication first began, humans could not tolerate drinking milk as adults. This is because the ability to digest lactose, the main carbohydrate present in milk, was present in young children but lost soon after the age of weaning: After weaning, production of lactase-phlorizin hydrolase (also simply called lactase or LPH)—the enzyme that breaks down lactose and makes milk digestible—tails off dramatically. But concurrently with the domestication of cattle, another cultural breakthrough occurred when people learned to ferment milk into cheese and yogurt. This fermentation dramatically reduced the amount of lactose, and thus created dairy products that could be digested. Anthropologists and archaeologists who have reconstructed human migration patterns after the last ice age believe that the added nutrients provided by dairy products like cheese and yogurt helped humans from the Middle East to move through and settle in southern Europe.

So far, our story is one of cultural change. But it turns to one of gene–culture coevolution about 7500 years ago near what is modern-day Hungary. At that time, a point mutation in a stretch of DNA that codes for the transcription of the *LCT* gene occurred. The *LCT* gene is associated with the production of the lactase enzyme that breaks down lactose, and this point mutation resulted in the ability of adult humans to digest milk by prolonging into adulthood the period over which the lactase enzyme breaks down lactose. Now, because of this genetic change, the cultural system of domestication already in place provided even larger benefits. Individuals with the *LCT* gene no longer had to ferment their dairy products, but instead could drink them fresh and obtain increased amounts of carbohydrates, fats, proteins, and calcium (Bersaglieri et al. 2004). Populations of humans with the *LCT* mutation spread north through Europe (**Figure 18.23**). Indeed, evidence from the sequencing of 8000-year-old human bone fragments from central and eastern Europe indicates that the frequency of the *LCT* mutation was very low at this time, perhaps as low as zero. This frequency rose to 25% about 3000 years ago and today is of the order 80% in these regions (Burger et al. 2007).

Further evidence for gene–culture evolution in the evolution of lactose tolerance can be found in (1) the strong historical and contemporary relationship that exists today between the frequency of dairy farming in an area and the frequency of the *LCT* mutation (Simoons 1969; Kretchmer 1971; Scrimshaw and Murray 1988) and (2) the evidence of strong artificial selection on the genes associated with milk production in cattle in areas where the frequency of the *LCT* mutation is high (Beja-Pereira et al. 2003).

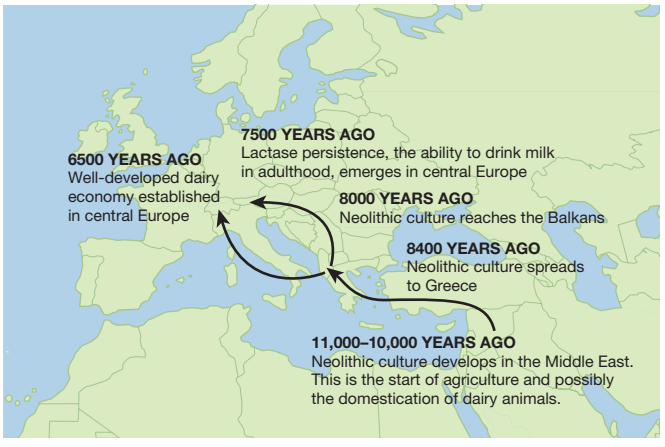

FIGURE 18.23 Lactose tolerance in humans. A schematic of the development of agriculture and the evolution of lactose tolerance in humans. Adapted from Curry (2013).

In this chapter, we have seen the many ways that coevolution has shaped the diversity of life around us and the complex interactions that often define between-species relationships, be they mutualistic, parasitic, predator–prey, or gene–culture interactions. In the next chapter of this book, we turn to an in-depth examination of our own (human) evolutionary history.

SUMMARY

1. Coevolution occurs when evolutionary changes to traits in one species cause natural selection to act in new ways on traits in another species, which in turn feed back to alter the nature of selection on traits in the first species, and so forth.
2. Mutualisms may evolve from initially neutral interactions between species or from interactions in which one species initially benefits and the other is initially unaffected in any way. A mutualism can even evolve from an initially parasitic relationship when the costs and benefits of that parasitic relationship change over time to favor the mutualism.
3. Under certain conditions, natural selection may favor communication between individuals of different species involved in a mutualistic relationship.
4. If the benefits of mutualism to both species are high and the mutualistic relationship has been in place over a long period of evolutionary time, the link between mutualists may lead to cospeciation, in which speciation in one species is associated with speciation in the other.
5. The two most common forms of antagonistic coevolution are that between predator and prey and that between parasite and host.
6. The dynamics of antagonistic coevolution can take the form of an evolutionary arms race.
7. Cospeciation may occur in parasite–host systems. Speciation in hosts can drive speciation in their parasites.
8. Natural selection can result in mutualism between a pair of species in some communities but antagonistic interactions between the same species in other communities. This leads to geographic variation in coevolutionary outcomes, known as mosaic coevolution.
9. Evolutionary biologists and anthropologists have begun examining gene–culture coevolution and its consequences, both within and between species. One of the most well-studied examples of gene–culture coevolution in our species is that of lactose tolerance in certain human populations.

KEY TERMS

antagonistic coevolution (p. 649)
aposematic coloration (p. 665)
Batesian mimicry (p. 665)
cospeciation (p. 659)
cultural evolution (p. 669)
cultural transmission (p. 669)
diffuse coevolution (p. 650)
gene–culture coevolution (p. 669)
mosaic coevolution (p. 667)
Müllerian mimicry (p. 665)
obligate mutualism (p. 660)

REVIEW QUESTIONS

1. What did studying the rate of nucleotide substitution in free-living versus mutualistic fungi allow Lutzoni and Pagel to test about the evolution of mutualism in lichens?
2. What is the difference between mutualistic and antagonistic coevolution?
3. What is diffuse coevolution?
4. In ant species that grow fungal gardens, what benefits do the ants receive? What benefits do the fungi receive?
5. With respect to mutualisms, what is "the cheater problem"?
6. What is cospeciation?
7. What are Batesian mimicry and Müllerian mimicry?
8. What is aposematic coloration?
9. What is mosaic coevolution?
10. What is gene–culture coevolution?

KEY CONCEPT APPLICATION QUESTIONS

11. Make the following argument: Compared to environments with abundant resources and little competition, mutualism is especially likely to evolve in environments that are especially harsh, with little food and much competition.
12. Why might microbes found in the guts of a series of host species and the host species themselves be an especially likely system in which to find cospeciation?
13. Why is an understanding of the natural history of the species being studied so critical to using the mosaic theory of coevolution to make specific, testable predictions?
14. Why do you suppose that communication between mutualistic species might speed up the pace of coevolutionary change?

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19

Human Evolution

◀ A Bornean orangutan (*Pongo pygmaeus*) touches a human's hand.

The Plateau of Tibet is sometimes described as the "roof of the world." This vast region, which borders the Himalayas to the southwest, is the largest and highest plateau in the world, with an average elevation of about 4500 meters above sea level (**Figure 19.1**). The plateau is home to more than 4.5 million people, mostly of the Tibetan ethnic group (**Figure 19.2**). More than half of these people live in cities and settlements above 3500 meters, and more than 600,000 live above 4500 meters: At this altitude crops cannot grow, and many people survive as nomadic yak herders on the high steppes (Wu 2001). At this great altitude, most of us would have a difficult time even breathing, as the partial pressure of oxygen is less than two-thirds of what we experience at sea level. Yet the Tibetans have been living, working, and raising their families here for thousands of years.

How do they do it? Accommodations for life at high altitudes occur on a number of timescales. On a timescale of days to weeks, humans undergo acclimatization, a suite of physiological changes including an increase in

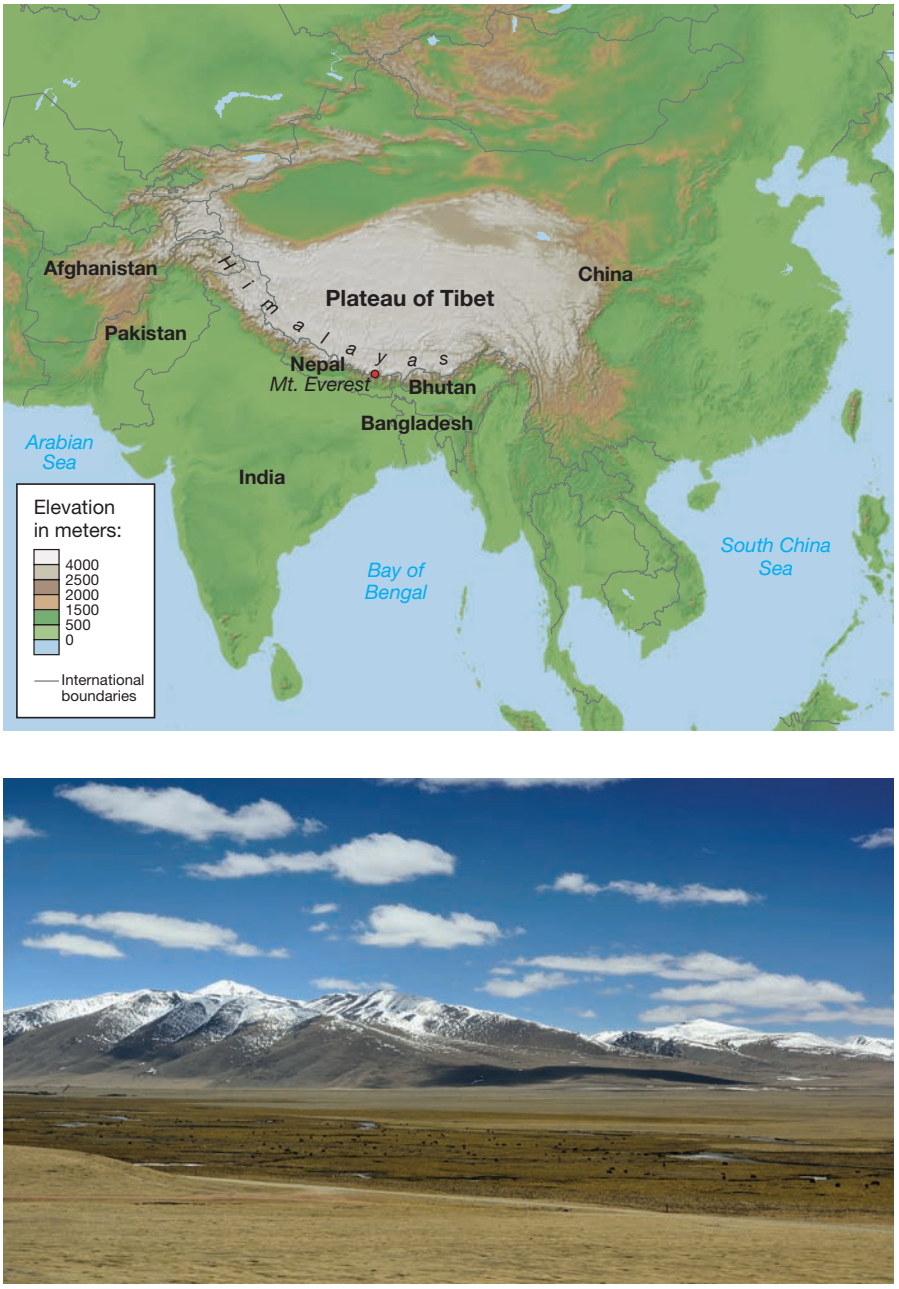

FIGURE 19.1 The Plateau of Tibet. The vast Plateau of Tibet, with an average elevation of about 4500 meters above sea level, is the highest region of the world. This is nearly 3 times as high as the "Mile-High City," Denver, which is at only 1600 meters in elevation.

the number of red blood cells that carry oxygen. On a developmental timescale, we see phenotypic plasticity: Individuals reared at high altitudes have a higher aerobic capacity than those reared near sea level. On an evolutionary timescale, high-altitude populations, including the Tibetan people, have evolved genetic adaptations that improve their physiological function in conditions of low oxygen.

In particular, the Tibetan people typically carry a variant allele at the *EPAS1* locus, which codes for a transcription factor that is active under conditions of low oxygen. This allelic variant contributes to the unusual high-altitude tolerance of Tibetans. Instead of increasing hemoglobin density to compensate for low oxygen,

individuals with this variant allele have low hemoglobin density at altitude—yet they manage efficient oxygen delivery through other mechanisms including increased blood flow (Beall 2007). Population genetic evidence strongly suggests that this is an adaptation. First of all, this *EPAS1* variant is extremely common among Tibetans but rare among the closely related Han Chinese who inhabit the lowlands beyond the Plateau of Tibet. Second, among the Tibetans, the variant allele appears to have been under extremely strong selection over the past 3000 years (Beall et al. 2010; Simonsen et al. 2010; Yi et al. 2010).

Intrigued by these findings, Emilia Huerta-Sánchez and her colleagues set out to uncover more about the history of the *EPAS1* allele in the Tibetan population (Huerta-Sánchez et al. 2014). Their findings were an enormous surprise.

FIGURE 19.2 The Tibetan people. The Tibetan people live at high altitudes across the Plateau of Tibet.

The team began by sequencing the region around the *EPAS1* gene in 40 Tibetans and 40 Han Chinese. Because the Tibetan and Han populations are closely related, allele frequencies tend to be similar in both populations across most of the genome. But around the *EPAS1* locus, the authors found strikingly different allele frequencies and haplotype patterns in Tibetans compared to those in most Han Chinese. This is consistent with the observation of strong selection for different *EPAS1* variants in the two populations. Moreover, computer simulations suggested that the extent of the genetic differences could not be explained by the fixation of a single beneficial allele and hitchhiking at surrounding loci. This haplotype must have been introduced by interbreeding with another population.

The next step was to figure out where the Tibetan haplotype came from. The researchers scanned the worldwide sample of human genomes in the 1000 Genomes Project (2012), as well as genomes from now-extinct members of the **hominin** clade; namely, humans and the extinct species more closely related to humans than to chimpanzees. They found a very surprising result: The only match to the dominant haplotype in the Tibetan population came from genome sequences of an extinct group known as the **Denisovan** hominins!

The Denisovans were more closely related to another *Homo* species, known as the **Neanderthals** (*Homo neanderthalensis*), than to modern humans. The Neanderthal–Denisovan lineage split about 600,000 years ago from the lineage leading to *Homo sapiens*, and then Neanderthals and Denisovans subsequently split about 400,000 years ago. They coexisted with the other *Homo* lineages until about 30,000 years ago, and we know from other lines of evidence that Denisovans and *Homo sapiens* interbred in Asia about 40,000 years ago (Meyer et al. 2012, 2013). It appears that this process of interbreeding provided the human population with a source of genetic variation that proved to be adaptive in modern human groups that migrated to high altitudes (**Figure 19.3**).

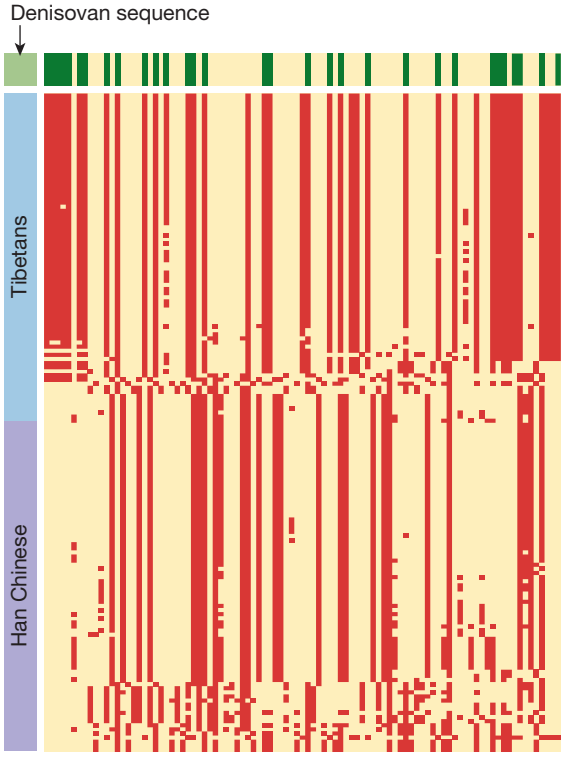

FIGURE 19.3 In the *EPAS*1 region of the genome, Tibetans share an extended haplotype structure with Denisovans. This haplotype structure is rare, though not entirely absent, among the Han Chinese. Huerta-Sánchez and colleagues inferred that this region of the genome was acquired from the Denisovans by interbreeding prior to the divergence of the Han and Tibetan populations, followed by strong positive selection in the Tibetans but not in the Han. In this diagram, each row represents the haplotype of a single individual; only polymorphic sites are shown. Tan represents the ancestral variants, and green or red represents derived ones. Adapted from Huerta-Sánchez et al. (2014).

Later in this chapter, we will revisit the Denisovans. For now, we note that genomic sequencing studies are revolutionizing our understanding of human origins and prehistory. Comparative genomics has resolved the evolutionary relationships among the great apes beyond a shadow of a doubt. Our growing technological capability to sequence ancient DNA is helping to fashion an increasingly detailed picture of hominin migration and interbreeding over the past 100,000 years. Yet genetic methods leave a huge gap between our divergence from the chimpanzee lineage about 5 million years ago and the movement of early *Homo sapiens* into Europe and Asia about 100,000 years ago. Thus, most of what we know of our evolutionary history during this intervening period comes from morphological and archaeological evidence.

In this chapter, we will address the following questions:

- How are humans related to other primate species and to the great apes in particular?
- What are the evolutionary radiations and extinctions that occurred in the lineage leading to humans after it diverged from the lineage leading to chimpanzees?
- When, where, and from what ancestral population did modern humans (*Homo sapiens*) first evolve?
- To what extent did humans interbreed with other hominin groups?
- How can we use the genetic structure of human populations to map the paths along which modern humans expanded out of Africa 60,000 years ago and colonized the entire globe?

19.1 Evolutionary Relationships among the Great Apes

In Chapter 1, we discussed how Darwin and Huxley used phenotypic characters to infer that humans are **hominoids**—members of a superfamily known as the Hominoidea. This clade, also known as the apes, consists of eight living genera: orangutans (*Pongo*), gorillas (*Gorilla*), chimpanzees (*Pan*), humans (*Homo*), and four gibbon genera. Genomic-scale sequence data definitively confirm Darwin and Huxley's conclusion.

However, phenotypic characters were not sufficient to resolve the relationships within the Hominoidea. Early molecular phylogenies were also inconclusive, in that different studies produced conflicting results. But today, genetic analyses using a large number of loci have shown beyond a shadow of a doubt that humans are the sister group to the two species of chimpanzee: the common chimpanzee (*Pan troglodytes*) and the bonobo (*Pan paniscus*). Gorillas are more distantly related, orangutans yet more so, and gibbons are the most distant (**Figure 19.4**). The nomenclature of the hominoidea is illustrated in **Figure 19.5**.

At the majority of loci, humans are more closely related to chimpanzees than to any other living species. At approximately 20% of loci, however, humans are more closely related to gorillas than to chimpanzees (Chen and Li 2001; Patterson et al. 2006; Prado-Martinez et al. 2013). How could this be, given that humans

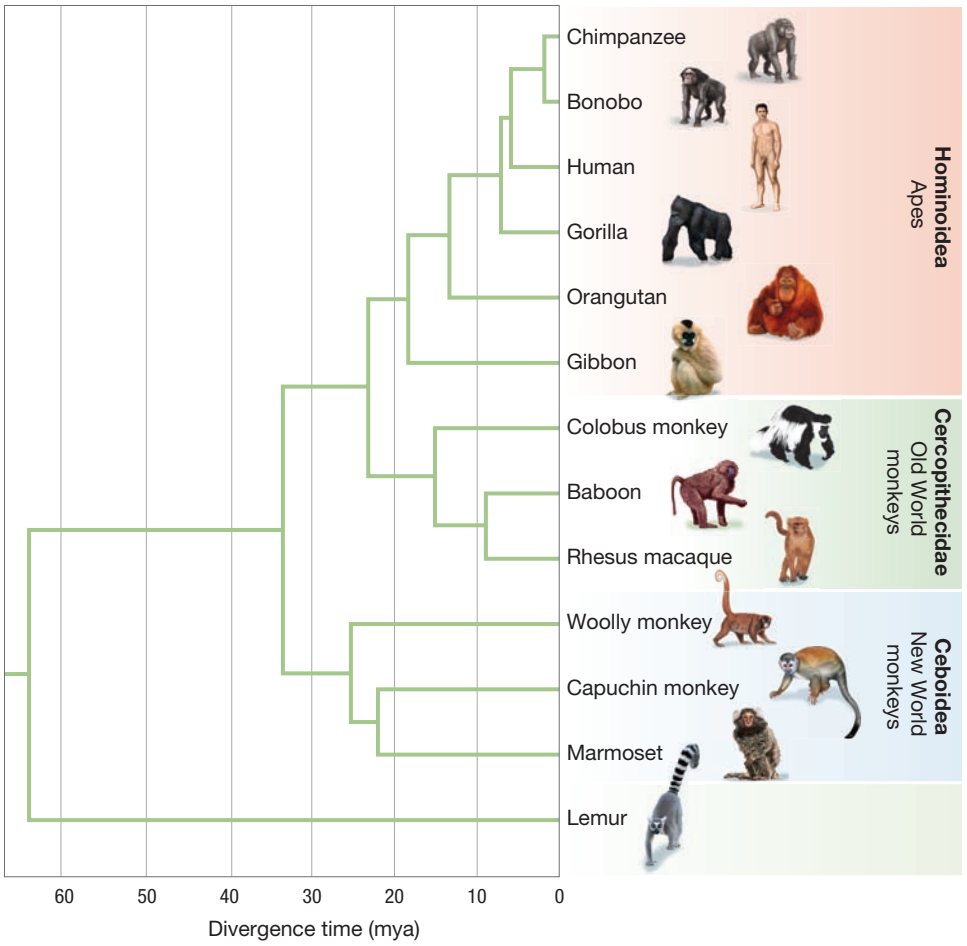

FIGURE 19.4 A phylogeny of the primates. Humans are part of the superfamily Hominoidea and are most closely related to the chimpanzee and bonobo. Also shown are the family Cercopithecidae (Old World monkeys) and the superfamily Ceboidea (New World monkeys). This phylogeny is presented as a chronogram, with the branch lengths indicating the divergence times in millions of years before the present (mya, million years ago). Adapted from Enard and Pääbo (2004).

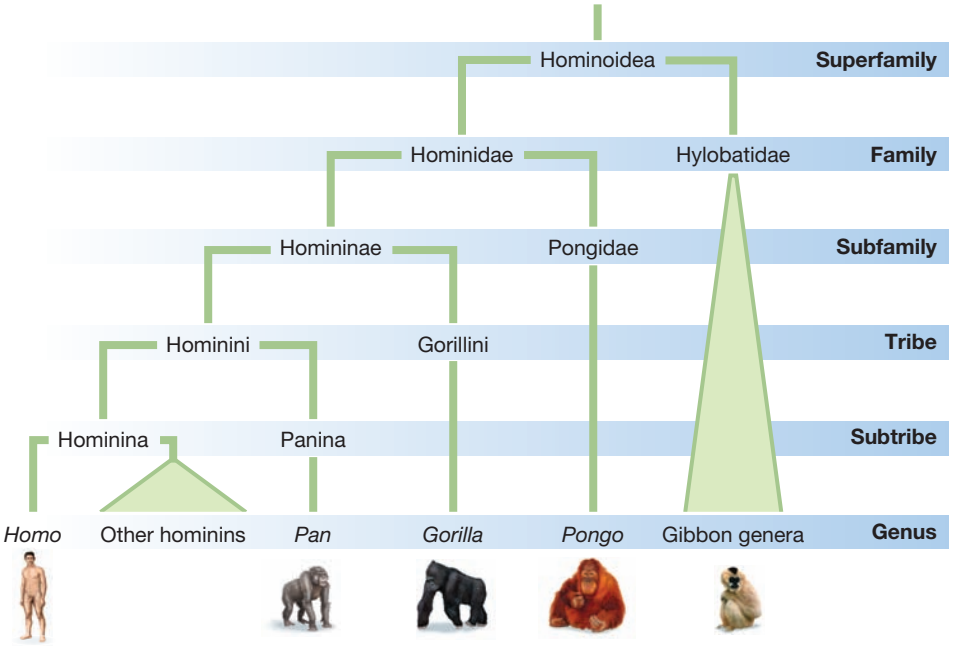

FIGURE 19.5 Hominoid nomenclature. Each clade along the branch leading to humans carries a name that is based on the Latin root *Homo* or *homin*, meaning "man." The subtribe Hominina consists of all Hominini more closely related to modern humans than to chimpanzees, including both the genus *Homo* (humans) and other archaic hominin genera such as *Australopithecus* and *Paranthropus.* Because our understanding of hominoid phylogeny has been in flux until recently, the nomenclature for these clades has changed a number of times and is still used in conflicting ways in the current literature. Adapted from Mann and Weiss (1996).

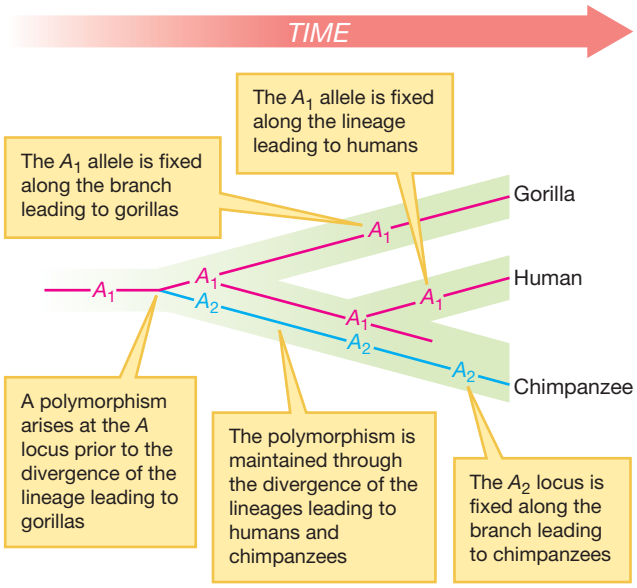

FIGURE 19.6 At some loci, humans may be more similar to gorillas than to chimpanzees. If a polymorphism is maintained in the population from the divergence of gorillas to the divergence of chimpanzees, humans and gorillas can end up sharing a common allele that differs from that in chimpanzees. Adapted from Enard and Pääbo (2004).

and chimpanzees share a more recent common ancestor? In Chapter 4, we learned of one possible reason that two distinct branches might share common characters: The similarities in gorillas and humans could be homoplasies or symplesiomorphies. But there is another process that also generates this pattern. This process is called **deep coalescence**, or incomplete lineage sorting, because the coalescent event between the alleles at this locus predates the speciation event separating the lineages of interest (Rogers and Gibbs 2014).

Figure 19.6 illustrates deep coalescence. The locus shown is polymorphic for A_1 and A_2 at the time of the split between the human–chimp and the gorilla lineages, and this polymorphism is maintained until the split between the human and chimp lineages. By chance the same allele, A_1, is fixed in the lineages leading to humans and in gorillas, while the other allele, A_2, is fixed in the lineage leading to chimpanzees. The result is that at this particular locus, humans and gorillas will share a common allele that is different from the allele that we observe in chimpanzees. Deep coalescence can be exacerbated by gene flow between incipient species that are not yet fully genetically isolated (Mailund et al. 2014). Indeed, some researchers have argued that gene flow of this kind may have occurred between humans and chimpanzees after the initial separation between these lineages. While these claims are controversial, if interbreeding had occured, this would further blur the phylogenetic signal that we use to resolve the human–chimpanzee–gorilla split (Patterson et al. 2006).

This example highlights the distinction that we drew in Chapter 8 between *species trees* and *gene trees*. Often, single-gene trees will closely reflect species trees. But along branches of the species tree that are short (that is, those that represent a relatively small number of generations) and wide (that is, those that reflect a large population size), we are particularly likely to observe deep coalescence, as in case B of **Figure 19.7**. Because the brown-shaded branch in case A is long and narrow, coalescence along this branch is likely, and deep coalescence beyond this branch is unlikely. By contrast, in case B, the brown-shaded branch is short and wide, making coalescence along the branch less likely and deep coalescence beyond this branch more likely.

Deep coalescence is particularly common in the human–chimpanzee–gorilla clade. This is because of a short, wide branch much as in case B in Figure 19.7.

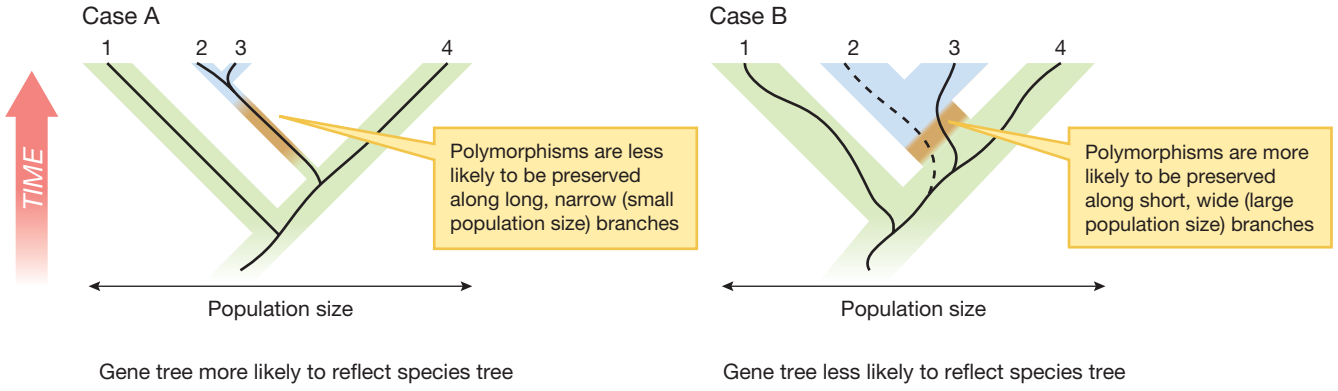

FIGURE 19.7 Deep coalescence is more likely along short, wide branches. Gene copies from species 2 and 3 are more likely to coalesce along the brown-shaded branch in case A than in case B. Thus, any single-gene tree is more likely in case A than in case B to reflect the species tree. Adapted from Maddison (1997).

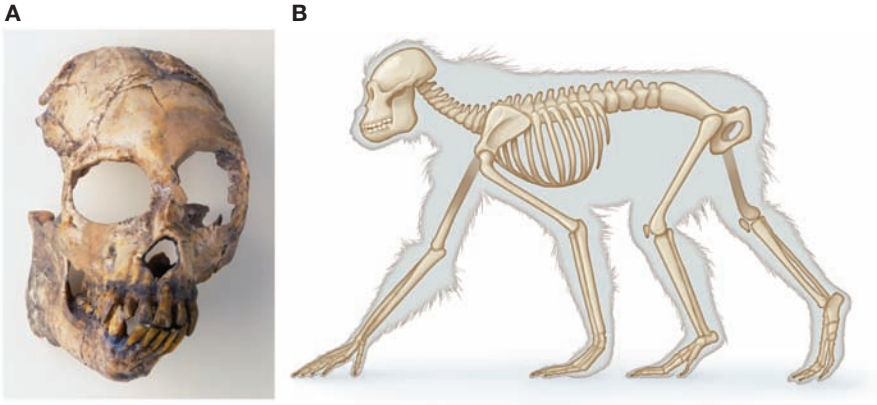

FIGURE 19.8 Proconsul africanus. *Proconsul* is considered by most researchers to be an early genus within the hominoid clade. **(A)** *Proconsul* skull. **(B)** *Proconsul* skeleton.

As illustrated in the chronogram in Figure 19.4, the split between humans and chimpanzees occurred quite soon after the split between gorillas and humans–chimps. As a result, a large number of loci remained polymorphic from the divergence between humans–chimps and gorillas right through to the divergence between humans and chimps. At well over 30% of loci, either humans and gorillas are sister groups, or gorillas and chimpanzees are sister groups—and thus the single-gene trees at these loci do not reflect the species tree for these three species.

Although genetic data firmly establish the phylogenetic relationships among humans and our closest living relatives, we know considerably less about the phylogeography of these species. While we know what the pattern of branching ancestry is, and we have a good estimate of when the speciation events occurred, we do not know precisely *where* the various hominoid species first arose. But we do have a decent picture of the *early* origin of this clade: The Hominoidea appear to have arisen in East Africa about 20 million years ago. We know this on the basis of fossils such as those of the genus *Proconsul* (**Figure 19.8**). But, while *Proconsul* was likely closely related to the common ancestor of all living apes, this genus of tailless, arboreal primates is anatomically different from extant ape species. Notably, *Proconsul* lacked the limb mobility that characterizes modern apes and allows them to swing by their arms from tree limbs (Larsen 2008).

Details of the origin of the great apes, the Hominidae, are less clear. Fossils dating to about the time of divergence of this clade—14 million years ago—have been found in Africa, Asia, and Europe alike. Where did these species arise? A sparse fossil record from this period until about 5 million years ago has hampered our progress in uncovering the answers (Hill and Ward 1988).

Many investigators suspect an East African origin for this clade, followed by subsequent migration into Europe and Asia (Andrews 1992). But other evidence is consistent with a Eurasian origin, so the topic remains an active area of investigation (Moya-Sola et al. 2009).

19.2 The Hominin Clade

Abundant genetic evidence from humans, gorillas, orangutans, and chimpanzees has allowed us to resolve the phylogenetic relationships of these species. It is much harder to resolve the phylogenetic history of the hominin clade. The main reason is that all species in this clade are extinct except for our own, and we have very limited information about the others. Until very recently, evolutionary biologists,

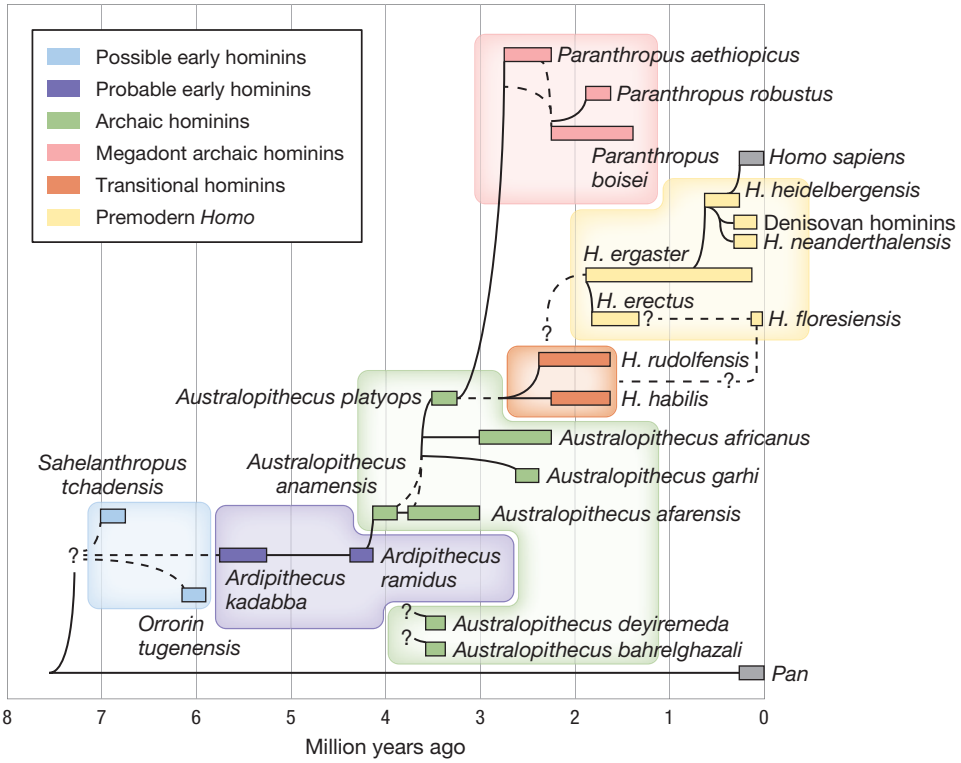

FIGURE 19.9 A chronogram of hominin evolution. Major *grades*—that is, groups of morphologically similar species that do not qualify as monophyletic clades—are indicated by color. Adapted from Strait et al. (2007) and Wood and Lonergan (2008).

paleontologists, and anthropologists have had to rely exclusively on fossil evidence to reconstruct the evolutionary history of the hominins. However, recent advances in sequencing ancient DNA, even from fossils as old as 700,000 years (Orlando et al. 2013), have driven an explosion in molecular phylogenetic approaches to understanding the complex evolutionary history of the hominin clade. As of 2015, whole-genome sequence data have been reported for Neanderthal samples at least 50,000 years old (Green et al. 2010); for a 40,000- to 80,000-year-old Denisovan hominin from southern Siberia (Reich et al. 2010; Meyer et al. 2012); and for various fossil remains of *Homo sapiens* including one genome from an individual who died about 45,000 years ago (Fu et al. 2014). Going back even further in time, researchers were able to sequence a mitochondrial genome from the femur of a 400,000-year-old hominin who was probably a member of the Denisovan lineage (Meyer et al. 2014).

As a result of the limited number of specimens found to date and the difficulty of determining species boundaries from fossil evidence, hominin classification remains an active area of scientific debate, and little consensus exists on exactly where to draw species boundaries. What is clear, however, is that the story of hominin evolution has not been a single linear progression. Rather, just as elsewhere in the tree of life, the story of this clade has been a story of branching evolution in which most species are now extinct. **Figure 19.9** shows a chronogram of hominin evolution, indicating the age of each fossil and the likely phylogenetic relationships among them. This figure illustrates the numerous branching speciation events that have occurred along the lineage leading to modern humans since its divergence from the lineage leading to chimpanzees.

During much, if not all, of this period, multiple hominin species coexisted. Only for the past 30,000 years, since the disappearance of the Neanderthals, Denisovans, and *Homo erectus,* have modern humans been the sole representatives of the hominin lineage across the main of Eurasia. A fourth hominin species, *Homo floresiensis,*

persisted even longer on the remote Indonesian island of Flores until perhaps as recently as 12,000 years ago.

The rise of the hominin lineage coincided with a change in the ecology of the African tropics about 6 million years ago. Cooling temperatures and seasonal patterns of rainfall caused huge expanses of the African continent to shift from tropical forest to savannah. While the chimpanzee and gorilla lineages remained in the forest, the Hominina moved onto the savannah and began to adapt to life in this new environment. One of the most important early changes in hominins was the evolution of **bipedal locomotion**—the ability to walk upright on two feet. A number of hypotheses have been proposed for why bipedal location was favored (Boyd and Silk 2009). Walking upright may have been more efficient energetically than the knuckle-walking gaits of chimpanzees. Standing upright may have helped hominins to keep cool in the unfiltered sunshine of the savannah: Standing upright, less surface area is exposed to overhead rays, and more surface area is exposed to cooling winds. Bipedal locomotion frees up the hands for carrying items and for plucking fruit from small trees. And while evolution cannot plan ahead, once bipedal locomotion evolves, the hands are released from selection for walking efficiency and can evolve toward greater manual dexterity.

Thus, the distinguishing features of the hominin lineage as a whole are *not* the large brains, language, and tool use that we associate with premodern and modern humans. These features evolved long after the divergence from chimpanzees. Rather, the principal distinguishing features of this lineage are changes in dentition, including the loss of the large canines seen in other apes (**Figure 19.10**), and changes in skeletal structure that facilitated the upright bipedal mode of locomotion discussed earlier (**Figure 19.11**). These skeletal changes include development of longer legs, the loss of an opposable (thumb-like) big toe, changes

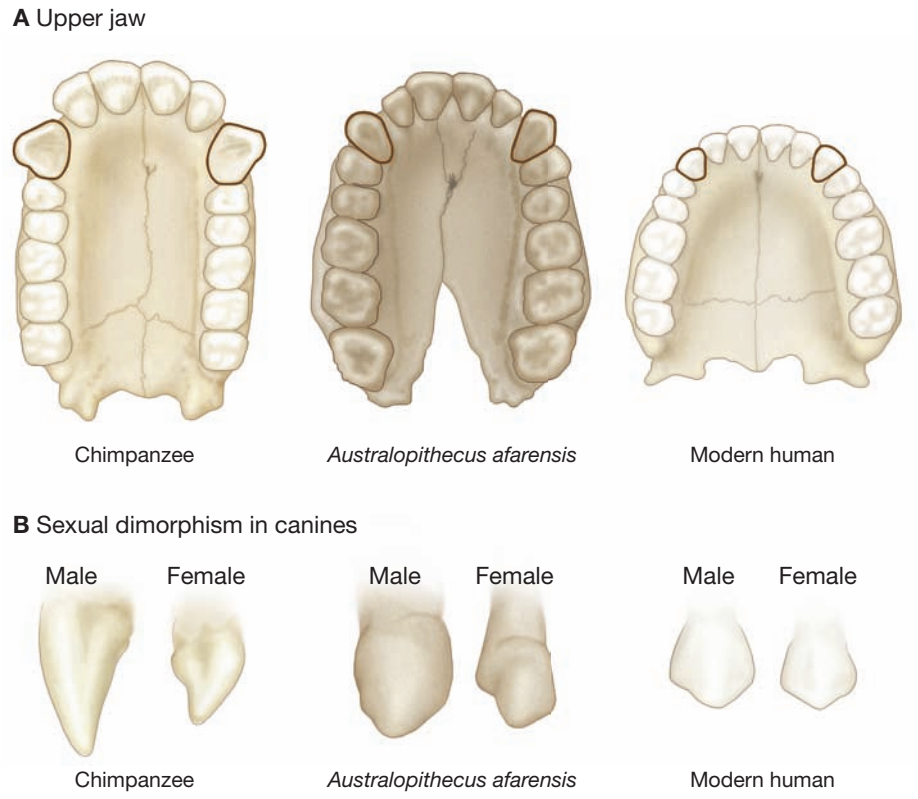

FIGURE 19.10 Large canines have been lost in the hominin lineage. The large canine teeth seen in the male chimpanzee (*Pan troglodytes*) and other great apes, highlighted in the upper jaw figures **(A)**, are substantially reduced in the archaic hominins and further reduced in modern humans **(B).** Adapted from Boyd and Silk (2009).

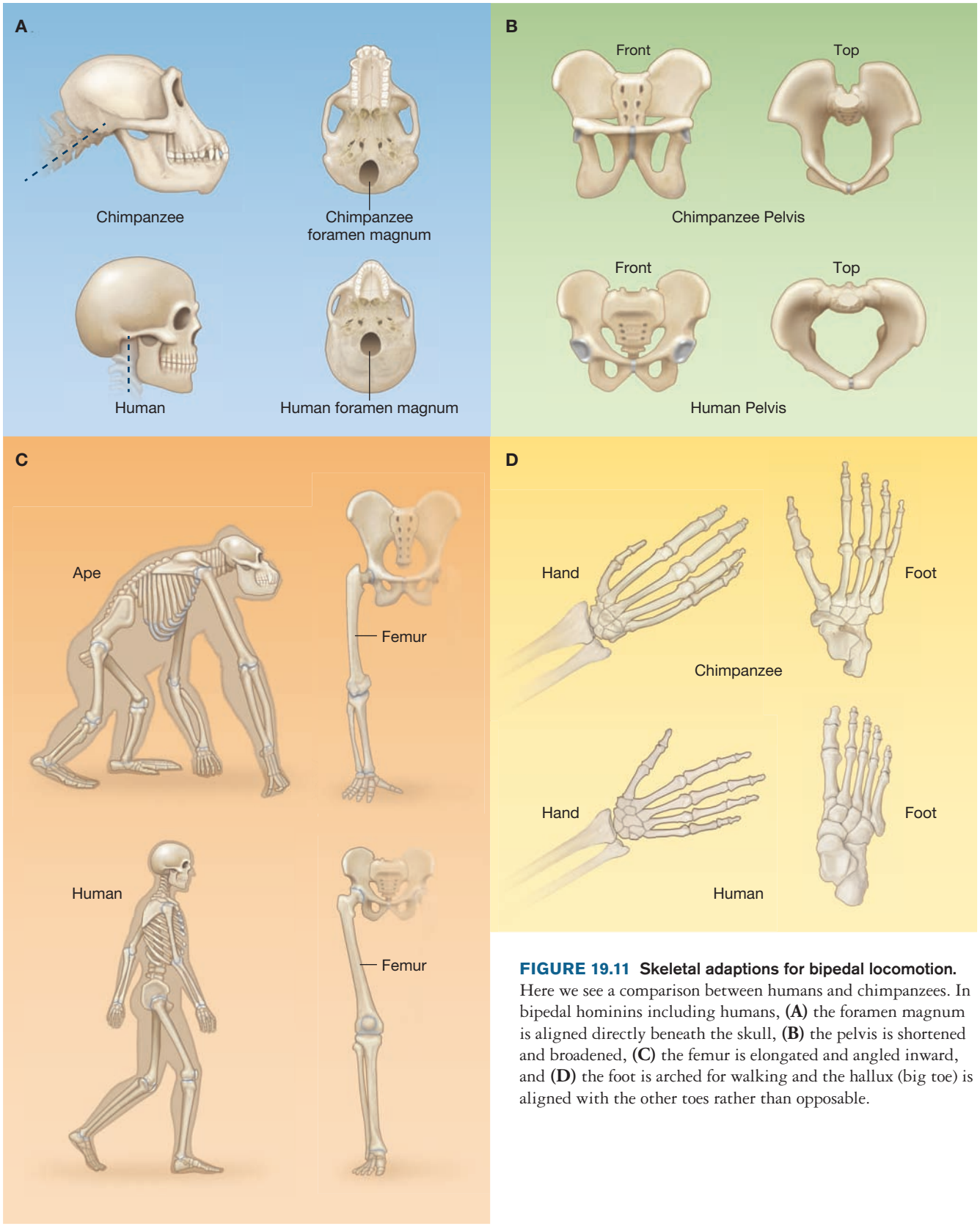

FIGURE 19.11 Skeletal adaptions for bipedal locomotion. Here we see a comparison between humans and chimpanzees. In bipedal hominins including humans, **(A)** the foramen magnum is aligned directly beneath the skull, **(B)** the pelvis is shortened and broadened, **(C)** the femur is elongated and angled inward, and **(D)** the foot is arched for walking and the hallux (big toe) is aligned with the other toes rather than opposable.

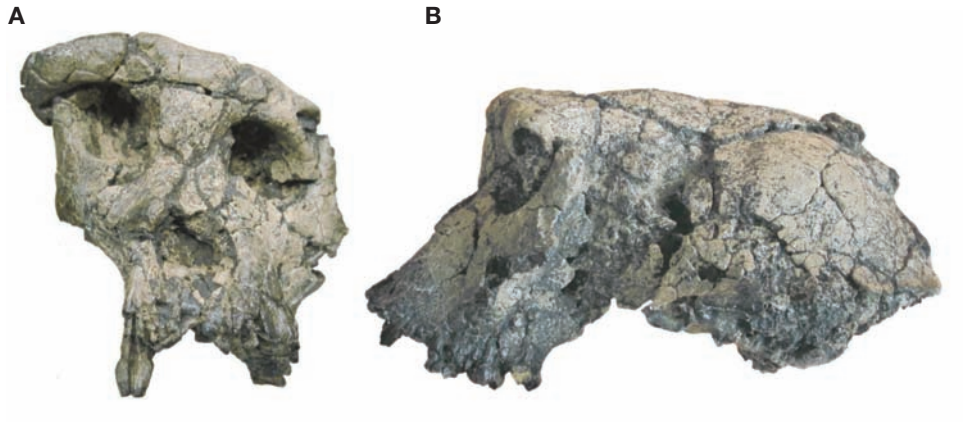

FIGURE 19.12 A fossilized skull of *Sahelanthropus tchadensis*. **(A)** In the front view, the distortion of the skull is apparent. **(B)** Side view of the skull.

in pelvic geometry and in the structure of the foot bones, and a shift of the foramen magnum—the opening in the base of the skull for the spinal cord—to directly below the skull (Larsen 2008; Boyd and Silk 2009).

The First Hominins

The earliest putative hominin fossils, those dating to soon after the divergence of humans and chimpanzees, are the most difficult to place definitively within the hominin lineage. For one thing, these fossils tend to be in poorer condition than other hominin fossils because of their relatively great age. For example, the fossilized skull of *Sahelanthropus tchadensis*, discovered in Chad in 2001 by Brunet and colleagues (Brunet et al. 2002), has been distorted by geological forces to the point that it is challenging to reconstruct the original skull geometry (**Figure 19.12**).

But there is an even bigger conceptual problem: Close to the human–chimpanzee divergence, it is very difficult to tell, based on morphological characters, whether a given fossil belongs to the hominin lineage or to the panin (chimpanzee) lineage. On the basis of the orientation of the skull and the dental anatomy, however, it is most likely that *S. tchadensis* lies along the hominin lineage. Another possible early hominin in this category is *Orrorin tugenensis*, for which a complete skull has yet to be discovered. Skeletal features of these species suggest an improved capacity for upright locomotion relative to ancestral forms.

Next to appear in the fossil record are a set of probable early hominins. Researchers have faced similar problems with the condition of these fossil remains, but advances in scanning and computer imaging have enabled sophisticated reconstructions of cranial shape based on skull fragments. **Figure 19.13** shows a set of skull fragments of the early hominin *Ardipithecus ramidus* after initial physical

FIGURE 19.13 Reconstructing an *Ardipithecus ramidus* skull by computer imaging. **(A)** Skull fragments after initial physical reassembly. **(B)** Computer reconstructions of cranial morphology derived from these fragments. The face of *A. ramidus* is considerably flatter than that of a chimpanzee (Suwa et al. 2009).

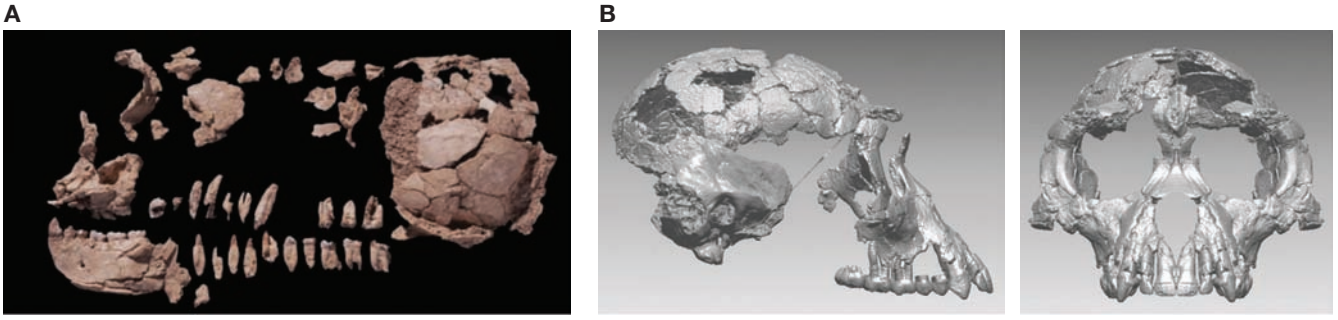

FIGURE 19.14 Three archaic hominins. These archaic hominin species had small brain sizes compared to humans, but they appear to have walked upright at least some of the time, and they may also have fashioned stone tools. **(A)** *Australopithecus platyops*. **(B)** *Australopithecus afarensis*. **(C)** *Australopithecus africanus*.

FIGURE 19.15 Very early hominin tool use. This stone tool was created by archaic hominins about 3.3 million years ago. It is one of many found at an archaeological site in West Turkana, Kenya. Photo courtesy of Sonia Harmand.

FIGURE 19.16 Oldowan chopper tools. The tools of the Oldowan industry were formed by hitting a large core stone with a hammer stone, causing flakes to break off from the core. Found in Meka Kunture, Ethiopia, the choppers shown here were produced about 1.7 million years ago.

assembly, and then as a computer-imaging reconstruction. Like *S. tchadensis* and *O. tugenensis*, *A. ramidus* had a small cranial capacity and some capacity for upright locomotion, but the foot morphology of *A. ramidus*, with a gripping opposable big toe, suggests that it was at least partially arboreal.

The Archaic Hominins

Archaic hominins appear next in the hominin lineage (**Figure 19.14**); these shared an increasing number of physiological features with the genus *Homo*. They appear to have been fully capable of bipedal locomotion. One of these species, *Australopithecus afarensis*, is well known in the popular consciousness as the 3.2-million-year-old fossil "Lucy." Other species of archaic hominins appear to have been present at the same time, including several other *Australopithecus* species: *A. platyops*, *A. africanus*, *A. bahrelghazali*, and *A. deyiremeda* (Guy et al. 2008; Haile-Selaisse et al. 2015).

Recent evidence suggests that one or more of the archaic hominin species fashioned stone tools and used them when scavenging for meat. For a long time, hominin tool use was only thought to go back to about 2.6 million years ago. Then in 2010, one research team found 3.4-million-year-old fossilized ungulate bones in Ethiopia. These bones had multiple cut marks that appear to have been created by stone tools (McPherron et al. 2010). At the time, no tools that old had been found before, and some researchers questioned whether these marks had truly been made by tools at all. In 2015, another group working in Kenya found numerous stone tools, mostly striking implements and anvils, dating to approximately 3.3 million years ago (**Figure 19.15**) (Harmand et al. 2015). While some debate remains about these early indications of tool use, later archaic hominins certainly fashioned stone tools for cutting, pounding, scraping, and boring. These tools, the first of the so-called *Oldowan industry*, date back as far as 2.6 million years ago (Semaw et al. 2003; Larsen 2008). Oldowan, or *mode 1*, tools, relatively simple in manufacture, included chipped flakes obtained by striking a core stone at an angle with another stone used as a hammer (**Figure 19.16**).

FIGURE 19.17 *Paranthropus* species. This clade was a side branch off of the hominin lineage leading to modern humans. Note the massive ridges at the top of the skull and broad, protruding cheekbones; these provided attachment points for huge chewing muscles. **(A)** *Paranthropus boisei*. **(B)** *Paranthropus aethiopicus*. **(C)** *Paranthropus robustus*.

As we mentioned, hominin evolution has not been single progression from a common ancestor with chimpanzees to modern humans, but rather a branching process of speciation just as we would expect with any other species. One of the most interesting side-branches comprises the so-called megadont archaic hominins. This group, sometimes known as the *robust australopithecines*, appears to form a proper clade: the genus *Paranthropus*. This morphologically unusual clade of hominins is characterized by huge muscle attachment regions on the skull that confer the distinctive top ridges and broad, protruding cheekbones on the skulls in **Figure 19.17**. These areas anchored the massive chewing muscles that *Paranthropus* possessed, and the protruding cheekbones allowed larger chewing muscles to pass beneath them. The teeth of these species were correspondingly large; hence, the name *megadont*, which means "big teeth." As a result, members of this clade were well suited for grinding low-quality food sources. While an evolutionary dead-end, *Paranthropus* was contemporaneous with the later archaic hominins discussed earlier, and even with some early members of the genus *Homo.*

The Genus *Homo*

Approximately 2.3 million years ago, the first members of the genus *Homo* appeared. These **transitional hominins** bridge the gap between some of the archaic hominins we considered in the previous subsection and the so-called premodern hominins that were the immediate ancestors to *Homo sapiens.* While both had brain sizes smaller than those of modern or premodern humans, *Homo habilis* and *Homo rudolfensis* had cranial capacities that were greater than those of the archaic hominins before them, as well as other morphological features that more closely resemble those of modern humans (**Figure 19.18**). They also appear to have made abundant use of tools.

Homo ergaster appeared in Africa and Europe and the stockier, heavier-skulled *Homo erectus* appeared in Asia about 1.9 million years ago. Whether these represent two separate species or regional forms of a single species, as some researchers believe, their body plans closely resembled modern humans and thus we consider them to be **premodern hominins** (**Figure 19.19**). Relative to archaic and transitional

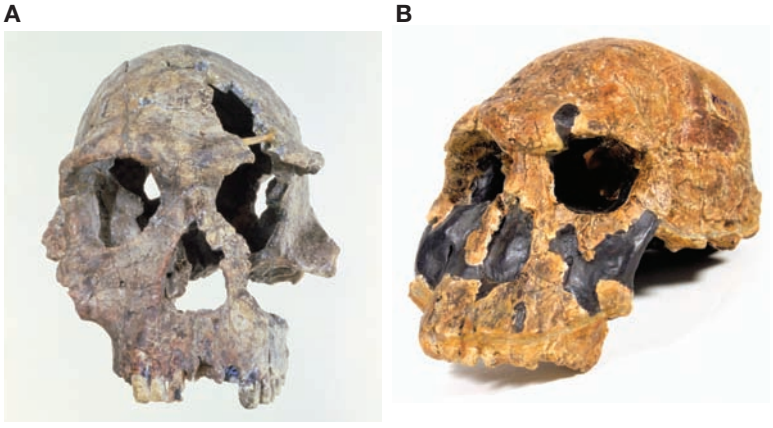

FIGURE 19.18 Transitional hominins. Skulls of the transitional hominins: **(A)** *Homo habilis* and **(B)** *Homo rudolfensis.*

FIGURE 19.19 Premodern hominins. **(A)** *Homo ergaster*, *(B) Homo erectus*, and **(C)** *Homo heidelbergensis. Homo heidelbergensis* appears much later in the fossil record and is a possible ancestor to both modern humans and Neanderthals.

FIGURE 19.20 An Acheulean hand axe. This tool, found in Europe and dating to about 400,000 years ago, exhibits a much more complex structure than that of Oldowan tools and demonstrates careful planning and substantial skill in construction.

FIGURE 19.21 A 200,000-year-old stone tool covered in pitch. This stone blade was created by *Homo neanderthalensis* about 200,000 years ago. The bottom of the blade is coated with an adhesive pitch fashioned by heating birch sap over a fire. This pitch would have been used to affix the stone to some type of handle.

hominins, *Homo ergaster* was taller—1.5 to 1.8 meters—with longer legs and a considerably larger brain. As indicated by the name—*Homo ergaster* means "working man"—this species created more elaborate tools than did previous hominins or *Homo erectus* in Asia. While the manufacture of Oldowan tools continued around the world, a new class of tools known as the *Acheulean industry*, or *mode 2*, tools appeared in Africa about 1.6 million years ago. These tools, known as *bifaces*, were formed by chipping away at a core stone to produce tools with sharp edges running along both sides (**Figure 19.20**).

In addition to fashioning and using stone tools such as hand axes, *Homo ergaster* and *Homo erectus* were making use of fire to cook food and provide warmth by 400,000 years ago at the very latest. Because it is difficult to distinguish between hominin-controlled fires and natural fire sources such as brush fires ignited by lightning strikes, the best early evidence for fire use comes from signs of fire deep within caves—unequivocal examples date back to 400,000 years ago (Karkanas et al. 2007; Roebroeks and Villa 2011). More recent hominin species also used fire in toolmaking. By 200,000 years ago, Neanderthals in Europe were using fire to create pitch from birch sap, with which they could attach stone tools to handles of other material (Mazza et al. 2006) (**Figure 19.21**). And by 164,000 years ago, early *Homo sapiens* were using fire to harden their stone tools (Brown et al. 2009).

Some researchers have proposed that our distant ancestors have been using fire for much longer—and that the use of fire played a very important role in shaping the course of hominin evolution. Anthropologist Richard Wrangham notes that raw foods, particularly raw tubers, take a long time to chew, are difficult to digest, and provide a poor source of calories. When cooked, the same foods offer large energetic stores that are easily consumed and readily processed by the digestive system. About 1.9 million years ago, the hominin lineage leading to modern humans underwent a number of morphological changes that appear to be associated with reduced nutritional challenge: a decrease in the size of the jaw and molars, a decrease in the size of the digestive tract, and an increase in female body mass. Most importantly, this period sees a massive growth in the size of the brain, imposing intense energy demands (Herculano-Houzel 2012) that would be difficult to meet on a diet of raw foods alone. Wrangham's *cooking hypothesis* posits that all of these changes, along with major shifts in life history strategies and behavior, were driven by the acquisition of fire technology in early *Homo ergaster* and the subsequent availability of a whole new range of high-energy foods (Wrangham et al. 1999; Wrangham 2009). Wrangham's hypothesis is intriguing but far from universally accepted. To date, one of the biggest challenges to this hypothesis is that we have very scant evidence that hominins controlled fire prior to 400,000 years ago. However, a recent study offers some hope in this regard: Burn traces in South Africa's Wonderwerk cave date to about 1 million years ago and suggest some use of fire by *Homo ergaster* at this early date (Berna et al. 2012).

Some time between 800,000 and 500,000 years ago, an additional species arose on the lineage leading to *Homo sapiens.* This species, known as *Homo heidelbergensis,* derived from *Homo ergaster* but had a higher and rounder cranium and a larger brain. Its facial structure was more similar to that of modern humans as well, though it retained a powerful brow ridge. *Homo heidelbergensis* initially created Acheulean tools, and numerous lines of evidence suggest that *Homo heidelbergensis* hunted big game to a greater degree than its predecessors. By 300,000 years ago, *Homo heidelbergensis* developed a new and more sophisticated toolmaking technology known as the

Levallois technique, or *mode 3* technology (**Figure 19.22**). These tools were more elaborately flaked and were sometimes attached to handles. Most researchers believe that *Homo sapiens* evolved from *Homo heidelbergensis* about 200,000–130,000 years ago.

Two additional *Homo* species have been discovered recently. In one of the most astonishing fossil finds of the past century, the skeleton of a tiny hominin was discovered in 2004 in the Liang Bua cave on the Indonesian island of Flores (Brown 2004; Morwood et al. 2005). After a period of initial skepticism—some anthropologists argued that the Flores individual was simply a deformed *Homo sapiens*—remains from several more individuals were found and determined to come from a new species, dubbed *Homo floresiensis* (**Figure 19.23**). The individuals of this species were very small, around a meter in height, and radiometric dating suggests that they went extinct only 12,000 years ago. Archaeological evidence suggests the presence of hominins on Flores since 840,000 years ago, but is unclear whether these early individuals were also *Homo floresiensis.*

FIGURE 19.22 Stone tools created using the Levallois technique. In this technique, a sloped base is prepared and then a thinner flake, the tool itself, is split off from the base.

FIGURE 19.23 *Homo floresiensis.* A comparison of skulls from *Homo floresiensis* (left) and *Homo sapiens* (right) reveals that *H. floresiensis* has the distinctive skull shape of the *Homo* genus, with a flat face and reduced dentition—yet this species has a skull that is tiny in comparison to the other members of the *Homo* clade.

Homo floresiensis walked upright, although their feet were very large compared to those of other *Homo* species, and the lack of a raised arch suggests an early divergence from the *Homo* lineage (Jungers et al. 2009). Their brains were tiny compared to those of other *Homo* species, and physiologically they shared many traits with archaic hominins such as *Australopithecus afarensis*. But their flat faces and overall skull geometry place them firmly within the genus *Homo*. Stone tools and charred, cut animal bones found alongside *Homo floresiensis* remains hint that they likely created tools, used fire, and hunted prey, including the *Stegodon* pygmy elephants that inhabited the island. Many anthropologists believe that their minute stature is the result of a common process known as *insular dwarfing*, in which species isolated on an island decrease in size as an evolutionary response to the combination of reduced food availability and reduced predation.

Numerous mysteries surround *Homo floresiensis*; among them perhaps the greatest is where this species fits into the hominin phylogeny. Researchers largely agree that *Homo floresiensis* is more distantly related to *Homo sapiens* than the Neanderthals or the Denisovans. The most likely hypothesis is that they are the descendants of *Homo erectus,* from the initial expansion of that species across Asia. On the basis of morphological analysis, one group of researchers has suggested that they may have branched off even earlier from the lineage leading to *Homo sapiens*—perhaps more than 2 million years ago from the transitional hominins *Homo habilis* and *Homo rudolfensis* (Argue et al. 2009). If so, was there an earlier expansion out of Africa than the expansion of *Homo ergaster* 1.8 million years ago or did *Homo floresiensis* arise in Africa and migrate subsequently? Yet other researchers have conjectured that *Homo floresiensis* even could be descended from archaic hominins such as *Australopithecus*. At present, we don't know which of these hypotheses is correct, but further fossil remains of this species or other *Homo* species in the area could help us reconstruct the story. Ancient DNA evidence would be even more useful, but to date researchers have been unable to extract intact DNA from the limited materials available, in part because the high temperatures of the island accelerate DNA degradation. Techniques for sequencing ancient DNA continue to improve, however, and researchers are

FIGURE 19.24 Skeletal remains of *Homo naledi*. More than 1500 bones, bone fragments, and teeth from at least 15 differents individuals were found in the Dinaledi Chamber of the Rising Star cave system.

optimistic that we will soon learn much more about the phylogenetic history of *Homo floresiensis.*

In September 2015, another remarkable fossil discovery was announced. Deep in South Africa's Rising Star cave system, researchers discovered skeletal remains from a previously unknown species, which they named *Homo naledi* (**Figure 19.24**). Rather than finding a few fragments of bone as is common in such situations, the researchers found extensive skeletal remains (Berger et al. 2015). In some ways, *H. naledi* resembles archaic hominins. Their limb morphology is similar in many ways to that of archaic hominins, and though this species stood about 1.5 meters tall and weighed about 45 kilograms, the brain is no bigger than that of *Australopithecus*. Yet the species displays a number of characteristics found only within the genus *Homo.* These include hands that were adapted for climbing but would also have been highly dexterous, human-like feet adapted for upright walking, a reduced jaw and teeth, and a cranial structure similar to that of *Homo erectus* and *Homo habilis*.

The *H. naledi* fossils are remarkable for their location as well. All of the skeletal remains were found well into the cave, at the bottom of a remote chamber connected to the rest of the cave system by a thin vertical shaft about 10 meters in length. (**Figure 19.25**). The resting positions of the

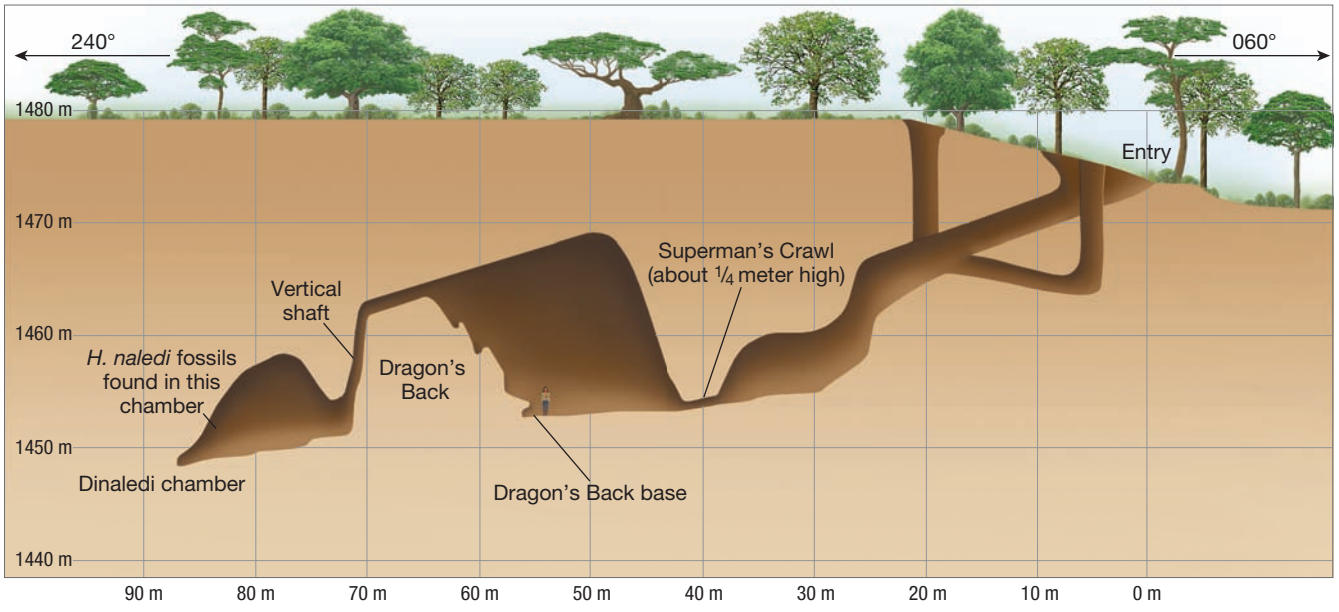

FIGURE 19.25 A deep site for the fossil remains of *H. naledi*. The *H. naledi* specimens were found deep into the Rising Star cave system, on the far side of an extremely narrow horizontal passage ("Superman's Crawl") and at the base of a chamber connected to a long, narrow, vertical shaft. (Image of investigator not to scale.)

bones reveal that the bodies were intact when they reached the chamber. There is no evidence that flooding or other geological processes delivered the remains to their current location, scavengers and predators could not reach this site, and the lack of unhealed fractures suggests that the individuals did not end up in the chamber by falling or other trauma. Unlike in most cave assemblages of hominin fossils, no remains from other large species are interspersed with the hominin bones, and there is no evidence of long-term habitation of the chamber. While other explanations cannot be ruled out, the team that made the discovery has hypothesized that the bodies were deliberately placed in the shaft and allowed to fall into the chamber, as if in a form of burial (Dirks et al. 2015).

Because the discovery of *H. naledi* is so recent, researchers have yet to determine the age of the fossil remains. Knowing the age is obviously crucial to understanding the phylogenetic relationships between this species and other members of the hominin clade. As with *Homo floresiensis*, we can anticipate that this story will unfold further in the near future as the fossils are dated and subjected to additional study.

19.3 The Emergence of Anatomically Modern Humans

With the innovations in physiology, toolmaking, and perhaps fire technology, the premodern hominins *Homo ergaster, Homo erectus,* and *Homo heidelbergensis* underwent a remarkable expansion, spreading from their origins in Africa throughout Europe and Asia. As these species spread, they took their toolmaking technologies with them, and we see the history of hominin migrations, as inferred from fossil evidence, reflected in the geography of hominin tools (**Figure 19.26**).

Models for the Evolution of Modern Humans

When, where, and how did the transition from premodern hominins to modern humans (*Homo sapiens*) take place? For many years, two contrasting hypotheses dominated the debate. The **multiregional hypothesis** suggested that hominins left Africa and colonized the rest of the Old World a single time, nearly 2 million years ago, as *Homo ergaster. Homo ergaster* populations in different parts of the world then diverged from one another morphologically, but modest gene flow among these geographically separated *Homo ergaster* populations prevented branching speciation. Gradually over the past 2 million years, these loosely associated populations evolved together first into *Homo heidelbergensis* and then into modern humans (**Figure 19.27A**) (Wolpoff et al. 1988, 2000).

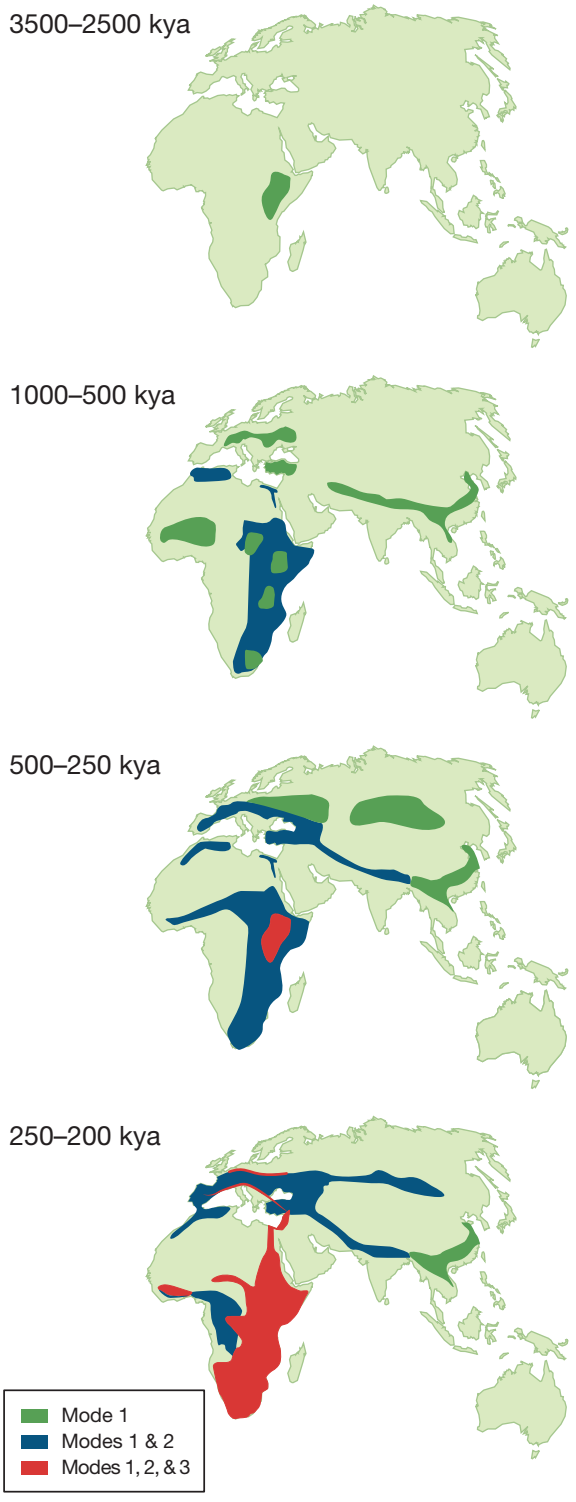

FIGURE 19.26 The spread of toolmaking technologies out of Africa. The mode 1, mode 2, and mode 3 technologies each arose in Africa and then spread across the globe as their makers migrated out of Africa in consecutive waves (kya, thousands of years ago). Adapted from Boyd and Silk (2009).

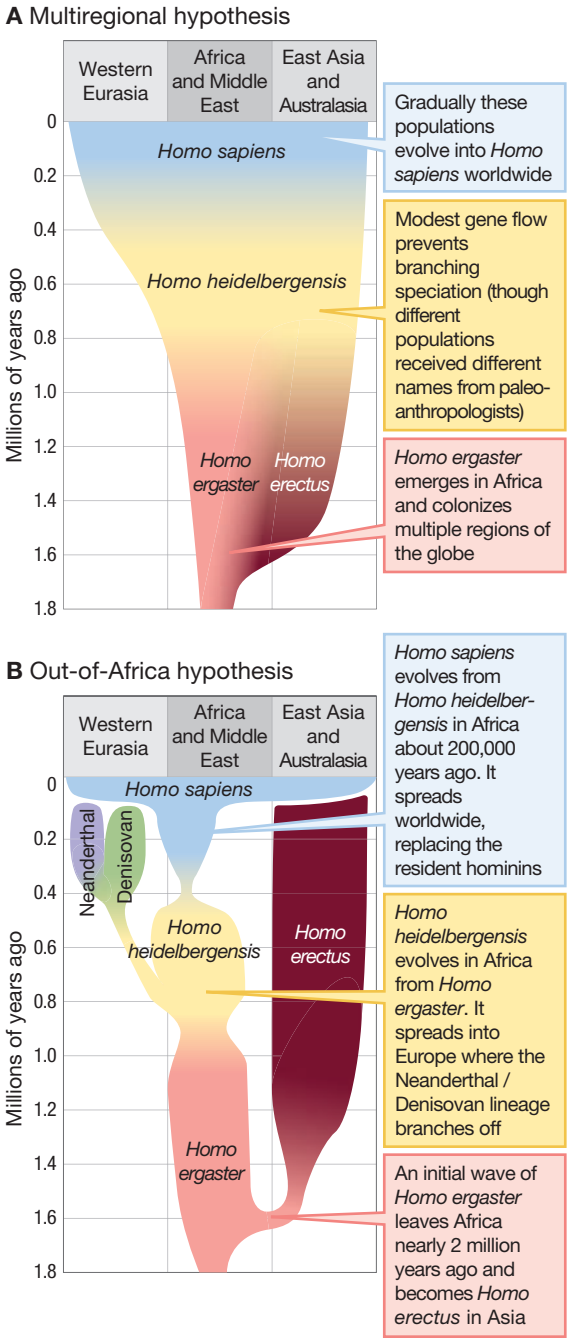

FIGURE 19.27 The classic multiregional and out-of-Africa models. Diagrams contrast **(A)** the multiregional model and **(B)** the out-of-Africa model of human evolutionary history. Adapted from Boyd and Silk (2009).

The original **out-of-Africa hypothesis** suggested that hominins left Africa and colonized the rest of the Old World in two major waves (Stringer and Andrews 1988), but this hypothesis has been updated to account for new evidence that suggests three waves of colonization (Templeton 2005). In the first wave, *Homo ergaster* arose in Africa, and as shown in **Figure 19.27B**, this species migrated through the Near East and into Europe and Asia (where many researchers would say it evolved into *Homo erectus*) nearly 2 million years ago. Subsequently, *Homo heidelbergensis* arose from *Homo ergaster* in Africa between 800,000 and 500,000 years ago. In the second wave, *Homo heidelbergensis* migrated into Europe and Asia where it evolved into *Homo neanderthalensis* and the Denisovan hominin about 400,000 years ago. In a third and final wave, *Homo sapiens* evolved from *Homo heidelbergensis* in Africa between 200,000 and 130,000 years ago. This species then migrated into Europe and Asia in one or more waves about 60,000 years ago, replacing the premodern hominins, such as *Homo erectus* and *Homo neanderthalensis,* from the two previous waves.

The key distinction between the multiregional and out-of-Africa models involves the fate of premodern hominin populations in Europe and Asia. The multiregional hypothesis predicts humans of European ancestral origin are descended from premodern hominins in Europe, humans of Asian ancestral origin are descended from premodern hominins in Asia, and humans of African ancestral origin are descended from premodern hominins in Africa. By contrast, the out-of-Africa hypothesis predicts that premodern hominin populations in Europe and Asia died out without contributing to the modern human gene pool, and that all modern humans are descended from premodern hominins in Africa alone.

The two models pose very different explanations for the differences among modern human populations. Under the multiregional model, differences among modern populations have their origins in geographic separations that have been maintained for 2 million years. Under the out-of-Africa model, differences among non-African human populations cannot predate the most recent migration out of Africa 60,000 years ago, and differences even between African populations cannot date back beyond the origin of *Homo sapiens* in Africa 200,000–130,000 years ago.

Evidence for the Out-of-Africa Model

A number of lines of evidence support the out-of-Africa model—albeit with a surprising twist that we will discuss shortly—over the multiregional model. First of all, archaeological evidence reveals that successive toolmaking technologies arose in Africa and spread in consecutive waves. Second, fossil evidence suggests gradual divergence of the premodern *Homo* species in their various locations in Africa, Europe, and Asia, with anatomically modern *Homo sapiens* arising first in

Africa some time between 200,000 and 130,000 years ago. After this event, we see an expansion of *Homo sapiens* beyond Africa about 60,000 years ago, eventually replacing the other *Homo* forms. This suggests migration and replacement, as in the out-of-Africa model (Strait et al. 2007).

Third, early genetic data, typically based on mitochondrial DNA (mtDNA), provided some of the strongest evidence for the out-of-Africa hypothesis. Recall from Chapter 12 that mitochondria have their own genome, which is nonrecombining and maternally inherited. These properties make it particularly straightforward to infer phylogeny from mtDNA sequence data. In a classic 1987 paper, Rebecca Kann and colleagues used a technique called restriction mapping to create a phylogenetic tree based on mtDNA from 147 humans worldwide. They found greater mitochondrial diversity within Africa than in the rest of the world combined, suggesting an African origin for modern humans. Using the molecular clock approach we discussed in Chapter 8, they were also able to estimate a coalescent time for the mtDNA of living humans. They estimated that the mitochondrial DNA in living humans coalesced to a single woman, a premodern hominin dubbed the **mitochondrial Eve**, who lived roughly 200,000 years ago in Africa (Lewin 1987; Kann et al. 1987).

A number of years later, Max Ingman and his colleagues looked at the full mitochondrial DNA sequence of 53 humans from around the world (Ingman et al. 2000). Their findings agreed with those of Kann and colleagues. They estimated that the coalescence time for human mitochondrial DNA is between 120,000 and 230,000 years ago, and the coalescence time for non-African mtDNA is about 38,500 years ago. Like Kann's team, Ingman and his colleagues found vastly more mitochondrial diversity within Africa than across the rest of the globe. This implies that—at least at the mitochondrial locus—non-Africans are a subclade of the larger phylogenetic tree for modern humans.

These observations tell us two important things: (1) the recent common ancestor of all human mtDNA dates to about the time of the emergence of *Homo sapiens* in Africa, not to about the time of the emergence of *Homo erectus*, and (2) non-African *Homo sapiens* share an even more recent common ancestor, dating to about the time that *Homo sapiens* replaced other *Homo* species around the globe.

The limitation to all of this work is that mitochondrial DNA is nonrecombining, and therefore the mitochondrial chromosome behaves as a single maternally inherited locus. As we know, single-gene trees do not always accurately reflect the corresponding species tree. The next step in understanding human origins was to look at a large number of nuclear loci and build phylogenies on the basis of this information. Much of the evidence from nuclear loci is consistent with an origin of modern humans in Africa about 200,000 years ago, followed by a more recent migration of *Homo sapiens* out of Africa about 60,000 years ago. In other words, much of the data supports the out-of-Africa model. Some of the data, however, tells a different and more complicated story, which we will explore in the next section.

KEYCONCEPT QUESTIONS

19.1 If all living humans can trace their matrilineal ancestry back to a single female who lived between 100,000 and 200,000 years ago, does that mean that our ancestral population necessarily went through such a tight bottleneck that the mitochondrial Eve was the only reproductive female of her time?

19.2 Suppose that in 100,000 years our descendants, future hominin scientists, continue to study human origins on the basis of genetic data. Explain why it is unlikely that they would agree with us that the mitochondrial Eve was a premodern hominin living in Africa somewhere between 100,000 and 200,000 BCE.

From *Homo heidelbergensis* to Modern Humans

Fossil and archaeological evidence allows us to sketch out a rough picture of hominin evolution from *Homo heidelbergensis* to modern humans. *Homo heidelbergensis* gave rise to two closely related sister hominin groups, the Neanderthals and the Denisovans. The evolutionary branch leading to modern humans split from the branch leading to Neanderthals and Denisovans about 600,000 years ago. Approximately 200,000 years later, Neanderthals and Denisovans split from one another. These groups and their possible immediate predecessors were present in Europe and central Asia no later than 300,000 years ago and persisted until about 30,000 years ago.

Compared to modern humans, Neanderthals were heavier, stronger, and more stocky, with a more pronounced brow ridge and larger eyes (**Figure 19.28**). Socially and culturally, however, they shared many characteristics with modern humans (Klein 2003). They made elaborate mode 3 tools and hunted large game. They cooked with fire. From fossil remains of individuals who had healed from severe and debilitating wounds, we know that they cared for their sick, injured, and elderly. From Neanderthal grave sites that have been found, we have learned that they buried their dead. The anatomy of their vocal tracts and some genetic evidence suggest that they were probably capable of some degree of speech.

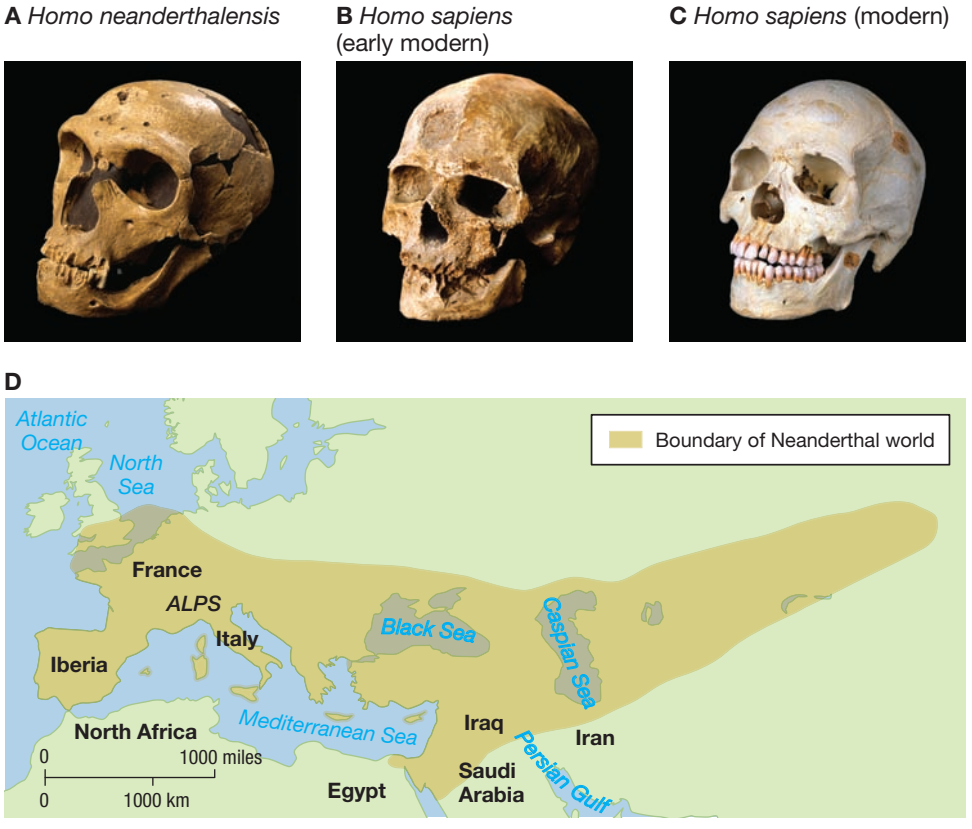

FIGURE 19.28 The Neanderthal. **(A)** A Neanderthal skull (*Homo neanderthalensis*), with skulls from **(B)** a European early modern human from about 30,000 years ago (*Homo sapiens*), and **(C)** a modern human (*Homo sapiens*) for comparison. Note the prominent brow ridge on the Neanderthal skull that is absent from the two *Homo sapiens* skulls. **(D)** A map of the proposed range of *Homo neanderthalensis*. Panel D adapted from Krause et al. (2007).

A second group, called the Denisovans, are known from a single finger bone and a couple of teeth found in a cave in the Altai mountains (Reich et al. 2010; Meyer et al. 2012). Despite this extremely scanty fossil record, we know a surprisingly large amount about the Denisovans, because researchers have been able to sequence the Denisovan genome from that single bone. From this genome sequence, coupled with several Neanderthal genomes and thousands of modern human genomes, we are developing an increasingly detailed understand of the relationship between these recent hominins.

We now turn to our own species. *Homo sapiens* appeared in Africa roughly 200,000–130,000 years ago. Compared to *Homo heidelbergensis, Homo sapiens* had a rounded cranium with a larger brain size, a pronounced chin, smaller teeth, and reduced brow ridges. Their bodies were more slender, with longer limbs and a shorter torso. About 60,000 years ago, they migrated out of Africa and throughout Europe and Asia, which at the time were inhabited by other *Homo* species. *Homo sapiens* and these other *Homo* species coexisted for thousands of years in some of these areas, as established by fossil evidence. About 50,000–40,000 years ago, some anthropologists argue, *Homo sapiens* underwent a rapid period of change in Europe, suddenly developing a suite of new behavioral and cultural traits, known as the Upper Paleolithic lifestyle. Other researchers argue that this process was much more gradual and largely took place in Africa; they claim that the rapid change observed in Europe is simply a result of the migration from Africa around this time (McBrearty and Brooks 2000) (**Figure 19.29**). Either way, the Upper

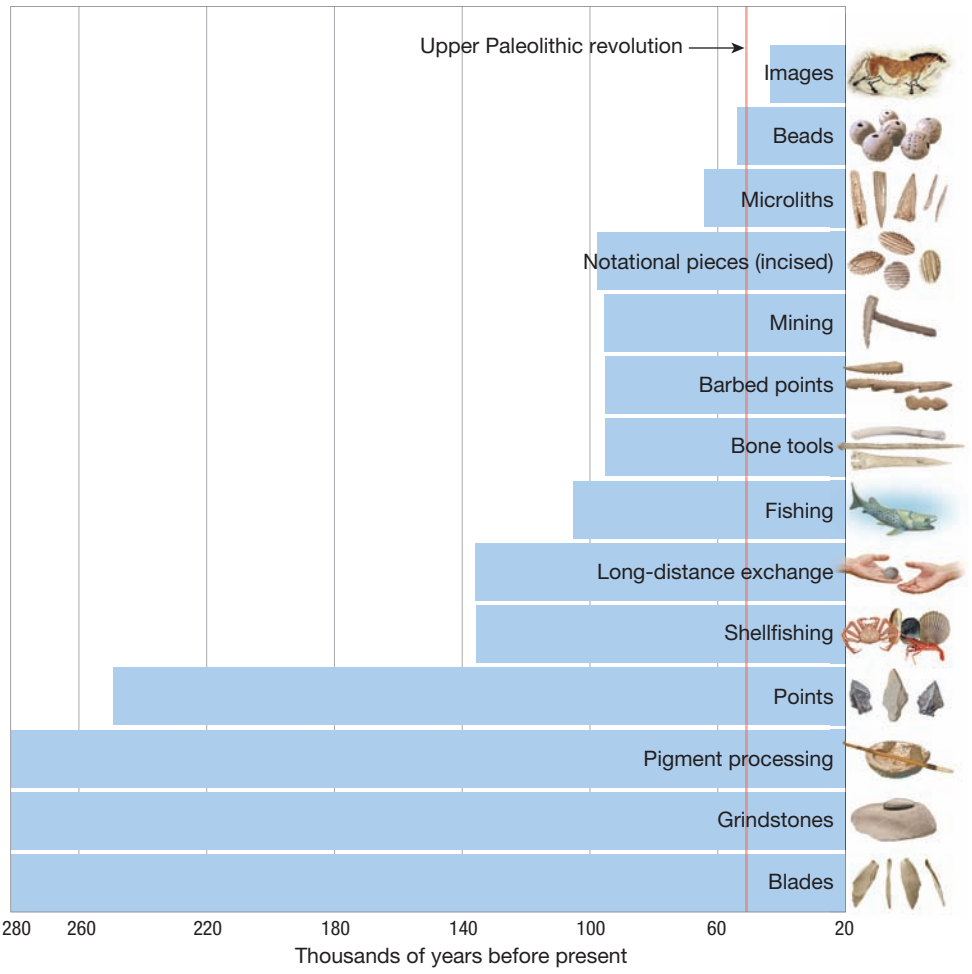

FIGURE 19.29 Timing of cultural and behavioral traits emerging in Africa. Many researchers believe that rather than humans undergoing a sudden dramatic behavioral and technological shift about 50,000 years ago—the so-called Upper Paleolithic revolution—humans in Africa developed advanced behaviors and technologies gradually over tens of thousands of years. Adapted from McBrearty and Brooks (2000).

Paleolithic culture involves a breadth of advanced cultural and behavioral traits. During this period we see the production of increasingly advanced *mode 4* tools. Upper Paleolithic humans used bone and ivory for tools. They created elaborate structures for shelter. They produced art and musical instruments, and they frequently hunted larger game. They buried their dead in a ceremonial manner, and they traded tools and materials across long distances (Johanson 2001). Although it is more difficult to ascertain, most researchers believe that language was also fully developed by this stage. At this point, *Homo sapiens* had become, physiologically and culturally, fully modern. With this new suite of traits, *Homo sapiens* rapidly replaced the other *Homo* species around the globe. With the possible exception of *Homo floresiensis*, these other *Homo* species were gone by about 30,000 years ago. **Figure 19.30** summarizes the evolutionary timeline for the genus *Homo.*

19.4 Interbreeding among Humans, Neanderthals, and Denisovans

We hinted in the previous section that work with nuclear DNA has revealed unexpected but fascinating aspects of hominin evolutionary history. In this section, we will explore one of the most exciting developments in the study of human genetics over the past decade: our growing understanding of how our gene pool has been shaped by interbreeding between humans and other hominins.

Interbreeding with Neanderthals

Fossil evidence suggests that interbreeding might have taken place between humans and Neanderthals. Some fossils appear to have morphological characters intermediate between *Homo sapiens* and *Homo neanderthalensis*, for example. But it is very difficult to tell for certain whether this is indicative of interbreeding or due to other unrelated causes. And it is yet more difficult from fossil evidence alone to ascertain whether these interbreeding events, if they did occur, have contributed to genetic variation in modern humans.

Genetic information can be more informative. In an early study, researchers extracted mtDNA from a number of Neanderthal bone fragments and also from bones of approximately the same age from ancient *Homo sapiens* (Serre et al. 2004). They found that the Neanderthals had a unique set of mtDNA genotypes that were not represented among ancient or modern humans. From this evidence alone, the authors could rule out the possibility of *extensive* interbreeding between humans and Neanderthals. But the mtDNA is effectively only a single locus among millions of loci, and, because mitochondria are maternally inherited, it represents only the history of descent through the female lineage. As a result, the researchers had no evidence of interbreeding—but they were unable to rule out the possibility that limited interbreeding had taken place.

To determine more conclusively whether any inbreeding had taken place, researchers needed a way to look at nuclear loci from Neanderthals. By sequencing the entire Neanderthal genome from ancient bone fragments, a large team led by Richard Green and Svante Pääbo made this possible on a large scale (Green et al. 2010).

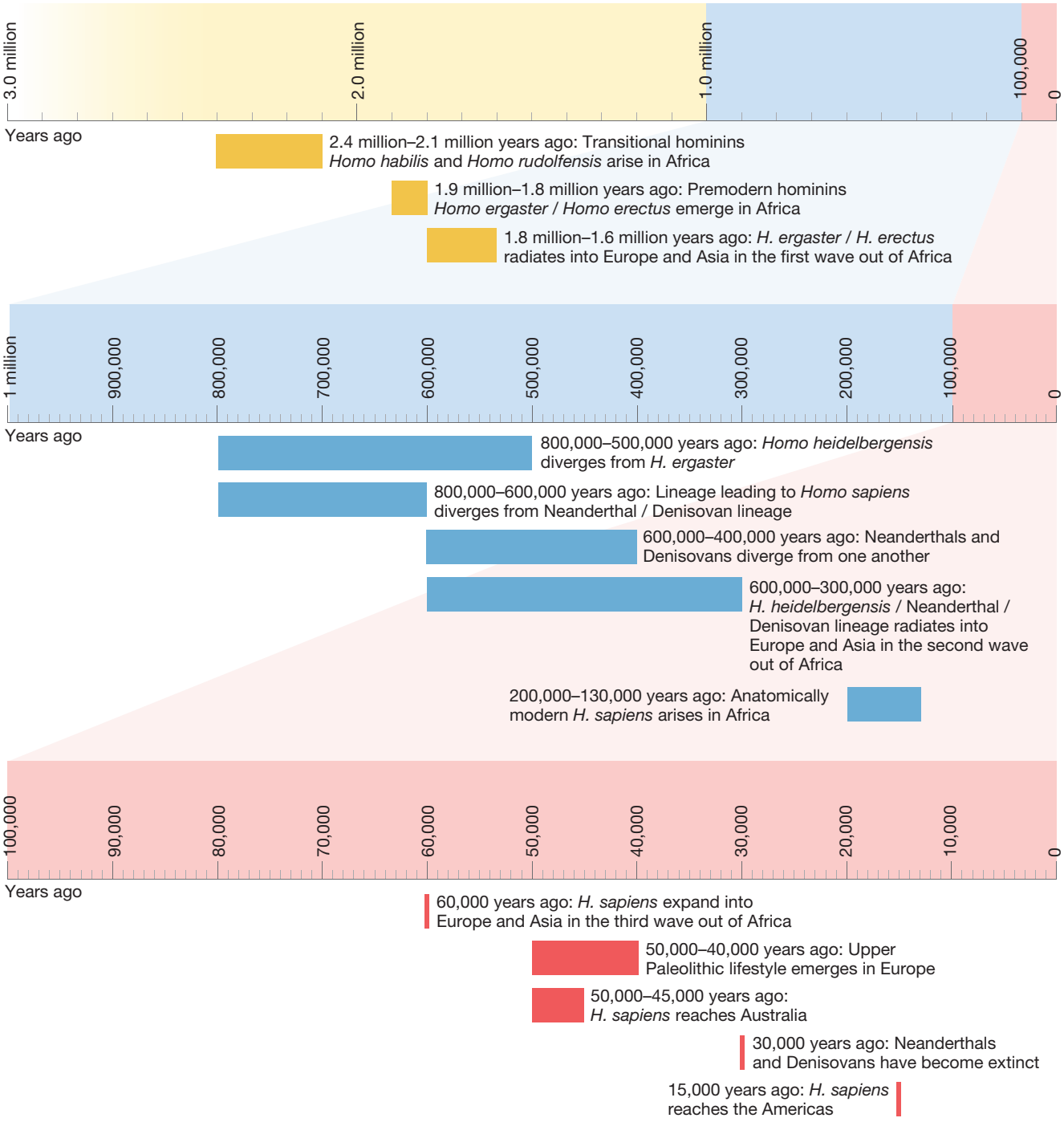

FIGURE 19.30 A timeline of evolutionary events within the genus *Homo*. The timing of evolutionary events in the *Homo* lineage, established by dating fossil remains and by inference from genetic sequence data, is a matter of great contention among anthropologists and subject to frequent revision as new discoveries are made and new dating techniques become available. This timeline indicates the approximate dates that we use in this book.

But even with genomic information about Neanderthals in hand, it remains tricky to infer interbreeding on the basis of nuclear DNA evidence. The problem involves the distinction between single-gene trees and species trees that we discussed earlier in this chapter for humans, chimpanzees, and gorillas. In particular, alleles

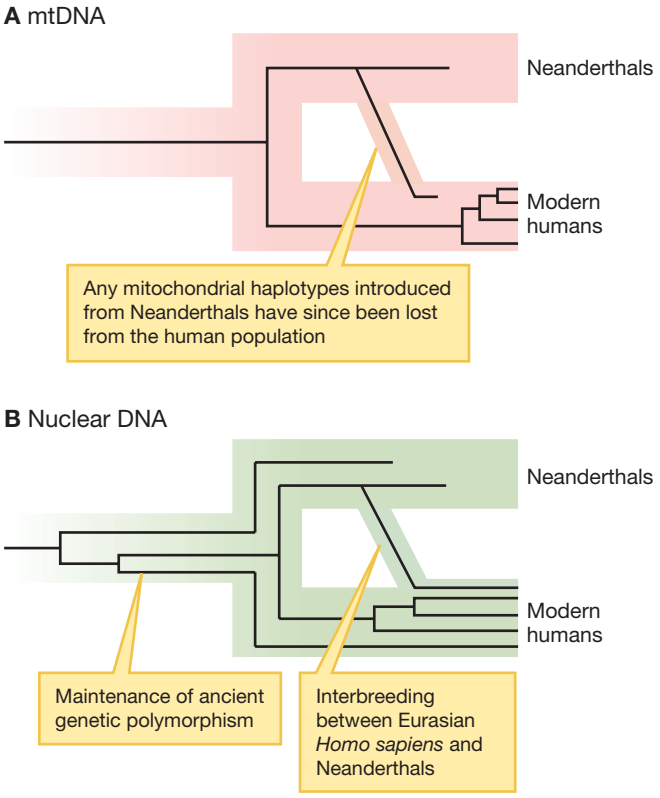

FIGURE 19.31 Gene trees and species trees for humans and Neanderthals. **(A)** The mitochondrial genome is effectively a single locus. At this locus, the gene tree reflects the main species tree that we would see if we ignored interbreeding. Despite interbreeding, any mtDNA haplotypes introduced from Neanderthals into the human population have since been lost. **(B)** Some loci in the nuclear genome have been polymorphic in humans since long before the divergence of Neanderthals and modern humans. At other nuclear loci, alleles have been transferred from Neanderthals to humans by interbreeding. As a result, at some nuclear loci, certain human alleles may be more closely related to Neanderthal alleles than they are to other human alleles. Adapted from Pääbo (1999).

shared by Neanderthals and a subset of humans need not derive from interbreeding—they instead could be the result of deep coalescence. As illustrated in **Figure 19.31**, a substantial fraction of the genetic variation in living modern humans reflects polymorphisms that date back to before the divergence of Neanderthals and *Homo sapiens.* For example, one study found that 13% of the common polymorphic deletions in the human population originated before these species diverged (Lin et al. 2015). As a result, even in the absence of interbreeding, we would expect to find that, at genetic loci with these old polymorphisms, certain individual humans may be more closely related to individual Neanderthals than they are to other individual humans (Pääbo 1999).

Given this complication, how could researchers distinguish between the results of interbreeding and the results of ancient genetic polymorphisms dating back prior to the human–Neanderthal divergence? Green and colleagues reasoned that if Neanderthals shared more alleles in common with humans who had descended from populations that overlapped with Neanderthals than with humans who had descended from populations that had never overlapped with Neanderthals, this would support the hypothesis that humans and Neanderthals had interbred. Given the Eurasian range of Neanderthals and the pattern of modern human migration out of Africa, we would expect humans who descend from Europeans and Asians to share more alleles with Neanderthals than do humans who descend from African populations.

This is precisely what Green and colleagues found. The genomes of African humans do not contain Neanderthal-derived DNA. But 1% to 4% of the genome of a typical individual non-African human is derived from Neanderthals. Moreover, different individuals possess a different 1% to 4% of the Neanderthal genome. Recent studies have revealed that an astounding 20% to 30% of the Neanderthal genome can be found somewhere in the human population (Sankararaman et al. 2014; Vernot and Ackey 2014), though no single human has anywhere near that fraction of Neanderthal-derived DNA.

The distribution of Neanderthal alleles in human populations has been influenced by selection. A few alleles acquired from Neanderthals—including alleles for skin pigmentation and structure—appear to have been selectively favored in some human populations (Vernot and Akey 2014). But most Neanderthal alleles introduced into human populations seem to have deleterious consequences. Some have nonetheless persisted and are associated with increased susceptibility to disease, including several autoimmune disorders. Many other Neanderthal-derived alleles appear to have been eliminated by purifying selection: Within the human genome, more of the genic regions from Neanderthals occur in noncoding parts of the genome than would be expected by chance (Sankararaman et al. 2014).

Gene Flow from Denisovans

Neanderthals were not the only hominin group to interbreed with humans. As we saw in the introduction to this chapter, Denisovans and humans also interbred where they met in Europe and Asia.

Denisovans interbred with additional hominin species as well. The principle of genetic equidistance that we discussed in Chapter 5 predicts that as sister groups, Denisovans and Neanderthals should be about equally distant from modern humans—but this is not the case. Instead, Denisovans are a significantly greater genetic distance from humans. This is best explained by a model in which Denisovans, but not Neanderthals or populations along the branch leading to modern humans, interbred with some other population of as yet unknown hominins (Prüfer et al. 2014).

On the basis of our new understanding of hominin interbreeding, **Figure 19.32** illustrates a revised model of human evolution. Many features of the original out-of-Africa model are retained. As in that model, modern human variation is largely derived from what was present in the population of *Homo sapiens* that first emerged in Africa less than 200,000 years ago. But the out-of-Africa model does not tell the entire story; elements of the multiregional model, or something like it, are observed as well. Not all genetic variation has been lost from *Homo* species that diverged prior to the emergence of *Homo sapiens* in Africa. And, as predicted by the multiregional model, different populations of modern humans have inherited different amounts of this genetic material from different sources. While gene flow has not been as

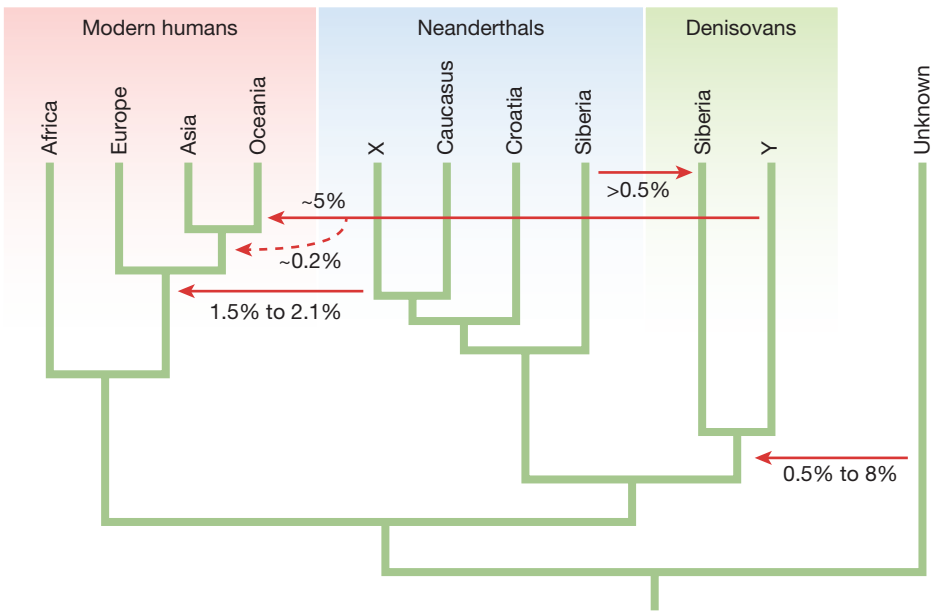

FIGURE 19.32 A model of gene flow between Neanderthals, Denisovans, and modern humans. The phylogenetic tree indicates the primary relationships among these species. Red arrows indicate gene flow from one species into another, with numbers indicating the percentage of the recipient species' genome that is composed of alleles from the donor species. Among modern humans, branch tips refer to populations of ancestral origin (Africa, Europe, Asia, and Oceania). Among Neanderthals and Denisovans, geographical labels refer to individual sequenced genomes, while X and Y refer to Neanderthal and Denisovan genomes inferred from contemporary human genomes. The "Unknown" represents an archaic hominin that split off from *Homo sapiens*/Neanderthals/Denisovans prior to their divergence. Adapted from Pääbo (2014).

extensive as envisioned in the original multiregional model, a limited amount of that variation has been passed from Neanderthals and Denisovans to populations of *Homo sapiens* in Eurasia and Oceania through interbreeding that took place some time within the past 100,000 years.

Since the turn of the twenty-first century, new archaeological discoveries and advances in genomic sequencing have radically altered our understanding of recent hominin history. Over the past hundred thousand years, multiple hominin species, varying substantially in stature and form, walked the Earth. They competed with one another for resources, and they presumably fought one another for land and food. Remarkably, some of the species, including *Homo sapiens*, interbred with one another—and their hybrid offspring survived to produce surviving offspring themselves. On one remote island, a descendant of *Homo erectus* or an even earlier species managed to hold out until 12,000 years ago. *Homo sapiens* ultimately displaced the others, but during the process, several other hominin species left their genetic mark on the human population. This story is a far cry from what scientists believed two decades ago—that the other hominins were rapidly outcompeted by *Homo sapiens* and disappeared almost without a trace. Instead, over the past hundred millennia the manifold interactions among species of the genus *Homo* have provided a drama worthy of the imagination of J. R. R. Tolkien.

19.5 Migration of Modern Humans

Molecular genetic approaches offer the promise of inferring phylogenetic history—and thus reconstructing the pattern of migrations by which modern humans came to inhabit the entire globe (excepting Antarctica) by 12,000 years ago. But doing this is not an easy task. The main reason is that human groups are not genetically distinct, and there are few alleles that are uniquely diagnostic of ancestral origin. Most alleles are found in multiple regions of the globe, and the minority of alleles that are found only in a single region tend to be very rare even in the population where they are found (Rosenberg et al. 2002). Statistical analyses have repeatedly found that the vast majority of genetic variation in humans occurs within, not between, populations. Early studies using protein polymorphisms found that at least 80% of their variation occurs within populations, and more recent studies using genomic data and improved statistical methodologies estimate that within-population variation composes 93% to 95% of total human variation (Lewontin 1972; Barbujani et al. 1997; Brown and Armelagos 2001; Rosenberg et al. 2002).

To put it another way, take two individuals from two different ancestral origins, say one Asian and one European. The genomes of these two individuals from different ancestral origins will on average differ only slightly more than the Asian would differ from another Asian or the European from another European. Moreover, this slightly greater difference is only observed on average; sometimes, the Asian and European would be more similar to one another than each would be to many members of the same ancestral group.

This may come as a surprise to some people. After all, we can often make a good guess about someone's ancestry from his or her appearance. Indeed, certain characteristic features, such as skin color and hair form, offer substantial clues to ancestral origin.

But while such features exist, they are the exception, not the rule. Leading population geneticists Marc Feldman, Richard Lewontin, and Mary Claire King explain:

> The genes underlying the phenotypic differences used to assign race categories are atypical, in that they vary between races much more than genes in general. Together, the iconic features of race correlate well with continent of origin but do not reflect *genome-wide* differences between groups. (Feldman et al. 2003, p. 374, emphasis added.)

As these authors point out, we can find individual loci that differ according to ancestral origin and thus allow us to infer population history—but these loci are not typical of human genetic variation. The social construction of *race* does not accurately reflect an underlying genomic reality.

Gene Trees for Modern Human Populations

Nonetheless, there are ways we can learn about human origins using genetic data. In the 1960s, Luca Luigi Cavalli-Sforza and Anthony Edwards realized that molecular genetic data could provide information about the phylogenetic history of human groups. In this era before genetic sequencing, molecular data were scarce, but one well-studied set of molecular characters were the various blood groups (the ABO group, the Rh group, and so forth). Because these characters are highly polymorphic within any given population and because each of the variants is observed in most populations, Cavalli-Sforza and Edwards could not construct a standard genetic phylogeny, in which the tree is inferred using characters associated with individuals. They could, however, build a tree using characteristics of *populations*; namely, the frequencies of the various blood types. The basic logic was that the longer the two populations had been separated, the more time there would have been for their blood type frequencies to diverge.

Cavalli-Sforza and Edwards used this reasoning to develop a number of statistical approaches for inferring phylogenies and constructed the first phylogenetic trees showing the relationships among human groups (Edwards and Cavalli-Sforza 1964; Cavalli-Sforza and Edwards 1965; Cavalli-Sforza 1966). Their original tree is shown overlaid on a map of the world in **Figure 19.33**. It is remarkable that with such crude data, Cavalli-Sforza and Edwards were able to generate unrooted trees not so different from those that we obtain using contemporary whole-genome approaches; compare Figure 19.33 with Figure 19.40 later in this section. However, they were not able to identify Africa as the origin point from which modern humans migrated across the globe. Instead they hypothesized that the deepest branching point in the phylogeny was the one separating East Asians from Europeans and Africans (Cavalli-Sforza 1966).

Subsequent studies used protein polymorphism data (Lewontin 1972) and later mitochondrial DNA sequence to map out the historical relationships among modern groups. As we discussed in Section 19.3, mitochondrial studies

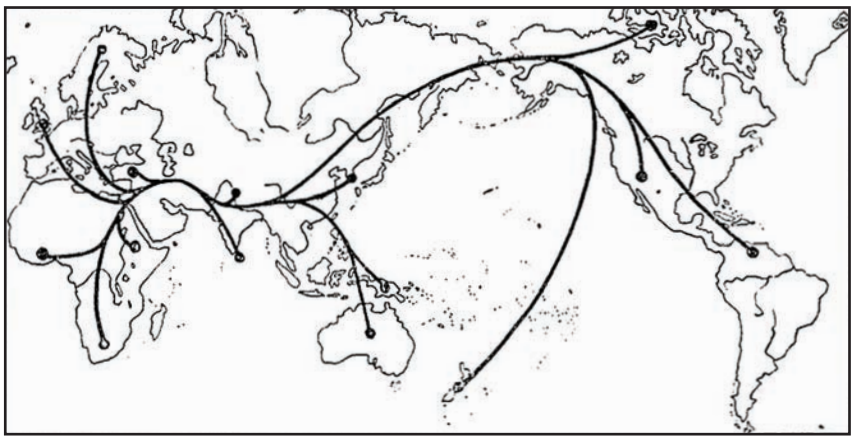

FIGURE 19.33 An early diagram of the relationships among human populations on the basis of blood type frequencies. Edwards and Cavalli-Sforza inferred the phylogenetic relationships among 15 human populations by applying a parsimony method to blood type frequency data in each population. The relationships they inferred are not so different from our current understanding, and when mapped onto the globe they reveal a pattern of migration similar to that of current models.

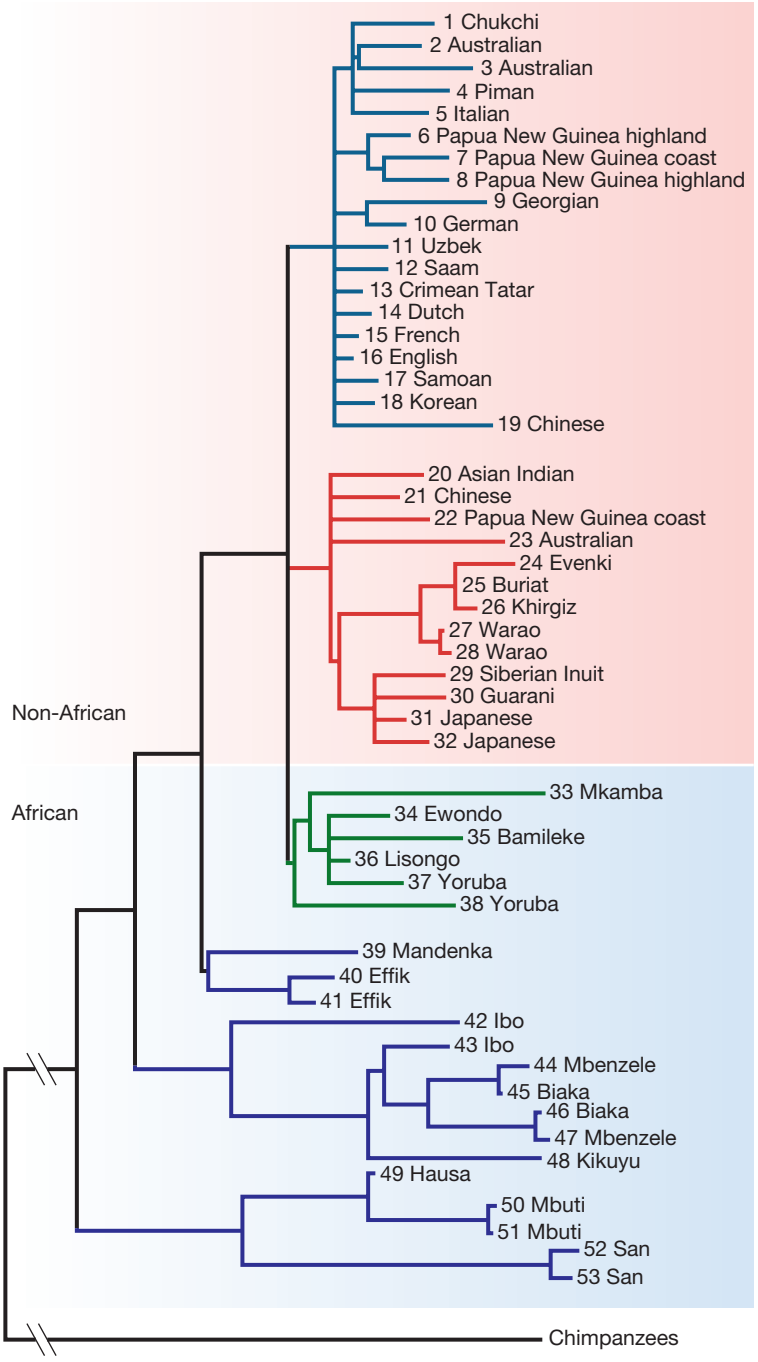

FIGURE 19.34 An mtDNA phylogeny of modern human populations. In this mtDNA phylogeny for 53 modern human populations, with chimpanzees as an outgroup, the deepest branches are located in Africa, with all non-African populations representing a fairly shallow pair of clades at the top of the tree. Because mtDNA is effectively a single locus, the phylogeny shown here is effectively a single-gene tree rather than a definitive representation of the true historical relationships among human groups. Adapted from Ingman et al. (2000).

revealed greater diversity within African populations than among all other non-African populations, suggesting an African origin for modern humans. **Figure 19.34** indicates the phylogenetic relationships among 53 contemporary human groups on the basis of mtDNA data (Ingman et al. 2000). Some aspects of the tree seem unlikely to reflect true demographic history; for example, the placement of Papua New Guinea groups in both major non-African clades. But given that an mtDNA phylogeny is effectively a single-gene tree, this is unsurprising. Migration presumably has been ongoing between groups. Deep coalescence is also likely to contribute to the incongruence between the mtDNA phylogeny and population's true history. Recall that deep coalescence is most common when phylogenetic branches are short and population sizes are large; both are likely to be the case for many of the relationships among these human populations.

Another uniparentally inherited, nonrecombining region of the genome is the Y chromosome, and researchers can create phylogenetic trees from the Y chromosome just as they do with mitochondrial DNA. Phylogenetic trees based on the nonrecombining portion of the Y chromosome likewise indicate an African origin of modern humans. An early Y chromosome phylogeny suggested that for the Y chromosome, the most recent common ancestor of current humans lived within the past 140,000 years, and the most recent common ancestor of non-African humans dates to about 50,000 years ago, consistent with the model of a recent migration out of Africa (Underhill et al. 2000). More recently, however, Y chromosomes with much older divergence times, about 250,000 years ago, have been found in a small number of individuals of African descent (Mendez et al. 2013; Karmin et al. 2015). While this could indicate an earlier origin of modern *Homo sapiens*, such is not necessarily the case. The estimated date for the Y chromosome could diverge from its true date because of demographic and selective processes, or the Y chromosome in question could have entered the human population more recently via interbreeding from an earlier hominin population in Africa.

Because a major region of the Y chromosome is nonrecombining, this chromosome also provides a stronger signal of demographic events than can be obtained from recombining autosomal loci. Comparing the diversity of the patrilineally transmitted Y chromosome to that of the matrilineally transmitted mitochondrial DNA, Monika Karmin and colleagues revealed a striking bottleneck in the effective population size at the Y chromosome, but not the mtDNA, beginning about 10,000 years ago (**Figure 19.35**). Most likely, this is the consequence of a dramatic increase in the variance in male reproductive success at this time. Karmin and colleagues hypothesize that this was a consequence of changes in human social organization associated with the advent of agriculture, also occurring about 10,000 years ago, perhaps in concert with wars of conquest.

Multilocus Studies of Population History

As we have discussed, single-gene trees such as those obtained from mtDNA and the Y chromosome will not exactly reflect population history, nor will they agree with one another. To get a much better picture of population history, we need a way of looking at multiple loci at the same time. While a number of

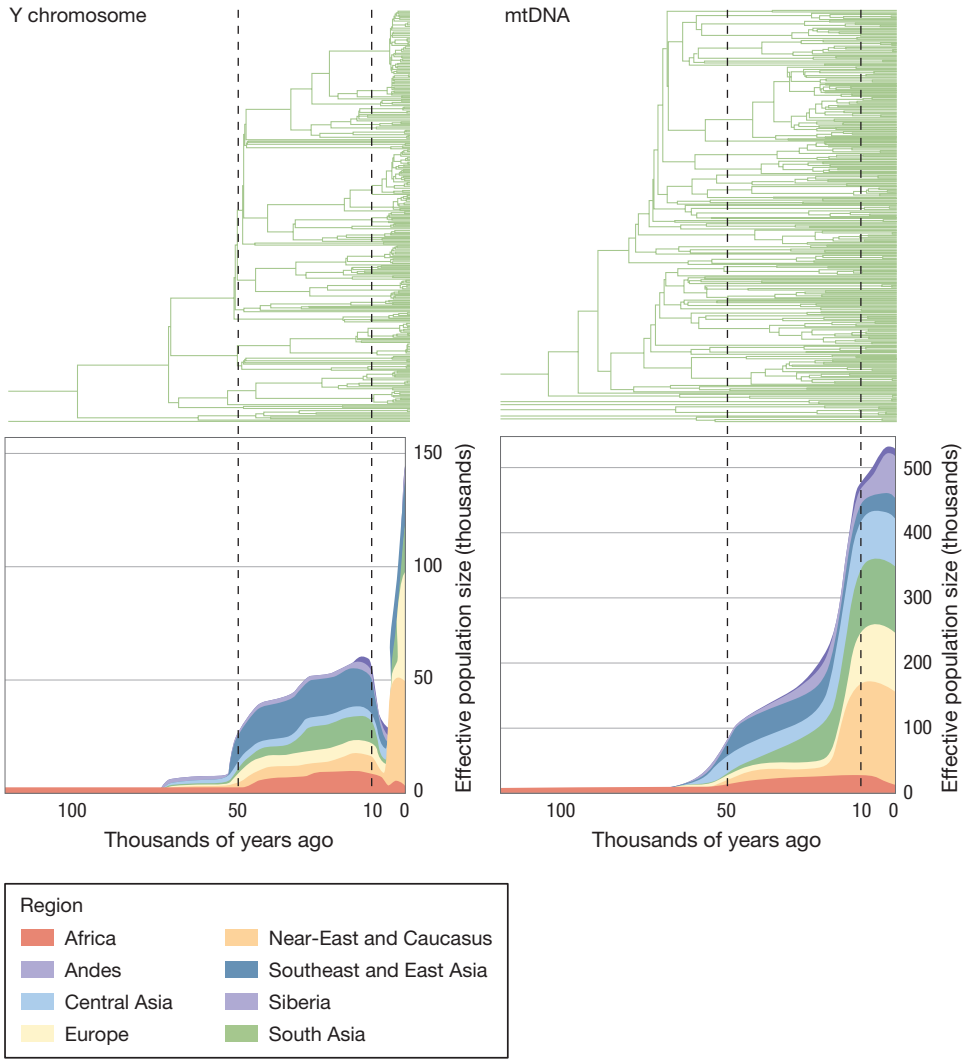

FIGURE 19.35 Phylogeny and effective population size for the Y chromosome and mtDNA. The upper panels show phylogenies for several hundred Y chromosome (left) and mtDNA (right) sequences from around the globe. The lower panels show estimated effective population size by region over time. Y chromosome diversity and effective population size increase dramatically during the migration out of Africa. About 6000 years ago, the effective population size for the Y chromosome was severely restricted, but no such pattern occurs for mtDNA, which at this stage has an effective population size 17 times larger than that of the Y chromosome. Adapted from Karmin et al. (2015).

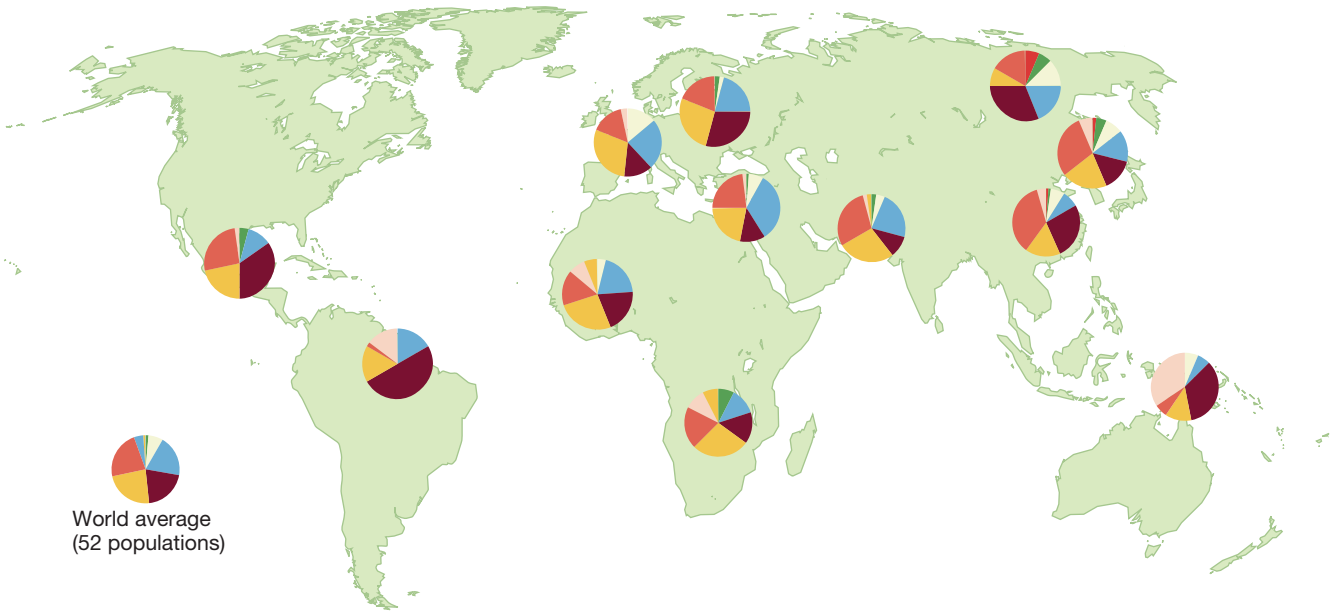

FIGURE 19.36 Frequencies of microsatellite alleles by continent of origin. At most microsatellite loci, including locus D13S149 shown here, most alleles are present in most geographic regions. Though allele frequencies vary from region to region, specific alleles are rarely diagnostic of specific regions. Adapted from King and Motulsky (2002) by permission of AAAS.

approaches have been proposed, a method known as ***structure*** (Pritchard et al. 2000) has proved particularly effective at uncovering the genetic signal of human migrations. The *structure* algorithm works by assuming that the set of genotypes under consideration comes from some specified number (k) of distinct groups, each with its own characteristic allele frequencies. A computational search algorithm simultaneously assigns individuals to populations and infers the allele frequencies for each population.

The *structure* approach is successful, despite the scarcity of genotypes specific to individual groups, because small allele frequency differences among populations can together distinguish ancestral origin if one considers a sufficiently large number of loci at the same time. As a consequence, even when we can statistically identify ancestral origin from an individual's genome, we have little power to predict genotype knowing only ancestral origin (Feldman and Lewontin 2008). We see this in striking fashion in **Figure 19.36**.

Noah Rosenberg and his colleagues applied the *structure* algorithm to sequence data from the Human Genome Diversity Project (Cann et al. 2002; Rosenberg et al. 2002). They used 377 microsatellite loci to cluster more than a thousand genotyped individuals from 52 different populations. Using this approach, they were able to identify the geographic regions from which individuals derived their ancestry. When the researchers specified that the algorithm should divide their samples into five clusters, the resulting clusters corresponded to major geographic regions: sub-Saharan Africa, Eurasia, East Asia, Oceania, and the Americas (**Figure 19.37**). An important aspect of this approach is that it can uncover **admixture**: Individuals can be ascribed partial ancestry from multiple source populations. In Figure 19.37, we see that several populations, including the Mozabite in North

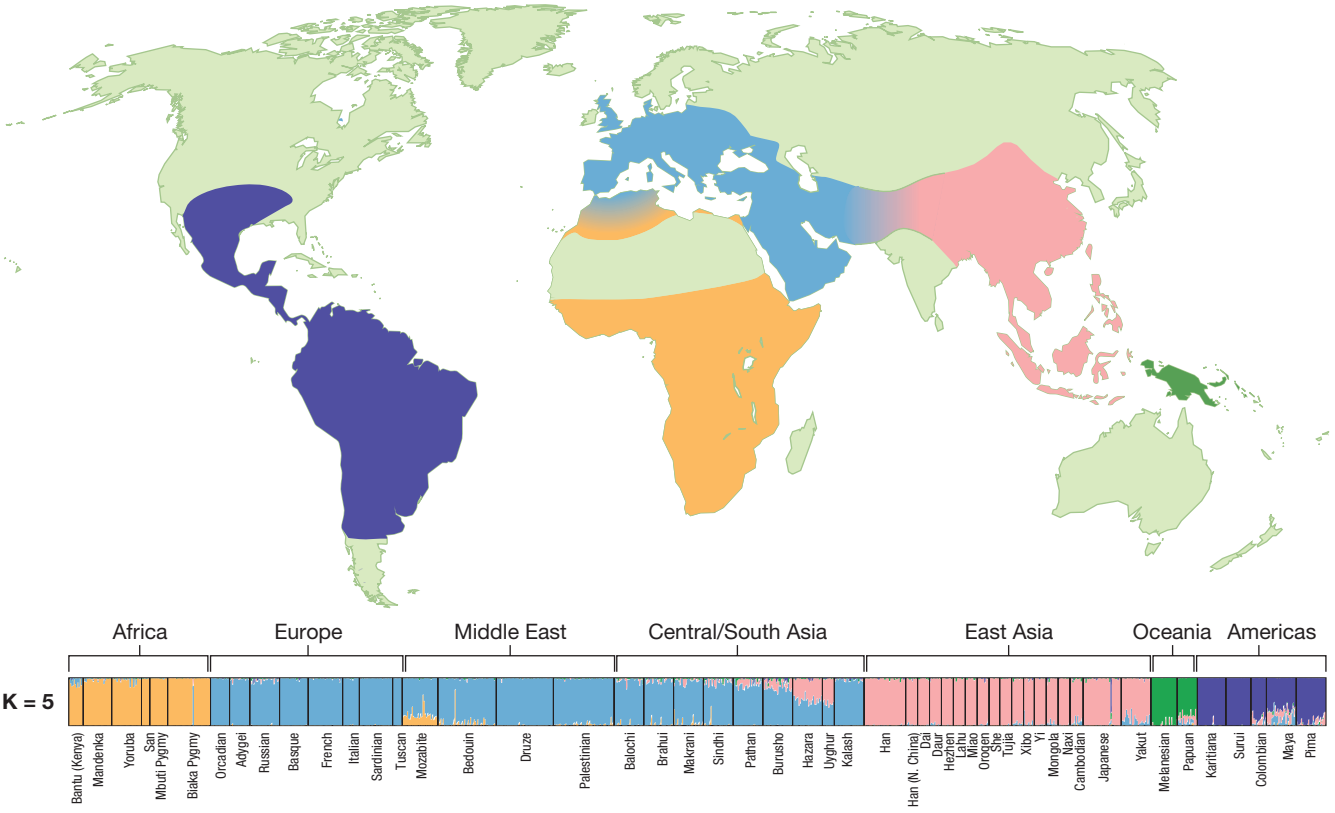

FIGURE 19.37 The *structure* algorithm reveals major divisions between ancestral groups. Applying *structure* to more than 1000 individuals from 52 populations with $k = 5$ recovers the geographic structure of these populations. The five major groupings correspond to sub-Saharan Africa, Eurasia, East Asia, Oceania, and the Americas. In the block diagram, each column represents a single human genotype. Some groups, such as the Mozabite in North Africa and the Hazara and Uyghur in central Asia, exhibit substantial admixture from neighboring regions. Adapted from Rosenberg et al. (2002).

Africa and the Hazara and Uyghur in central Asia, have genotypes that are admixtures of those from neighboring regions.

The same approach can also identify regional differences at a finer scale. For example, **Figure 19.38** shows the results when the algorithm is applied to Eurasia alone and programmed to find four clusters: Europe, the Middle East, and central Asia are three of the regions that are separated. The fourth cluster is something of a surprise. The Kalash ethnic group, who reside in the remote Kush mountains of North Pakistan, form a fourth cluster, indicating their substantial genetic and presumably historical differentiation from populations in the rest of the region.

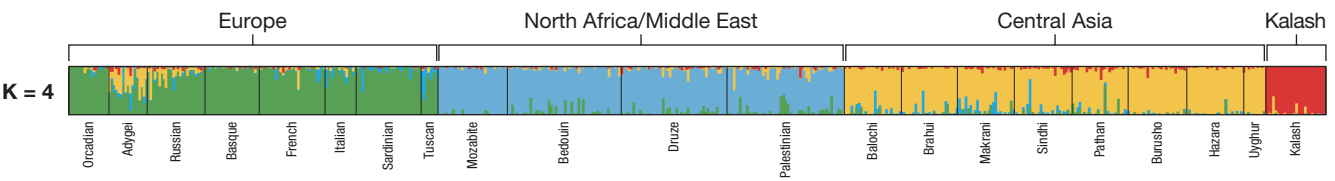

FIGURE 19.38 Applying structure to reveal genetic structure within Eurasia. When the Eurasian genotypes are separated into four populations, they divide cleanly by geography: Europe, North Africa and the Middle East, Central Asia, and a fourth region occupied by the Kalash people, an ethnic group in the remote Kush mountains of Northern Pakistan. Adapted from Rosenberg et al. (2002).

The success of *structure* demonstrates that large multilocus data sets can reveal the phylogeography of human populations. Even though allele frequencies vary gradually over space, geographic barriers to dispersal generate enough discontinuity in genotype distributions that we are able to reconstruct geography from genotype data (Rosenberg et al. 2005).

Researchers have been able to use a similar approach to create phylogenetic trees showing the relationships among human groups. Jun Li and colleagues used an alternative statistical approach to infer ancestry and detect admixture (Tang et al. 2005; Li et al. 2008) on the basis of a genome-wide sample of 650,000 loci exhibiting single nucleotide polymorphism (SNPs). When separating their 938 sampled individuals into five clusters, their results closely matched those from the Rosenberg study. When grouping their data into seven clusters, they further revealed a split between Europe and East Asia and revealed extensive admixture in the Middle East (**Figure 19.39A**).

Using the allele frequencies at each SNP, Li and colleagues were also able to construct a maximum likelihood phylogenetic tree for human populations (**Figure 19.39B**). The tree reveals a number of important aspects of the history of human migrations. First, the root of the tree is located in Africa, supporting the hypothesis of an African origin for modern humans. Second, the phylogeny shows strong geographic structure: geographically close groups tend to cluster together. Notably, this structure suggests that the human expansion across the globe occurred in a single gradual expansion rather than, for example, separate migrations from Africa each independently colonizing different regions. We can infer this from the successive nesting of clades. The clades from Oceania, the Americas, and East Asia are nested within a larger clade that includes all of central Asia. The central Asian clade and a European sister clade is nested with a clade including populations from the Middle East. And the Middle Eastern clade is nested within the clade of African populations. This indicates a gradual expansion from Africa, through the Middle East, into Europe and into central Asia, and then from central Asia into East Asia, Oceania, and the Americas.

This recent African origin and successive migration around the world has created a distinctive pattern of genetic variation among human populations. In Chapter 8, we looked at the *leading edge expansion* of the black spruce into formerly glaciated regions of Canada: Because only a subset of the population migrated into the new area, we observed substantially less genetic diversity within the newly settled population relative to the source. *Homo sapiens* have gone through a similar process, sometimes referred to as a *serial founder effect,* as a subset of humans migrated first out of Africa about 60,000 years ago and then into Europe, Asia, Oceania, and the Americas (Henn et al. 2012). Several times during this process, a small subpopulation on the leading edge of the advancing human wave sent out founders into an adjacent geographic region. Each successive expansion resulted in reduced genetic diversity in the newly colonized region relative to the source region because of the founder effect.

Li and colleagues were able to see this loss of diversity clearly from their data. They calculated expected heterozygosity (H_e) for haplotype blocks along an arbitrarily chosen chromosome (chromosome 16) for each population in their sample. They found that expected heterozygosity decreased with distance from Ethiopia, a purported origin for the human migrations in their study (**Figure 19.39C**). This

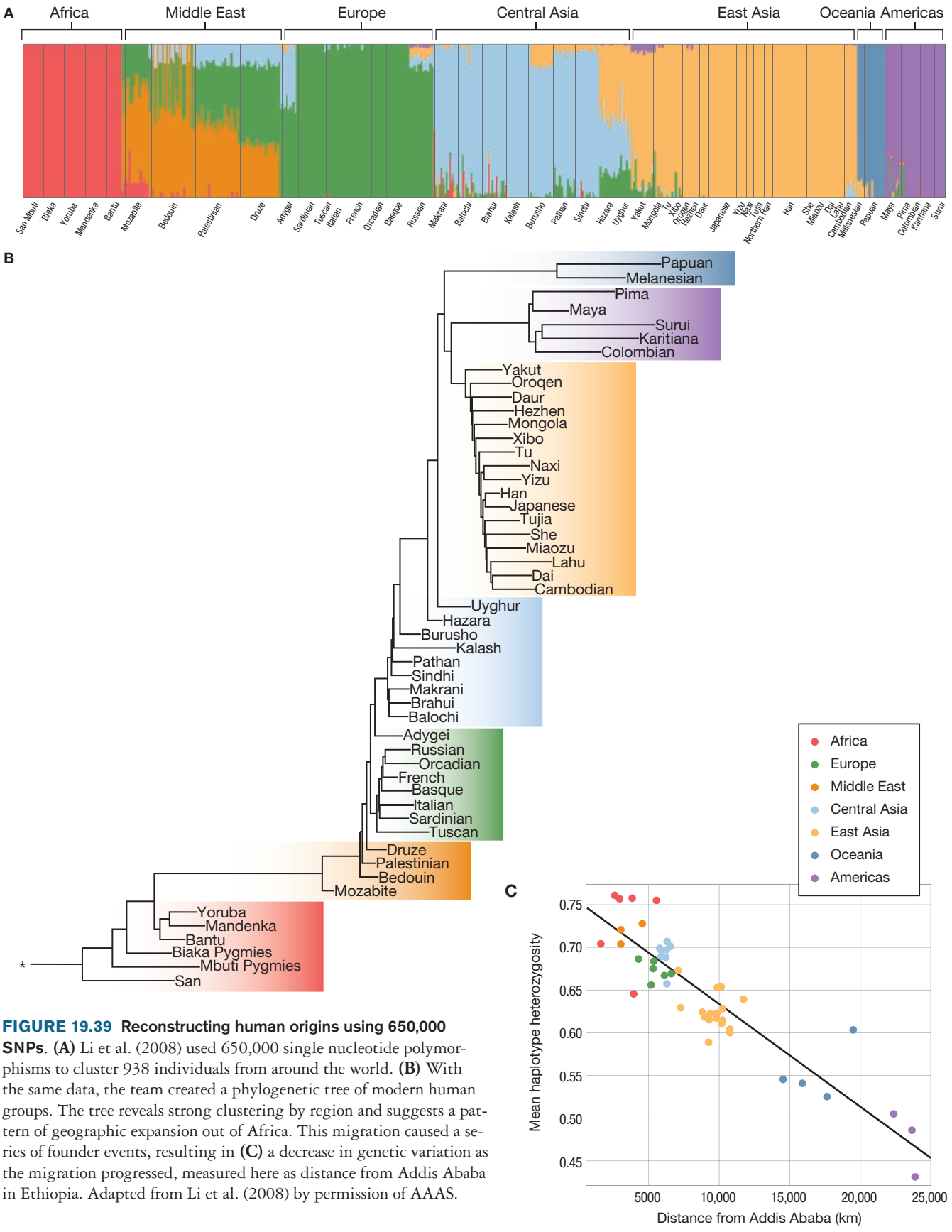

FIGURE 19.39 Reconstructing human origins using 650,000 SNPs. **(A)** Li et al. (2008) used 650,000 single nucleotide polymorphisms to cluster 938 individuals from around the world. **(B)** With the same data, the team created a phylogenetic tree of modern human groups. The tree reveals strong clustering by region and suggests a pattern of geographic expansion out of Africa. This migration caused a series of founder events, resulting in **(C)** a decrease in genetic variation as the migration progressed, measured here as distance from Addis Ababa in Ethiopia. Adapted from Li et al. (2008) by permission of AAAS.

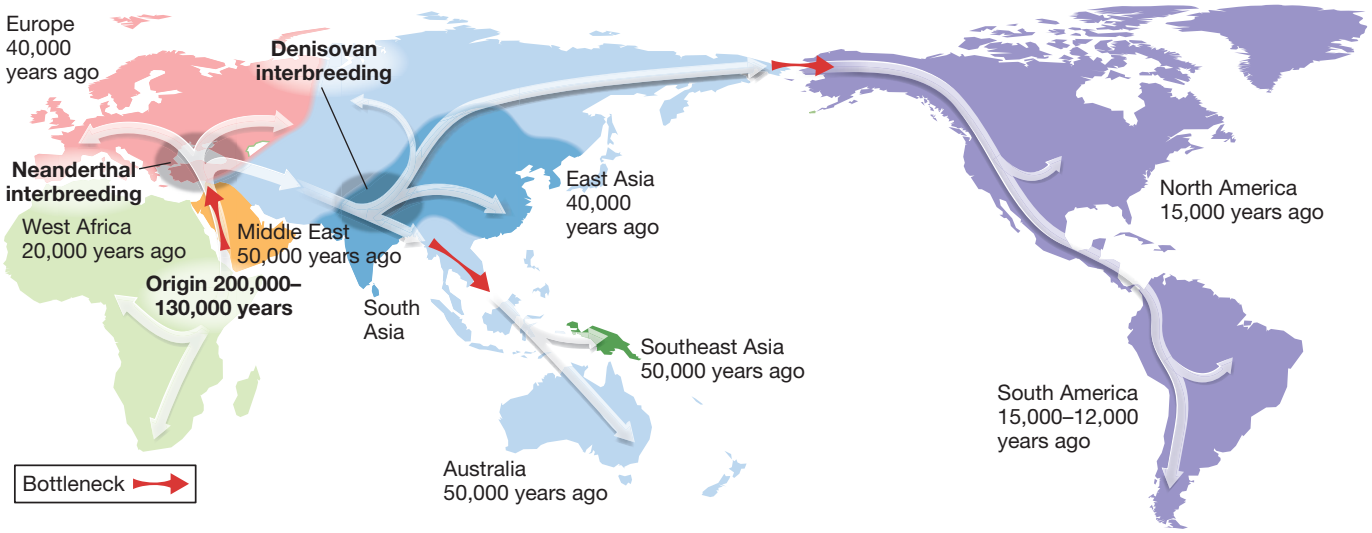

FIGURE 19.40 The expansion of modern humans across the globe. Bringing together the many sources of evidence we have discussed, this map summarizes the major migrations of modern *Homo sapiens* during the global expansion from 70,000 to 12,000 years ago. Adapted from Henn et al. (2012) and Comas (2014).

pattern appears time and again in studies of human diversity. As we would predict from a model of successive expansions, human diversity decreases with distance from East Africa (Prugnolle et al. 2005; Ramachandran et al. 2005; Deshpande et al. 2009).

Putting together what we have explored so far, we can diagram the major migrations underlying the human expansion around the globe, beginning with the origin of *Homo sapiens* in Africa 200,000–130,000 years ago, expanding out of Africa 60,000 years ago, experiencing a series of founder effects, interbreeding with Neanderthals and Denisovans, and ultimately spanning the globe by 12,000 years ago (**Figure 19.40**). How remarkable that this map has been retained in the collective genetic makeup of our species and now is ours to uncover with the power of genetic sequencing and phylogenetic reconstruction.

Host–Pathogen Coevolution

Thus far, we have explored how researchers have used human genetic sequences to reconstruct the history of modern human migrations, but this is not the only source of information available. Another way to test our hypotheses about human migrations is to study the evolutionary histories of the human-specific pathogens that have coevolved with our species. But this is not as straightforward as it may sound. The problem is that most pathogens infect individuals for only a short period of time and then spread *laterally,* from the infected person to an unrelated individual. Such pathogens can readily move from one human population to another based on a single migration event. Other pathogens move back and forth between human populations and nonhuman reservoirs. Yet others go through waves of epidemic expansion and tight bottlenecks that destroy past phylogenetic signals (Holmes et al. 2008). In any of these cases, the evolutionary history of such pathogens is unlikely to reflect the evolutionary history of their hosts. But for pathogens that are human-specific and are primarily transmitted

vertically, from parents to offspring, we might expect their evolutionary history to reflect that of the human populations that they infect.

One such pathogen is *Helicobacter pylori,* a bacterium that colonizes the stomach and is found in about half of the human population worldwide (**Figure 19.41**) (Suerbaum and Michetti 2002). Once an individual is colonized, he or she often remains colonized by the same strain for decades. Moreover, *H. pylori* is primarily transmitted within families, and often vertically from parents to offspring. Humans appear to be the primary hosts of *H. pylori*, and while other species can be infected, no transmission from other species has been recorded. As a consequence, *H. pylori* strains are transmitted in parallel with human genes, and thus patterns of genetic diversity within this pathogen are expected to mirror patterns of genetic diversity within humans. An additional advantage of working with *H. pylori* is that this species engages in recombination at an unusually high rate. As a result, linkage disequilibrium is low, and this can improve our ability to infer evolutionary history from genetic data.

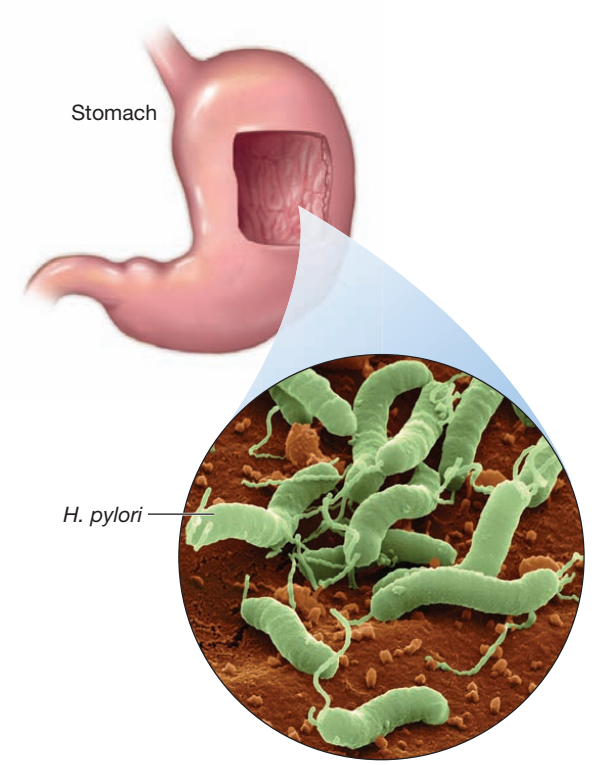

FIGURE 19.41 ***Helicobacter pylori.*** The helical bacterium *Helicobacter pylori* penetrates the stomach's mucosal layer and infects the stomach lining. The bacterium infects about half of the world's human population and is responsible for more than half of peptic ulcers and stomach cancers. Infection is typically lifelong, and the bacterium is usually transmitted within families.

To determine whether geographic patterns of *H. pylori* reflect those of their human hosts, researchers sequenced regions of the *H. pylori* genome from 769 samples taken from people living in 51 regions around the world (Falush et al. 2003; Linz et al. 2007). They found distinct genetic clusters corresponding to continent-scale geography. But unlike the sharp divisions that *structure* reveals for human genotypes, the *H. pylori* genotypes reveal a continuous gradation of genetic composition when moving from one region to the next (**Figure 19.42**).

Indeed, the genetics of *H. pylori* have been useful in resolving some smaller-scale details of early human migrations in India, Southeast Asia, and the Pacific (Wirth et al. 2004; Moodley et al. 2009; Breurec et al. 2011). They also reveal more recent patterns of movement, including the colonial expansion out of Europe, Chinese trade routes into Southeast Asia, and the forced relocations from West Africa to the Americas due to the slave trade (Moodley and Linz 2009).

Just like human genetic diversity, *H. pylori* genetic diversity decreases with distance from East Africa, suggesting an African origin and subsequent migration around the globe for the bacterium (**Figure 19.43**). Using a simulation model, researchers have inferred that *H. pylori* expanded out of Africa between 54,500 and 61,500 years ago—in close agreement with estimates of when modern humans expanded beyond Africa based on human genetic data (Linz et al. 2007). In a separate study, many of the same biologists used coalescent models to look back even further into the history of this pathogen. They inferred that *H. pylori* first originated in humans about 100,000 years ago. The most closely related *Helicobacter* species, which infects large felines, actually split off from *H. pylori* well after this date, about 50,000 years ago. This means that humans introduced the pathogen to big cats rather than vice versa (Moodley et al. 2012).

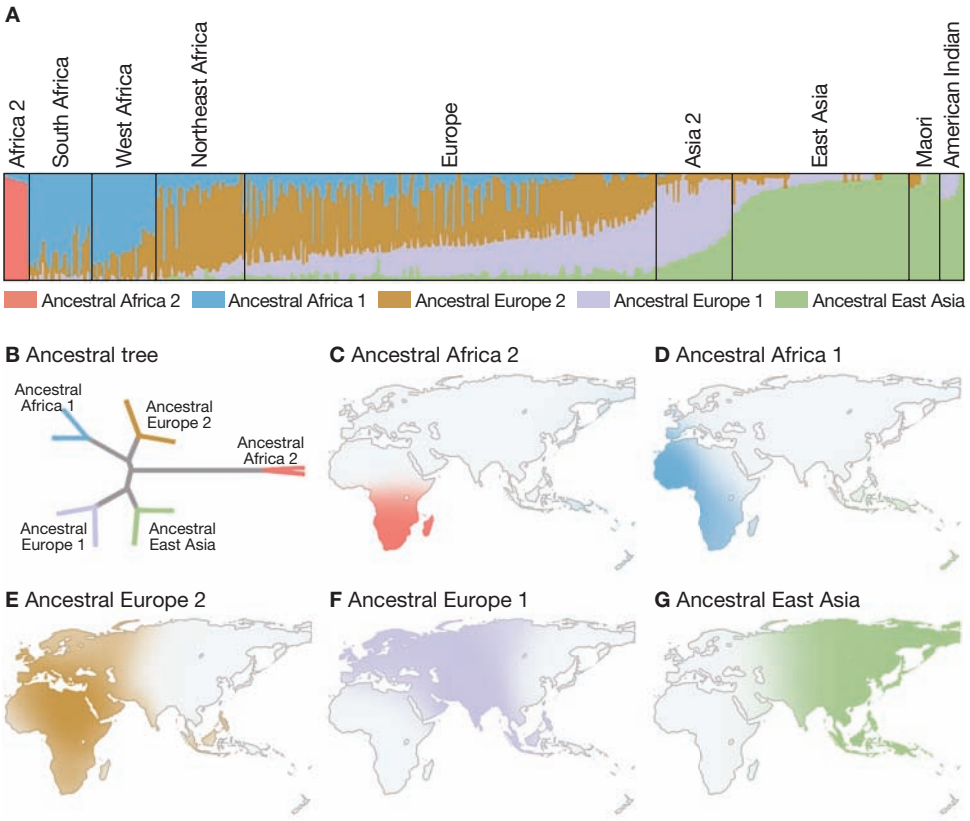

FIGURE 19.42 Ancestral *H. pylori* genotypes as revealed by *structure*. **(A)** Applied to *H. pylori* sequence data, the *structure* algorithm reveals five major source populations (indicated by color) with phylogenetic relationships illustrated in **(B)**. The labels atop the diagram in panel A refer to the regions or populations where the actual samples were obtained. The Ancestral Africa 2 **(C)** and Ancestral Africa 1 **(D)** strains originated in South Africa and West Africa, respectively. Ancestral Europe 2 **(E)** originated in North Africa and spread northward as modern humans expanded, while Ancestral Europe 1 **(F)** originated from central Asia and was brought back into Europe via westerly migration. Ancestral East Asia **(G)** originated in East Asia and spread into both Oceania and the Americas. Adapted from Linz et al. (2007).

KEYCONCEPT QUESTION

19.3 What types of viruses are likely to have a population genetic structure that reflects the history of human migrations around the globe? What types of viruses are unlikely to have a population genetic structure similar to that of their hosts? Provide an example of each.

Helicobacter pylori is not the only pathogen that has coevolved with human hosts such that its phylogenetic structure reflects that of human populations. A whole-genome analysis of *Mycobacterium tuberculosis,* the bacterium responsible for human tuberculosis, closely mirrors the patterns observed in *H. pylori* (Comas et al. 2013).

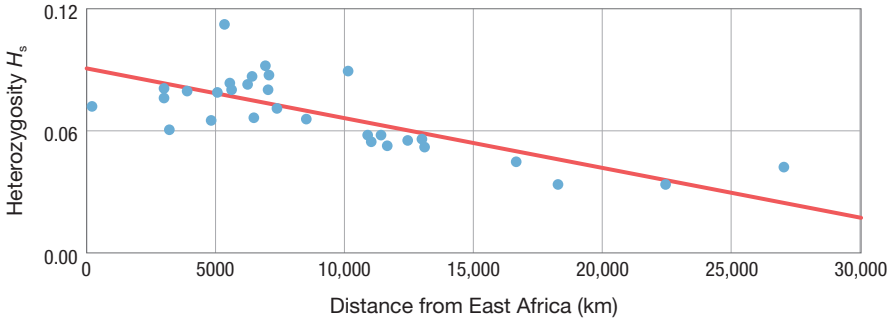

FIGURE 19.43 Heterozygosity of *H. pylori* subpopulations decreases with distance from East Africa. Here, the researchers measured the heterozygosity of the subpopulation (H_s) rather than expected heterozygosity of individuals (H_e) because *H. pylori* is haploid. Adapted from Linz et al. (2007).

This study suggests an African origin for *M. tuberculosis* about 70,000 years ago, followed by global expansion with migrating human populations. Moreover, a phylogeny of more than 200 *M. tuberculosis* genomes reveals a remarkable concordance with the human mtDNA phylogeny (**Figure 19.44**). Statistical models based on *M. tuberculosis* genome diversity indicate that the disease persisted at relatively low density for 60,000 years before exploding as human population densities increased roughly 11,000–6,000 years ago during the Neolithic transition.

We are also developing a better understanding of how our species has been involved in coevolutionary associations—such as those we discussed in Chapter 18—with macroparasites such as bedbugs, tapeworms, and lice. Many of these associations appear to be very old. Bedbugs were likely acquired from bats and evolved to become hominin-specific pathogens before the divergence of *Homo sapiens* from other hominins (Balvín et al. 2012). Hominids appear to have acquired tapeworms long before the origins of *Homo sapiens,* perhaps due to the shift to

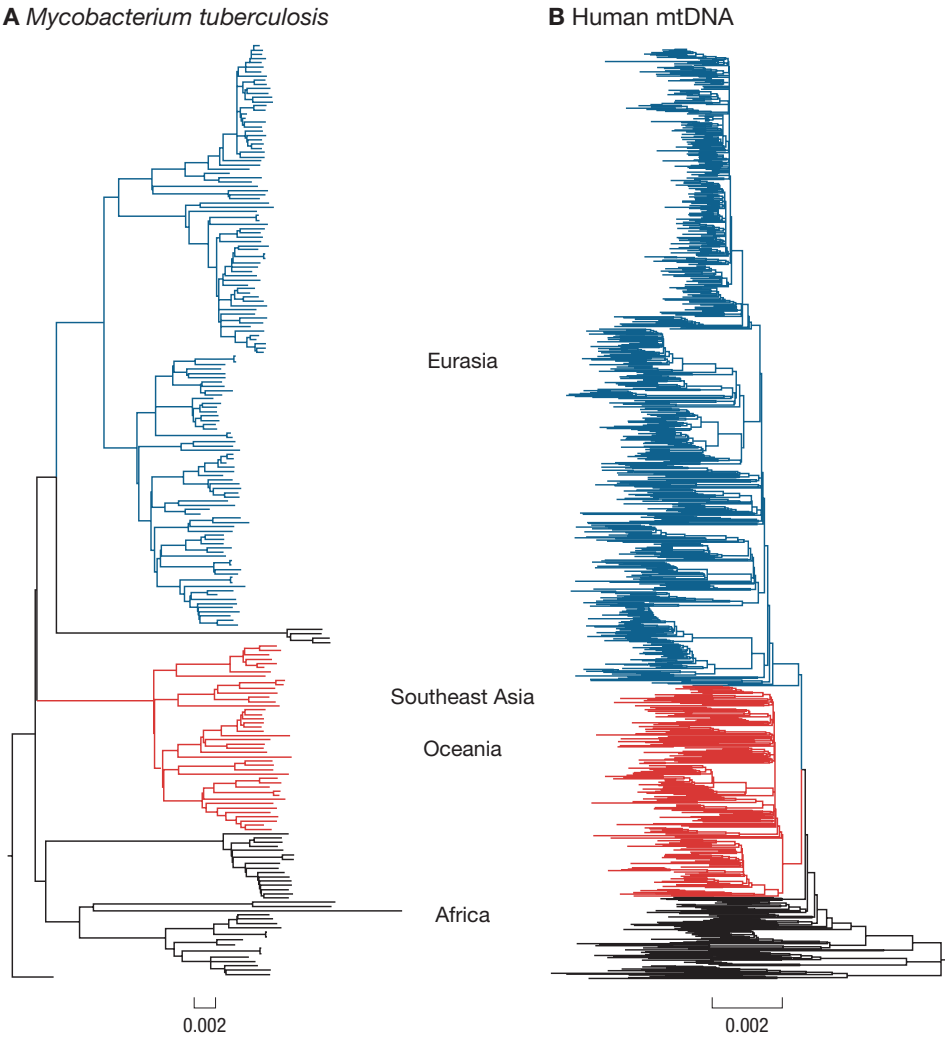

FIGURE 19.44 The phylogeny of *Mycobacterium tuberculosis* parallels the human mtDNA phylogeny. A phylogeny of 220 *M. tuberculosis* isolates from around the world **(A)** closely reflects the geographic structure of a phylogeny of nearly 5000 human mitochondrial genomes **(B)**. Adapted from Comas et al. (2013).

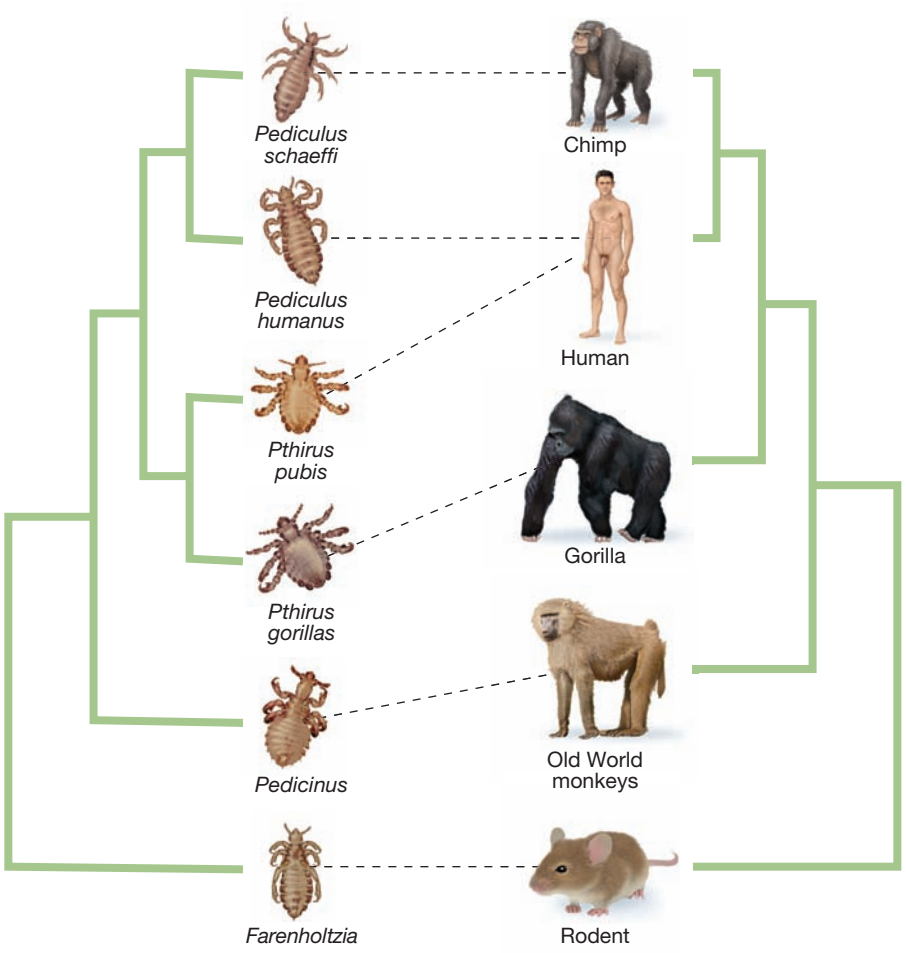

FIGURE 19.45 Coevolution of lice and primates. Louse species have cospeciated with humans, chimps, and gorillas—but a gorilla *Pthirus* louse jumped to humans about 3.3 million years ago and subsequently speciated into the human pubic louse *Pthirus pubis.*

a meat-based diet. Humans subsequently transmitted their tapeworms to the domesticated pigs with which we associate these parasites, rather than vice versa as previously believed (Hoberg et al. 2001; Perry 2014). Because two ecological types of the human louse *Pediculus humanus*—head lice and clothing lice—diverged between 83,000 and 170,000 years ago, researchers have hypothesized that humans first began wearing clothes during that time window (Toups et al. 2011). Pubic lice (*Pthirus pubis*), meanwhile, did not speciate on their human hosts but instead were acquired from gorillas about 3.3 million years ago, long after the divergence of those two species (Reed et al. 2007) (**Figure 19.45**).

As we have seen, examining the evolutionary history of our parasites and pathogens is only an indirect way of inferring human evolutionary history. This approach relies on tight coevolutionary dynamics between host and pathogen. To resolve history at the species level, we need cospeciation; to resolve history at finer scales, we require barriers to pathogen transmission that reflect within-species restrictions on gene flow. If we want to uncover human genetic history, we will typically do better by working directly with human genetic data. Yet pathogen work such as we have described here is powerful in that it cross-validates human genetic studies. All of the results that we have considered in this section, from single-gene trees to multilocus studies to studies of pathogen genetics, fit together like

the pieces of a puzzle. Together these findings support the out-of-Africa hypothesis, albeit in a modified form that allows for limited gene flow from Neanderthals and Denisovans, much more strongly than any individual study could.

In this chapter, we have explored the evolutionary origins of our own species. We have found that just like any other species, our phylogenetic history is not a single progression from early hominin to *Homo sapiens,* but rather an intricate tree with many side branches that were lost to extinction. We have seen how recent advances in the genomic sequencing of ancient DNA reveal the complex web of gene flow between hominin species, and we have described the ways in which researchers are using genetic data to retrace the migrations of modern humans around the globe and to understand the histories of contemporary human populations. In the next chapter, we turn away from the past and look at how a better understanding of evolutionary biology can help us in the here-and-now by improving research and practice in the field of medicine.

SUMMARY

1. Genomic evidence reveals that humans are members of the primate superfamily known as the Hominoidea, or the apes.
2. Among living species, chimpanzees are most closely related to humans. Yet at about 20% of loci, humans are more closely related to gorillas due to a phenomenon known as deep coalescence.
3. The hominin clade includes both humans and extinct species more closely related to humans than to chimpanzees. Hominins are distinguished by a reduction of the jaw and teeth and by adaptations for bipedal locomotion. This clade is not a single progression of species, but rather a branching tree with multiple and diverse hominin species coexisting for most of its evolutionary history.
4. Some time after 2.4 million years ago, the first members of the genus *Homo,* known as the transitional hominins, arose from archaic hominin ancestors. These species had larger brains than previous hominins and developed the ability to construct and use stone tools. They were followed by the premodern hominins, including *Homo ergaster*, with yet greater cranial capacity and longer limbs.
5. The multiregional hypothesis suggests that hominins left Africa and colonized the rest of the Old World in a single event nearly 2 million years ago. Worldwide, *Homo* populations diverged from one another morphologically, but modest gene flow among the populations prevented branching speciation. Gradually, these loosely associated populations evolved together into modern humans.
6. The out-of-Africa hypothesis posits multiple hominin radiations out of Africa. Under this hypothesis, *Homo sapiens* evolved in Africa less than 200,000 years ago, migrated beyond Africa around 60,000 years ago, and replaced other hominins around the globe by 30,000 years ago. Fossil evidence, archaeological evidence, and mitochondrial DNA sequence data all support the out-of-Africa model.
7. The out-of-Africa model does not tell the whole story, however. After migrating out of Africa, *Homo sapiens* interbred with at least two other *Homo* species, the Neanderthal and the Denisovan people. Today, humans of non-African origin carry a small amount of DNA from these other *Homo* species.
8. The majority of variation between human individuals is within-population variation: On average the genetic differences between individuals of different ancestral origins are only slightly greater than the genetic differences between individuals with the same ancestral origin.
9. By working with large numbers of loci simultaneously, researchers can use advances in genetic sequencing and phylogenetic inference to infer the ancestral origin of individuals and reconstruct the historical relationships among ancestral groups. Analyses of this nature help us sketch out a story of human migrations around the globe.
10. The genetic history of certain pathogens, particularly those that are vertically transmitted from parents to offspring or shared primarily within families, reflects the genetic history of human populations and can be used to learn more about our evolutionary past.

KEY TERMS

admixture (p. 706)
archaic hominins (p. 688)
bipedal locomotion (p. 685)
deep coalescence (p. 682)
Denisovan (p. 679)
hominin (p. 679)
hominoid (p. 680)
mitochondrial Eve (p. 695)
multiregional hypothesis (p. 693)
Neanderthal (p. 679)
out-of-Africa hypothesis (p. 694)
premodern hominins (p. 689)
structure (p. 706)
transitional hominins (p. 689)

REVIEW QUESTIONS

1. What is the difference between a hominin and a hominoid? Which of these labels applies to modern humans?
2. Why are we unable to determine definitively the evolutionary relationships between humans, chimpanzees, and gorillas by analyzing a single locus such as the mitochondrial chromosome?
3. List two important anatomical features that distinguish hominins from other hominoid lineages.
4. How might the ability to use fire have had a massive impact on the trajectory of hominin evolution?
5. Why does the fact that mitochondrial Eve was an African hominin who lived about 200,000 years ago support the out-of-Africa hypothesis over the multiregional hypothesis?
6. At some genetic loci, certain individual humans are more closely related to individual Neanderthals than they are to other individual humans. Why is this not by itself sufficient evidence to demonstrate interbreeding between humans and Neanderthals?
7. Why do researchers hypothesize that the Denisovans, but not the Neanderthals, interbred with some other more distant hominin species?
8. Why is a phylogeny created using the mitochondrial genome only a partially accurate reflection of the phylogenetic relationships among modern human populations?
9. Why does the genetic diversity of human populations decrease with distance from Africa?
10. Why is *Helicobacter pylori* a particularly useful bacterial species to use in inferring the population structure of its human hosts?

KEY CONCEPT APPLICATION QUESTIONS

11. Only when extensive genetic data became available were scientists able to definitively resolve the relationship between humans, chimpanzees, and gorillas. Explain why the relationship between these three species was a particularly difficult challenge to solve.
12. The highly polymorphic *HLA* alleles in humans reflect polymorphisms that have been conserved since before the divergence of humans and chimpanzees approximately 5.5 million years ago. Explain how this can be consistent with the observation that the most recent common ancestor of all human mitochondrial genomes—mitochondrial Eve—dates to around 200,000 years ago.
13. Genetic analysis of the bacterium *Streptococcus mutans*, which causes dental caries (cavities), suggests that this species originated in humans about 10,000 years ago. Propose a hypothesis for why this pathogen emerged at that time in human history.
14. Nonscientists sometimes speculate that modern technology has brought human evolution to a halt. Explain why this is incorrect.

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20

Evolution and Medicine

◀ Rock art from the prehistoric Barrier Canyon Culture in Buckhorn Wash of the San Rafael Swell, Utah.

Folklore has it that fever can bring about prophetic dreams, visions, and epiphanies. One could even argue that, in a sense, the subject of this entire book—the theory of biological evolution—arose in part through a feverish epiphany. Alfred Russel Wallace, who developed the theory of evolution by natural selection in parallel with Charles Darwin, described how he came to realize the role of natural selection:

> At that time I was suffering from a rather severe attack of intermittent [malarial] fever at Ternate in the Moluccas, and one day while lying on my bed during the cold fit, wrapped in blankets, though the thermometer was at 88°F . . . there suddenly flashed upon me the idea of the survival of the fittest—that the individuals removed by [disease, famine, and the like] must be on the whole inferior to those that survived. In the two hours that elapsed before my ague fit was over I had thought out almost the whole of the theory, and the same evening I sketched the draft of my paper, and in the two succeeding evenings wrote it out in full, and sent it by the next post to Mr. Darwin. (Wallace 1891, p. 20)

But whether or not fever brings epiphanies—or more likely bizarre dreams—fever makes one feel miserable. Fever can even be life threatening if body temperature rises too high. Fortunately, fever is usually easy to remedy. A number of common over-the-counter drugs have *antipyretic* effects: Aspirin, acetaminophen, and ibuprofen all reduce or eliminate fever with a minimum of side effects in most patients. Thus, it makes perfect sense, on first consideration, that we should treat fever whenever we can. Doing so is easy, and it relieves suffering or worse.

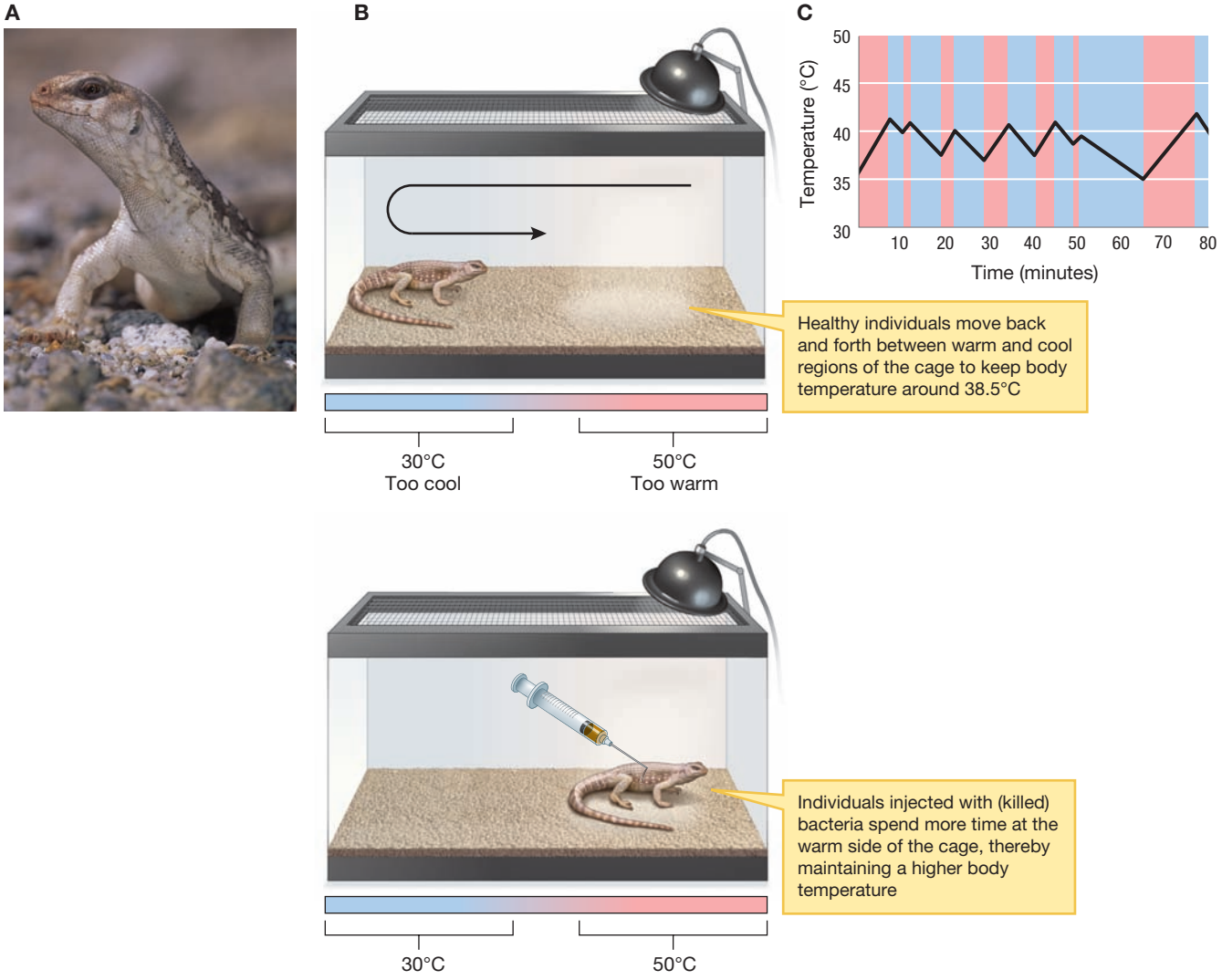
But if we consider what fever is *for* in the first place, we might begin to wonder about the wisdom of treating it. There is overwhelming evidence that fever, like cough and diarrhea, is one of the body's evolved defenses against infection by pathogens (Nesse et al. 2006). Might there be times when it is best to let a fever run its course rather than treat it? Or is it reasonable to block the fever response even if it has evolved as a defense against pathogens? We begin by describing one particularly striking example of fever in another species, and we will return to answer these questions in more detail later in this chapter.

Perhaps unsurprisingly, fever is common among vertebrate species. Fever is not restricted to *endotherms*, the so-called warm-blooded species that actively regulate their body temperatures. In a classic experiment, Linda Vaughn and her colleagues demonstrated that a cold-blooded *ectotherm*, the desert iguana (*Dipsosaurus dorsalis*), induces fever behaviorally in response to bacterial infection by moving to warmer locations within its habitat (Vaughn et al. 1974). To demonstrate this, Vaughn and her colleagues constructed environmental chambers with regions that were kept at two different temperatures: one below the thermal optimum for the lizards, and one above the thermal optimum. They found that healthy lizards thermoregulate by moving between the two areas, as shown in **Figure 20.1**.

Vaughn and her colleagues then stressed a group of lizards by injecting them with bacteria. To avoid causing infections that would kill the lizards, the researchers killed the bacteria with heat. Although the dead bacteria were not able to reproduce, they could nonetheless stimulate an immune response in the injected lizards. To make sure that any change in the behavior of the lizards was due to the bacteria rather than to the handling process and the physical injection, the researchers compared the individuals injected with bacteria to a control group that had been injected with saline solution. They found that both groups used behavioral thermoregulation, but the lizards injected with bacteria had a shift in their preferred temperature. Compared to the controls, lizards injected with bacteria stayed on the warm side of the enclosure until reaching a higher body temperature. Similarly, the lizards injected with bacteria were quicker to leave the cool side of the enclosure than were the controls.

This work provides compelling evidence that the lizards respond to bacterial infection with what is called *behavioral fever*. Subsequent studies have shown behavioral fever in other ectothermic vertebrates, including a wide range of reptiles, fish, and amphibians (Reynolds et al. 1976; Covert and Reynolds 1977; Monagas and Gatten 1983). A number of invertebrate species have also been shown to elevate their body temperatures in response to infection (Thomas and Blanford 2003).

These results suggest that fever may somehow be advantageous in dealing with infection. Both endothermic and ectothermic vertebrates induce fever, albeit by very different mechanisms, in response to infection. This might give



us pause as we think about the purpose of fever and the medical implications of treating it. We will return to the issue of fever in Section 20.2. There we will see why, defensive role notwithstanding, treating fever may cause little or no harm in most cases.

In this chapter, we will explore the new and rapidly growing field of evolution and medicine. Throughout, a unifying theme will be that evolutionary biology informs medical science by providing explanations for how vulnerabilities to disease have evolved—and that this in turn helps us both to understand the proximate mechanisms responsible for disease and to generate hypotheses about how disease can be treated. We will look at six different classes of explanation for disease vulnerability, and we will explore case studies for four of them. In the course of discussing these four cases, we will resolve the following questions:

- Why can we safely treat most fevers despite the fact that fever appears to be an evolved defense?
- What is the role of the immune system in host–pathogen coevolution?

FIGURE 20.1 Thermoregulatory behavior in the desert iguana *Dipsosaurus dorsalis*. **(A)** *Dipsosaurus dorsalis*. **(B)** Vaughn observed how uninfected *D. dorsalis* thermoregulated by moving between the cool end and the warm end of its enclosure, whereas infected *D. dorsalis* spent more time at the warm end of its enclosure. **(C)** A graph showing temperature regulation behavior for an uninfected lizard. The curve goes up and down as the lizard thermoregulates, moving to the cool end of the chamber when it reaches too high a body temperature and to the warm end of the chamber when it reaches too low a temperature. Panel C adapted from Vaughn et al. (1974).

- How have physiological structures that evolved several hundred million years ago left us vulnerable to choking, and how has selection within the past hundred thousand years exacerbated this problem?
- Why do organisms age, decline, and die?

Although these examples encompass only a small sampling of the ways in which evolutionary biology can contribute to medical research and practice, they will provide a sense of the intimate connections between evolutionary history, evolutionary processes currently in operation, and the practice of human medicine.

20.1 Vulnerability to Disease

Few philosophical problems are more vexing—or have attracted more commentary—than the question of human suffering. "If the immediate and direct purpose of our life is not suffering," wrote the philosopher Schopenhauer, "then our existence is the most ill-adapted to its purpose in the world" (Schopenhauer 1851, cited in Nesse 2001). *Why* does the human condition involve such heavy doses of pain, misery, anxiety, and sadness? In this chapter, we will look at science's answers to these questions. In doing so, we first need to be clear as to exactly what we mean by the question "why?"

Levels of Explanation

The Nobel prize–winning biologist Niko Tinbergen distinguished among four different types of answers that can be given to a "why" question in biology: (1) proximate explanations, (2) developmental explanations, (3) evolutionary explanations, and (4) phylogenetic explanations. Although Tinbergen developed this distinction to apply to explanations for behaviors, we can apply them equally well to explanations for illness or disease. Proximate explanations tell us about the immediate mechanism that precipitated a particular pathology. Developmental explanations tell us how the pathology came about over the course of the organism's lifetime. Evolutionary explanations tell us how natural selection and other evolutionary processes interact to leave the body vulnerable to a particular pathology. Phylogenetic explanations look at a species' evolutionary history and explain where in this evolutionary history such vulnerabilities came about.

Medicine largely deals with the first two levels of explanation, for good reason: Proximate and developmental explanations associate disease with factors *that we have the power to change*. Much of clinical medicine is *reactive*: It aims to respond to a problem and to correct that problem to whatever degree is possible. This aim places a significant premium on a proximate understanding of disease. If we are to intervene to eliminate or eradicate disease, we must understand the proximate contributors to that disease, and then alter them. Similarly, *preventative medicine* commonly considers the developmental explanations of disease in order to intervene before illness begins. How do a patient's lifestyle and life experience shape disease vulnerability? How can changes in lifestyle reduce the probability of illness later in life?

Beyond the obvious importance of proximate and developmental explanations for medicine, researchers and clinicians alike are beginning to recognize the utility

of evolutionary and phylogenetic explanations as well. An understanding of these factors deepens our understanding of the defenses and weaknesses of the human body. Moreover, explanations at the evolutionary and phylogenetic levels can suggest new hypotheses about mechanisms and consequences at the proximate and developmental levels. Evolutionary thinking can guide medical research on mechanisms of disease and lead us to new methods of preventing or treating illness.

We also need to consider what is the *object* of evolutionary explanation. What is it about disease that we aim to explain? Illness itself is rarely an adaptation; rather, we aim to understand the *vulnerability* to illness. How did these vulnerabilities evolve, and why have such vulnerabilities persisted despite the operation of natural selection?

KEYCONCEPT QUESTION

20.1 Propose a proximate explanation and an evolutionary explanation for why we sneeze.

Six Explanations for Vulnerability to Disease

Randy Nesse and George Williams proposed six classes of evolutionary explanation for vulnerability to disease in their 1994 book *Why We Get Sick* (Nesse and Williams 1994, Nesse 2005). All of these types of explanation are based on principles we have already studied in previous chapters. But in this chapter, we will use those principles to understand human vulnerabilities to disease. It is important to recognize that these types of explanation are not mutually exclusive. As we will see explicitly in our discussion of choking, multiple explanations may contribute to a single vulnerability.

The first two explanations revolve around the fact that evolution by natural selection may not be fast enough to solve certain problems.

1. Humans are locked in a coevolutionary arms race with their pathogens, most of which evolve much more rapidly than do humans.
2. Natural selection has not had time to catch up with rapid changes in the environment.

In each of these cases, there may be heritable genetic variation for disease susceptibility in the population, and less susceptible variants may be favored. Thus, in principle, natural selection could reduce the vulnerability to disease. Yet, susceptibility to disease may remain in the population simply because selection has not had time to eliminate it.

In the first case, vulnerabilities remain because pathogens provide a moving target, evolving rapidly to escape whatever mechanisms evolve in the host to prevent or eliminate infection. For example, the human immune system has a specific receptor named TLR5 that detects the presence of bacteria by binding to the flagellum that many bacterial species use for movement. But a number of human pathogens, including *Campylobacter jejuni* and *Helicobacter pylori* (**Figure 20.2**), have evolved a modified flagellum that is not recognized by TLR5 (Andersen-Nissen et al. 2005).

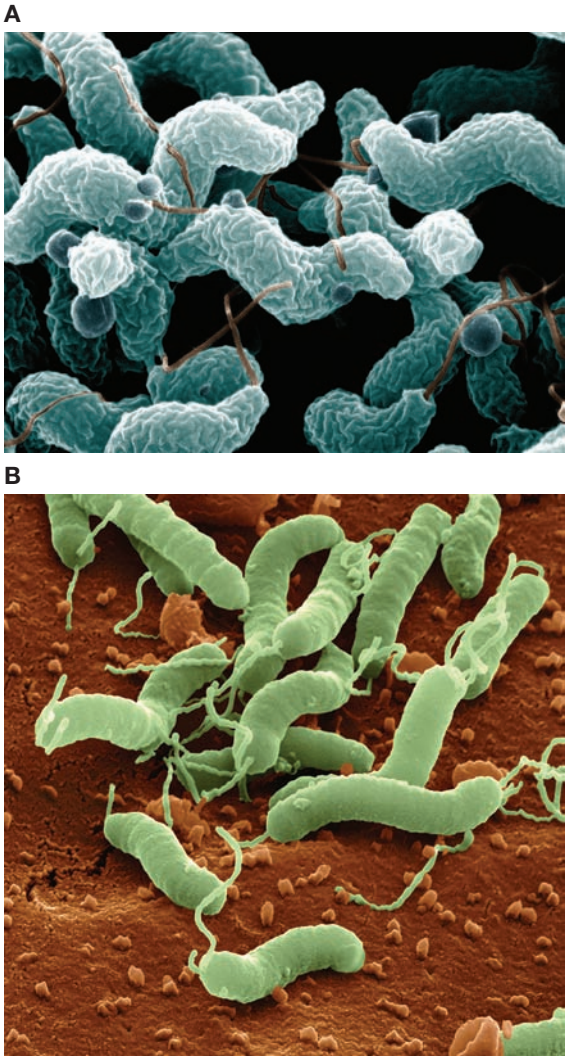


FIGURE 20.2 The flagellated bacteria *Campylobacter jejuni* and *Helicobacter pylori*. **(A)** *Campylobacter jejuni* is a common source of food-borne infection and a major cause of diarrheal disease in humans. **(B)** *Helicobacter pylori* colonizes the stomach and upper small intestine of more than half of the world's population; this species causes stomach ulcers in up to 20% and stomach cancer in up to 2% of carriers. Both have evolved modified flagella that are not recognized by the human immune receptor TLR5, which detects the flagella of other bacterial species.

In the second case, vulnerabilities remain because relatively recent environmental changes create a *mismatch* between our bodies and our current environment. This may be a particularly important mechanism for disease vulnerabilities in humans because human cultural innovations have radically changed human life and human diets over the past 30,000 years. For example, most contemporary humans in developed nations spend much of their time doing "near work," in which the eye is focused on a book, computer, or other object less than 2 feet away. But the feedback systems that fine-tune the shape of the growing eye evolved to function in an environment where the individual spent much of the time with the eye focused on distant objects. The result is an increased susceptibility to developing myopia—shortsightedness—in modern populations (Angle and Wissmann 1980; Foster and Jiang 2014).

The next two explanations pertain to limits on what evolution can do, even given huge amounts of time.

3. The laws of physics and the nature of biology impose trade-offs on what an organism can do.
4. Natural selection lacks foresight, so that sometimes we are stuck with historically contingent relics of our past.

In the first of this pair of explanations, we note that many aspects of our physiology reflect compromises in function. For example, thicker bone structure would reduce the number of fractures that we suffer, but it would come at the expense of nimbleness and speed. Higher metabolic rates might improve numerous aspects of physiological function, but they would do so at the cost of increased nutrient demands. In the second of this pair of explanations, we note that evolution is a historically contingent process: Our current anatomy evolved by a gradual process of modification that occurred without the benefit of foresight. For example, when the basic tetrapod body plan shared by all terrestrial vertebrates was evolving, evolution had no way to plan ahead and ensure that this body plan would be an appropriate foundation for some future bipedal hominid. The transition to upright locomotion freed up our hands and improved the energetic efficiency of our gait—but at substantial cost. In humans, the entire weight of the upper body is supported by the spinal column—which in turn must be curved to balance the torso properly. The result is enormous pressure and strain upon the lower vertebrae, leading to chronic back pain in a large fraction of the adult population (Strassmann and Dunbar 1999).

The last two explanations for vulnerability to disease are focused on what it is that natural selection actually favors: not health and well-being, but rather reproductive success.

5. Natural selection favors reproductive success, even at the expense of vulnerability to disease.
6. Some defenses, such as fever, nausea, and anxiety, may be unpleasant to experience, but they are beneficial adaptations rather than maladies.

In each of these explanations, reactions or symptoms that we label as disease because they are unpleasant may not be maladaptive from a fitness perspective. In the first of this pair, we need to recognize that natural selection does not maximize health at age 70 or even survival at age 16; rather, it maximizes *expected lifetime reproductive success*. Thus, phenomena such as physical decline associated with old age or risk-taking behavior associated with adolescence may be adaptive if the alleles responsible also contribute to reproductive success. In the second of this pair, we need to distinguish between symptoms that are unpleasant but beneficial as defenses—vomiting, fever, or itching—and truly maladaptive defects such as chronic pain.

20.2 Fever

Having laid out the big picture regarding why we are vulnerable to disease, we now turn to a set of case studies illustrating some of the reasons for these vulnerabilities. In this section, we return to the example with which we began this chapter: the phenomenon of fever and the issue of how we should treat it.

The proximate mechanisms responsible for triggering mammalian fever are relatively well understood. When immune cells recognize the presence of a pathogen, they release signaling chemicals known as *cytokines*. Among many other functions, cytokine signals stimulate the brain region known as the hypothalamus, which is responsible for regulating many of the body's physiological systems. The signals induce a shift in the body's thermal setpoint for temperature regulation, driving an increase in body temperature and inducing fever (**Figure 20.3**). For example,

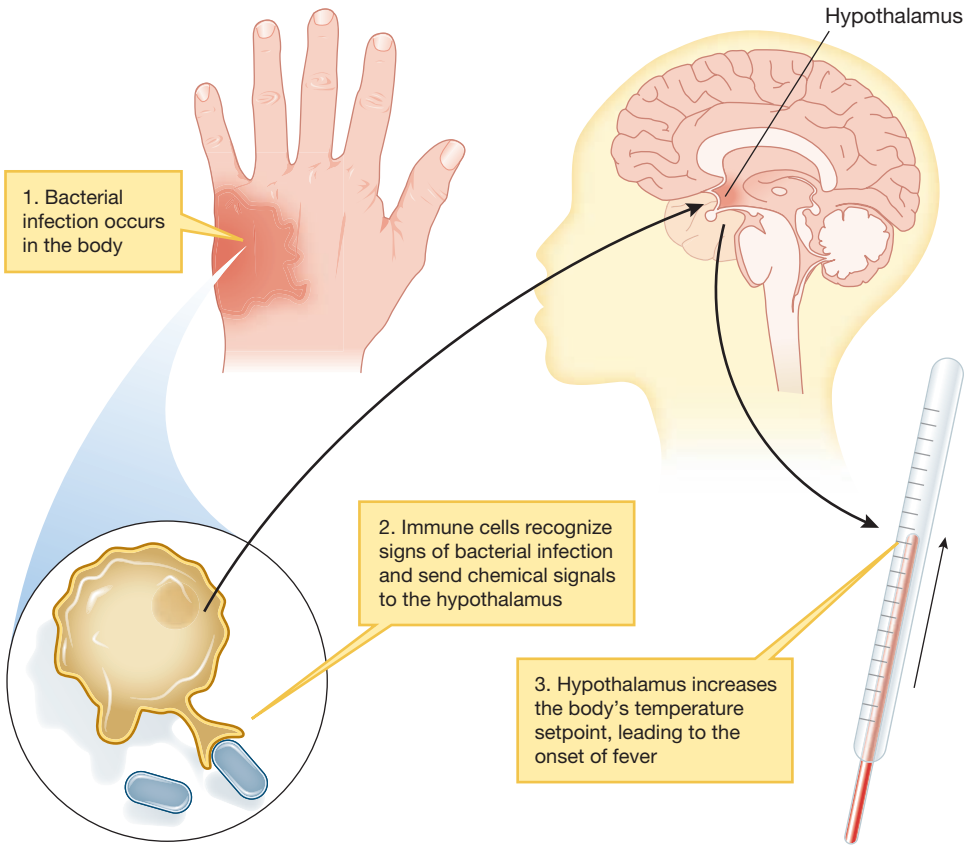


FIGURE 20.3 Proximate mechanism for fever. Immune cells recognize a pathogen and send chemical signals to the hypothalamus in the brain, which increases the body's thermal setpoint, leading to fever.

when components of the bacterial cell wall are bound by immune cells known as macrophages, the macrophages produce the fever-inducing signals. The presence of bacterial cell wall components is a strong indicator of bacterial infection, and thus this pathway illustrates a mechanistic coupling between indications of pathogen challenge and the fever response.

Consequences of Fever

Correlational studies indicate that in humans, higher fevers are associated with more severe infections. From studies that control for infection severity in animal models, it appears that the presence of fever is also correlated with better disease outcomes. A number of studies of human patients have shown that patients who receive fever-reducing antipyretic drugs such as aspirin, acetaminophen, or ibuprofen recover *less* quickly from viral infection (Hasday et al. 2000). This indicates that fever may play a beneficial role in shortening the duration of infection. Studies of patients with bacterial sepsis (a severe and very dangerous full-body inflammatory response induced by bacterial infection) have often, but not always, revealed a higher survival rate in patients that exhibit fever than in patients without fever. It is important to recognize that this observed correlation does not provide direct evidence of causation. Survival rates could be lower in patients without fever because, for example, the inability to mount a fever response could be indicative of more severe illness.

As we described in the introduction of this chapter, numerous nonhuman species exhibit a fever response as well (**Figure 20.4**). In many of the species, manipulative studies are possible. Animals infected with bacterial pathogens and then treated with antipyretic drugs have more rapid bacterial proliferation and higher mortality rates than individuals allowed to develop a normal fever response. Similar results have been observed for viral infections. Because manipulating temperature by pharmaceutical means may cause additional side effects that make it hard to interpret the experimental results, researchers have also conducted experiments in which they manipulate body temperature directly. These studies tend to show comparable benefits to elevated body temperature. Taken together, the correlational studies in humans and manipulative studies in other vertebrates strongly suggest that fever is an adaptation for defense against pathogens.

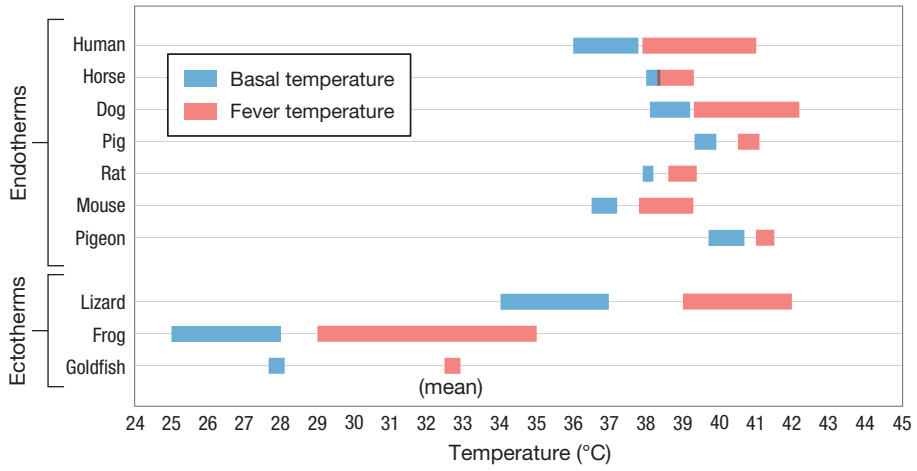


FIGURE 20.4 Basal and fever temperatures in vertebrate species. Fever has been documented in many vertebrates, including both endotherms and ectotherms. Adapted from Hasday et al. (2000).

If fever conferred only benefits, we might expect endotherms to have higher body temperatures all the time, ill or otherwise. But fever has its costs. For one thing, the metabolic costs of fever are significant. Simply running a fever of 2°C to 3°C above normal temperature causes a 20% increase in metabolic rate, and the shivering response sometimes used to elevate temperature can increase metabolic rate up to sixfold over baseline (Kluger et al. 1996, 1998;

Hasday et al. 2000). This increased metabolic load may be especially expensive in precisely the situations in which it occurs: when individuals are already stressed by infection. By up-regulating the immune system, fever may also exacerbate the tissue damage due to aspects of the immune response. Indeed, some experimental studies in mice suggest that while increased body temperature increases the rate at which bacteria are eliminated from the bloodstream, it also increases the probability of death. The fact that fever is phylogenetically so widespread despite its high cost provides another strong line of evidence that fever is an adaptation.

Although we understand the proximate mechanisms for how fever is induced and although multiple lines of evidence suggest that fever is an adaptation, we do not have a good understanding of precisely why fever seems to be beneficial. Researchers have proposed a number of hypotheses. One idea is that the temperature increase may harm the pathogen more than the host. Temperature increases due to fever indeed reduce the growth rates of many microorganisms, but some bacteria grow at comparable rates whether at normal or fever temperatures. Another hypothesis is that temperature increases may up-regulate some immune defenses and increase the rate at which immune cells both proliferate and act against pathogens. A third possibility is that fever may also trigger the expression of *heat shock proteins*, which help cells deal with intracellular damage, and may thus be useful during infection (Singh and Hasday 2013).

The Smoke Detector Principle

Given the costs associated with fever, why are fevers so common? And if fevers are evolved defenses, why can we treat them without severe consequences? To answer these questions, we need to think about the decision to trigger a defensive reaction. Ideally, the body would initiate an unpleasant or costly defensive reaction only when such a reaction was absolutely necessary. For example, the body would only mount a fever response when it was challenged by a pathogen that could not be cleared without fever. But there is an information problem here. At the time a bacterial infection is detected, the body doesn't know just how severe a particular infection may turn out to be—or, in some cases, whether an infection is really present at all.

Moreover, for fever as for other defensive responses, some kinds of mistakes are far more costly than others. A *false positive*, in which a defensive response is initiated in the absence of a threat, typically imposes only a modest energetic cost, whereas a *false negative*, in which no defensive reaction is imposed even in the presence of a threat, can be extremely costly or even fatal. This is the *principle of asymmetric harm*—a false positive is a minor nuisance whereas a false negative can be a catastrophe. As a result, we expect evolution to tune our defensive responses so that they will be invoked too often, rather than too seldom.

Randy Nesse has compared this problem to that faced in the design of a smoke detector (Nesse 2001). Nesse points out that no one wants a smoke detector that detects only some or even the majority of fires: A smoke detector needs to raise the alarm each and every time there is a fire, even at the cost of the occasional false alarm when cooking bacon. Here again, the underlying principle is that false positives are inexpensive compared to false negatives. Indeed, given that false

positives are so much cheaper than false negatives, an optimally designed smoke detector may be in error (of the false-positive sort) 99% of the time that it triggers an alarm, as long as it is almost never in error when it does not sound.

Applying this logic to fever, we can make a number of predictions. First, the cost of a modest fever—some energetic expense, some discomfort and associated downtime—is far less than the cost of failing to produce a fever when it is needed to clear an infection. Thus, we might expect that fevers should be relatively common, and most should be unnecessary. If so, this means that in most cases, we should be able to intervene safely, reducing fever with antipyretic drugs. Medical technologies—most notably antibiotics and rehydration therapy—and improvements in nutritional status further reduce the risk of death by infection relative to what it would have been throughout much of our evolutionary history. Thus, even many of those infections that might historically have been lethal without a fever response can now be safely controlled without fever. This is not to say that treating most fevers with antipyretic drugs is necessarily a good idea. Further research will be needed to resolve that issue. But it does provide an explanation for why we can often safely interfere with fever, despite its role as an evolved defense against pathogens.

KEYCONCEPT QUESTION

20.2 Anxiety appears to be an evolved mechanism to help us avoid or escape from dangerous situations. Apply the smoke detector principle to explain why—even if levels of anxiety have been optimized by natural selection—many actual instances of anxiety that people experience would be unnecessary. That is, explain why many or most episodes of anxiety may not be associated with real danger.

20.3 Coevolutionary Arms Races between Pathogens and Hosts

Infectious disease medicine aims to help us deal with challenges from pathogens ranging from viruses such as influenza and human immunodeficiency virus (HIV) to bacteria such as *Staphylococcus aureus* to eukaryotic parasites such as the malaria parasite (*Plasmodium falciparum*) and parasitic helminth worms. Throughout this book, we have looked at some of the evolutionary considerations that arise in this area of medicine, most notably the evolution of antimicrobial resistance to the drugs that we use against these pathogens. In this section, we will step back and consider more generally why natural selection has not solved the problem for us already. Why are we vulnerable to pathogens in the first place? Why hasn't natural selection provided us with impenetrable immune defenses?

To answer this question, we return to a subject from Chapter 18: the phenomenon of coevolutionary arms races. Such arms races are particularly important in the evolution of hosts and pathogens. Pathogens are selected to do whatever furthers their own reproduction and transmission, and this often involves exploiting the host. Hosts are selected to minimize the harm caused by

pathogens: This is often best accomplished by eradicating the pathogen entirely from the host's body.

At first glance, multicellular hosts appear to have a marked disadvantage in this coevolutionary arms race. Such hosts are typically much larger than their pathogens, with two important coevolutionary consequences.

First, pathogens usually have far shorter generation times than their hosts. For example, the human generation time is of the order 20 years, whereas many bacterial pathogens have generation times of the order an hour or two. This is a 100,000-fold difference in generation times! As a result, natural selection can act extremely rapidly on pathogens relative to hosts. In the time it takes an individual human to go from birth to sexual maturity, bacterial pathogens can go through more generations than there have been in the entire evolutionary history of the *Homo* genus.

Second, pathogens have much larger population sizes than their hosts. A single bacterial infection may consist of billions of cells. A patient infected with HIV may produce more than 100 billion HIV virions per day during the period of peak viral load. Globally, this translates into vast population sizes. Worldwide, there are an estimated 10^{20} *Escherichia coli* living in the gastrointestinal tracts of humans alone, and the population sizes of enteric anaerobes are several orders of magnitude larger (Whitman et al. 1998; Tenaillon et al. 2010). Large pathogen populations that have rapid turnover are able to generate a great deal of genetic variation by mutation, and thus they can generate ample raw material on which natural selection may sort. Moreover, as we saw in Chapter 8, in a large population, natural selection acts more efficiently than drift and can consistently fix alleles conferring smaller fitness benefits. One factor counteracting this population size advantage is that the effective population size of some microbial pathogens can be much smaller than the census population size, due to the tight bottlenecks in transmission from one patient to another coupled with strong selection imposed by immune systems. The worldwide effective population size of *E. coli* is estimated to be around 10^8 cells (Lynch and Conery 2003). More dramatically, the worldwide effective population size of the measles virus is estimated at about 4000 virions and that of the influenza A virus at around a mere 500 virions (Bedford et al. 2011).

With the odds stacked so badly against hosts, how can they possibly keep up? Across the tree of life, hosts have evolved immune systems that isolate pathogens, minimize the harm that they cause, and, if possible, eliminate them from the body. Perhaps the best known of these is our own, the vertebrate adaptive immune system, but there are many others. Bacteria use so-called *restriction–modification systems* to identify and eliminate viral nucleic acids. Bacterial CRISPRs (clustered regularly interspaced short palindromic repeats) confer a form of immune memory against mobile genetic elements (Horvath and Barrangou 2010; Wiedenheft et al. 2012). RNA interference appears to have originated in early eukaryotes as a system for silencing viral gene expression. Plants have extensive systems of nonspecific or "innate" immunity, as do multicellular animals. Among social insects, colony recognition systems and the associated response to intruders can even be viewed as a colony-level immune system. In the remainder of this section, we will focus on the human immune system, but comparable analysis is possible for any of these other systems as well.

Immune Strategies

The human immune system must (1) recognize and (2) eliminate or incapacitate microbial pathogens. Because pathogens draw on such a large pool of genetic variation and evolve so rapidly, a host population could never keep up if it had to match every adaptive substitution in the pathogen population with an adaptive substitution of its own. Instead, immune systems rely on a number of tactics that limit the ability of pathogen populations to outrun their hosts by virtue of rapid natural selection. There are at least three ways that host immune systems can do this.

Detecting Characteristic Components of Pathogens

Perhaps the most straightforward strategy for dealing with rapidly evolving pathogens is to target those components of the pathogen that are distinct from any products of healthy cells and that cannot easily be changed in the course of pathogen evolution. Fortunately for us, bacteria have a number of such components. Our innate immune response detects the presence of pathogens using *pattern recognition receptor* molecules that bind to common components of pathogens known as *pathogen-associated molecular patterns,* or PAMPs. These receptors recognize highly conserved components of pathogens, such as the peptidoglycan polymer that makes up bacterial cell walls (**Figure 20.5**), the lipopolysaccharide molecules in Gram-negative bacterial cell membranes, and the flagellin protein of bacterial flagella. These elements are structurally essential to the various bacteria that use them, and their structures appear to be so highly conserved that bacteria are unable to evolve variants that pass undetected by the pattern recognition receptors (Janeway 1989; Kumar et al. 2011).

Finding Infected Cells

Pattern recognition receptors also target viruses, but free virus particles are tricky to deal with for a number of reasons. First, viruses have few conserved external structures that an immune system could use to identify them. Second, many viruses

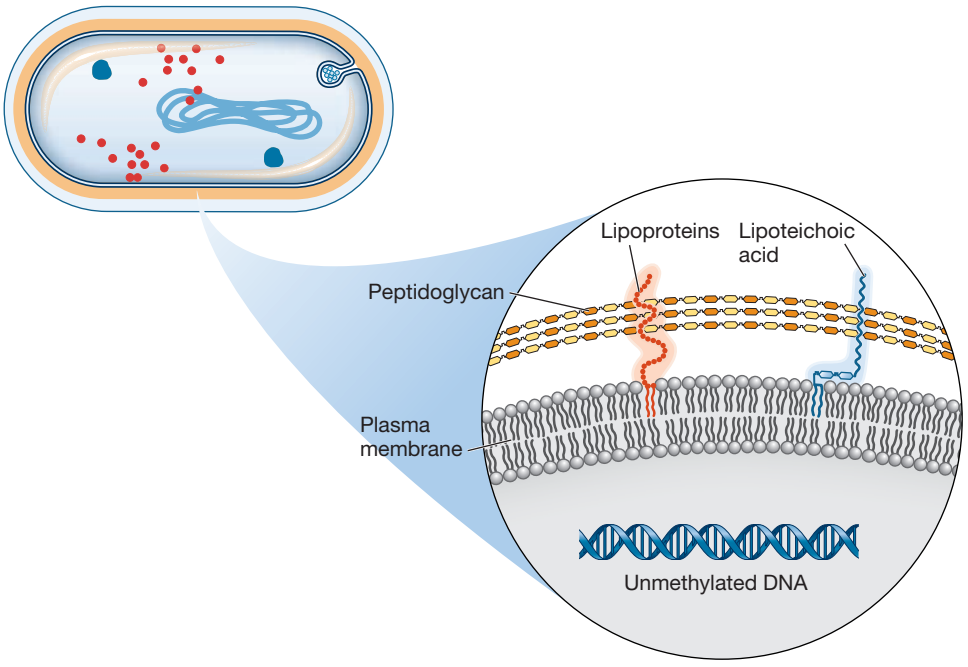


FIGURE 20.5 Pathogen-associated molecular patterns (PAMPs). One way in which immune systems detect the presence of pathogens is with receptors that bind to common and highly conserved pathogen components known as PAMPs. Shown here are a number of PAMPs in a Gram-positive bacterium: elements of the peptidoglycan layer, membrane lipoproteins, lipoteichoic acid, and unmethylated cytosine–guanine base pairs in bacterial DNA. Adapted from Wardenburg et al. (2006).

are produced by budding from a host cell. Such viruses are therefore wrapped in a membrane layer that is structurally the same as the host cell membrane, save for the inclusion of a few viral transmembrane proteins. Third, viruses replicate within host cells, and thus eliminating free virus is not sufficient to clear an infection. Therefore, the immune system must also locate and deal with infected host cells that serve as sources that produce additional virions.

That said, viruses are not without distinctive molecular characteristics. Most notably, properly functioning eukaryotic cells should not contain long double-stranded RNAs, whereas cells infected by viruses do. Double-stranded RNAs occur as either viral genomes or intermediates in the process of viral genome replication. As such, the innate immune system responds aggressively to such double-stranded RNAs within a cell. Other viral nucleic acids and some viral coat proteins are detected by pattern recognition receptors as well (Thompson et al. 2011).

To find other cues that reveal viral pathogens, host immune systems must learn, one way or another, to recognize the molecular signs associated with viral infection of a cell. Vertebrate adaptive immune systems do exactly this. They learn, during the lifetime of a single individual, to detect the cues associated with pathogens or pathogen-infected cells. The basic mechanism is known as **clonal selection**, and we present a simplified picture of the process here.

Prior to pathogen infection, the immune system produces an enormous, highly diverse repertoire of T cells that recognize infected cells and B cells that produce

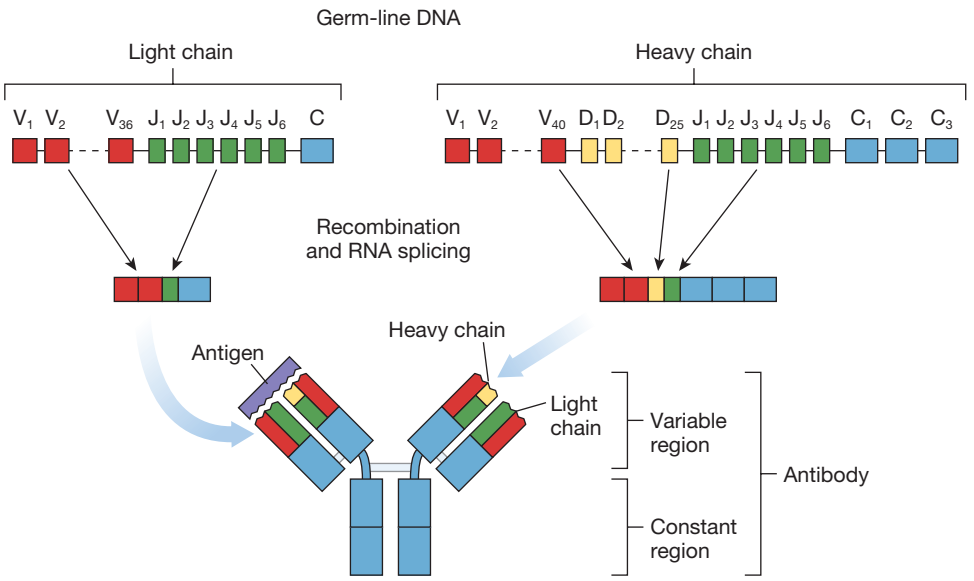


FIGURE 20.6 V(D)J recombination generates a diverse repertoire of antibodies. An antibody protein is composed of a heavy chain and a light chain. The heavy chain is produced by randomly selecting one of roughly 40 variable (V) segments, one of 25 diversity (D) segments, and one of 6 joining (J) segments by the process of somatic recombination. These are appended to three constant (C) segments that play a structural role in the protein. The light chain comprises one of 36 variable segments, one of 6 joining segments, and a single constant segment. The variable region, where the antigen binds, is thus highly diverse across cells. The T-cell receptors used to recognize infected cells are produced by a similar process. Adapted from Rajewski (1998).

antibodies. Each T cell or B cell recognizes some unique *antigen*, often a small section of a pathogen protein. Rather than separately encoding each B-cell antibody and T-cell receptor in the genome, the antibodies and receptors are produced through a random process of somatic recombination known as *V(D)J recombination* (**Figure 20.6**). Much as a combination lock can specify 10,000 different codes using only 40 digits (four wheels, each with the numbers 0–9), the immune system can potentially create millions of different receptors by combining a relatively small number of receptor subunits in different ways.

Because these receptors have been created by a process of random recombination, many will bind to *self* proteins; that is, those formed by the host itself. In a screening process, the cell lines with receptors that bind to self proteins are deleted shortly after they are produced. The end product is a vast diversity of immune receptors, all specific for proteins that are not produced by the host itself. If one of these binds to an antigen, that antigen probably shouldn't be there. If there are also signs of cellular damage in the vicinity, the antigen is probably a component of a pathogen and will trigger an immune response.

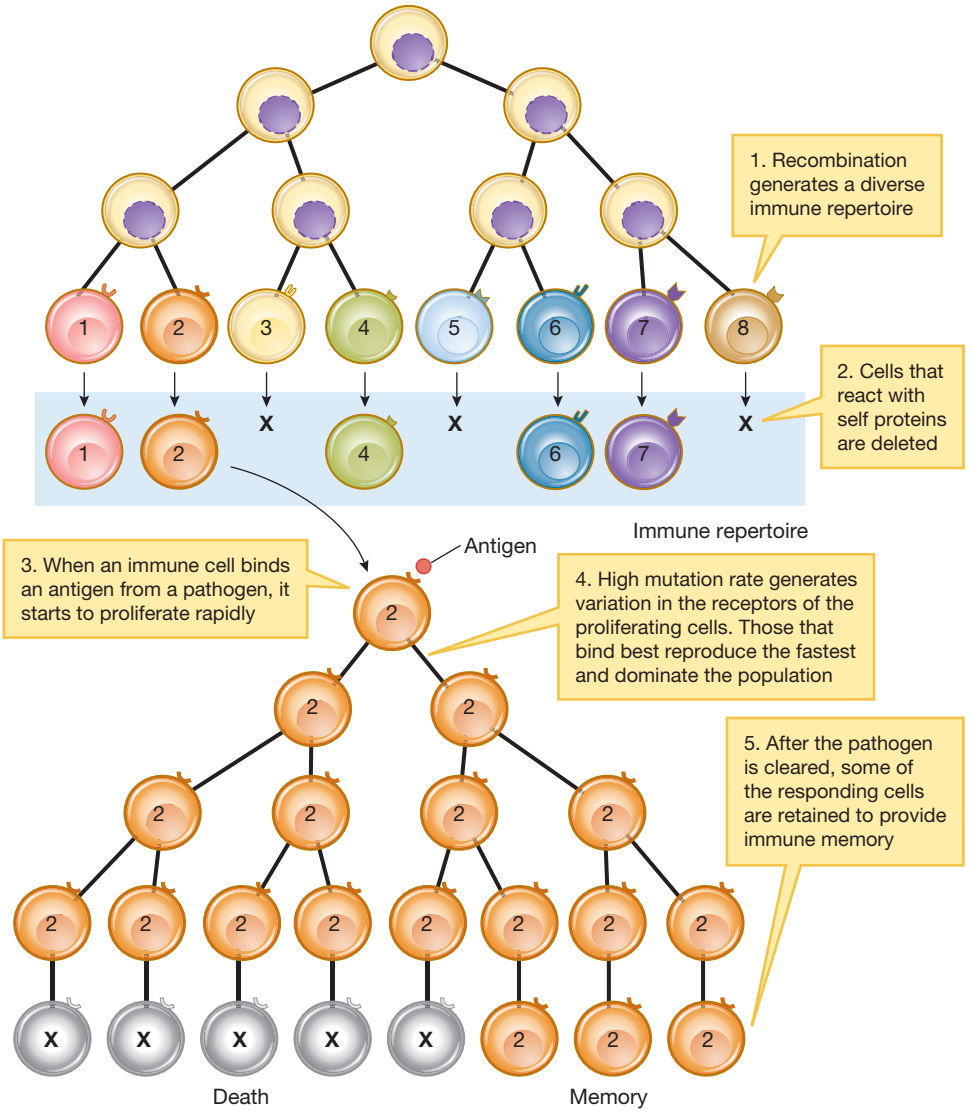


FIGURE 20.7 Clonal selection and clonal expansion. The immune repertoire features roughly a million receptor types, and the process of clonal expansion results in a 1000-fold increase in the number of pathogen-specific immune cells, but the basic process is shown here using a far smaller number of cells. Adapted from Bergstrom and Antia (2006) and Goldsby et al. (2000).

When an immune cell is triggered by interaction with an antigen, it begins to proliferate rapidly in a process known as **clonal expansion** (Figure 20.7). By the process of clonal expansion, the immune system creates a large number of cells that specifically react with the antigen that has been detected; these cells can then eradicate the pathogen.

This process already has much in common with natural selection: A good fit between receptor and target is developed by first randomly producing large amounts of variation and subsequently selecting on this variation.

In addition, proliferating B cells generate further variation and undergo additional rounds of selection. During the clonal expansion of a B-cell lineage, an improved match to the pathogen is achieved through a process known as **affinity maturation**. Like the basic process of clonal selection, affinity maturation works by generating variation and then selecting upon it, but in this process just those B cells that most closely recognize the antigen are used as the starting point. In these rapidly dividing cells, the coding sequence that specifies the structure of the immune receptor undergoes mutation at a much higher rate than the rest of the genome, rapidly generating further variation in the receptor's structure. Those receptor variants that most effectively bind to the antigen proliferate at a faster rate, outcompeting less effective variants of the receptor. The result is a gradual improvement in the match of the receptor to the antigen target over the course of clonal expansion.

The key point about the entire system is that vertebrates are fighting fire with fire—or rather, they are fighting selection with selection. Vertebrate *organisms* do not have short enough generation times or large enough populations to have any hope of matching pathogens in their rate of evolution. But vertebrate *cells* can reproduce rapidly and form large populations. Thus, the vertebrate adaptive immune system sets up its own internal selective process on its immune cells. It generates a massive amount of diversity and then stages its own selective competition to find immune cells that recognize a specific pathogen and to subsequently refine the binding ability of these cells. The internal selective process on individual cells operates on a timescale comparable to that of pathogen evolution, thereby enabling vertebrates to keep pace with their rapidly evolving antagonists.

KEYCONCEPT QUESTION

20.3 In Chapter 3, we explored how natural selection requires three key components: variation, inheritance, and differential reproductive success. On the basis of these criteria, could we say that the adaptive immune system uses a form of natural selection—on a population of cells rather than a population of individuals—to mount a response to pathogens? Explain.

Creating Variation through Sexual Reproduction

If there are weaknesses to a defense system such as the adaptive immune system, rapidly evolving pathogens will find them and exploit them. How do immune systems deal with this threat? According to the Red Queen hypothesis that we described in Chapter 16, sexual reproduction may be an adaptation for generating large amounts of variation in host lineages, and thus preventing pathogens from

specializing on any particular host genotype. Sex may be particularly useful for dealing with pathogens that are transmitted *vertically*, from parent to offspring. If hosts reproduce asexually, their pathogens will already be well adapted to exploit their offspring, because those offspring are genetically identical to their parent. By contrast, if hosts reproduce sexually, the offspring will be genetically different from the parents, and thus less susceptible to pathogens that have been successful in the parents.

Evolution of Pathogens to Subvert Immune Systems

Pathogens for their part evolve remarkably sophisticated and effective ways to avoid being eliminated by their hosts' immune systems (Finlay and McFadden 2006). Some of these mechanisms help the pathogens avoid detection by immune systems, but this is not the only way that pathogens deal with immune challenge. Another approach is to subvert the function of the immune system by sabotage or subterfuge (Bergstrom and Antia 2006).

Viral, bacterial, and protozoan pathogens use a wide repertoire of subversive tactics. (Tumor cells do something similar [Vinay et al. 2015].) Many microbes have evolved methods to down-regulate the responses of the innate immune system's pattern recognition receptors (Roy and Mocarski 2007). The human immunodeficiency virus not only down-regulates the expression of host major histocompatibility complex (MHC) molecules involved in recognizing an infected cell, it also induces programmed death in uninfected immune cells (Evans and Desrosiers 2001). The poxviruses—large DNA viruses responsible for diseases such as chickenpox and smallpox—have numerous ways of tampering with the signaling molecules that immune cells use to coordinate and regulate their activity. These viruses produce enzymes that degrade the immune system's chemokine signals (chemical signals controlling the replication and migration of cells to fight infection) before they reach their destination, and they produce molecules that block the host's chemokine signal receptors. They produce false chemokine signals that stimulate some receptors, and they produce decoy receptors that divert the true signals away from their intended targets (Liston and McColl 2003). Some viruses even turn RNA-directed components of the host's immune system against the host itself, using this system to silence certain host genes and thereby render the host more susceptible to the pathogen (Pfeffer et al. 2004; Wang et al. 2004). Bacteria also subvert host responses. They tap into the inhibitory pathways that the host uses to keep its own inflammatory response under control. Among other tactics, bacteria produce surface proteins that bind and activate inhibitory signal receptors and secrete enzymes that degrade immune signaling molecules (Van Avondt et al. 2015). Finally, pathogenic protozoa attack the antibody-producing branch of the immune system at many points, inhibiting B-cell development, tricking the host into diluting the pool of pathogen-specific B cells with large populations of low-affinity B cells, and inhibiting the proper formation of immune memory (Nothelfer et al. 2015).

Because internal pathogens have already invaded the body, they are well positioned to tamper with the immune system's communication and coordination pathways. Intracellular pathogens can go yet further: having invaded individual host cells, they can readily manipulate host gene expression. To deal with these

challenges, immune systems must be able to function robustly despite targeted misinformation and other forms of "information warfare." How they do this remains an open research question, although hypotheses are starting to emerge. Multiple redundant defenses, fail-safe devices rather than feedback control, cross-validation of signals, and distributed rather than centralized decision making all appear to be mechanisms for defending against internal subversion by pathogens (Bergstrom 2009).

Effects of Immune Systems on Pathogens

Pathogens have driven the evolution of immune systems, and immune systems have driven the evolution of pathogen countermeasures. We have looked at examples of each of these. And if the Red Queen hypothesis for sex is correct, the influence of pathogens on hosts has been far greater than simply forcing hosts to have immune systems. If this theory is correct, pathogens are ultimately responsible for the large-scale patterns of host evolution: Pathogens have driven the evolution of sex, a principal mechanism by which most multicellular eukaryotes generate genetic variation on which natural selection can operate.

The influence of immune systems on the large-scale patterns of pathogen evolution is no less significant. Recent phylogenetic studies of microbial pathogens have revealed that the nature of immune selection on a pathogen population often has a strong influence on the phylogenetic structure of that pathogen.

Some viral pathogens, such as measles, generate a very strong immune response that confers lifelong immunity. These pathogens cannot readily evolve **escape variants** that can dodge the immune memory of previously infected individuals by changing a few key epitopes—components of proteins that are recognized by the immune system. Such pathogens have the sort of classic phylogenies that we might expect to see in nonpathogen species (**Figure 20.8A**). Other viruses, such as the human influenza A virus, readily evolve escape variants capable of reinfecting previously infected individuals. These escape variants enjoy a tremendous selective advantage in a population of hosts with immunological memory against previous strains. The result is a distinctive phylogenetic pattern, known as a **cactus-shaped phylogeny**, in which most clones are lost in any given year, and the lineage is continued by one or at most a small number of escape variants (**Figure 20.8B**). These phylogenies typically have no deep branches, but instead each has a single trunk with only very minor twiggy lineages—the spines of the metaphorical cactus—branching off from it.

FIGURE 20.8 Phylogenies of measles and influenza. **(A)** The measles virus generates lifelong immunity, so there is no immune selection for escape variants. Shown here is a gene tree for the measles nucleocapsid gene. **(B)** The influenza virus can evolve escape variants that get around immunological memory, reinfecting hosts that have previously had the disease. Such escape variants are strongly favored by immune selection: This process gives rise to the characteristic cactus-shaped phylogeny of the influenza hemagglutinin gene shown here. Adapted from Grenfell et al. (2004) by permission of AAAS.

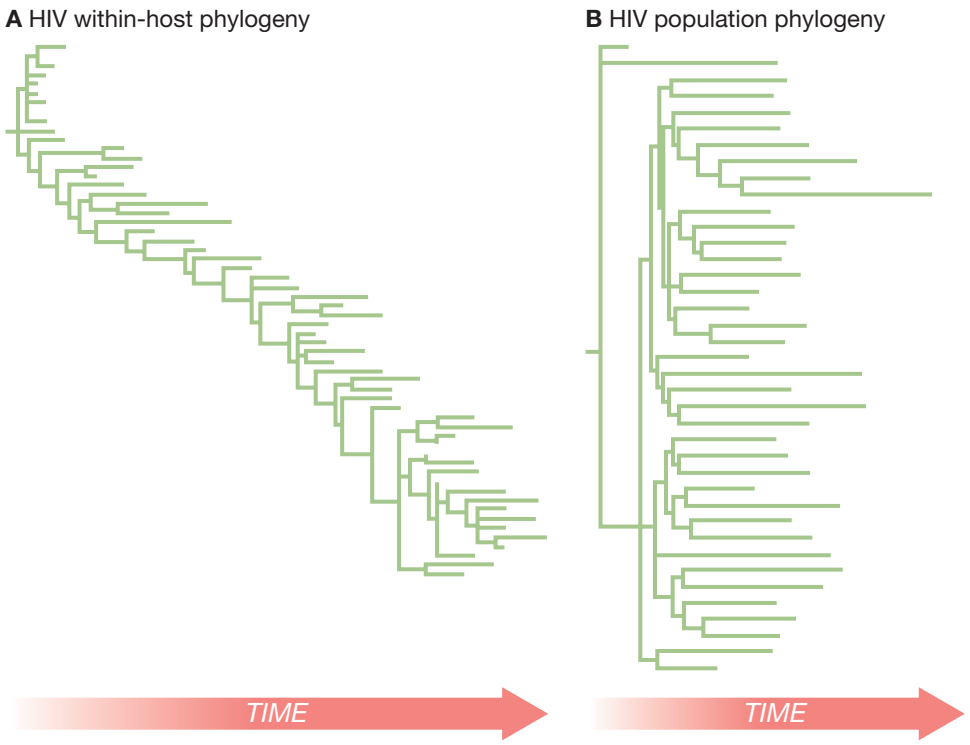


FIGURE 20.9 Within-host and population-wide phylogenies of HIV. **(A)** Within a single host, HIV has a cactus-like phylogeny. **(B)** By contrast, at the population level, the phylogeny of HIV is more conventionally shaped, though with deep branches due to its rapid epidemic expansion. Adapted from Grenfell et al. (2004) by permission of AAAS.

Viruses that cause long-term infection, such as HIV, provide an opportunity to compare phylogenetic structure within a single host to phylogenetic structure across a population of hosts. Within a host, strong immunological selection drives a series of escape variants to high frequency, generating a cactus-like phylogeny reminiscent of the population-level phylogeny of influenza (**Figure 20.9A**). Looking at the population as a whole, however, immunological selection plays very little role. The result is that at the population level, HIV has a more conventional branching phylogeny, as illustrated in **Figure 20.9B**. The shape of the HIV phylogeny also reveals something about the recent demographic history of this pathogen. The branch tips are very long compared to what we would expect in a constant-size population, reflecting the epidemic expansion of HIV over the past 30 years.

20.4 Phylogenetic Constraint and Vulnerability to Choking

On January 13, 2002, the 43rd president of the United States of America nearly met with a premature and tragic end. The evolutionary cause was a "mistake" that evolution had made more than 300 million years previously; the proximate cause was a pretzel. President George W. Bush was watching a football game alone but for the company of his dogs, Barney and Spot, when he choked on a pretzel. He passed out, fell forward, and according to his own recollection awoke on the floor to find the two dogs staring at him with concern.

Choking accidents such as this one are surprisingly common. According to the National Safety Council, choking is the fourth leading cause of accidental death (after automobile accidents, poisoning, and falls) in the United States. Looking

at our anatomy, the reason has been obvious to thinkers as far back as Aristotle, who noted the unfortunate intersection of the trachea with the esophagus as a cause of choking (Aristotle's *On the Parts of Animals* 3:3; Held 2009). **Figure 20.10** illustrates that the route that air takes through the nasal cavity to the trachea and into the lungs actually intersects the route that food or water takes through the mouth to the esophagus and into the stomach.

A structure known as the *epiglottis* has evolved as a partial work-around to this problem. As shown in Figure 20.10, the epiglottis functions as a trapdoor over the larynx and trachea. When we breathe, the epiglottis is raised, allowing free passage of air into the lungs. When we swallow, the epiglottis is pushed downward over the opening and thus prevents food or water from entering the trachea and lungs.

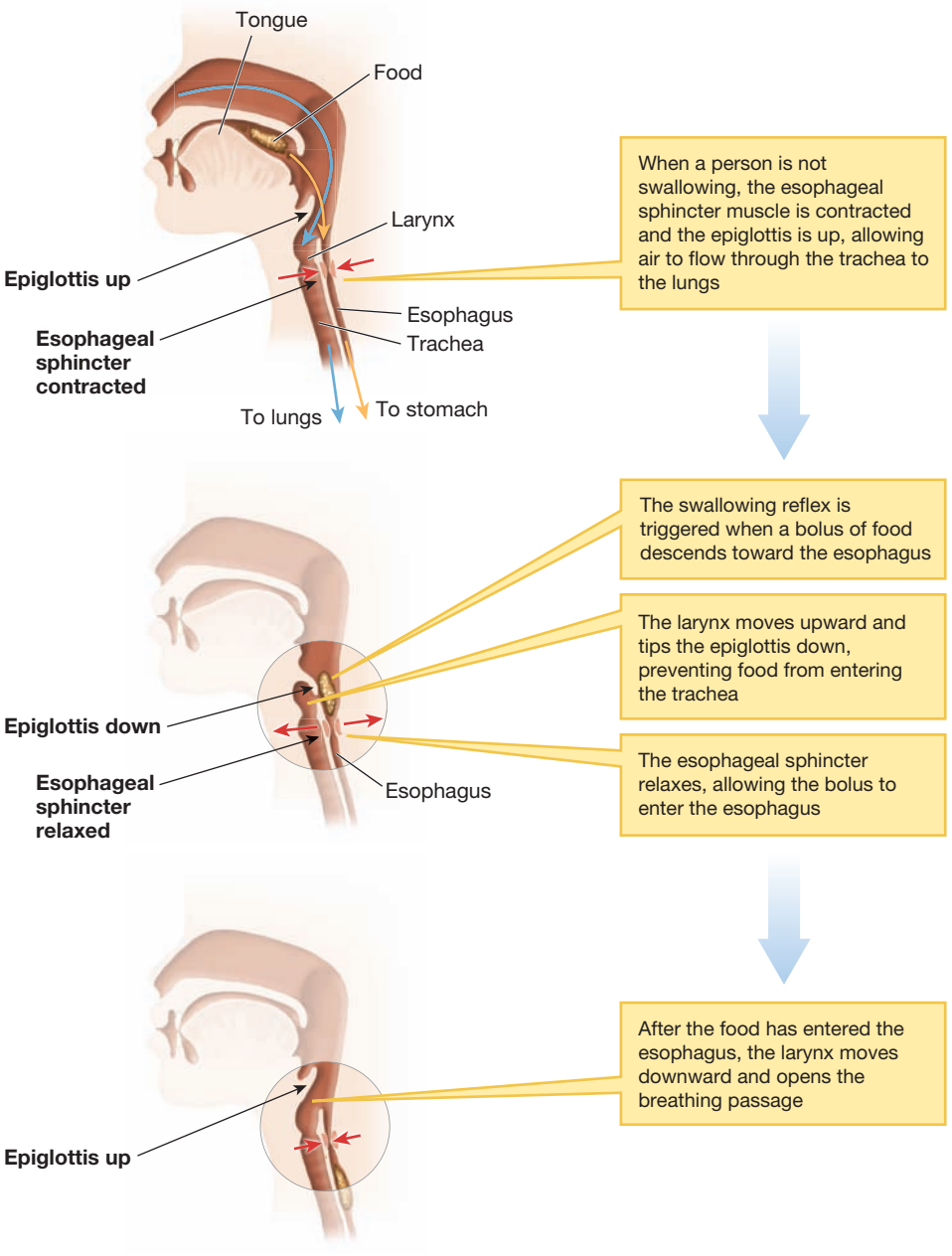


FIGURE 20.10 Anatomy of the throat, trachea, and esophagus. The pathway through which air passes from the nose to the trachea and lungs crosses the pathway through which food or water passes from the mouth to the esophagus and stomach. By closing down like a trap door, the epiglottis provides a safeguard against accidently taking food into the trachea. Adapted from Othman (2010).

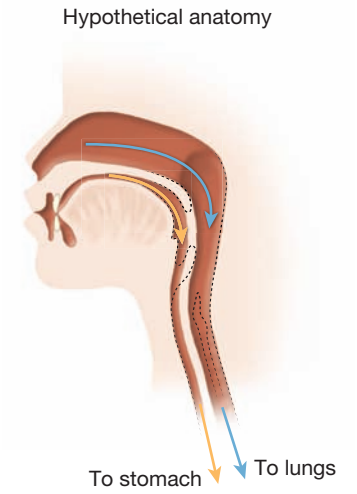
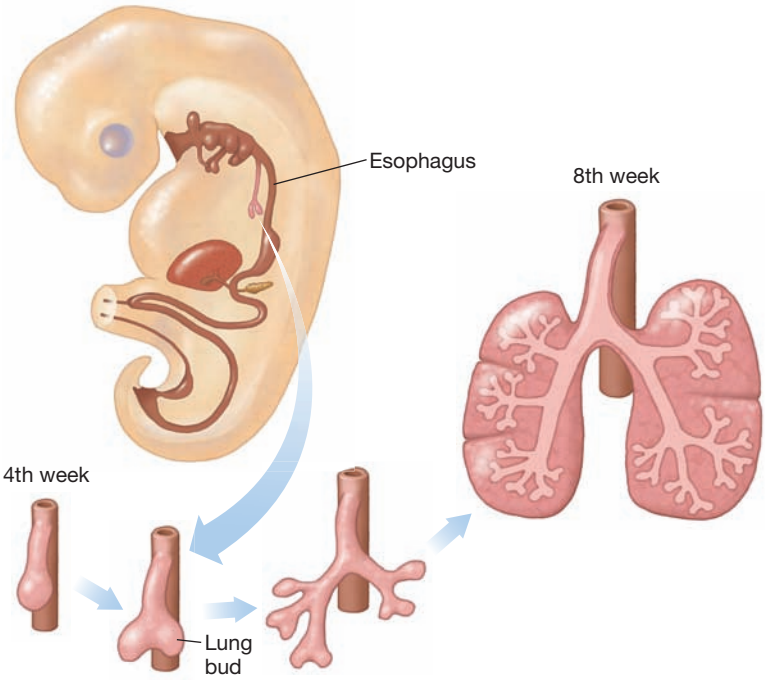


FIGURE 20.11 A hypothetical design for a trachea and esophagus that do not intersect. Shown here is a hypothetical anatomy in which the airway and the route of food are entirely separate, and choking on food is impossible. Adapted from Held (2009).

FIGURE 20.12 The lungs develop from esophageal tissue. In a developing embryo, lungs are formed when a respiratory bud extrudes from the developing esophagus.



Even with the epiglottis as a safety mechanism, this is a poor design at best. In principle, there is very little reason that the path of food and water should have to intersect the airway. **Figure 20.11** shows a much more sensible alternative, in which the two are kept entirely separate. Admittedly, one benefit of the current structure is that the mouth provides a backup airway if the nose becomes clogged—but a broader nasal opening or second redundant pathway could readily solve the problem as well. Another benefit, as we will see, is that the current structure facilitates complex vocalizations, including human speech.

So how did we end up in this mess? This is a consequence of **phylogenetic constraint**, in which the legacy of our evolutionary past limits the course of further evolution and results in seemingly suboptimal structures (Liem 1988; McKitrick 1993; Held 2009). Lungs arose very early in primitive fish as a pouch of esophagus or gut tissue that probably served to trap gas bubbles and thereby to capture additional oxygen in low-oxygen environments. Fish gulped air through the mouth to fill these pouches, and thus they had to be connected to the mouth and digestive passageway. Moreover, when lungs first arose as an offshoot of the throat, there was no choking risk because lungs merely provided a backup to the gills as a primary source of oxygen. Later in the tetrapod vertebrate lineage, however, gills were lost, and the lungs became the sole source of oxygen. But because the lungs arose as an extension of the esophagus rather than as a separate organ system and because developmentally lungs are formed from the esophageal tissue, this breathing apparatus could not readily be decoupled from the feeding apparatus from which it arose (**Figure 20.12**).

The problems created by this morphological configuration are exacerbated in humans, relative to other mammals, by the descended larynx that facilitates human speech. In most nonhuman mammals, the larynx is positioned high in the throat, and the epiglottis when raised meets the soft palate (Laitman and Reidenberg 1993). This blocks off the mouth cavity when the trachea is exposed, allowing air to flow through the airway in one unbroken channel while food or water flows around the epiglottis at the same time (**Figure 20.13**). But humans are different: We vocalize by controlling the flow of air through the mouth cavity, shaping the cavity with the lips, teeth, and tongue. This requires free airflow from the trachea through the mouth. Accordingly, the human larynx and epiglottis are positioned much lower, so that even when the trachea is exposed, air has an unimpeded path through the mouth.

Here we see how one particular vulnerability—the human vulnerability to choking—can be understood by considering more than one of Nesse's six explanations. The basic vulnerability, in which the airway and path of food cross one another, arose in an early vertebrate ancestor prior to the evolution of the lungs as the primary

breathing organs. This organization could not readily be reversed later in the evolutionary process, and we are stuck with this anatomical inefficiency as a relic of our evolutionary history. Humans are further vulnerable because of a trade-off: Speech requires slow, controlled flow of air from the lungs through the mouth cavity, and thus humans face a trade-off between communication ability and choking risk. But perhaps we should not be too discouraged by this. For all of the problems that our feeding anatomy gives us, Lewis Held points out that we don't have it as bad as cephalopods such as octopuses and squid. In these organisms, the brain wraps around the esophagus (**Figure 20.14**), so that each bite of food must pass through the middle of the brain, and too large a morsel can have disastrous consequences (Held 2009).

KEYCONCEPT QUESTION

20.4 We have seen how the vulnerability of humans to choking is the consequence of phylogenetic constraint in evolution. When the structures that would someday become lungs first evolved, evolution lacked the foresight to head off a future choking risk once lungs became the sole way of breathing. Can you think of another example of how phylogenetic constraint and evolution's lack of foresight have left the human body vulnerable to disease, injury, inefficiency, or malfunction?

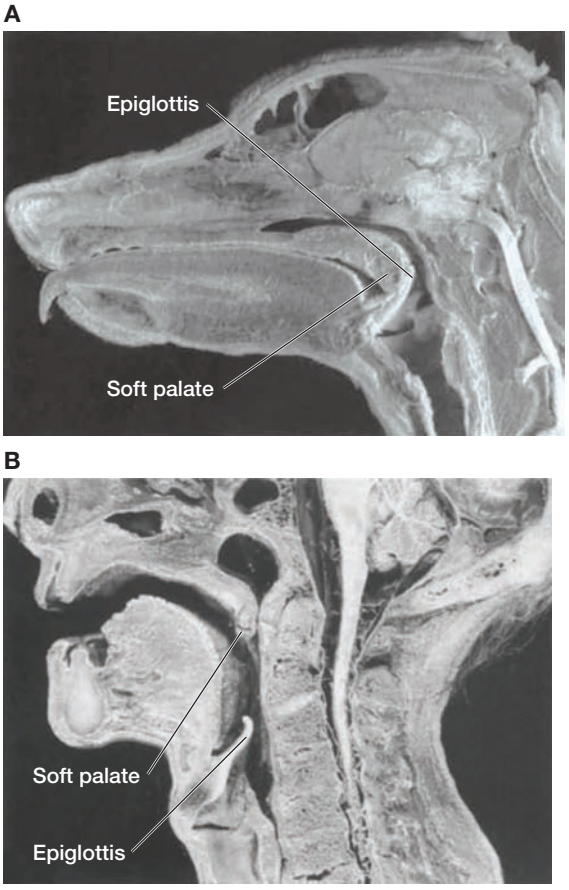


FIGURE 20.13 A descended larynx exacerbates the choking hazard in humans. **(A)** In nonhuman mammals, such as the dog shown here, the epiglottis reaches the soft palate, effectively sealing off the mouth cavity whenever the trachea is open. **(B)** The larynx is positioned much lower in all humans except for infants. This facilitates speech production by the passage of air through the mouth, but it comes at the expense of not blocking the flow of material from the mouth, even when the tracheal opening is exposed (Laitman and Reidenberg 1993).

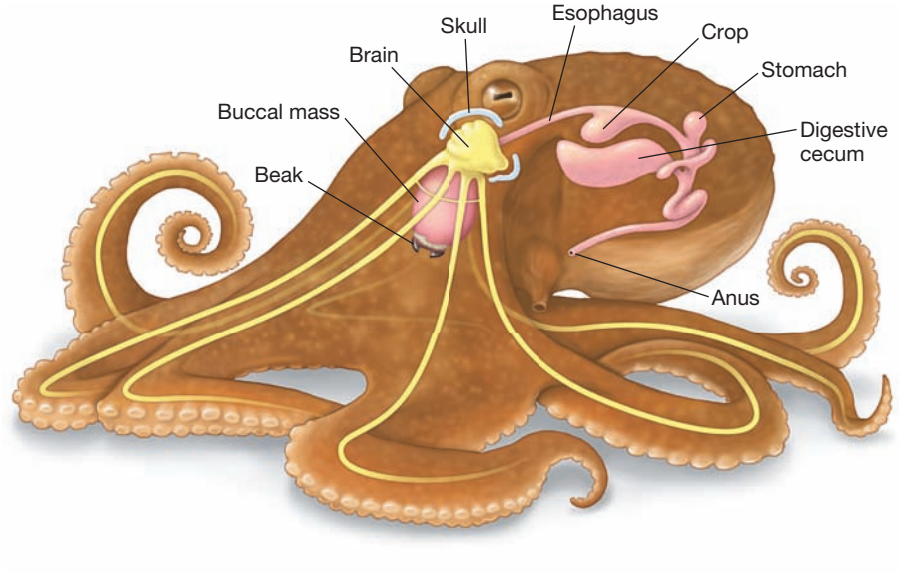


FIGURE 20.14 The precarious physiology of the octopus. The octopus brain wraps around the esophagus so that each bite of food must pass through the middle of its brain.

20.5 Senescence

Senescence refers to a general decline in the physical functioning or performance of living organisms with age. Typically, the process of senescence results in an increase in the mortality rate and a decrease in fecundity—that is, ability to produce offspring—with age. While the eventual consequence of senescence is death, the two are not synonymous. Rather, senescence refers to the general process of decline that ends in death. Thus, while average life span or maximum life span are correlates of the senescence rate, senescence has effects reaching beyond longevity (Ackermann and Fletcher 2008). We see these effects dramatically in the decline in human athletic performance with age (**Figure 20.15**).

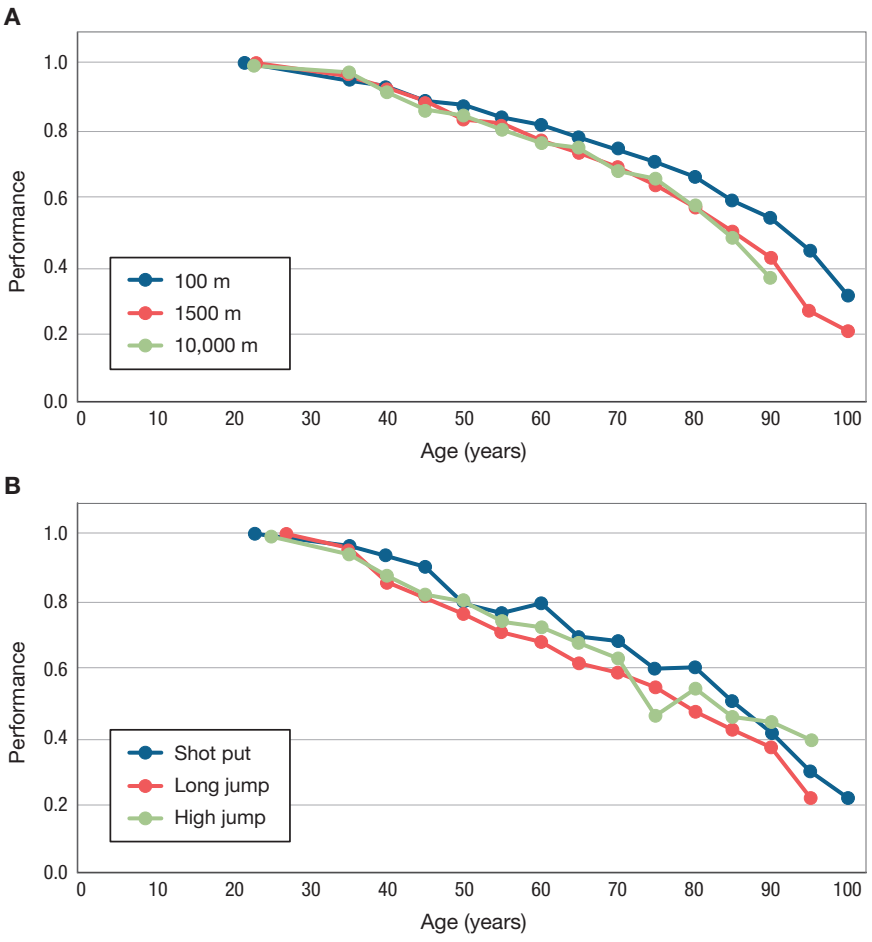


FIGURE 20.15 Decline in maximal human physical performance with age. Here, maximum physical performance is measured by world record times in **(A)** track and **(B)** field events. Performance is scaled relative to the world record time or distance for any age. In track events, performance is quantified as average speed; in field events, performance is quantified as distance or height. Note that, as world record times, these represent the limits of human performance. Thus, the falloff in performance of average individuals with age is likely to be substantially greater than that shown in the graphs. Adapted from Nesse and Williams (1994), Austad and Finch (2008), Track and Field News (2010), and World Masters Athletics (2011).

Vulnerability to Senescence

Senescence is a very general phenomenon among multicellular organisms. **Figure 20.16A** shows the general increase in age-specific mortality for humans, chimpanzees, and porpoises. **Figure 20.16B** shows age-specific mortality for water fleas (*Daphnia*) and fruit flies (*Drosophila*). While the timescales vary over several orders of magnitude, the general pattern is the same in all cases: The rate of mortality accelerates as individuals age. **Figure 20.17** shows fertility—the actual number of offspring produced—as a function of age for humans and for *Drosophila*. Again we see a similar pattern: Fertility declines dramatically as individuals age beyond reproductive maturity. (Human females go through menopause, and thus they show a particularly strong pattern in this regard; this phenomenon appears to be quite rare in other species.)

How can we explain these patterns? Why are humans, like almost all other multicellular species, vulnerable to senescence? How did this vulnerability evolve? It is important to be clear about what precisely is our target of evolutionary explanation. Senescence is not an evolved developmental program; it is not an adaptation in its own right. Rather, it is a by-product of other physiological adaptations. Thus, what we want to explain is not the evolution of senescence, but rather why we have not seen the evolution of adaptations that prevent aging. In other words, why has evolution left the body vulnerable to aging and age-related death?

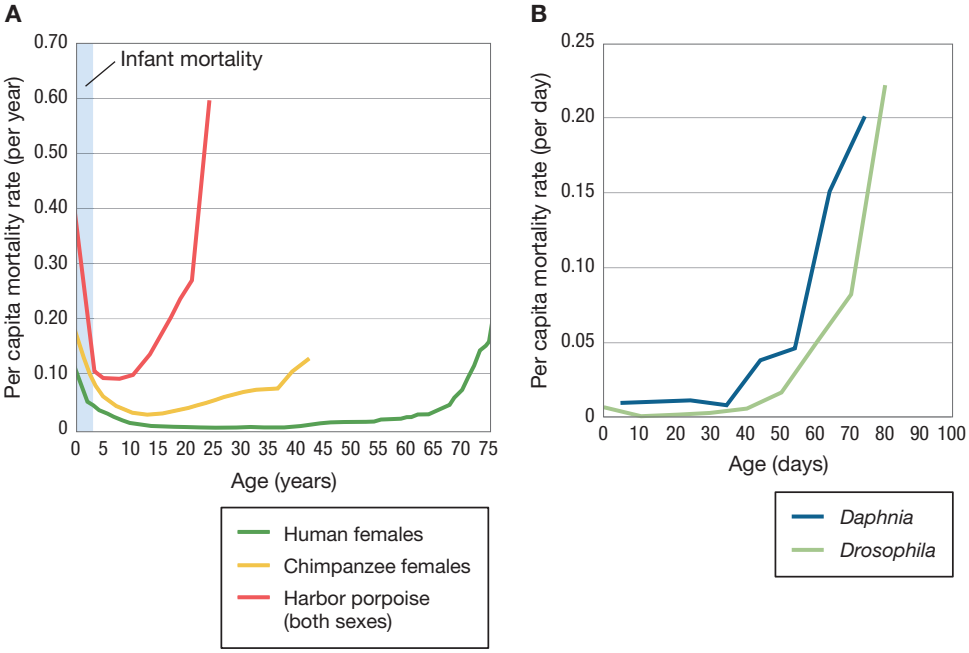


FIGURE 20.16 Age-specific mortality in five species. Age-specific mortality—that is, the per capita probability of death at a given age—shows a characteristic increase at older ages across a wide range of species. Here we show five examples. **(A)** Age-specific mortality in human females in an Ache hunter-gatherer population, chimpanzee (*Pan troglodytes*) females, and harbor porpoises (*Phocoena phocoena*) of both sexes. In these three species, rates of infant and juvenile mortality are high but decline dramatically into adulthood before rising dramatically as individuals reach old age. Adapted from Kaplan et al. (2000) and Moore and Read (2008). **(B)** Age-specific mortality in water fleas (*Daphnia*) and in fruit flies (*Drosophila melanogaster*). Adapted from Nisbet and Murdoch (1995) and Snoke and Promislow (2003).

Rate-of-Living Hypothesis for Senescence

One possible explanation is that senescence is simply unavoidable. Just as machines wear down over time and eventually break down completely, so do the bodies of living beings. Selection may result in a slowing down of senescence, but there is only so much that selection can do in this respect. This type of explanation is sometimes known as the **rate-of-living hypothesis** for senescence, because it posits that senescence is a consequence of physical wear and tear.

The rate-of-living hypothesis for senescence makes two strong testable predictions. First, if selection has already done everything possible to slow

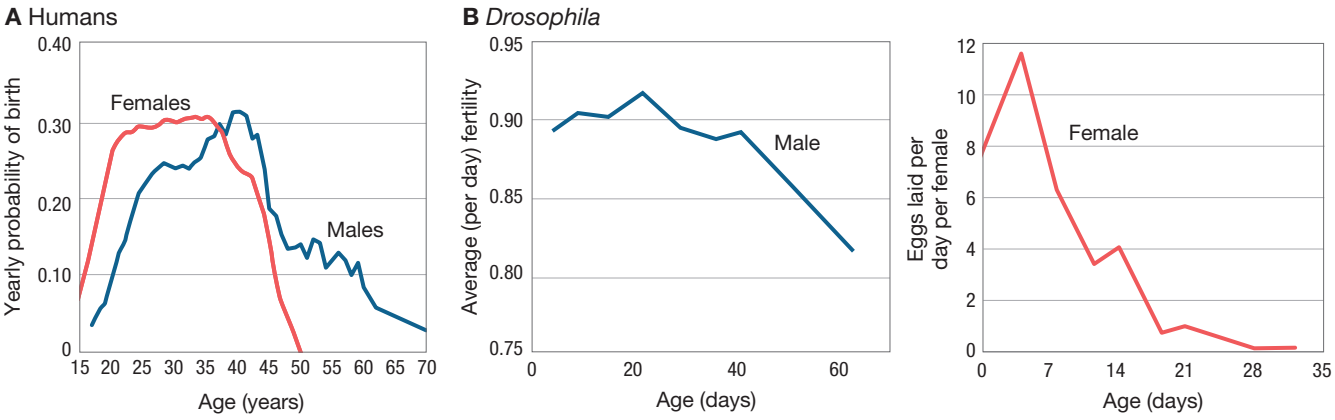


FIGURE 20.17 Age-specific fertility. Age-specific fertility—that is, the per individual birth rate—shows a characteristic decline at older ages across a wide range of species. **(A)** In an Ache hunter-gatherer human population, female age-specific fertility drops dramatically because of menopause as individuals reach their forties. Male age-specific fertility also falls off with age, although less rapidly. Adapted from Hill and Hurtado (1996). **(B)** Age-specific male and female fertility in *Drosophila melanogaster*. Female fertility is determined by the number of eggs laid by the female. Adapted from Snoke and Promislow (2003) and Tatar et al. (1996).

TABLE 20.1

Heritability of Life Span

Species	Heritability (h^2)
Caenorhabditis elegans	0.34
Drosophila melanogaster	0.06–0.09
Mus musculus	0.29
Homo sapiens	0.17–0.35

Adapted from Austad and Finch (2008).

the pace of senescence, there should be little or no remaining genetic variability in the rate of senescence. But biologists have uncovered ample evidence to contradict this prediction. In such model organisms as fruit flies (*Drosophila melanogaster*), nematode worms (*Caenorhabditis elegans*), and mice (*Mus musculus*), researchers have identified scores of known *longevity mutations* that confer slower rates of senescence (Tatar et al. 2003). Even in humans, a few alleles—for example, the *APOE2* allele—are known to contribute to greater longevity (Christensen et al. 2006).

Furthermore, the heritability of life span is substantial in many species. **Table 20.1** lists the observed ranges of heritabilities for humans and model organisms from a number of empirical studies. From this, we again can conclude that there is considerable genetic variation for life span in these species. Consonant with these observations, researchers have found that it is possible to increase life span through artificial selection in a number of model species (**Figure 20.18**).

Second, the rate-of-living hypothesis predicts a strong inverse correlation across species between metabolic rate and life span. The faster an organism's metabolism, the faster its physical structures should wear out and break down and the faster it should senesce. More specifically, intracellular damage due to oxidative stress should increase with metabolic rate. Thus, the rate-of-living hypothesis predicts that longevity and metabolic rate should be inversely correlated. Indeed, comparing across species, we do see a general trend in this

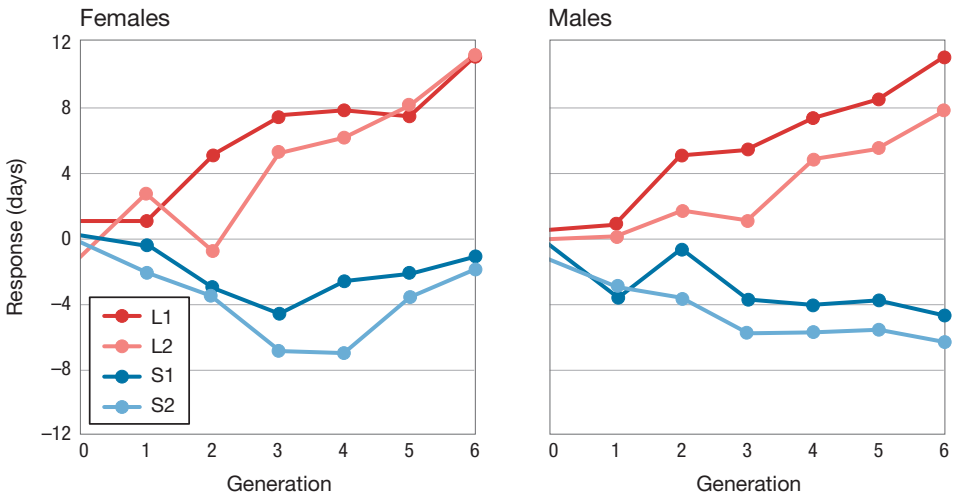


FIGURE 20.18 Artificial selection influences life span in *Drosophila*. Bas Zwaan and colleagues selected for short life span in two lines (S1 and S2) of *Drosophila melanogaster* and for long life span in two other lines (L1 and L2). The *y* axis indicates the difference in the life span of each treatment group relative to the life span of unselected control lines. Life span changes significantly in the direction of selection in each case. This indicates the presence of substantial genetic variation associated with the rate of senescence. Adapted from Zwaan et al. (1995).

direction (Figure 20.19). But a close look at the data leaves us with a number of reasons to be skeptical that the rate-of-living hypothesis provides a complete explanation for differences in senescence rates. These include the following: (1) longevity mutants—that is, mutants that live longer than do wild-type individuals of the same species—do not necessarily show reduced metabolic rate relative to wild-type individuals; (2) within a species, longevity and metabolic rate are not associated; (3) frequent exercise increases metabolic rate but does not decrease longevity; and (4) as illustrated in Figure 20.19, birds typically have much longer life spans than mammals of comparable basal metabolic rate (Hulbert et al. 2007).

Thus, the testable predictions of the rate-of-living model are not well supported by the available data. While metabolic rate may influence the rate at which oxidative stress and other forms of damage accumulate, the notion that damage is responsible for senescence provides us with only a proximate explanation. We have seen that there is substantial variation in the ability to withstand and repair such damage within and across species, and we need an evolutionary explanation for these observations.

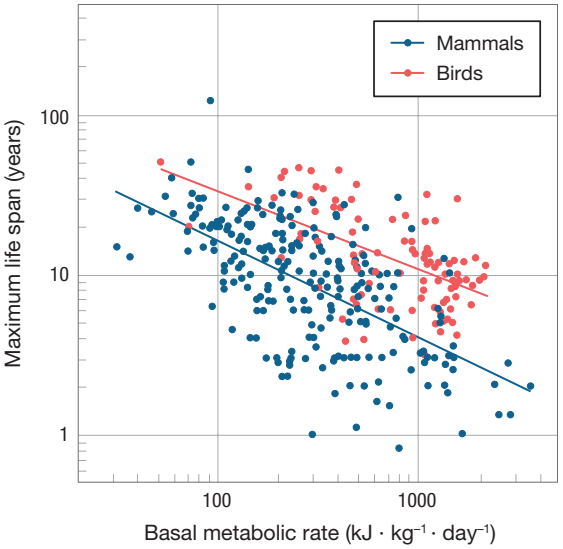


FIGURE 20.19 An inverse relationship between metabolic rate and life span. This plot of basal metabolic rate against maximum life span, on logarithmic axes, shows an inverse relationship between the two variables. But the broad scatter observed and the different curves for birds and mammals indicate that differences in metabolic rate only partially explain differences in life span. Adapted from Hulbert et al. (2007).

KEYCONCEPT QUESTION

20.5 Critique the following claim: "All vertebrate organisms senesce, therefore senescence must be an adaptation for something—we just need to figure out what it is an adaptation for."

An Evolutionary View of Senescence

For senescence, as for many other biological phenomena, J. B. S. Haldane was the first to provide a cogent evolutionary explanation (Haldane 1941). Haldane wanted to understand why Huntington's disease, a genetic disease caused by a mutation in a single gene, was so prevalent in the population—at a frequency of approximately 1 in 10,000 in the United States—despite its debilitating effects. If Huntington's disease is caused by a deleterious mutation, why isn't this mutation eliminated from the population through the action of natural selection? The answer, Haldane reasoned, has to do with the age at which Huntington's disease begins to manifest symptoms. Symptoms typically arise only once an individual is in his or her mid-to-late forties, *after* the vast majority of reproduction has already occurred, and after the disease allele has already been passed on to the next generation.

Selection on Early-Acting and Late-Acting Mutations

In general, senescence is a simple consequence of the fact that selection operates more strongly on traits that appear at young ages than on traits that appear at old ages because of *extrinsic mortality*; that is, causes of death other than senescence. The idea is that even in the absence of senescence, a population would have fewer

old individuals than young ones because individuals face a continual risk of death due to accidents, pathogen infections, and attacks by predators. As a result, traits that appear early in life will be selected upon in most members of the population, whereas traits that appear late in life will be subject to natural selection only in those members of the population that survive sufficiently long.

Let's look at a hypothetical example. Suppose members of a small rodent species have a survivorship rate of 98% per day. That is, they face a 2% chance of dying each day throughout their lives, but otherwise they do not suffer from mortality or senescence. **Figure 20.20** shows the **survivorship curve**—the fraction of surviving individuals as a function of age—for this species. Moreover, suppose that individuals of this species reach reproductive maturity in 30 days and produce an average of one offspring every week thereafter. The average reproductive success of an individual in this population is therefore proportional to the number of days beyond 30 that the individual lives. In this particular example, the average reproductive success is about 3.8 offspring produced.

Now compare two different deleterious mutations, each of which decreases survivorship from 98% to 96% per day for a period of 30 days. A *late-acting* mutation decreases survivorship from day 150 to day 180. An *early-acting* mutation decreases survivorship from birth until reproductive maturity at 30 days. Which of these deleterious mutations imposes a greater fitness cost?

Because fecundity is unchanged by either mutation, we can answer the question by comparing the survivorship curves illustrated in **Figure 20.21**. The average reproductive success is proportional to the shaded area in each case. As shown by the comparison figure, the shaded area—and thus the average reproductive success—is larger for individuals with the late-acting mutation than for individuals with the early-acting mutation. Individuals with the late-acting mutation have an average reproductive success of about 3.7 offspring—only a slight decline in fitness from the wild type. By contrast, individuals with the early-acting mutation suffer a huge drop in average reproductive success to only about 2.1 offspring.

Why do we see this difference, given that the deleterious effect of the mutation lasts only 30 days in either case? Put simply, it is a matter of how likely an individual is to suffer from each type of mutation. The early-acting mutation takes effect immediately at birth, and thus each individual must survive its ill effects simply to reach reproductive maturity. By contrast, an individual suffers from the late-acting mutation only if it survives to 150 days of age—and this occurs only about 5% of the time.

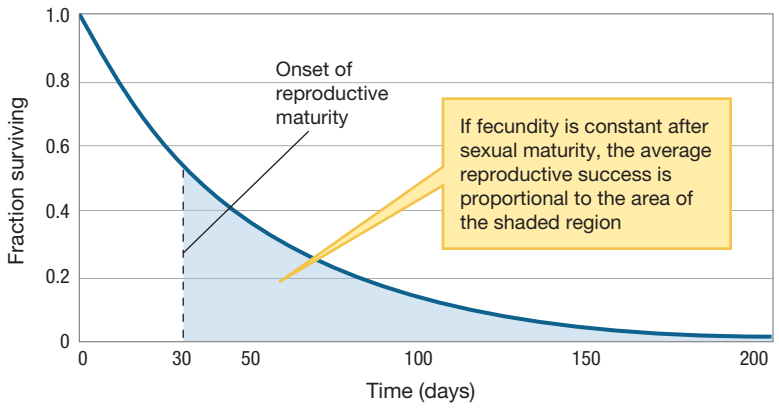


FIGURE 20.20 A survivorship curve. A survivorship curve indicates the fraction of individuals who survive to reach a given age. In this example, individuals reach maturity at 30 days and reproduce at a constant rate thereafter. The average reproductive success is proportional to the average reproductive life span; that is, the average number of days lived beyond 30. This quantity is indicated by the area under the shaded curve.

For this reason, natural selection operates more strongly on mutations that reduce survival early in life than on those that reduce survival later in life. A comparable argument can be crafted for mutations that reduce fecundity: These will be more strongly selected against when they act early in life than when they act later in life. This is the key insight behind our understanding of the evolution of aging. Because of extrinsic mortality, natural selection selects strongly against variants that have decreased survival or reproduction early in life,

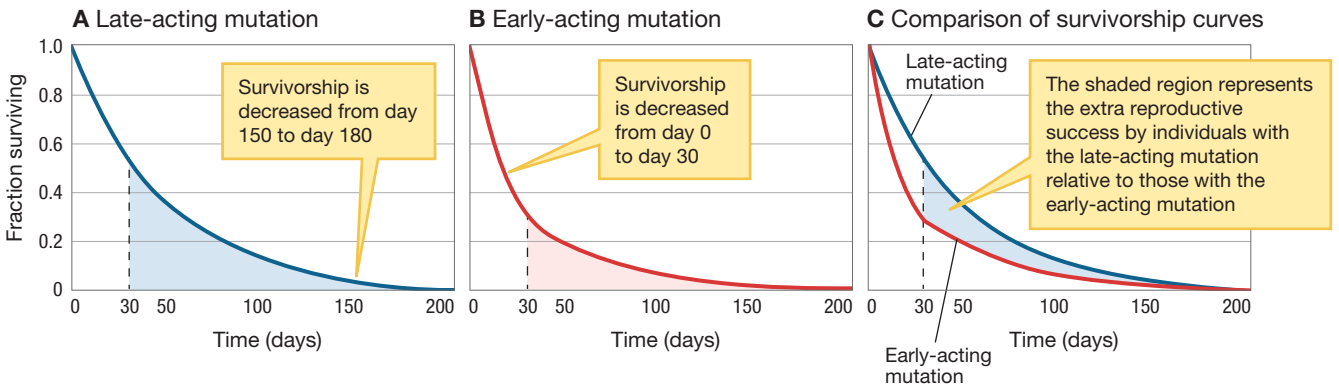


FIGURE 20.21 The fitness cost of a deleterious mutation depends on when during the life span it acts. **(A)** If a deleterious mutation reduces survivorship late in life, reproductive success (indicated by the shaded region) declines only slightly, whereas **(B)** if such a mutation reduces survivorship early in life, reproductive success declines dramatically. **(C)** The difference between the shaded regions in the two cases represents the additional fitness cost if a deleterious mutation acts early rather than late.

but selects only weakly against variants with decreased survival or reproduction later in life. Yet, a critical question remains: Just because selection is weaker on traits that manifest late in life, why can't selection also eliminate deleterious traits that appear later in life?

The Mutation Accumulation Hypothesis

One answer is that, as we saw in Chapter 8, in a finite population natural selection is not effective at eliminating mutations that have very small fitness effects. The **mutation accumulation hypothesis** proposes that for late-life traits, selection is simply not strong enough to purge deleterious mutations (Medawar 1946, 1952). As a result, mutations that have deleterious effects later in life build up in the genome, whereas mutations with deleterious effects early in life are eliminated by natural selection. The consequence is that individuals that live long enough will be plagued by a suite of late-acting deleterious mutations; senescence is the consequence of the effects of these mutations.

The Antagonistic Pleiotropy Hypothesis

Some mutations may have multiple effects at different points in the life cycle. Peter Medawar imagined what would happen if a single allele had beneficial effects early in life but deleterious effects later in life. He noted that such an allele could easily be favored by selection. Because natural selection acts more strongly on traits that manifest early in life, "a relatively small advantage conferred early in the life of an individual may outweigh a catastrophic disadvantage withheld until later" (Medawar 1952, p. 49). The evolutionary biologist George Williams called this explanation the **antagonistic pleiotropy hypothesis** (Williams 1957). In Chapter 3, we considered the basic phenomenon of antagonistic pleiotropy; that is, an allele that has beneficial effects on one trait or in one context may also have deleterious effects on another trait or in another context. Age-specific antagonistic pleiotropy might be responsible for senescence. A pleiotropic allele that confers even modest benefits at a young age might be favored despite having major deleterious consequences later in life.

We can again turn to our hypothetical example to illustrate antagonistic pleiotropy. Suppose that a new mutation gives rise to an allele that increases survivorship from 98% to 99% per day during the first 30 days of life, at the cost of a drastic decrease in survivorship to a mere 80% per day after 120 days. A survivorship curve for the allele is shown in **Figure 20.22A**. To see whether the allele is favored relative to

the wild type, we compare the area under the curve in Figure 20.22A to that in Figure 20.20. **Figure 20.22B** overlays the two curves to facilitate the comparison. The green-shaded area, where the survivorship curve for the new allele lies above the survivorship curve for the wild type, represents the increased reproductive success due to the early-life benefits of the new allele. The gold-shaded area, where the survivorship curve for the new allele lies below the survivorship curve for the wild type, represents the lost reproductive success due to late-life costs. Because the former area is much larger than the latter, the new allele confers higher net reproductive success, and thus it will be favored by natural selection. In this particular example, individuals with the new allele have an average reproductive success of around 2.3 offspring, compared to 1.9 offspring for the wild type.

The antagonistic pleiotropy hypothesis states that if a number of new mutations with similar effects on fecundity or on survivorship were to be fixed in the population by natural selection, organisms would experience a large decay in function later in life because of the collective effects of these alleles. It is important to recognize, however, that the deleterious mutation accumulation hypothesis and the antagonistic pleiotropy hypothesis are not mutually exclusive. It is entirely possible, and indeed entirely likely, that populations will accumulate both late-acting deleterious alleles because of drift and antagonistic pleiotropic alleles because of selection.

Under evolutionary theories of aging, senescence should always be manifested as a generalized deterioration, rather than the result of deterioration of one single bodily system. Moreover, Williams notes that the accumulation of antagonistic pleiotropic mutations creates positive feedback in favor of further such mutations. Not only does extrinsic mortality reduce the strength of selection later in life, but also the late-life decline of survival and fecundity due to senescence further reduces any selection for late-acting benefits.

Williams' predictions of generalized deterioration underlying senescence have often been interpreted as fatal to our dreams of extending the human life span. Williams himself takes this view: "Basic research in gerontology has proceeded with the assumption that the aging process will ultimately be explicated through the discovery of one or a few physiological processes." If this were the case, we could potentially overcome aging by learning to control these few processes. Unfortunately, Williams observes, an evolutionary theory of aging predicts

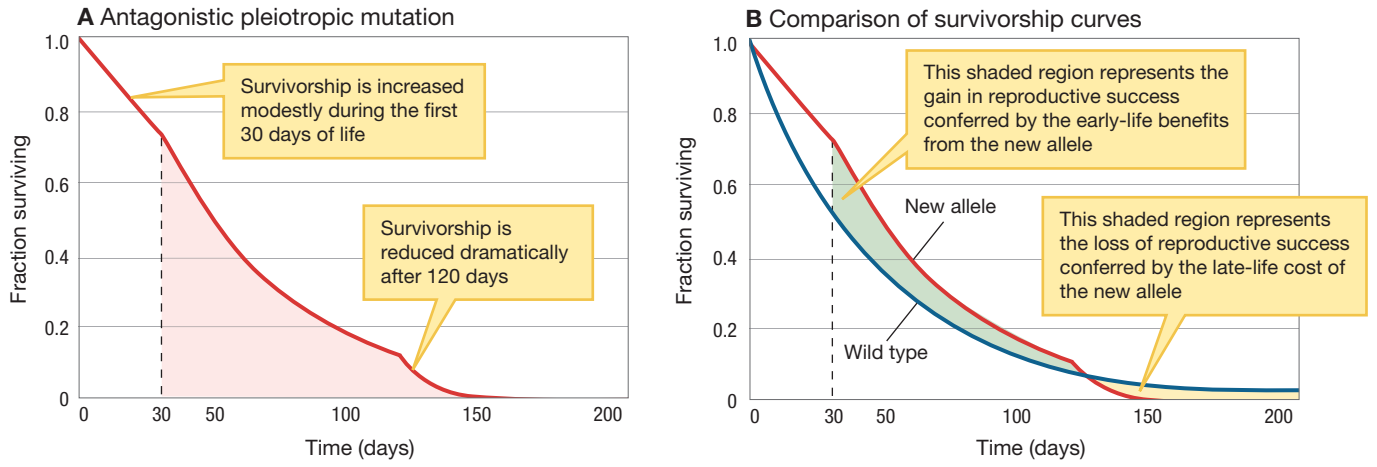


FIGURE 20.22 An antagonistic pleiotropic allele confers a fitness advantage. **(A)** A new allele has antagonistic pleiotropic effects, increasing survivorship early in life, but reducing survivorship later in life by a much greater fraction. **(B)** Comparing the survivorship curves, we see that the early benefits of the new allele outweigh its later costs.

otherwise, and his antagonistic pleiotropy model "banishes the 'fountain of youth' to the limbo of scientific impossibilities where other human aspirations, like the perpetual motion machine . . . have already been placed by other theoretical considerations" (Williams 1957, p. 407).

However, evolutionary theories of aging do not necessarily predict that all bodily systems should fail at the same time (**Box 20.1**). And as solid as Williams' logic appears, pessimism about life extension on theoretical grounds should be tempered by the weight of empirical evidence. In the United States, life expectancy at birth increased from 47.3 years in 1900 to 76.8 years in 2000. That is a staggering increase of 7 hours per day across the entire twentieth century. Worldwide, we see the same in developed nations (Oeppen and Vaupel 2002; National Center for Health Statistics 2015) (**Figure 20.23**). Importantly, expected life span at birth has increased approximately linearly with time for many decades and shows no sign of slowing. Prior to 1950, most of the increase in life span was due to decreases in early mortality; after 1950, the increase has been driven by increases in survival beyond retirement age (Oeppen and Vaupel 2002). This trend offers hope that we may be able to reduce the rate at which human aging progresses, even if we cannot overcome the logic of Williams' theory.

The Rate of Senescence Should Increase with the Rate of Extrinsic Mortality

The mutation accumulation hypothesis and the antagonistic pleiotropy hypothesis agree in a number of their testable predictions. Both predict that the higher the rate of extrinsic mortality in a population, the more rapidly members of the population should senesce. This is because as extrinsic mortality increases, expected life span decreases—even ignoring any possible effects of senescence—and selection against late-appearing deleterious phenotypes is reduced. Comparative studies that estimate senescence rates by measuring maximum longevity tend to confirm this prediction across a range of species. Groups of organisms that are protected in one

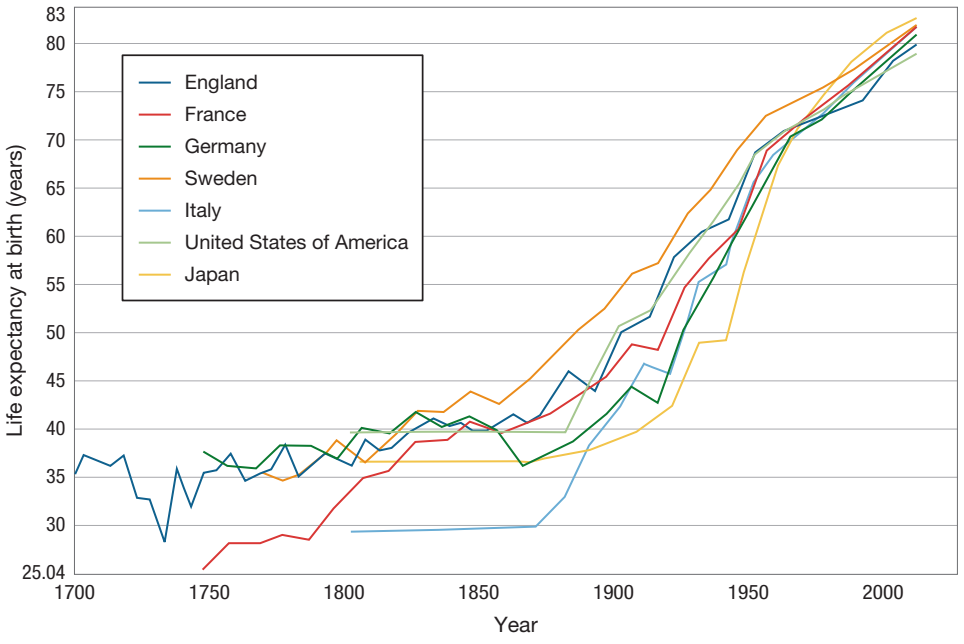


FIGURE 20.23 Life span at birth in developed nations has increased dramatically over the past 150 years. Despite numerous prognostications of inherent limits to human life span, life span at birth has approximately doubled over the past century and a half, and the current trends give little reason to expect this increase to stop. Adapted from Roser (2015).

BOX 20.1 Do We Expect All of the Body's Systems to Break Down at Once?

In a humorous 1858 poem "The Deacon's Masterpiece, or the Wonderful One Hoss Shay," Oliver Wendell Holmes Sr. described a carriage so artfully constructed as to have no weakest link. The carriage ran smoothly for exactly a hundred years. But then one day, as the parson took a drive, the carriage shuddered terribly, and the parson found himself sitting abruptly on a pile of rubble and dust in the middle of the road (**Figure 20.24**).

> What do you think the parson found,
> When he got up and stared around?
> The poor old chaise in a heap or mound,
> As if it had been to the mill and ground!
> You see, of course, if you're not a dunce,
> How it went to pieces all at once,—
> All at once, and nothing first,—
> Just as bubbles do when they burst.

FIGURE 20.24 The one-hoss shay disintegrated all at once. In this illustration from the original poem, the parson sits atop a pile of rubble when, after 100 years of service, every piece of his carriage breaks at exactly the same time. Illustration by Howard Pyle.

In a remark accompanying the poem, Holmes discussed how one might craft such a cab:

> Observation shows us in what point any particular mechanism is most likely to give way. In a wagon, for instance, the weak point is where the axle enters the hub or nave. When the wagon breaks down, three times out of four, I think, it is at this point that the accident occurs. The workman should see to it that this part should never give way; then find the next vulnerable place, and so on, until he arrives logically at the perfect result. (Holmes 1858, p. 4)

From an evolutionary perspective, we might expect our bodies to function like the "one-hoss shay," running smoothly for years before multiple organ systems veer simultaneously toward failure (Nesse 1988). Natural selection would play the role of the workman, favoring increases in the durability only of those parts that are first to fail.

But this is not what we observe. In practice, not all of the body's subsystems are equally likely to fail with age. Failures of the heart, the cerebral vasculature, or the tumor suppressor systems that prevent the growth and spread of cancers cause vastly more human fatalities than do failures of comparably complex and equally critical systems such as the liver or kidneys.

Why do some systems appear to be so vulnerable, while others appear to be "overbuilt" with respect to the stresses placed upon them during the human lifetime? At first glance, one might conclude that this pattern reflects a mismatch between current environmental conditions and those conditions under which our organ systems evolved. Perhaps human hearts today face greater resistance from atherosclerotic arteries; perhaps modern causes of hormonal dysregulation increase the risk of stroke; perhaps human life spans today exceed the duration of protection provided by our evolved tumor suppressor genes.

way or another from sources of external mortality such as predation indeed have longer life spans than related groups that lack such protection. Shelled organisms such as turtles outlive species without shells; venomous organisms outlive those without venom; flying species outlive those that cannot fly. For example, bats can escape predators by flying; as a result, bats experience lower extrinsic mortality

It would be a mistake to conclude that we *require* a mismatch explanation to account for these differences in failure rates. In a fascinating paper, Robert Laird and Thomas Sherratt (2010) explain why. They lead with a story about Henry Ford—perhaps a recasting of Holmes' poem—that ought to be true even if it isn't:

> Henry Ford, it is said, commissioned a survey of the car scrap-yards of America to find out if there were parts of the Model T Ford which never failed. His inspectors came back with reports of almost every kind of failure: axles, brakes, pistons—all were liable to go wrong. But they drew attention to one notable exception, the kingpins of the scrapped cars invariably had years of life left in them. With ruthless logic Ford concluded that the kingpins on the Model T were too good for their job and ordered that in future they should be made to an inferior specification. (Humphrey 1983, as quoted in Laird and Sherratt 2010, p. 3)

This story about Henry Ford is often invoked as metaphor for how natural selection should not "overbuild" some systems while leaving others vulnerable. Indeed, it is true that natural selection will act to improve further the reliability of a system that never fails in the first place. It is also true that if bodily systems always failed in the same sequence, at any given time selection would act only to improve the longevity of the weakest link. But it is simply false that when different systems each fail at a different rate, optimal design will *equalize* the failure probabilities of various components.

Rather, optimal design should balance the *marginal return*—the gain in life span per unit cost—of increased investment into each system. For example, suppose that when the heart and lungs failed at similar rates, it would be cheaper to reduce further the failure rate of the heart than to reduce the failure rate of the lungs. In this case, the optimal design—and the one that would be favored by natural selection—would be one in which the marginal returns on investments into heart and lungs are equal. In such a design, the heart would fail with lower probability than the lungs (**Figure 20.25**).

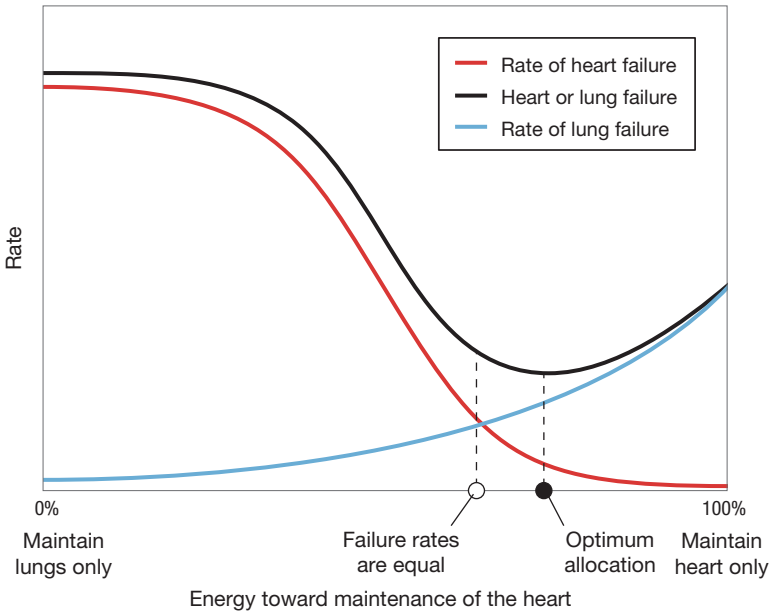


FIGURE 20.25 Optimal allocation does not imply equal failure rates. In this example, an organism evolves to allocate energy between maintenance of the heart and maintenance of the lungs. The probability of heart failure (red curve) decreases as more effort is allocated to heart maintenance. The probability of lung failure (blue curve) increases as more effort is allocated to the heart and thus diverted away from the lungs. The total rate of system failure (black curve) is given by the sum of the red and blue curves. The optimal allocation, indicated by the solid circle, minimizes the total rate of system failure. This involves a heavier allocation to the heart than that equalizing the failure rates of the two systems (open circle). Adapted from Laird and Sherratt (2010).

The moral of the story for evolution and medicine is that we cannot leap quickly from the observation that failures in a small number of bodily systems result in the majority of human mortality to the conclusion that these failures arise from mismatch between the conditions of human evolution and the current human environment.

Adapted from Bergstrom (2010).

than that of comparably sized flightless mammals. Indeed, bats have longer life spans than other mammals of similar sizes (**Figure 20.26**).

In a classic experiment, Steve Austad tested the prediction that senescence is proportional to extrinsic mortality. He examined rates of senescence in two populations of opossums (*Didelphis virginiana*) that for many generations had

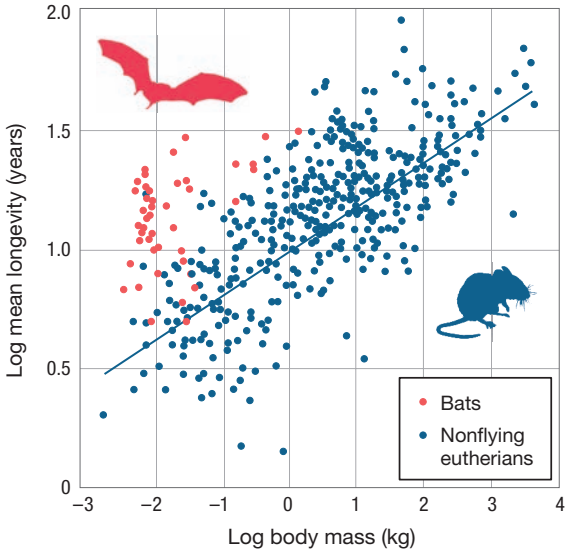


FIGURE 20.26 Bats senesce slower than do flightless mammals. Flightless mammals show a close relationship between body mass and longevity; this relation is approximately linear on a log–log plot. Bats lie well above the trend line for flightless mammals, indicating that they live much longer relative to their body size. Adapted from Austad and Fischer (1991).

faced very different levels of extrinsic mortality (Austad 1993). A mainland population had experienced high levels of predation by bird and mammal predators. By contrast, an isolated population on Sapelo Island, 5 miles off the coast of Georgia, had experienced much lower predation pressure because there were no large predators on the island. Austad reasoned that, if the evolutionary theories of aging are correct, selection should have favored slower rates of senescence in the island population than in the mainland population. By contrast, if the rate-of-living hypothesis was responsible for the phenomenon of senescence, both populations should senesce at comparable rates.

Austad attached radio collars to opossums in each population in order to monitor their mortality rates. He also recaptured individuals intermittently to assess their fecundity and physiological condition. Austad's results provided strong evidence for the evolutionary explanation of senescence. Relative to individuals in the mainland population, island individuals had lower age-specific mortality (**Figure 20.27**) and lower rates of physiological decline as measured by the breaking time of the collagen fibers in the tail. Age-specific fertility also declined more quickly on the mainland than on the island, as we would expect under evolutionary explanations of senescence.

KEYCONCEPT QUESTION

20.6 In his classic 1957 paper on the evolution of senescence, George Williams noted that some species, such as carp, increase continually in size and also in fecundity over the course of their lifetimes (Williams 1957). He predicted that such species would not senesce as fast as species that do not increase in fecundity beyond reproductive maturity. Explain the reasoning behind this prediction.

The Disposable Soma Hypothesis

The strength of the antagonistic pleiotropy hypothesis is that, according to the hypothesis, senescence is a consequence of natural selection on correlated traits (early and late survival and fecundity) rather than simply a side consequence of mutations on which selection is so weak that they cannot be eliminated. But with this strength comes a significant challenge. We need to be able to explain why we would expect to see antagonistic pleiotropy with respect to age, and this is not at all obvious. After all, most of the adaptations we have considered throughout this book—cryptic coloration, for example—should be beneficial irrespective of age. Williams' theory requires a large class of alleles that are beneficial early in life but harmful later in life. Why would such alleles exist?

The **disposable soma hypothesis** provides an answer to this puzzle. It suggests that these antagonistic pleiotropic relationships between beneficial effects early in life and deleterious effects later in life are the result of a fundamental trade-off. The disposable soma hypothesis was first framed narrowly, as a trade-off between growth, on one hand, and repair of the transcriptional and translational machinery within cells on the other (Orgel 1963; Kirkwood 1977). The basic

idea is that once organisms have evolved a distinction between germ-line cells and somatic cells, these two types of cells face different requirements. The transcriptional and translational machinery within the germ-line cells, which are passed from generation to generation, is selected to avoid degradation and decay. Otherwise, the genes carried in these germ-line cells would not be transmitted faithfully to future generations. But matters are different in somatic cells. At some point, energy that could be invested in ensuring transcriptional and translational fidelity might better be invested in promoting rapid growth, even at the cost of such fidelity. While the structure and function of the genetic machinery within somatic cells would then degrade over an organism's lifetime, the whole process could be reset in the next generation, as new and perfectly intact somatic cells would be produced from the more carefully preserved germ-line cells.

Today, the disposable soma hypothesis is typically interpreted in a more general manner. It is seen as pertaining to any trade-off between investment in *reproduction* and investment in *repair*. Any allocation of resources toward immediate reproductive benefit and away from repair and regeneration is an allocation that privileges the germ line while relinquishing the soma to the ravages of entropy. In other words, the disposable soma hypothesis focuses on the trade-offs between early fecundity and later survival. Why not preserve the soma as well as the germ line? We have already seen the answer that evolutionary theories provide: The presence of extrinsic mortality means that sooner or later, any given soma's luck will run out. Given this, selection will not tend to favor the investments in repair that would confer indefinite survival of the entire soma, as there is no selective benefit to investing in repairs that the organism will not live long enough to need (Williams 1957; Kirkwood and Austad 2000).

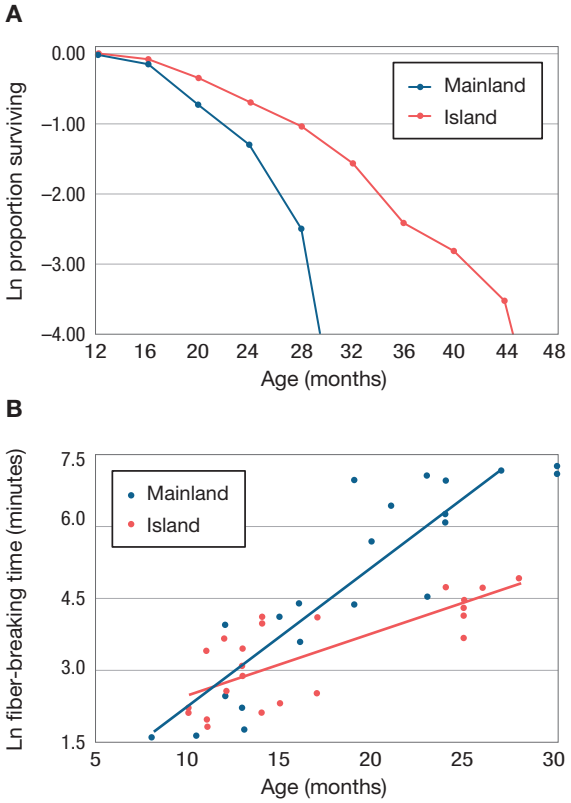


FIGURE 20.27 Senescence in island and mainland opossums. **(A)** Mortality rate increases with age in both populations, as indicated by the increasingly steep slope of the survival curves with time, but opossums from the island population outlive those from the mainland by a considerable margin. **(B)** The amount of collagen fiber cross-linking—a symptom of aging—can be measured by suspending the fibers in a urea solution. Fibers with less cross-linking (that is, those that have aged less) break more quickly. Here we see that cross-linking both increases with age and increases more rapidly in mainland opossums than in island individuals. Adapted from Austad (1993).

Aging in Bacteria

In an ingenious experiment, Martin Ackermann and his colleagues showed that the trade-off between repair and reproduction occurs even in organisms that lack a germ–soma distinction (Ackermann et al. 2003). They reasoned that if the evolutionary perspective on aging is correct, even bacteria should senesce, provided that they divide in a way that clearly differentiates a new daughter cell from an older mother cell (Partridge and Barton 1993). We usually envision bacteria as dividing symmetrically into two similar daughter cells, rather than as dividing into an older mother and a younger daughter cell. But Ackermann and his colleagues found a bacterium with an unusual life cycle: *Caulobacter crescentus* divides asymmetrically with a clear mother–daughter distinction. The bacterium begins life as a free-swimming *swarmer cell* propelled by a flagellum, but later it matures to become a *stalked cell*, anchoring itself to a surface using an attachment known as a *holdfast* (**Figure 20.28**). After attaching, stalked cells never return to the swarmer state; instead, they undergo repeated cell divisions, with the newly formed cell taking on the swarmer state while the original cell retains its holdfast.

An evolutionary perspective on aging would predict that the stalked cells should exhibit senescence. Because stalked cells face extrinsic mortality and do not revert

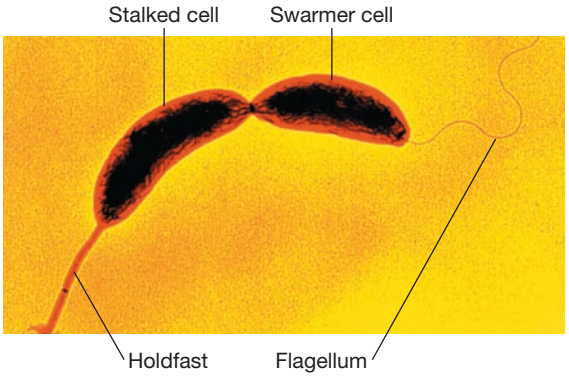


FIGURE 20.28 Stalked cells of *Caulobacter crescentus* cells produce swarmer cell daughters. Here, a swarmer cell with its flagellum is about to bud off from a stalked cell, which is attached to the substrate by a holdfast.

to the swarmer form, evolutionary logic suggests that they should at some point stop investing in repair and instead invest in extra production of swarmer cell offspring. The expected consequence is senescence: Stalked cells should experience higher mortality and/or lower rates of reproduction as they age.

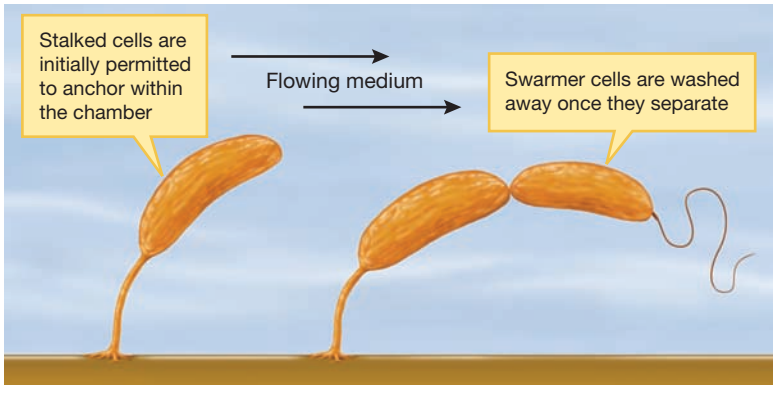
To test this prediction, Ackermann and his colleagues devised a way to measure the reproductive rate of stalked cells over time. They allowed cells to anchor themselves in a chamber, with liquid medium flowing past, and filmed the cells over a couple of weeks under a microscope. Because of the flowing medium, newly formed swarmer cells were washed away, and they did not clutter the chamber, allowing the researchers to note the times at which each stalked cell underwent cell division (**Figure 20.29**).

Ackermann filmed the bacteria over a period of more than 300 hours subsequent to anchoring, and he measured the rate at which stalked cells produced new swarmer offspring. His results provided strong evidence of senescence: Reproductive output declined substantially over the course of the experiment, and the rate of decline accelerated for older cells (**Figure 20.30**). To be certain that this decline was the result of senescence rather than a consequence of changing experimental conditions, he also measured the rate of reproductive output of swarmer cells produced at 250 hours into the experiment. These cells were rejuvenated: They reproduced at the rates observed at the start of the experiment, not at the reduced rates found in aged mother cells.

With this study, Ackermann and his colleagues showed that senescence can occur in bacteria, at least when they divide asymmetrically, as does *C. crescentus*. Subsequent work indicates that this phenomenon may be much more general. Even bacteria that appear to divide symmetrically, such as *E. coli*, may actually distribute new and old components of the cytoplasm to two different cell poles during division, thereby producing an aged "parent" and a younger "daughter." Indeed, older bacteria with older cytoplasmic components appear to have decreased rates of replication (Lindner et al. 2008). Again, the trade-off between repair and reproduction arises: Rather than repairing old cytoplasmic components, these structures can be segregated together into a senescing parent while new components are synthesized for a rejuvenated offspring (Ackermann et al. 2007).

These studies have substantially advanced our understanding of what it means to grow old. The trade-off between reproduction and repair has often been framed as a consequence of the distinction between the germ line, which is potentially eternal, and the somatic cells, which are disposed of in each generation. But this new work has demonstrated compellingly that a reproduction–repair trade-off can occur even without the germ–soma distinction, and thus even unicellular organisms such as bacteria can senesce. Ultimately, evolutionary hypotheses for senescence posit that aging occurs because—like inexpensive consumer electronics—our bodies, cells, and intracellular components are cheaper to replace than to repair.

FIGURE 20.29 Measuring reproductive rate in *Caulobacter crescentus*. By filming stalked cells dividing in a flow chamber, Ackermann and his colleagues could measure the rate of reproduction as a function of each cell's age; that is, time since forming a stalk.



In this chapter, we have seen a number of ways in which the principles of evolutionary biology can contribute to our understanding of medicine. By necessity, we have merely scratched the surface of this vibrant and rapidly emerging area of research. Numerous other examples and applications are being explored, and doubtless many more will be discovered as this research area continues to expand.

Having finished our discussion of evolution and medicine, we have reached the end of this volume. Thousands of observations and experiments, as well as mathematical and conceptual models, have demonstrated that the theory of evolution—descent with modification—explains the diversity of life on our planet, both present and past. No other scientific theory comes remotely close to explaining so much of what we know about the diversity of life. And so, more than 150 years after Charles Darwin wrote *On the Origin of Species*, we can think of no more appropriate way to draw to a close than in the same spirit that Darwin concluded his revolutionary book: contemplating the grandeur of a universe in which "from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

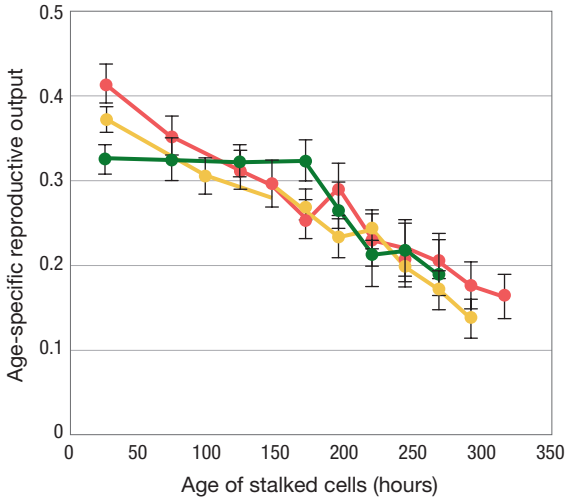


FIGURE 20.30 The asymmetrically dividing bacterium *Caulobacter crescentus* undergoes senescence. Age-specific reproductive output, shown here for three replicate experiments, incorporates both the probability of survival and the rate of cell division. Adapted from Ackermann et al. (2003) by permission of AAAS.

SUMMARY

1. In biology, the question "Why do we see a particular trait or phenomenon?" can be answered at multiple levels. Proximate explanations specify immediate mechanisms, developmental explanations specify changes that occur during an individual's lifetime, evolutionary explanations specify how selection and other evolutionary processes have shaped a trait, and phylogenetic explanations specify when and where in the history of life the trait arose.
2. An evolutionary perspective on human disease does not ask why disease is evolutionarily advantageous, but rather it asks why evolution has left the body vulnerable to disease.
3. Nesse and Williams distinguished six different evolutionary explanations for vulnerability to disease: (1) coevolutionary arms races; (2) not enough time for selection to catch up with environmental conditions; (3) trade-offs; (4) historical contingency and phylogenetic constraint; (5) selection favors reproductive success at the expense of health and well-being; (6) some symptoms are possibly defenses rather than pathologies. These explanations need not be mutually exclusive.
4. Fever appears to reduce the duration and severity of microbial infection, but usually it can be treated without major negative consequences.
5. The smoke detector principle suggests that defenses will tend to be overly sensitive because the cost of a false alarm is much less than the cost of failing to respond to a true threat.
6. Immune systems help hosts cope with pathogens that typically evolve far more rapidly, but pathogens evolve ways of subverting immune responses.
7. Selection due to immune responses can have a major impact on the phylogenetic structure of viral clades.
8. Evolution is unable to plan ahead for future contingencies; as a result, organisms may be susceptible to problems that could have been avoided by structuring the anatomy in a different way. Human susceptibility to choking provides an example.
9. Organisms senesce because natural selection is strong on traits that are manifest early in life but weak on traits that appear later in life. The mutation accumulation hypothesis suggests that drift leads to an accumulation of alleles with deleterious effects later in life. The antagonistic pleiotropy hypothesis suggests that alleles with beneficial effects early in life but deleterious effects later in life will be favored by selection and therefore accumulate in genomes. The disposable soma hypothesis focuses on a trade-off between investment in reproduction and in repair.

KEY TERMS

affinity maturation (p. 733)
antagonistic pleiotropy hypothesis (p. 745)
cactus-shaped phylogeny (p. 735)
clonal expansion (p. 733)
clonal selection (p. 731)
disposable soma hypothesis (p. 750)
escape variants (p. 735)
mutation accumulation hypothesis (p. 745)
phylogenetic constraint (p. 738)
rate-of-living hypothesis (p. 741)
senescence (p. 740)
survivorship curve (p. 744)

REVIEW QUESTIONS

1. Briefly describe Tinbergen's four levels of explanation as applied to disease.
2. Nesse and Williams' six explanations for disease can be sorted into three groups of two based on a common theme: coevolutionary arms races and mismatch; trade-offs and lack of foresight; evolution maximizes reproductive success and some symptoms are actually defenses. Briefly describe the principle unifying each pair.
3. List two sources of evidence that fever is an adaptation.
4. Why would we expect most pathogens to be able to evolve far more quickly than their hosts?
5. What is affinity maturation?
6. What is a cactus-shaped phylogeny, and when might we see such a phylogeny for a pathogen species?
7. Which two of Nesse and Williams' six causes of disease contribute to the human vulnerability to choking?
8. How is senescence defined?
9. Contrast the rate-of-living hypothesis for senescence with the evolutionary hypotheses for senescence proposed by Medawar and Williams.
10. What trade-off does the *disposable soma hypothesis* focus on?

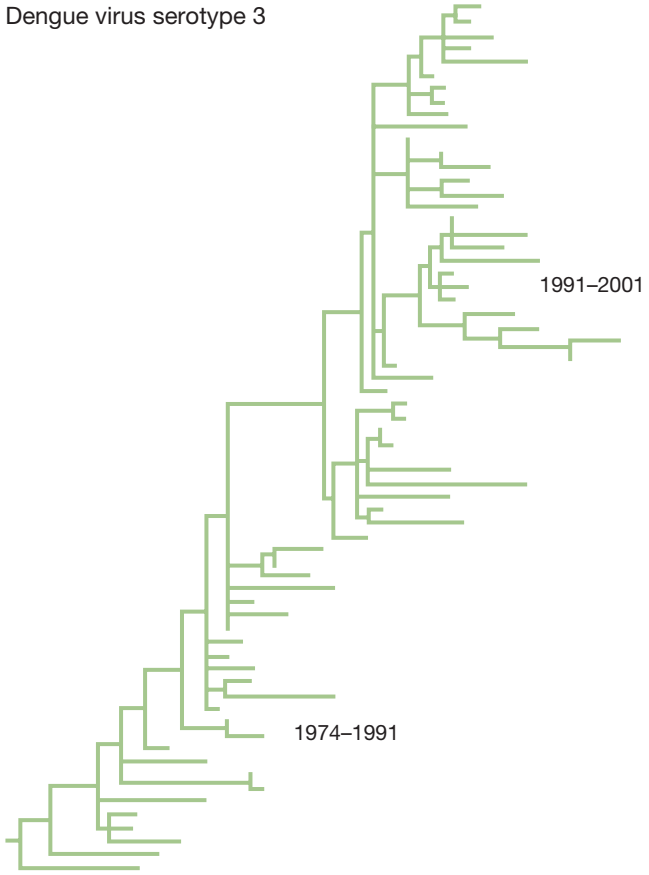
KEY CONCEPT APPLICATION QUESTIONS

11. In a 1994 paper published in the *Annals of Internal Medicine*, Philip Mackowiak attempted to explain fever (the febrile response) as follows:

> If one considers the consequence of the febrile response and its mediators only from the point of view of the host, there can be no reconciliation between its reported capacity for benefit at certain times and harm at others. However, if one views the febrile response from the perspective of the species, its salutary effects on mild to moderately severe infections and its pernicious influence on fulminating infections become less paradoxic—that is, if one accepts preservation of the species rather than survival of the individual as the essence of evolution. An evolutionary process driven by such a principle might lead to sacrifice of the individual if it poses a threat to the species. In this context, the febrile response and its mediators might have evolved both as a mechanism for accelerating the recovery of infected individuals with localized or mild to moderately severe systemic infections and for hastening the demise of hopelessly infected individuals, who pose a threat of epidemic disease to the species. (Mackowiak 1994, p. 1039)

In other words, Mackowiak proposed that fever may be harmful to individuals who manifest it, but beneficial to the species in that it prevents transmissible pathogens from spreading rapidly through the population. On the basis of your understanding of evolution by natural selection, critique this explanation.

12. The smoke detector principle explains why many defenses may be overly sensitive in the sense that they are often triggered in the absence of threat. Use the same logic to explain why many defenses may be larger in magnitude than is usually needed. Why, for example, does the average T-cell response lead to the production of a much larger number of pathogen-specific T cells than are needed to clear the average infection?
13. The following figure (adapted from Adams et al. 2006) shows a phylogeny of dengue virus serotype 3. On the basis simply of the structure of this phylogeny, would you guess that infection by the dengue virus confers long-term immunity? Explain.



14. Feedback control allows a response to be finely regulated by signals or cues from the environment. Explain why feedback control might be a dangerous way to regulate immune responses when pathogens are present.

15. Two mutations arise in a population of mice. The first increases fecundity by 20% during the first month after sexual maturity (but not beyond), whereas the second increases fecundity by 20% during the third month after sexual maturity (but not beyond). Which mutation will be more strongly favored by natural selection? Explain.

16. Classify each of the following explanations as proximate, developmental, evolutionary, or phylogenetic.

    a. Fever is an adaptation that facilitates clearance of viral and bacterial infections.

    b. Coronary artery disease develops over time due to a gradual lifelong buildup of fatty deposits on the inner walls of the coronary arteries.

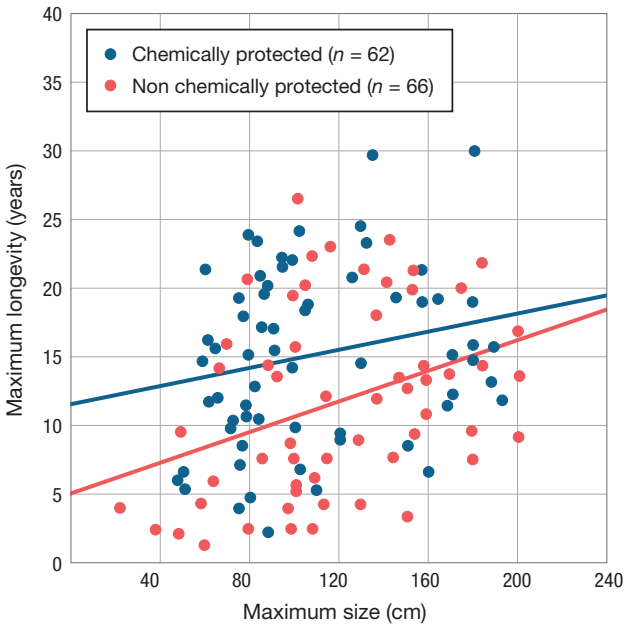
    c. The severe diarrhea associated with cholera results from the action of the cholera toxin on the cells of the intestinal lining, causing them to excrete water and electrolytes into the intestine.

    d. Most mammals synthesize their own vitamin C and are not susceptible to scurvy, but scurvy susceptibility arose in the primate lineage when the gene for gulonolactone oxidase (involved in vitamin C biosynthesis) was inactivated about 61 million years ago in the primate lineage.

    e. The sickle cell allele, responsible for sickle cell anemia, persists in many equatorial populations because in heterozygotes it protects against infection by the malaria parasite.

    f. Gout is caused when an excess of uric acid in the blood leads to the formation of uric acid crystals within the joints.

17. The scatterplot below (adapted from Blanco and Sherman 2005) indicates longevity and size for chemically protected (venomous) and non chemically protected (non-venomous) snakes. Interpret these results in light of evolutionary theories of senescence.



SUGGESTED READINGS

Gluckman, P., and N. Hansen. 2006. *Mismatch: Why Our World No Longer Fits Our Bodies*. Oxford University Press, Oxford. An exploration of how recent rapid changes in our environment leave our bodies mismatched with our circumstances of life, with a particular focus on early development.

Nesse, R. M., and G. C. Williams. 1994. *Why We Get Sick: The New Science of Darwinian Medicine*. Times Books, New York. More than 20 years ago, this book spurred current interest in the application of evolutionary biology to medicine. While somewhat dated, it remains an engaging and enjoyable read.

Omenn, G. S. 2010. Evolution and public health. *Proceedings of the National Academy of Sciences of the United States of America* 107(suppl 1): 1702–1709. A review of the many ways that evolutionary biology can contribute to improved public health practice.

Shubin, N. 2009. *Your Inner Fish: A Journey into the 3.5-Billion-Year History of the Human Body*. Vintage, New York. In this gripping book, paleontologist Neil Shubin explores the field of evo–devo (Chapter 13) in order to explain how our bodies reflect the legacy of our evolutionary history.

Sompayrac, L. 2008. *How the Immune System Works*, 3rd Ed. Wiley-Blackwell, Malden, Massachusetts. This short primer on immunology explains the fundamental logic behind the operation of the immune system without getting bogged down in the intricate details of immune function.

ANSWERS TO KEY CONCEPT QUESTIONS

Chapter 1

1.1 One possibility is that by understanding how natural selection has shaped traits in species we are trying to preserve, we can make inferences about the conditions of the environment in which these species evolved and use such inferences to create a modern environment in which our targeted species can thrive. Another possibility is that by thinking about rates of evolution, we can estimate whether any particular species will be able to adapt sufficiently rapidly to keep up with the effects of global climate change.

1.2 One way to follow up on Harcourt and colleagues' results would be to test their hypothesis in other taxa, ideally taxa that are phylogenetically distant from primates. For example, there are about 10,000 species of birds. Birds display tremendous variation in mating systems and would be ideal to test the hypothesis that selection more strongly favors large testes relative to body weight in multi-male breeding systems.

1.3 When we use

$$k\frac{N}{m} + (1 - k)\frac{N}{f}$$

to represent the number of grandoffspring as in Box 1.1, we are assuming an equal cost to producing males and females. If males are twice as expensive to produce as females, we must build in this extra cost. For the energy it takes to produce two females, a parent can only produce the equivalent of one male. Let us think of k as the fraction of reproductive effort devoted to producing males. The fraction of offspring is then $k/2$ males and $(1 - k)$ females. They have reproductive successes N/m and N/f as before. Thus, the total number of grandchildren to parents who invest a fraction k of their reproductive effort into males and a fraction $(1 - k)$ into females is equal to

$$k\frac{N}{2m} + (1 - k)\frac{N}{f}.$$

1.4 Without a sound theoretical base and good observational and experimental skills, there would have been nothing for Charlat and his team to test. Nonetheless, to test Fisher's model, they had to be on the islands at just the right time to catch the sex ratio when it was so far away from 1:1. Sex ratios that are so strongly skewed tend not to stay around for long; either the population goes extinct or some mutation brings the sex ratio back to 1:1. They did not go to the islands planning to find the skewed sex ratio they observed. Nor were they guaranteed that the butterfly population would acquire a mutation returning the sex ratio to even—but it did. They got lucky.

Chapter 2

2.1 The formation of a hypothesis is just one step in what is known as the scientific method. If a hypothesis is not falsifiable, that is, if a hypothesis cannot, in principle, be shown to be false, then that hypothesis can never be rejected, no matter what the data demonstrate. The key to determining whether a hypothesis is falsifiable is to ask yourself the question: "In principle, what evidence would be sufficient to make me reject this hypothesis?" If the answer is "nothing," the hypothesis is not falsifiable.

2.2 The inheritance of acquired characteristics supposes that traits acquired during the lifetime of an organism are passed down to its offspring. In the case of a blacksmith, we see his muscles getting larger the more he works, and we see that his sons have large muscles. One might be fooled into thinking that the former led to the latter. Instead, one of a number of possible explanations here is that blacksmiths' sons are more likely to become blacksmiths themselves—and as blacksmiths, these sons will also grow larger muscles through repeated hard work at the forge. What is inherited (though not genetically) is the profession of blacksmithing, rather than the size of the muscles.

2.3 Any number of examples might work here. For example, artificial selection for dogs that are used in herding sheep would involve systematically, generation after generation, selecting dogs that were best at circling sheep flocks and keeping them in tight formation.

2.4 Robert used a variational process here similar to that used in artificial selection. His additions are akin to random mutations, and his process of deleting what he did not like was the sorting process that produced a more enjoyable musical experience for him.

Chapter 3

3.1 Any selection process requires variation, heritability, and fitness difference. If these three prerequisites for selection are in place for culture traits, then a process analogous to natural selection could operate in principle. Clearly cultural traits vary. Mechanisms such as social learning—learning from others—and teaching each provide mechanisms for transmission of traits across generations. Some cultural traits are more fit than others in the sense that some are more likely to be transmitted. (Simply increasing the fitness of its bearers does not make a cultural trait more fit, unless that also improves the trait's own chances of transmission.)

3.2 Hooves and horseshoes are both beneficial in that both provide a durable surface that treads against the ground. But to be an adaptation, a trait or feature must have been shaped by natural selection. Hooves have evolved by natural selection for this function, so they are considered adaptations. Horseshoes do benefit horses by preventing cracked hooves, but the trait of having shod hooves is obviously not the direct consequence of natural selection, nor is it genetically inherited. Thus, we would not call horseshoes an adaptation.

3.3 One theory for why some diseases are especially prevalent in older individuals is that they are, in part, the result of antagonistic pleiotropy in genes that have positive fitness effects when individuals are young but negative fitness effects when individuals get older. We will explore this hypothesis in considerable detail when we discuss senescence in Chapter 20.

3.4 The mistake that this argument makes is to assume that exaptations are not produced by natural selection. In fact, exaptations, like adaptations, are the products of natural selection. In the case of exaptations, the function of the trait has changed over time, but natural selection was responsible both for the original function and the subsequent shift in function.

Chapter 4

4.1 On the tree of life, fungi are much closer to humans than are bacteria. As a result, humans have more biochemical similarities with fungi, and toxins that affect fungi are more likely to be harmful to human hosts as well.

4.2 Snakes, turtles, and crocodilians are reptiles. But the descendants of their common ancestor include birds, which are not reptiles.

4.3 Shrublike/treelike species are monophyletic in the Commelinids. They are not monophyletic in the Dipsacales; rather, they are paraphyletic because the common ancestor of these species—at the root of the tree—has herbaceous descendants. The Apiales fail to be monophyletic for the same reason.

4.4 Endothermy is likely to be analogous. Either it was gained twice, once in birds and once in mammals, or it was gained once prior to the split between mammals/reptiles and birds and was lost three times in snakes, turtles, and crocodilians. The latter requires a greater number of evolutionary events and thus is less likely.

4.5 There are many possible answers here. Examples include "Penguins and loons are sister groups," "Spoonbills, storks, and flamingos form a monophyletic group," etc.

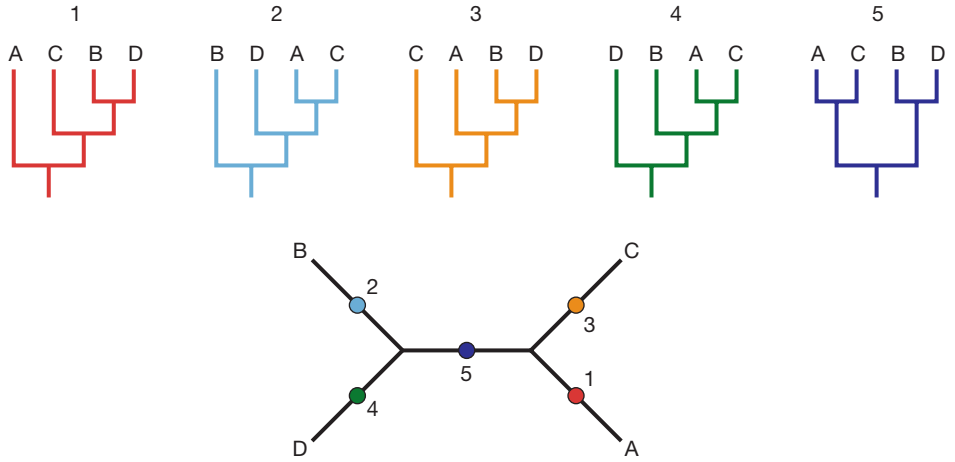
4.6 In Figure 4.32A, the group "shoebills and herons" is already a monophyletic group, so it is the smallest monophyletic group with both shoebills and herons as members. In Figure 4.32B, the smallest monophyletic group containing both shoebills and herons is "shoebills, pelicans, spoonbills, and herons."

Chapter 5

5.1 Many answers are possible here. For example, researchers can use phylogenetic analysis to estimate the time of onset of an epidemic, to figure out whether an outbreak has one or multiple sources, to determine where the epidemic came from (if from a zoonotic—non-human—source), to estimate

rates of transmission, to detect the evolution of more highly transmissible pathogen strains, and to infer geographic patterns in disease origin and spread. For the interested reader, Pybus and Rambaut (2009) provide an accessible review of the many ways in which phylogenetic analysis can help in managing an ongoing epidemic.

5.2 The unrooted four-species tree in Figure 5.20A has five branches. By placing the root on each of these branches, we will get five different rooted trees. The illustration below shows the rooted tree that arises from each of the five root locations.



5.3 The most strongly supported clades are {Mouse, Rat}, {Pig, Cow}, Eutheria, Theria, Metatheria, and Prototheria, each with 100% bootstrap support. The most weakly supported clade is the {Human, Lemur, Colugo, Tree shrew} clade with 39% bootstrap support.

5.4 Figure 5.34 hints at evolutionary relationships but does not explicitly show the branching pattern of relatedness among the species illustrated. There is no way to see from this tree which species are sister species, which groups are monophyletic clades, and so forth. The vertical axis specifies time as in a chronogram, but the horizontal axis reflects geographic location rather than being arbitrary as in a standard phylogeny. While the labeled species or taxa on a standard phylogeny are at the branch tips only, here intermediate (ancestral) species are labeled throughout the tree.

Chapter 6

6.1 Linked characters do not assort independently. If Mendel had worked with linked characters in his studies of the inheritance of multiple characters, he would not have observed the clean ratios of offspring phenotypes that he did and he would not have been able to deduce the law of independent assortment.

6.2 In incomplete dominance, the phenotype is a blend of phenotypes, but the heritable elements themselves—the alleles—do not blend. With particulate inheritance and incomplete dominance, individuals with intermediate phenotypes can have offspring with the more extreme phenotypes. Under blending inheritance, this is not the case.

6.3 Two *different* mutations arose *separately* in the lineages leading to each species. Thus, the increased ability to bind oxygen is not a homologous trait in the two species. Rather, it is an example of convergent evolution.

6.4 Unlike animals, plants commonly undergo polyploidization events in which the entire genome is doubled. Whenever this type of genome duplication occurs, it results in a new species with an even haploid number of chromosomes. No comparable process can generate a new species with an odd number of chromosomes. (In their 2000 paper, Otto and Whitton constructed a mathematical model to illustrate this phenomenon and used it to estimate rates of genome duplication.)

Chapter 7

7.1 Violations of any or all of the Hardy–Weinberg assumptions could be responsible. For example, the population could be under natural selection, it could be receiving migrants from other populations, or there could be assortative mating with individuals more likely to mate with others of the same genotype.

7.2 $A1$ will be fixed more quickly when it is recessive. This may seem counterintuitive, given that recessive alleles take longer to reach fixation when they start out rare. But the key to this problem is to notice that here, the $A1$ allele doesn't start out rare; it starts at frequency 1/2. So, whether it is dominant or recessive, $A1$ will initially increase rapidly in frequency. We can see this in Figure 7.13. The important thing is what happens once it becomes very common.

If $A1$ is dominant, then once it reaches high frequency, selection against $A2$ will nearly stop, as almost all $A2$ alleles will appear in $A1A2$ heterozygotes that exhibit the higher-fitness $A1$ phenotype. But if $A1$ is recessive, then even as it becomes very common, selection continues against $A2$ because any $A2$ allele will appear in an individual that exhibits the lower-fitness $A1A2$ phenotype. Figure 7.13 illustrates this phenomenon as well.

7.3 One straightforward way to tell the difference would be to set up population cages with different starting allele frequencies. Let them run for only one generation, and measure the fitnesses of each genotype over a single generation. If the fitnesses measured for each genotype depend on the genotype frequencies in that cage, this suggests frequency dependence. If the fitnesses measured for each genotype are independent of the genotype frequencies in the cage, this suggests overdominance.

7.4 At mutation–selection balance, the deleterious allele will be present at frequency μ/s when dominant and at frequency $\sqrt{\mu/s}$ when recessive. Because μ/s is between 0 and 1 (at least, when this approximation of the mutation–selection balance for a dominant allele holds), its value will be less than the square root of its value. Thus, the deleterious allele is more frequent when recessive.

Intuitively, this makes sense. When a deleterious allele is recessive, it is selected against only when it appears in homozygous form—which is seldom, for a rare allele. Thus, a deleterious recessive allele is only occasionally selected against in the population. When dominant, a deleterious allele is always selected against, and thus selection acts more strongly against it.

Chapter 8

8.1 There are a number of possible answers here. For example, (1) this population might exhibit positive assortative mating, such that individuals are more likely to mate with others of the same genotype; (2) this population might be subdivided into a structured set of subpopulations, with restricted mating between subpopulations; or (3) this population might be experiencing selection, specifically underdominance.

8.2 Recall the definition of expected heterozygosity: the fraction of heterozygotes expected under the Hardy–Weinberg model, given allele frequencies. The decrease in expected heterozygosity due to drift does not appear as a deviation from the Hardy–Weinberg model. Rather, it appears as a change in the allele frequencies in the population such that fewer heterozygotes are expected under the Hardy–Weinberg model; namely, one allele becomes more common, and the others become more rare.

8.3 Surprisingly, N_e can exceed N. We can see this most easily if we think of N_e as the size of the Wright–Fisher population that would undergo a comparable loss of expected heterozygosity. In the Wright–Fisher model, gametes are drawn at random for the next generation from a gamete pool. Some individuals ultimately contribute many gametes to the next generation, while others contribute none. If some mechanism (for example, the actions of a breeder) were to even out the contributions to the next generation and make them more equal, expected heterozygosity would decline more slowly than under the Hardy–Weinberg model. Imagine, for example, that every parent contributed exactly two gametes to the next generation. Then, expected heterozygosity would not decline at all—corresponding to an N_e value of infinity. This is not a mere theoretical possibility: Templeton and Read (1994) provide an example of a managed population of Speke's gazelles in which N_e exceeds N.

8.4 The coalescent process slows down dramatically as we get to a small number of individual gene copies. Recall that for a population of k gene copies, the expected time required to get back to a single pair ($k = 2$) is $2N$ generations, while the final coalescent event takes an additional $2N$ generations. Thus, for the population described in this question, we expect coalescence from 1000 down to 10 ancestral gene copies to take fewer than $2N$ generations. We then expect coalescence from the last 10 ancestral copies down to 1 copy to take more than $2N$ generations. Therefore, it takes longer to go from the last 10 to 1 than it takes to go from the initial 1000 down to 10.

8.5 Under the neutral model, we would typically expect the rate of fixation in a population to be equal to the rate of mutation in an individual. In this case, however, the rate of fixation at the A locus is much lower than the rate of mutation at that locus. This suggests that the A locus is under stabilizing selection; that is, that most new mutations at this locus are selected against.

Chapter 9

9.1 Linkage disequilibrium without physical linkage: We have seen that linkage disequilibrium is produced by several processes including natural selection. Here's one example: Imagine that *A* and *B* loci are on separate chromosomes and thus physically unlinked, the dominant *A* and *B* alleles are rare, and the *AB* phenotype is lethal. Then all gametes would be *Ab*, *aB*, or *ab*, and by Equation 9.1 the coefficient of linkage disequilibrium *D* would be negative.

Linkage disequilibrium in equilibrium: We know that linkage disequilibrium is broken down by recombination at a rate *rD* per generation, and that it is produced by various processes including natural selection. When these processes balance one another, we have a nonzero value of linkage disequilibrium that does not change over time.

9.2 Increasing the amount of epistasis increases the degree to which gene interactions are important in determining fitness. Put another way, increasing the degree of epistasis increases the context dependence of each allele. In such circumstances, a given allele will have widely varying consequences depending on the rest of the genetic background. Thus, we expect a more rugged landscape.

9.3 When we estimate narrow-sense heritability as the slope of the regression line between the average behavior of the two parents and the behavior of the offspring, we are assuming that any correlation between parents and offspring is due entirely to genetics and not due to environment. By removing the offspring from their parents, the researchers minimize the possibility that the environmental effects of parents' rearing behavior is responsible for some of the correlation between parents and offspring.

9.4 The definition of narrow-sense heritability is given by Equation 9.12:

$$h^2 = \frac{V_A}{V_A + V_D + V_I + V_E}$$

By the definition of variance, the *V* terms in this equation are all non-negative. Therefore, the value of h^2 can range from a minimum of 0 (when V_A is 0 and at least one of the other variances is positive) to a maximum of 1 (when V_A is positive and the other variances are all 0).

Nonetheless, it is possible for the breeder's equation to result in negative estimates of heritability when the selection response goes in the opposite direction of selection. We will see one example of this later in the chapter in Figure 9.46, where the 10-year rolling estimates of h^2 several times drop below 0. It is also possible for the breeder's equation to result in h^2 estimates greater than 1 if the selection response happens to be larger than the selection differential.

Chapter 10

10.1 We expect greater linkage disequilibrium between loci S and C of the hepatitis B virus.

The hepatitis B virus is encoded by a single genetic segment containing both the S and C loci, so we expect recombination between these loci to be rare even in patients infected with multiple strains. The influenza virus, meanwhile, is composed of eight separate segments, and the HA and NA loci are on different segments. Thus, in a cell infected with multiple strains of influenza, new reassortments (recombinants) of the HA and NA loci should be readily produced. Recombination breaks down linkage disequilibrium, so we expect less linkage disequilibrium between these two loci of the influenza virus.

10.2 Conjugation involves an elaborate series of events including the production of conjugative pili and a conjugative junction; a conjugative plasmid includes the genes that are responsible for these processes. These genes directly benefit the plasmid, and thus conjugation is readily seen as an adaptation. Transformation can occur by accident (for example, when a cell is electroporated in the lab), but naturally competent species have active DNA uptake mechanisms as well as mechanisms to prevent uptake of interspecific DNA. Again, the sophistication of these mechanisms suggests that they are likely to be adaptations. Transduction, by contrast, occurs through the action of the phage. But it is not beneficial to the phage; rather, it is an accident in which the phage takes up host DNA and thus fails to produce a viable bacteriophage. Because transduction does not benefit the phage, it is highly unlikely to be an adaptation.

10.3 Both LINE-1 and *Alu* elements reproduce themselves within the genome at possible costs to the host organism, and thus are selfish genetic elements. We could see *Alu* elements as hyperselfish because they are nonautonomous. In addition to free riding on the host genome, they free ride on the transpositional ability of adjacent LINE-1 elements. As a result, they need not encode their own

transcriptional machinery, and thus can get by with very short genomes. This seems to have helped them spread to more than a million copies in the human genome.

10.4 A number of answers are possible. The mutation spectrum influences the GC content and codon usage bias. Transposition drives the proliferation of transposons throughout the genome, contributing substantially to genome composition and facilitating ectopic recombination. Centromeric regions may expand via centromeric drive and subsequently cause rapid evolution of centromeric histone proteins such as CenH3.

Chapter 11

11.1 Many answers are possible here. For example, crystals share many of the characteristics of life—growth and structural organization—but they are not alive.

11.2 This sort of data helps evolutionary biologists understand the abiotic stage upon which natural selection operated during the early evolution of life.

11.3 In the process of abiogenesis, very simple life emerges a single time from nonliving material, after which it starts to reproduce directly in a process of *biogenesis* (life giving rise to life). In the process of spontaneous generation, complex life-forms are created over and over, de novo, from nonliving matter rather than being produced through biogenesis.

11.4 All modern organisms are DNA-based; therefore, their common ancestor LUCA is likely to have been DNA-based as well.

11.5 In the Sumper experiment, unlike the Spiegelman experiment, the presence or absence of a selective agent—acridine orange—was experimentally manipulated. In the presence of the selective agent, types well-suited to that agent evolved; in its absence, other types evolved.

11.6 The evolution of DNA-like structures that were able to act as enzymes occurred after only a few rounds of replication. This is a blink of the eye in evolutionary time. One way to think of this experiment is as a proof of principle—a sort of feasibility test—for the evolution of DNA in the RNA world.

Chapter 12

12.1 The extraordinary number of studies on humans, especially in the medical sciences, means that we probably have developed a more complete catalog of cell types in humans than of cell types in other species. As a result, we may be prone to overestimating the number of cell types in humans relative to that in other species.

12.2 Protocells were made of formerly autonomous units—self-replicating RNAs and simple micelles, for example—that joined together and started to replicate together, leading to a shared reproductive fate. This allowed for economies of scale. With a membrane to separate inside from outside, new possibilities arose for acquiring and processing nutrients. It became possible to synthesize additional catalytic molecules such as proteins to accelerate the reproductive process. Specialization would have occurred as well: Different RNAs could catalyze different internal reactions, for example. The hypercycle model we described in Chapter 11 illustrates a further form of specialization that becomes possible within an enclosed protocell.

12.3 During the transition from unicellularity to multicellularity, organisms were faced with a new challenge. Though multicellularity has many associated benefits, most are dependent on the ability of cells within an organism to coordinate their structures during development and their activities thereafter. In a multicellular organism, cells need to exchange information in ways that promote survival and reproduction. Increases in the efficiency of cell–cell signaling pathways would thus be favored by selection.

12.4 Investigators in this system might "knock out" or "knock in" certain genes associated with cell–cell cohesion and with apoptosis to examine the transition to multicellularity.

Chapter 13

13.1 Both abiotic components (temperature, acidity, and so on) and biotic components (competition from siblings in a clutch, parasites found in the uterus, food sources from the mother) might lead to natural selection favoring some embryonic traits over others. For example, in cases where multiple embryos are developing simultaneously, any trait that might help an embryo sequester nutrient resources better than its siblings would be favored by natural selection.

13.2 In especially harsh environments, where survival and reproduction decrease quickly with age, the acceleration of the appearance of reproductive traits—progenesis—might be favored.

13.3 People tend to like cute, baby-like features in animals, as they are less threatening. Making Mickey Mouse's features more and more paedomorphic made him cuter and cuter, and hence more and more popular.

13.4 The genes associated with the homeobox are primarily involved in the early development of body construction. As we learned in the discussion of von Baer's law, early body construction is the anchor on which almost all other developmental processes rest. As such, it would likely be especially resistant to change, because most change would lead to body plans that were inferior to what was already present.

13.5 Neofunctionalization, and to some extent subfunctionalization, are processes akin to what tinkerers do. A duplicate copy of a gene provides the evolutionary process with a spare gene copy, the raw material for tinkering via mutational change and, ultimately, solving some problem that was difficult to solve before.

Chapter 14

14.1 If a species is a group of populations that have a shared past and will have a shared future on a phylogenetic tree, then from a historical perspective, one could argue that purebreds have had different evolutionary histories from one another (for varying amounts of time, depending on the breed—some breeds have been under human control for hundreds to thousands of years). If humans continue to control breeding in these breeds, then each will have its own separate future evolutionary fate. As a result, each may eventually be considered different species under the evolutionary species concept. If humans do not continue to control breeding in these breeds, then each will likely not have a unique evolutionary future, and hence would not be considered species under the evolutionary species concept.

14.2 Molecular genetic data allow evolutionary biologists to study thousands or millions of characters instead of a few dozen as when using morphological data. If convergent evolution has occurred at some of these characters, the misleading signal from those characters will be hopefully be swamped by reliable signal from many other characters. Put another way, while it is possible that very small stretches of DNA will be similar across species because of convergent evolution, it is extremely unlikely for longer stretches of DNA. Moreover, using molecular data we can identify more reliable pinpoint characters—such as synonymous sites or non-coding regions—that should not be under selection and thus should not be subject to convergent evolution.

14.3 The fact that individuals look and act differently across populations and are well adapted to their particular environments suggests that they might be classified as different species under the phenetic species concept and possibly under the ecological species concept as well. However, the ease with which matings occur between members of the different populations when they are brought into contact suggests that under the biological species concept, speciation is not complete.

14.4 Any number of answers would be acceptable here. Two possibilities: (1) Genetic drift will operate more strongly on the small isolated populations in the peripheral isolate model than on the larger populations assumed under the vicariance model. (2) Environments on islands may be more homogenous than in the larger areas assumed in the vicariance model, and so selection might act more efficiently on islands because of the relative environmental homogeneity there.

Chapter 15

15.1 A number of answers would be correct here. For example, we might look for marks on fossil bones that indicate that animals were hunted by humans using weapons that the archeological record shows were produced during this period.

15.2 The **Signor–Lipps effect**—the lag between the date of the last known fossil and the date of actual extinction—might lead to misidentification of the magnitude of extinction, if, for one reason or another, the distribution of dates we have for the last known fossil in a taxon are spread out far more than the actual dates of extinction in that taxon.

15.3 The vertical axis of van Valen's plot in Figure 15.34 represents the number of species of a given age. Far more species have gone extinct than are living today. Therefore for any species age, we observe a larger value for extinct taxa than for living ones. One might also note that the data for living species are *right-censored*—that is, their values are artificially low because we don't know how long they will persist into the future.

15.4 As we learned in Chapter 2, Lyell's work on gradual change in geology had a strong influence on Darwin's view of evolutionary change as a slow and gradual process. Thus Lyell would have been a phyletic gradualist. (For historical accuracy, we note that Darwin's work had little effect on Lyell, who never accepted evolutionary principles. But had Lyell come around to evolutionary principles, he would surely have been a gradualist.)

Chapter 16

16.1 Phylogenetic evidence reveals that the earliest living organisms would have reproduced asexually (though perhaps with considerable horizontal gene transfer). So we might expect asexual reproduction in eukaryotes and asexual reproduction in bacteria is a symplesiomorphy. But phylogenetic evidence also suggests that the earliest *eukaryotes* reproduced sexually.

Furthermore, we generally think of complexity as a derived state. From a mechanistic perspective, sexual reproduction is generally regarded as being more complex (for example, having more steps in the process and more essential genes) than asexual reproduction. In that sense, it is surprising that the less complicated mode of reproduction is derived from the more complicated mode.

16.2 Any number of answers might work here. For example, in territorial species, there are costs of establishing and defending territories that may be critical for attracting mates. As another example, one or both sexes may be prone to injury during the mating process.

16.3 The median value in a group is the value of the "middle" individual if they are arranged in order. Thus, the median reproductive success of males is the reproductive success of the male who has higher success than one-half of the males and lower success than the other half. The same holds for females. The bitterling data reveal that most males have low reproductive success, while a small number have very high success. Thus, the median reproductive success of males is relatively low. By contrast, most bitterling females have intermediate reproductive success, and therefore the median reproductive success of females takes an intermediate value. In other words, females have a higher median reproductive success than males. We expect this pattern to be typical of most vertebrate species because, as stated earlier in the chapter, it is common for many males to have low success and a few to have high success, whereas most females have intermediate reproductive success.

16.4 In this case, the very trait that females use to select between males (intersexual selection) is the competition between males that is the defining characteristic of intrasexual selection.

Chapter 17

17.1 The definition of cooperation is based on costs and benefits to the participants, not on the presence of a nervous system or any specific cognitive abilities.

17.2 Inclusive fitness is concerned with kin that share genes identical by descent. The everyday definition of kin and family is quite broad and includes in-laws and spouses, neither of which, barring inbreeding, are genetic kin. Indeed, the English language obscures the distinction between genetic and non-genetic kin with words such as "aunt" and "uncle." Your aunt may be the sister of your mother or father, related to you by $r = 1/4$, or she may be the wife of the brother of your mother or father, and thus unrelated to you entirely. The terms "niece" and "nephew" have the same breadth of use.

17.3 If the chicks are full sibs, we plug the values into Hamilton's rule, with $r = 0.5$, $b = 0.5$, and $c = 0.2$. In this case, $r \times b$ is greater than c, and we expect this allele to increase in frequency. For half-sibs, $r = 0.25$, $b = 0.5$, and $c = 0.2$, and so $r \times b$ is less than c, and we do not expect the new allele to increase in frequency.

17.4 If individuals can recognize genetic kin, they can differentially allocate acts of altruism to such kin rather than unrelated individuals. At a finer level, natural selection might also favor kin recognition in which individuals dispense altruism to close kin rather than more distantly related kin. Kin recognition should be especially favored by selection when kin are not clustered together in space (if they were, rules of thumb like "if it is in your nest, treat it as kin," might suffice).

17.5 As a parent ages, the number of offspring it can expect to produce in the future—what is known as residual reproductive success—decreases. This favors allocating more resources to current offspring, which reduces parent–offspring conflict by better aligning the interests of parent and offspring.

Chapter 18

18.1 One such experiment might be a long-term study in which investigators experimentally reduced predation pressure on the acacia trees from mammalian predators (for example, through mesh enclosures) and examined whether acacias produced less of the resources the ants feed on.

18.2 All else equal, the parties involved in Müllerian mimicry have the same interests—namely, that the predator learn to recognize them as unpalatable. In this respect, at least, interactions between these species should take the form of mutualism. The situation is very different in Batesian mimicry, where it is to the disadvantage of one species (the unpalatable species) that another species (the palatable species) mimics its signal to a predator. As such, we might expect more antagonistic interaction effects.

18.3 Imagine that there was an insect predator or a microbial parasitic species that targeted the eggs of *G. politella*. Then, depending on the intensity of predation/parasitism and the geographic distribution of predation/parasitism, the costs and benefits to the woodland star of hosting *G. politella* eggs might change and affect the type of mosaic evolution we expect to observe across communities.

18.4 Sex-linked inheritance associated with alleles on the Y chromosome might also produce this result.

18.5 The rate at which cultural evolution occurs could affect the rate of genetic evolution because the rapidity with which cultural evolution can operate could set up strong selection pressure on traits subject to genetic evolution, speeding up the rate of the latter.

Chapter 19

19.1 Not at all. Because it does not recombine, the mitochondrial chromosome behaves as a single locus. As we saw when we explored coalescent theory in Chapter 8, irrespective of population size or growth, all current alleles at a given locus will coalesce to a single allele at some point in the past. Mitochondrial Eve is the individual in which that coalescence resided. Most likely, there were many other reproductive females in the population at the time. It just happens that none of their mitochondrial chromosomes have been passed on continually until the present day. By contrast, some of these other females most definitely did contribute nuclear alleles that persist today. Mitochondrial Eve is the ancestor of all living humans at the mitochondrial locus, but not at other loci.

19.2 The mitochondrial Eve can only be defined with respect to a given human population. When we talk about mitochondrial Eve today, we are referring to the historical female carrying the most recent common ancestor of all mitochondrial chromosomes in the current world population of humans. But 50,000 years ago, there most likely was additional mitochondrial diversity in the human population, and so the mitochondrial Eve at that time would have been an earlier individual than the current mitochondrial Eve. Similarly, if we look 100,000 years into the future, some of the mitochondrial diversity present today will likely be lost. Thus, the mitochondrial Eve of that day will be a more recent individual than the mitochondrial Eve of the present. Indeed, some reproductive female living today could, in principle, be the mitochondrial Eve for the human population 100,000 years from now.

19.3 The viruses with population genetic structures that are most likely to reflect those of humans would be human-specific viruses that create persistent lifelong infections and are often acquired from close relatives. One virus that fits the description very well is the herpes simplex virus 1 (HSV-1) responsible for cold sores. Indeed, Andreas Kolb and colleagues found that HSV-1 population genetic structure does allow us to reconstruct the history of human migration around the globe (Kolb et al. 2013). A lesser-known virus, the very common and usually harmless JC virus, also matches the description well: It is transmitted from parents to offspring and creates persistent infections. However, there is some controversy over whether the population genetic structure of this virus does reflect the history of human migrations (Shackelton et al. 2006).

The viruses with population genetic structures that are least likely to reflect those of humans would be those that are typically acquired from nonhuman animal hosts, such as rabies virus or West Nile virus, and those that cause short-term infections with rapid epidemic spread such as measles or smallpox. Another excellent example would be the influenza A virus, which jumps among multiple host species, undergoes rapid worldwide epidemic spread, and, as we will see in Chapter 20, has a "cactuslike" phylogeny with very shallow branches.

Chapter 20

20.1 Many answers are possible because this question asks one to propose answers at the proximate and evolutionary levels, not necessarily to find the correct answers. That said, the proximate explanation for why we sneeze is something like the following: Irritants in the mucosal membrane of the nasal passages stimulate histamine production. This causes local nerves to send a signal to the brain, which in turn triggers the sneeze reflex. The evolutionary explanation is most likely that the sneeze reflex evolved due to the benefits it confers as a mechanism of clearing pathogens and other irritants from the nose, by expelling at high velocity the mucus in which they are trapped.

20.2 The cost of being anxious when no threat is present will be relatively low; namely, the extra stress and energetic costs associated with the anxiety response. The cost of not being anxious when a threat is present can be very high, because the individual may fail to take action to evade the threat and may be severely injured. As a result, the optimal anxiety response can afford numerous false positives as long as it avoids almost all false negatives.

20.3 It is reasonable to consider the adaptive immune response as using a form of natural selection on a population of cells. All three of the requirements are met. Immune cells exhibit extensive variation, produced through the process of V(D)J recombination. There is inheritance: When cells divide, their daughter cells share the same specific immune receptor as the parent. Finally, through the process of clonal expansion, cells have differential reproductive success according to whether—and how well—they bind to dangerous antigens present in the body.

20.4 Many answers are possible here. For example, as mentioned earlier in the text, the high prevalence of lower back pain among adults results from a spine that evolved in our tetrapod ancestors to be slung between the forelimbs and hind limbs, but now supports the weight of the torso as a vertical column. The need to give birth through the pelvic canal is a phylogenetic constraint inherited from tetrapod ancestors that becomes a significant problem for humans because of the concomitant narrowing of the pelvis and greater cranial size. Our visual "blind spot," inherited from our early vertebrate ancestors, is an unavoidable consequence of having our retinal nerve fiber layer on top of the retinal cells rather than behind them as in an octopus. In the human eye, the thick optic nerve has to enter the eye somewhere, and this entrance point, without retinal cells, is our blind spot.

20.5 Just because a trait is universal, we cannot assume that it is adaptive. All turtles fall when dropped, for example, but being subject to the law of gravity is clearly not an adaptation. Similarly, most diseases or pathologies are not adaptations. What we aim to explain, as evolutionary biologists, is why evolutionary processes have left us vulnerable to various pathologies, not why these pathologies are adaptations.

20.6 In a species such as carp, the increase in fecundity with age offsets some of the decrease in probability of survival. Even without senescence, the chance that a given individual remains alive decreases with age, but individuals will produce more offspring per time period if they do survive to an older age. As a result, selection continues to act strongly on survival at older ages, and alleles that promote senescence are unlikely to be fixed.

Let's look at an example. Consider a species that has high juvenile mortality and thus survives to age 5 with probability 1/100. This species starts reproducing at age 5 and produces 100 offspring per year at this age, gradually increasing to 200 offspring per year after 10 years. The expected number of offspring produced at age 5 is the probability of survival to age 5 times the number of offspring: $1/100 \times 100 = 1$. The expected number of offspring produced at age 10 is the probability of survival to age 10 times the expected number of offspring produced to age 10. If the probability of survival from age 5 to age 10 is 1/2, the expected number of offspring at age 10 will be $1/100 \times 1/2 \times 200 = 1$. In other words, even accounting for extrinsic mortality, the expected number of offspring produced at age 5 and the expected number produced at age 10 are the same. As a result, selection operates as strongly on mutations that reduce survival at age 10 as it does on mutations that reduce survival at age 5.

GLOSSARY

abiogenesis The emergence of life from a nonliving precursor. (Chapter 11)

adaptation A trait that increases an organism's fitness and which is the result of the process of natural selection for its current primary function. (Chapter 3)

adaptive landscape A heuristic representation of fitness as a function of genotype or phenotype. Adaptive landscapes are commonly used by biologists to envision the course of evolutionary change. Also known as fitness landscapes. (Chapter 9)

additive genetic effects Genetic contributions to phenotype for a polygenic trait, in which the effects of each allele sum together to determine phenotype. (Chapter 9)

admixture A combination of genetic material from two previously separated populations. (Chapter 19)

affinity maturation A selective process by which immune receptors develop an improved match to a pathogen during clonal expansion. (Chapter 20)

alleles Gene variants; that is, alternate forms of the same gene. (Chapter 6)

allopatric speciation Speciation that occurs when incipient species are geographically isolated from one another. (Chapter 14)

altruism An action that has the immediate consequence of reducing an individual's own fitness while increasing the fitness of another. (Chapter 17)

amino acids Specified by nucleotide triplets, these molecules are the building blocks of proteins. (Chapter 6)

anagenesis Gradual modification of form over evolutionary time, without branching speciation. *See also* cladogenesis. (Chapter 15)

analogous trait A trait that is similar in two different species or taxa, not because of common descent, but rather as a result of natural selection operating in similar ways along separate evolutionary lineages. (Chapter 4)

anisogamy A reproductive system in which at least two different kinds (sizes) of gametes—such as eggs and sperm—are produced. *See also* isogamy. (Chapter 16)

antagonistic coevolution An evolutionary relationship in which evolutionary changes in each species decrease the fitness of the other species. (Chapter 18)

antagonistic pleiotropy A phenomenon in which a single gene has multiple phenotypic consequences with opposing effects on fitness. *See also* pleiotropic genes. (Chapter 3)

antagonistic pleiotropy hypothesis The hypothesis that senescence is largely due to the evolutionary accumulation of antagonistic pleiotropic alleles that increase survival or reproduction early in life at the cost of deleterious effects late in life. (Chapter 20)

antibiotic resistance The ability of microbes to survive and reproduce in the presence of antibiotics. (Chapter 1)

apomixis A form of asexual reproduction in which an unfertilized gamete undergoes a single mitosis-like cell division, producing two daughter cells that have an unreduced number of chromosomes and that are genetically identical to those of the mother. (Chapter 16)

aposematic coloration Warning coloration that functions to alert predators that a potential prey item is venomous or unpalatable. (Chapter 18)

archaic hominins Early species branching off from the lineage leading to humans subsequent to the human–chimpanzee split. These include numerous *Australopithecus* species. (Chapter 19)

artificial selection The process of human-directed selective breeding aimed at producing a desired set of traits in the selected species. (Chapter 1)

asexual reproduction The production of offspring from unfertilized gametes. (Chapter 16)

assortative mating A mating pattern in which individuals with similar phenotypes or genotypes mate with one another. (Chapter 7)

automixis A form of asexual reproduction in which haploid gametes are produced via meiosis, and then diploidy is restored by one of several asexual mechanisms. (Chapter 16)

autonomous transposons Small, selfish genetic elements—transposons—that encode the ability to move themselves around and/or replicate themselves within the genome. (Chapter 10)

background extinction A standard or "baseline" process of extinction occurring outside a period of mass extinction. (Chapter 15)

background selection A process by which neutral or beneficial alleles are lost because they are physically linked to nearby deleterious alleles. Background selection decreases genetic variation relative to what would be expected in a neutral model. (Chapter 9)

bacteriophage Small viruses that infect and reproduce within bacteria. (Chapter 10)

balanced polymorphism A stable equilibrium in which more than one allele is present at a locus. (Chapter 7)

balancing selection Natural selection that leads to an intermediate phenotype or to a stable equilibrium in which more than one allele is present. *See also* balanced polymorphism. (Chapter 7)

Batesian mimicry Mimicry in which a palatable species resembles an unpalatable species. *See also* Müllerian mimicry. (Chapter 18)

Bayesian inference A statistical approach often used to model evolutionary processes. Bayesian inference selects as "best" the tree that is most probable given both the observed data and some prior assumptions about possible trees. (Chapter 5)

biological species concept An approach to determining species boundaries in which a species is composed of actually or potentially interbreeding individuals. In the biological species concept, reproductive isolation determines species boundaries. (Chapter 14)

bipedal locomotion Walking upright on two legs. (Chapter 19)

bootstrap resampling A statistical technique for quantifying how strongly a data set supports a given phylogeny. (Chapter 5)

breeder's equation The equation $R = h^2S$, relating the selection response R to the selection differential S and the narrow-sense heritability h^2. (Chapter 9)

broad-sense heritability (H^2) The fraction of the phenotypic variance that can be attributed to genetic causes of any type and thus is potentially heritable. (Chapter 9)

Burgess Shale A fossil bed in British Columbia, Canada, containing extensive fossil evidence from the Cambrian explosion. (Chapter 15)

cactus-shaped phylogeny A phylogeny with short branches off a primary "backbone." Cactus-shaped phylogenies are observed in some infectious pathogens for which most clones are lost in any given year and a lineage is continued by one or at most a small number of escape variants. (Chapter 20)

Cambrian explosion The relatively rapid evolution of extensive phenotypic diversity during the early part of the Cambrian period (543 million to 490 million years ago). (Chapter 15)
carbonization A process of fossil formation wherein thin layers of carbon are laid down on sandstone or shale. (Chapter 15)
catastrophism The theory that the geology of the modern world is the result of sudden, catastrophic, large-scale events. (Chapter 2)
centromere drive Selection at the level of the chromosome that favors mutations to centromeres that increase their chance of segregating to the oocyte instead of to the polar bodies. (Chapter 10)
characters Measurable aspects of an organism. Characters may be anatomical, physiological, morphological, behavioral, developmental, molecular genetic, and so on. (Chapter 4)
chromatin DNA wrapped around histone proteins on chromosomes. (Chapter 6)
chromosomal deletion A mutation involving the loss of a section of a chromosome. (Chapter 6)
chromosomal duplication A mutation involving the duplication of a section of a chromosome. (Chapter 6)
chromosomal sex determination A sex determination system in which the sex of an individual is determined by the combination of sex chromosomes it possesses. (Chapter 1)
chronogram A phylogenetic tree on which absolute time is denoted. (Chapter 4)
***cis* regulatory elements** DNA sequences that modify the expression of other genes that are nearby on the chromosome, often by acting as binding sites for transcription factors. (Chapter 6)
clade A taxonomic group including an ancestor and all of its descendants. (Chapter 4)
cladogenesis Modification of form associated with branching speciation. *See also* anagenesis. (Chapter 15)
cladogram A phylogenetic tree in which cladistic (historical evolutionary) relationships are represented but in which branch lengths do not indicate the degree of evolutionary divergence. *See also* clade, phylogram. (Chapter 4)
cline A spatial gradient in the frequency of phenotypes or genotypes. (Chapter 14)
clonal expansion The process by which a specific immune cell is stimulated by an antigen and rapidly proliferates to create a large population of antigen-specific cells that can eradicate a pathogen. (Chapter 20)
clonal interference An overall reduction in the rate at which beneficial alleles are fixed in asexual populations due to competition among alternative beneficial mutations. (Chapter 9)
clonal selection The process by which a large repertoire of immune receptors is first generated via somatic recombination, and then cells bearing receptors that closely match an epitope on an invading pathogen go through rapid replication to produce a large population of pathogen-specific T cells or B cells. (Chapter 20)
coalescent point The point on a gene tree that delineates the gene copy that is the most recent common ancestor of the genes being studied in a population. (Chapter 8)
coalescent theory A theory developed to study the gene–genealogical relationships in a population by tracing the ancestry of gene copies backward from the present through a finite population. (Chapter 8)
codon usage bias A bias in which certain codons occur more frequently than others that specify the same amino acid. (Chapter 10)
codons A sequence of three consecutive nucleotides specifying an amino acid product. (Chapter 6)
coefficient of linkage disequilibrium (*D*) A measure of nonrandom association between alleles at two different loci. The coefficient of linkage disequilibrium D between two loci is defined as the difference between the actual frequency of a haplotype and the expected frequency of that haplotype if there were no association between alleles at one locus and alleles at the other locus. (Chapter 9)
coefficient of relatedness A measure of the extent to which individuals share alleles that are identical by descent. (Chapter 17)
coevolution The process in which evolutionary changes to traits in species 1 drive changes to traits in species 2, which feed back to affect traits in species 1, and so on, back and forth, over evolutionary time. (Chapter 3)
colinearity An arrangement of genes along a chromosome in which the relative position of a gene on the chromosome corresponds to the relative position of the body part that the gene regulates. (Chapter 13)
comparative anatomy The study of trait structure and function by comparing anatomical structures across species. (Chapter 1)
conjugation A process of horizontal gene transfer by which a donor bacterium passes a copy of a plasmid to a recipient bacterium. (Chapter 10)
conventional signals Signals that take their meaning from an arbitrary convention rather than from the cost of producing them. (Chapter 17)
convergent evolution The process in which natural selection acts in similar ways in different taxa, driving the independent evolution of similar traits in each taxon. *See also* analogous trait. (Chapter 4)
cooperation The process by which two or more individuals each receive a net benefit from their joint actions. (Chapter 17)
cooperator An individual that acts in a way that makes cooperation possible. (Chapter 17)
Cope's rule The observation that mammalian clades tend to increase in body size over evolutionary time. (Chapter 15)
cospeciation Concurrent occurrence of speciation in both partners of an interspecific mutualism. (Chapter 18)
costly signaling theory The theory that individuals with conflicting interests can communicate honestly using signals that are costly, or at least costly if untrue. *See also* handicap principle. (Chapter 17)
coupling Linkage disequilibrium in which the coefficient of linkage disequilibrium D is positive. *See also* repulsion. (Chapter 9)
crossing-over The physical exchange of segments of DNA on homologous chromosomes during meiosis. (Chapter 6)
cultural evolution The process by which culturally transmitted traits change over time. (Chapter 18)
cultural transmission The transmission of information from one individual to another by teaching or social learning. (Chapter 18)
C-value paradox The observation that differences in genome size measured in base pairs do not correlate with the phenotypic complexity of an organism. (Chapter 10)

deep coalescence The process in which alleles at a given locus fail to coalesce in between two successive speciation events, resulting in some cases in a gene tree that do not accord with the species true. (Chapter 19)
Denisovan An extinct group within the genus *Homo,* known by only two teeth and a finger bone. On the basis of genome sequence data, it is known that Denisovans and the lineage leading to modern humans interbred. (Chapter 19)
derived trait A trait that over evolutionary time has changed form or state from the ancestral form or state. (Chapter 4)
descent with modification The evolutionary process by which species change over time. (Chapter 1)
differential reproductive success The difference in the expected number of surviving offspring that can be attributed to having one particular genotype or phenotype instead of another. This is one component of natural selection. (Chapter 3)
diffuse coevolution A coevolutionary relationship that involves more than two species that affect one another. (Chapter 18)

direct fitness The expected number of viable offspring an individual produces. *See also* inclusive fitness, indirect fitness. (Chapter 17)

directional selection A process in which selection drives phenotype in a single direction or in which selection drives allele frequencies in a single direction toward fixation of a favored allele. *See also* balancing selection. (Chapter 7)

disassortative mating A mating pattern in which individuals with dissimilar phenotypes or genotypes mate with one another. (Chapter 7)

disposable soma hypothesis The hypothesis that senescence results from a necessary trade-off between investment in reproduction and investment in repair. (Chapter 20)

distribution of fitness effects The distribution of fitness effects of random mutations to a wild-type genome. (Chapter 6)

divergent evolution The process in which natural selection operates in different ways in each of two or more taxa that share a recent common ancestor, leading to different traits in these taxa. (Chapter 4)

dominance effects Interactions between two alleles at the same locus in determining phenotype. (Chapter 9)

dominant An allele A_1 is said to be dominant over another allele A_2 if its effects on phenotype mask those of A_2; that is, if the A_1A_2 heterozygote manifests the same phenotype as the A_1A_1 homozygote. (Chapter 6)

ecological species concept A diagnostic definition of species under which it is stated that a lineage of individuals occupying the same niche is a species. (Chapter 14)

economy of scale An economy of scale arises when a group of individuals can perform some task more efficiently than a lone individual or when a larger group can perform the task more efficiently than a smaller group. (Chapter 12)

effective population size The size of an idealized population (no migration, mutation, assortative mating, or natural selection) that loses genetic variation because of genetic drift at the same rate as the population under study. (Chapter 8)

efficiencies of specialization Benefits that arise when agents can specialize in different individual tasks and then share the outputs, rather than requiring each agent to be able to perform every necessary task. (Chapter 12)

endemic Found in only one specific area of the world. (Chapter 15)

endosymbiosis A mutually beneficial relationship in which one organism lives within the body—often within the cells—of another. (Chapter 12)

enhancer A section of DNA that lies outside of a gene but is involved in upregulating that gene's expression. (Chapter 13)

epigenetic inheritance Heritable (across cell generations or even through the germ line) mechanisms such as DNA methylation that alter gene expression without changes in DNA sequence. (Chapter 6)

epistasis The phenomenon in which alleles at two or more loci interact in nonadditive ways to determine phenotype. (Chapter 9)

escape variants Variant forms of a pathogen that are not recognized by the immunological memory of previously infected hosts. (Chapter 20)

eusociality A form of extreme sociality involving reproductive division of labor and the cooperative rearing of offspring. (Chapter 17)

evo–devo (evolutionary developmental biology) The subdiscipline within evolutionary biology that deals with the evolution of developmental pathways and the role that developmental changes have played in the evolution of life's diversity. (Chapter 13)

evolution Broadly defined as any instance of change over time. More specifically, in a biological context, it is the process of descent with modification that is responsible for the origin, maintenance, and diversity of life. (Chapter 1)

evolutionary arms race A form of coevolution in which the species involved each evolve countermeasures to the adaptations of the others; most often associated with host–pathogen and predator–prey coevolution. (Chapter 3)

evolutionary genomics The study of how the composition and structure of genomes have evolved and are evolving. (Chapter 10)

evolutionary radiation A burst of rapid speciation in a taxon, often associated with entering a new, relatively unoccupied niche. (Chapter 15)

evolutionary species concept According to this view, a species is a lineage that maintains a unique identity over evolutionary time. (Chapter 14)

evolutionary synthesis The collected efforts, primarily in the 1930s and 1940s, of evolutionary biologists, systematists, geneticists, paleontologists, population biologists, population geneticists, and naturalists in shaping modern evolutionary theory to show that a Darwinian view of small-scale and large-scale evolution alike is compatible with the mechanisms of genetic inheritance. Also known as the modern synthesis. (Chapter 2)

exaptation A trait that currently serves one function today but which evolved from a trait that served a different function in the past. (Chapter 3)

exons Stretches of DNA that code for protein products. *See also* introns. (Chapter 6)

expected heterozygosity Denoted as H_e, this is the fraction of heterozygotes expected under the Hardy–Weinberg model, given the allele frequencies in the population. (Chapter 8)

experimental evolution An experimental approach that examines evolutionary change in real time, often but not always by studying microbial populations in the laboratory. (Chapter 2)

extinction The loss of all individuals in a species. (Chapter 1)

fecundity A measure of the ability to produce offspring. (Chapter 7)

Fisher process A process of sexual selection in which some females express a preference for some male trait, and simply because of this preference, selection favors both the male trait and further female preference for it. (Chapter 16)

fitness A measure of reproductive success relative to the average reproductive success in a population. (Chapter 1)

fitness peaks Combinations of traits associated with the greatest fitness values on an adaptive landscape. (Chapter 9)

fitness valleys Combinations of traits associated with lower fitness values on an adaptive landscape. (Chapter 9)

fixation In population genetics, an allele is said to go to fixation in a population when it replaces all alternative alleles at the same locus; that is, when its frequency reaches 1. (Chapter 7)

fossil The remains or traces of a once-living organism. This term is usually used for remains that are greater than 10,000 years old. (Chapter 15)

fossil record The history of life on Earth as recorded by fossil evidence. (Chapter 15)

founder effect A change in allele frequencies that results from sampling effects that occur when a small number of individuals derived from a large population initially colonize a new area and found a new population. (Chapter 8)

frameshift mutation A mutation in which the addition or deletion of base pairs causes a shift in reading frame. Unless an addition or deletion involves a number of base pairs that is a multiple of 3, it will cause a frameshift mutation. (Chapter 6)

free rider An individual that takes advantage of others' cooperative or prosocial behavior without contributing any effort or expense to itself. (Chapter 17)

frequency-dependent selection A form of selection in which the fitness associated with a trait or genotype is dependent upon the frequency of that trait or genotype in a population. (Chapter 7)
frequency-independent selection A form of selection in which the fitness associated with a trait is not directly dependent upon the frequency of that trait in a population. (Chapter 7)

gametes The sex cells of an organism. In animals, sperm and eggs are gametes. (Chapter 6)
GC content The fraction of nucleotides in a gene, chromosome, or genome that are G or C rather than A or T. (Chapter 10)
GC skew A measure of whether G nucleotides or C nucleotides are overrepresented on either the leading or lagging strand of the chromosome. GC skew is typically measured as the (G – C)/(G + C) ratio of nucleotides along one strand of the chromosome. (Chapter 10)
gene A sequence of DNA that specifies a functional product. (Chapter 6)
gene–culture coevolution The interaction between genetic and cultural evolutionary change in which each drives the other. (Chapter 18)
gene duplication A new duplicate copy of a gene that is produced by mutation, or the process of producing such a copy. (Chapter 3)
gene expression The process by which a gene produces a functional product (often a protein). (Chapter 1)
gene family An ensemble of similar genes formed when a precursor gene undergoes duplication, and then the precursor or derivative genes undergo further duplications to produce multiple copies within the genome. (Chapter 13)
gene sharing The phenomenon in which a protein has more than one function and is expressed in more than one part of the body. (Chapter 3)
genetic code The way in which 20 different amino acids (and a stop signal) are specified by the 64 possible nucleotide triplets or codons. (Chapter 6)
genetic distance A measure of the genetic divergence between populations. (Chapter 5)
genetic draft A source of randomness in the evolutionary process due to the happenstance of the genetic background on which a new mutation arises. Some mutations will rise to high frequency because they arise on a favorable background; others will be lost because they arise on an unfavorable background. (Chapter 9)
genetic drift Random fluctuation in allele frequencies over time due to sampling effects in finite populations. (Chapter 8)
genetic equidistance principle The principle that if molecular evolution proceeds at the same constant rate over time in different lineages, all members of a clade should be genetically equidistant from an outgroup to the clade. (Chapter 8)
genetic hitchhiking The process by which a neutral or even disadvantageous allele is able to "ride along" with a nearby favorable allele to which it is physically linked, and thus increase in frequency. (Chapter 9)
genomic imprinting A phenomenon in which alleles are expressed differently when inherited from the mother than when inherited from the father. (Chapter 12)
genotype Either the combination of alleles that an individual has at a given locus or the combination of alleles that an individual has at *all* loci. (Chapter 6)
genotype space A conceptual model in which similar genotypes occupy nearby points on a plane. Adaptive landscapes are often illustrated in genotype space. *See also* adaptive landscape, phenotype space. (Chapter 9)
germ cells Germ cells are cells that will become specialized for reproduction and develop into gametes that pass DNA to the next generation. (Chapter 12)
G-value paradox The observation that despite seemingly large differences in organismal complexity, multicellular eukaryotes tend to have very similar numbers of protein-coding genes. *See also* C-value paradox. (Chapter 10)

handicap principle The hypothesis that the cost of producing signals ensures that they will be reliable, or "honest." *See also* costly signaling theory. (Chapter 16)
haplodiploidy A genetic system in which one sex is diploid and the other sex is haploid. (Chapter 17)
haplotype A haploid set of alleles, i.e., one at each locus. (Chapter 9)
haplotype blocks Stretches of the genome where recombination is infrequent and linkage disequilibrium is high. (Chapter 10)
Hardy–Weinberg equilibrium Given a set of allele frequencies, the expected set of genotype frequencies that will be observed under the Hardy–Weinberg model. (Chapter 7)
Hardy–Weinberg model A null model for how genotype frequencies relate to allele frequencies in large populations and how they change over time in the absence of these evolutionary processes: natural selection, mutation, migration, assortative mating, and genetic drift. (Chapter 7)
heterochrony Changes in the rate and timing of development. (Chapter 13)
heterozygote advantage *See* overdominance. (Chapter 7)
heterozygotes Individuals with two different alleles at a given locus; for example, A_1A_2. (Chapter 6)
histones Structural proteins around which DNA is wound. (Chapter 6)
homeobox A conserved 180-base-pair sequence present in homeotic genes in widely differing species. (Chapter 13)
homeotic genes Genes that determine the identity and positioning of anatomical structures during development. (Chapter 13)
hominin A member of the clade comprising humans and the extinct species more closely related to humans than to chimpanzees. (Chapter 19)
hominoid A member of the clade sometimes known as "apes," comprising orangutans (*Pongo*), gorillas (*Gorilla*), chimpanzees (*Pan*), humans (*Homo*), and four gibbon genera. (Chapter 19)
homologous trait A trait shared by two or more species because those species have inherited the trait from a shared common ancestor. (Chapter 4)
homoplasy A trait that is similar in two species because of convergent evolution rather than common ancestry. (Chapter 4)
homozygotes Individuals with two copies of the same allele at a given locus; for example, A_1A_1 or A_2A_2. (Chapter 6)
horizontal gene transfer (HGT) The transfer of genetic material from one organism to another organism that is not its offspring. Also called lateral gene transfer. (Chapter 10)
***Hox* genes** A set of genes that direct anterior-to-posterior positioning of body structures during the developmental process in animals. (Chapter 13)
hybrid zone An area in which diverging populations encounter each other, mate, and potentially produce hybrid offspring. (Chapter 14)
hypercycle model A model for the evolution of early life involving multiple types of replicators, each of which facilitates the replication of another in cyclical fashion. (Chapter 11)
hypothesis Proposed explanation for a natural phenomenon. Scientists are interested in hypotheses that generate testable predictions. (Chapter 2)

identical by descent When two or more gene copies are identical because of shared descent through a recent common ancestor. (Chapter 7)
inbreeding Mating with genetic relatives. (Chapter 7)
inbreeding depression A decrease in fitness that results from individuals mating with genetic relatives. *See also* inbreeding. (Chapter 7)

inclusive fitness The sum of indirect and direct fitness, inclusive fitness serves as a measure of the total contribution that an individual makes toward producing copies of its genes in the next generation, whether in its own descendants or in those of its relatives. (Chapter 17)
incomplete dominance The situation in which the heterozygote at a given locus expresses a phenotype intermediate to that of the two associated homozygotes. (Chapter 6)
independent contrasts A technique for accounting for shared common ancestry when using the comparative method to access evolutionary trends and patterns. (Chapter 5)
indirect fitness The incremental effect that an individual's behavior has on the fitness of its genetic relatives. (Chapter 17)
inheritance Transmission down across generations. (Chapter 3)
inheritance of acquired characteristics The hypothesis that traits acquired during the lifetime of an organism are passed on to its offspring. This idea was championed by Jean-Baptiste Lamarck. (Chapter 2)
intersexual selection Processes in which individuals of one sex select among individuals of the other sex as mates. (Chapter 16)
intrasexual selection Processes in which members of one sex, most often males, compete with each other for mating access to the other sex. (Chapter 16)
introns Noncoding stretches of DNA that interrupt protein-coding regions known as exons and that are excised before translation. (Chapter 6)
inversion A mutation in which the orientation of a stretch of a chromosome is reversed. (Chapter 6)
isogamy A reproductive system in which all individuals produce gametes of the same size. *See also* anisogamy. (Chapter 16)

K–Pg mass extinction A mass extinction (formerly known as the K–T mass extinction) that occurred about 65 million years ago at the boundary between the Cretaceous and Paleogene periods. (Chapter 15)

last universal common ancestor (LUCA) The population of organisms at the base of the tree of life. All living things today are descended from this one lineage. (Chapter 11)
lateral gene transfer *See* horizontal gene transfer (HGT). (Chapter 10)
latent variation The range of potential phenotypic variants that could be produced given the current genetic variation in the population, but are not observed because the necessary combinations of alleles are not realized in the population. (Chapter 9)
law of independent assortment Mendel's principle stating that which allele is passed down to the next generation at one locus is independent of which allele is passed down at other loci. This law holds only for pairs of unlinked loci, such as loci on different chromosomes. (Chapter 6)
law of segregation Mendel's principle that each individual (of a diploid species) has two gene copies at each locus and these gene copies segregate during gamete production. Thus, at each locus only one gene copy goes into each gamete, and an offspring receives one gene copy at each locus from each parent. (Chapter 6)
law of superposition The principle that, barring some kind of disturbance, fossils found lower down in the sediment at a particular locality will be older than those found closer to the surface. (Chapter 15)
leading edge expansion The process by which a species expands into a previously unoccupied area. The individuals colonizing the new area will tend to come from the populations nearest this region, and as a result populations in the newly colonized area will tend to exhibit a reduced genetic relation to those in the source population. (Chapter 8)
life history strategy The way that an organism invests time and resources into survivorship and reproduction over its lifetime. (Chapter 3)
LINE-1 elements Long interspersed elements. A common class of transposable elements, these autonomous transposons make up approximately 17% of the human genome. (Chapter 10)
linkage disequilibrium The presence of statistical associations between alleles at different loci. (Chapter 9)
locus The physical location of a gene on a chromosome. (Chapter 6)
long-branch attraction The tendency of some phylogenetic inference methods to incorrectly infer too close a relationship among rapidly evolving taxa. (Chapter 5)

major transitions Fundamental changes and developments in the organization of living things that have occurred over the history of life. (Chapter 1)
many eyes hypothesis The hypothesis that group living provides an advantage in the ability to detect predators. (Chapter 12)
marker gene A neutral gene with readily observable phenotypic consequences that can be used to track different experimental lines; for example, in microbial evolution experiments, such genes can be used to track different bacterial strains. (Chapter 3)
mass extinction A large-scale extinction of many taxa over a relatively short period of evolutionary time. (Chapter 15)
mating systems The mode or pattern of reproductive pairing in a population. Mating systems include monogamy, polygyny, and polyandry. (Chapter 1)
maximum likelihood A statistical approach often used to model the evolutionary process. This approach selects as "best" the phylogenetic tree that would have the highest probability of generating the observed data. (Chapter 5)
meiosis Process of cell division that reduces chromosome number in half and leads to the production of four gamete cells. (Chapter 6)
meiotic drive alleles *See* segregation distorters. (Chapter 17)
methodological naturalism An approach in which the world is explained solely in terms of natural, rather than supernatural, phenomena and processes. (Chapter 2)
minimal gene set The hypothetical minimal number of genes thought necessary to allow for cellular-based life. (Chapter 11)
mitochondrial Eve The individual woman who is the coalescent point for the mitochondrial DNA of all living humans; she is thought to have lived about 200,000 years ago in Africa. (Chapter 19)
mobile genetic elements Inessential or accessory genetic elements that can be horizontally transferred among genomes. These include plasmids, prophages, and transposons. (Chapter 10)
modern synthesis *See* evolutionary synthesis. (Chapter 2)
molecular clock A technique for assigning relative or absolute age based on genetic data. In their simplest form, molecular clock methods assume that substitutions at neutral loci occur in clocklike fashion, and so researchers use genetic distances between populations to estimate the time since divergence. (Chapter 8)
molecular mutualism When different molecules act such that they increase each other's rate of replication. *See also* mutualism. (Chapter 11)
monophyletic group A group that consists of a unique common ancestor and each and every one of its descendant species, but no other species. (Chapter 4)
mosaic coevolution A situation in which the same two species interact mutualistically in some communities but antagonistically in others. (Chapter 18)
Müllerian mimicry Mimicry in which unpalatable species resemble one another. *See also* Batesian mimicry. (Chapter 18)
Muller's ratchet A process by which the number of deleterious mutations builds up irreversibly over evolutionary time in asexual populations. (Chapter 16)

multicellularity The state in which a single individual is composed of multiple cells. (Chapter 12)

multiregional hypothesis The hypothesis for human origins stating that 2 million years ago, hominins left Africa and colonized the Old World a single time, as *Homo erectus.* These populations in different parts of the world diverged from one another morphologically, but because of modest gene flow among them, speciation did not occur. Gradually over the past 2 million years, populations together evolved into modern humans. (Chapter 19)

mutation A change to the DNA sequence. (Chapter 1)

mutation accumulation hypothesis The hypothesis that senescence occurs because natural selection is not strong enough to purge deleterious mutations associated with traits that are expressed only late in life. (Chapter 20)

mutation–selection balance An equilibrium frequency of deleterious mutations in which these deleterious mutations are maintained at a positive frequency in a population because of a balance between ongoing deleterious mutation and the purging effect of natural selection. (Chapter 7)

mutualism An ecological interaction in which different individuals, often of separate species, act so as to increase each other's fitness. (Chapter 11)

narrow-sense heritability (h^2) The fraction of the total phenotypic variation that is due to additive genetic variation and thus is readily accessible to natural selection. (Chapter 9)

natural history The study of organisms in their natural environments. (Chapter 2)

natural selection The evolutionary process by which beneficial alleles increase in frequency over time in a population because of increased survival and reproductive success of individuals carrying those alleles. Natural selection is the consequence of variation, inheritance, and differential survival. (Chapter 1)

Neanderthal A premodern *Homo* group, closely related to *Homo sapiens*, which lived in Europe and Central Asia until about 30,000 years ago. (Chapter 19)

nearly neutral theory The hypothesis that most polymorphisms and most substitutions, if not strictly neutral, are only mildly deleterious—and that because of relatively small population sizes, natural selection is unable to purge these deleterious variants. (Chapter 8)

neofunctionalization An evolutionary process in which duplicated genes diverge, and one copy takes on a new function. (Chapter 13)

neural crest cells Stem cells that, in vertebrates, migrate throughout the body and contribute to the development of numerous important organs, including the heart, brain, and teeth. (Chapter 13)

neutral mutations Mutations that do not affect fitness, either because they have no effect on phenotype or because the change in phenotype they induce has no fitness consequences. (Chapter 1)

neutral theory The hypothesis that at the molecular level of DNA sequence or amino acid sequence, most of the variation present within a population and most substitutional differences between populations are selectively neutral. (Chapter 8)

niche construction The process by which an organism shapes its own environmental conditions. (Chapter 5)

node A branch point on a phylogenetic tree, representing an ancestral population or species that subsequently divided into multiple descendant populations or species. (Chapter 4)

nonautonomous transposons Small, selfish genetic elements—transposons—that do not encode the genes necessary to catalyze their own movement and replication, but instead rely on the presence of autonomous transposons for these purposes. (Chapter 10)

noncoding DNA DNA that does not specify an expressed product such as a protein, tRNA, or mRNA. (Chapter 10)

nonsense mutation A mutation that creates a stop codon where there was not one previously. (Chapter 6)

nonsynonymous mutation A mutation in a gene that changes the amino acid sequence of the protein that gene encodes. (Chapter 6)

norm of reaction A curve that represents the phenotype expressed by a given genotype as a function of environmental conditions. (Chapter 3)

nuclear genome The set of chromosomes contained in the eukaryotic nucleus. (Chapter 10)

obligate mutualism A mutualism in which each partner requires the other for successful survival and/or reproduction. (Chapter 18)

observed heterozygosity The fraction of individuals in the population that are heterozygous at a given locus. (Chapter 8)

odds ratio testing A statistical technique for quantifying how strongly a data set supports a particular hypothesis. Applied to phylogenetics, odds ratio testing is sometimes used to determine how strongly the data support the hypothesis that a given group represents a monophyletic clade. (Chapter 5)

ontogeny The development of an organism. (Chapter 13)

outgroup A distantly related group with a known evolutionary relationship to the taxon being studied. Outgroups are used in rooting phylogenetic trees. (Chapter 4)

out-of-Africa hypothesis This alternative to the multiregional hypothesis posits that hominins left Africa and colonized the Old World in multiple waves, first as *Homo erectus* approximately 2 million years ago, second as *Homo heidelbergensis* 600,000 years ago, and third as *Homo sapiens* approximately 100,000 years ago. Under this hypothesis, *Homo sapiens* then replaced the resident hominin species, and thus all regional differences among non-African humans are no more than 100,000 years old. (Chapter 19)

overdominance A form of frequency-independent selection in which heterozygote genotypes have higher fitness than the corresponding homozygote genotypes. (Chapter 7)

paedomorphosis The appearance of traits seen in the juvenile stage of an ancestral species during the adult stage of a descendant species. (Chapter 13)

paleomagnetic dating The method of estimating fossil dates based on shifts in Earth's magnetic field by measuring the alignment of metal particles in the substrate in which the fossil was found. (Chapter 15)

paralogs A pair of genes within a genome that share common ancestry due to a gene duplication event. (Chapter 13)

parapatric speciation The process of speciation that occurs when diverging populations have distributions that abut one another. (Chapter 14)

paraphyletic group A group that includes the common ancestor of all its members but does not contain every species that descended from that ancestor. (Chapter 4)

parent–offspring conflict Conflict that arises because the genetic interests of offspring and their parents are not perfectly aligned. (Chapter 17)

parsimony An approach to selecting the best phylogenetic tree given some set of character data. Parsimony methods assume that the best tree is the one that requires the fewest character changes to explain the data. (Chapter 5)

periodic selection A process in which a series of clones carrying beneficial mutations successively go to fixation in an asexual population. (Chapter 9)

peripheral isolate model A form of allopatric speciation in which a population is split into geographically isolated populations that differ substantively in size, with one large population and one or several smaller populations. *See also* vicariance model. (Chapter 14)

phenetic species concept An approach to determining species boundaries in which species are identified as clusters of phenotypically similar individuals or populations. (Chapter 14)

phenotype The observable physical, developmental, and behavioral characteristics of an organism. (Chapter 1)

phenotype space A conceptual model in which similar phenotypes occupy nearby points on a plane. Adaptive landscapes are often illustrated in phenotype space. *See also* adaptive landscape, genotype space. (Chapter 9)

phyletic gradualism model The hypothesis that new species arise by a gradual transformation of an ancestral species through slow, continual change. *See also* punctuated equilibrium model. (Chapter 15)

phylogenetic constraint The fact that the legacy of human evolutionary past limits the course of further evolution and results in seemingly suboptimal structures such as the intersection of the trachea and esophagus. (Chapter 20)

phylogenetic distance methods Methods of constructing phylogenetic trees based on measurements of pairwise "distances" between species, where distance is a measurement of morphological or genetic differences between species. (Chapter 5)

phylogenetic diversity A measure of diversity quantified by summing lengths of all branches in a phylogenetic tree. (Chapter 1)

phylogenetic event horizon The point in the history of life beyond which phylogenetic analysis is uninformative because there are no surviving descendants from ancestors before this point. *See also* last universal common ancestor (LUCA). (Chapter 11)

phylogenetic species concept An approach to determining species boundaries in which a species is defined as the smallest monophyletic group that shares a unique derived character absent from all other groups on the phylogeny. (Chapter 14)

phylogenetic systematics An approach to classifying organisms based on their evolutionary histories. (Chapter 4)

phylogenetic tree A visual representation, in the form of a bifurcating tree, of the evolutionary relationship between species, genera, families, and higher taxonomic units. (Chapter 1)

phylogeny The branching pattern of relatedness among populations (or occasionally, individuals) in a group or taxon. (Chapter 4)

phylogeography The use of phylogenetic and population-genetic tools to study the geographic distributions of populations or species. (Chapter 5)

phylogram A phylogenetic tree in which the length of each branch represents the amount of evolutionary change that has occurred along that branch. (Chapter 4)

physical linkage The occurrence of two or more loci on the same chromosome. Physical linkage causes alleles at linked loci to segregate together (in the absence of recombination) into the gametes. (Chapter 9)

plasmids Circular extrachromosomal genetic elements common in bacteria and some other microorganisms. (Chapter 10)

pleiotropic genes Genes that affect more than a single trait. (Chapter 3)

polarity The order in which different variants of a trait evolved over evolutionary time. (Chapter 4)

polygenic traits Traits that are affected by many genes simultaneously. (Chapter 9)

polyphyletic group A group that does not contain the common ancestor of its members and/or all descendants of that common ancestor. (Chapter 4)

polytomy A node on a phylogenetic tree that has more than two branches arising from it. Polytomies are often used to represent uncertainty about phylogenetic relationships on a phylogenetic tree. (Chapter 4)

population A group of individuals of the same species that are found within a defined area and, if they are a sexual species, interbreed with one another. (Chapter 2)

population bottleneck A brief period of small population size. Population bottlenecks reduce genetic diversity and can accelerate changes in allele frequencies due to genetic drift. (Chapter 8)

population genetics A subdiscipline in evolutionary biology that investigates how allele frequencies and genotype frequencies change over time. (Chapter 7)

positive selection Selection favoring a beneficial allele. (Chapter 8)

postcopulatory sexual selection Sexual selection that occurs after matings have taken place. Sperm competition is one form of postcopulatory sexual selection. (Chapter 16)

postzygotic isolating mechanisms Reproductive isolating mechanisms that occur after fertilization and conception, often leading to embryos that may not develop fully to birth or to sterile offspring. *See also* prezygotic isolating mechanisms, reproductive isolating mechanisms. (Chapter 14)

prebiotic soup An ensemble of simple organic molecules suspended in liquid water prior to the emergence of life on Earth. (Chapter 11)

prebiotic soup hypothesis The idea that the earliest life emerged in a "souplike" liquid environment, drawing upon energy from cosmic rays, volcanic eruptions, and Earth's own internal heat. (Chapter 11)

premodern hominins Nonhuman hominin species that arose within the past 2 million years along the lineage leading to *Homo sapiens.* These species, which had larger brains and made more elaborate tools than their predecessors, include *Homo ergaster*, *Homo erectus*, *Homo heidelbergensis,* Neanderthals, and Denisovans. (Chapter 19)

prezygotic isolating mechanisms Reproductive isolating mechanisms that prevent mating from occurring or that prevent fertilization from occurring if such a mating does occur. *See also* postzygotic isolating mechanisms, reproductive isolating mechanisms. (Chapter 14)

promoter A short DNA sequence before the transcribed region of a gene, to which the RNA polymerase binds to initiate transcription. (Chapter 6)

prophages Viral genomes that insert themselves into bacterial chromosomes. Prophages can subsequently be excised from the genome and initiate viral replication within the bacterial cell. (Chapter 10)

protocell A simple cell-like entity that predated cellular life-forms in the history of life. (Chapter 11)

pseudoextinction A phenomenon in which a population changes by anagenesis over evolutionary time, until it is so different from the ancestral population that it is reclassified as a new species. (Chapter 15)

pseudogene A nonfunctional and typically untranslated segment of DNA that arises from a previously functional gene. (Chapter 8)

punctuated equilibrium model The hypothesis that major evolutionary changes, including speciation, do not occur through a slow, gradual process. Instead, stasis—the absence of change—is the rule during the vast majority of a lineage's history. But when evolutionary change does occur in lineages, it is rapid and typically leads to branching speciation (cladogenesis). *See also* phyletic gradualism model. (Chapter 15)

purifying selection Selection against deleterious mutations. (Chapter 8)

QTL mapping A technique for identifying the regions of the genome in which quantitative trait loci occur. *See also* quantitative trait loci (QTLs). (Chapter 9)

quantitative genetics A mathematical approach to the population genetic study of continuously varying traits. (Chapter 9)

quantitative trait loci (QTLs) Loci responsible for quantitative—that is, continuously varying—traits. (Chapter 9)

radiocarbon dating A technique for dating geological strata by using the decay rate of carbon-14 to nitrogen-14. (Chapter 15)

radiopotassium dating A technique for dating geological strata by using the decay rate of potassium-40 to argon-40. (Chapter 15)

rate-of-living hypothesis The hypothesis that senescence is an inevitable consequence of accumulated physical wear and tear. (Chapter 20)

realized heritabilities Narrow-sense heritability values estimated by using values of the selection differential and selection response in the breeder's equation. (Chapter 9)

recapitulation The appearance of traits in the juvenile stage of a descendant species that were expressed in the adult stage of an ancestral species. (Chapter 13)

recessive An allele A_1 is said to be recessive to another allele A_2 if its effects on phenotype are masked in the heterozygote; that is, if the A_1A_2 heterozygote manifests the same phenotype as the A_2A_2 homozygote. *See also* dominant. (Chapter 6)

reciprocal altruism The hypothesis that altruistic behavior can be maintained evolutionarily if individuals exchange acts of altruism. (Chapter 17)

recombination hotspots Small regions of the genome that are particularly prone to serving as locations of crossover. (Chapter 10)

Red Queen hypothesis The hypothesis that sexual reproduction is an adaptation allowing hosts to generate sufficient genetic variation to keep up with their pathogens and parasites in the coevolutionary arms race. This hypothesis predicts that the level of parasitic infection will be related to the frequency of sexual versus asexual reproduction. (Chapter 16)

regulatory elements Stretches of DNA involved in controlling levels of gene expression. (Chapter 6)

reproductive character displacement The situation in which a reproductive trait is less similar across two populations in sympatry than in allopatry. (Chapter 14)

reproductive isolating mechanisms Mechanisms that prevent gene flow between populations. (Chapter 14)

repulsion Linkage disequilibrium in which the coefficient of linkage disequilibrium D is negative. *See also* coupling. (Chapter 9)

ribozymes RNA molecules with enzymatic function. (Chapter 11)

ring species A situation in which populations that are unable to interbreed directly are nonetheless connected indirectly by gene flow through a chain of other populations. (Chapter 14)

RNA world A hypothetical early stage in the history of life in which RNA was the fundamental unit upon which life was based, fulfilling both an informational role (much as DNA does today) and a catalytic role (much as protein-based enzymes do today). (Chapter 11)

root The basal (most ancestral) lineage on a phylogenetic tree. (Chapter 4)

rooted tree A phylogenetic tree in which the root is indicated and thus the direction of time is specified. (Chapter 4)

runaway sexual selection An extreme case of the Fisher process of sexual selection in which positive feedback between genes that code for male traits and genes that code for particular mating preferences in females drives the evolution of highly exaggerated male traits and strong female preferences for them. (Chapter 16)

saltationism The hypothesis that evolutionary change occurs primarily as a result of large-scale changes. (Chapter 2)

secondary reinforcement The process by which two populations begin to diverge in allopatry but complete the process of speciation in sympatry when matings between individuals in these populations produce hybrid offspring with reduced fitness. (Chapter 14)

segmentation genes Genes associated with patterning of the body segments during development. (Chapter 13)

segregation distorters Alleles that bias the process of meiotic segregation in their own favor, increasing their representation to more than half the gametes produced by an individual. Also known as meiotic drive alleles. (Chapter 17)

selection coefficient A measure of the strength of natural selection for or against a specific phenotype or genotype. (Chapter 7)

selection differential (S) In quantitative genetics, the difference between the mean trait value of the individuals who reproduce and the mean trait value of all individuals. (Chapter 9)

selection response (R) In quantitative genetics, the difference between the mean trait value of the offspring population and the mean trait value of the parental population. (Chapter 9)

selective breeding A process in which humans decide which plants or animals in a population are allowed to breed. *See also* artificial selection. (Chapter 1)

selective sweep A phenomenon in which a selected allele goes to fixation, carrying with it alleles at physically linked loci. *See also* genetic hitchhiking. (Chapter 9)

selectively neutral Alternative alleles are selectively neutral when there is no fitness difference between them. (Chapter 8)

selfish genetic elements Stretches of DNA, such as transposons, that act primarily to ensure their own survival and replication within a genome, even at a fitness cost to the organism. (Chapter 10)

senescence General decline in the physical functioning or performance of living organisms with age. (Chapter 20)

sensory bias model Model for the evolution of elaborate traits by sexual selection, in which a preexisting bias in the perceptual system of one sex favors members of the other sex who display a particular trait. (Chapter 16)

sequence divergence A measure of the extent to which two DNA sequences differ from one another. (Chapter 5)

sex ratio The ratio of males to females in a population. (Chapter 1)

sexual conflict A phenomenon in which selection operates differently on males and females, typically with respect to mating behavior. (Chapter 16)

sexual reproduction Joining together of genetic material from two parents to produce an offspring that has genes from each parent. Typically, sexual reproduction involves both recombination between homologous chromosomes and outcrossing—mating between genetically different individuals. (Chapter 16)

sexual selection A form of natural selection that refers to selection for traits and behaviors that confer mating success (as opposed to survival). (Chapter 16)

sib–sib conflict Conflict that occurs when the genetic interests of siblings are not perfectly aligned, typically because each sibling is selected to seek a disproportionate share of parental resources. (Chapter 17)

Signor–Lipps effect The lag between the last observed fossil of an extinct species and the actual date of extinction. This effect can cause paleontologists to date an extinction earlier than it actually occurred. (Chapter 15)

silencers DNA sequences that suppress the expression of genes. (Chapter 13)

silent mutation *See* synonymous mutation. (Chapter 6)

SINE elements Short interspersed elements. A common class of transposable elements in humans, these nonautonomous transposons are incapable of independent replication but rather rely on genes encoded by autonomous transposons elsewhere in the genome. (Chapter 10)

sister taxa Two taxa that are immediately derived from the same ancestral node on a phylogenetic tree. (Chapter 4)

somatic cells Cells specialized in the maintenance and growth functions of an organism. (Chapter 12)

speciation The process by which new species arise from previously existing species. All models of speciation involve some type of breakdown of gene flow across populations. (Chapters 1, 14)
species selection A process of differential speciation and/or extinction between lineages that may drive some of the macroevolutionary trends observed across taxa. (Chapter 15)
spontaneous generation The now-disproved hypothesis that complex life-forms can arise, *de novo*, from inorganic matter. (Chapter 2)
structure An algorithm (and software package) used to infer population structure from genetic sequence information at multiple loci. (Chapter 19)
struggle for existence Darwin's idea that organisms are continually in competition for resources. (Chapter 2)
subduction The process in which one tectonic plate slides under another and moves toward Earth's mantle. (Chapter 15)
subfunctionalization A molecular evolutionary process by which gene duplication produces gene copies that diverge over evolutionary time and divide the work initially undertaken by the gene before duplication. (Chapter 13)
substitution The process in which a new allele arises by mutation and is subsequently fixed in a population. (Chapter 8)
survivorship curve The fraction of surviving individuals as a function of age. (Chapter 20)
sympatric speciation A process of speciation in which diverging populations are not geographically separated. (Chapter 14)
symplesiomorphy A derived trait that has arisen so recently that it appears in only one of two sister taxa. Evolutionary biologists try to avoid using symplesiomorphies in phylogenetic reconstruction. (Chapter 4)
synapomorphy A derived trait that is shared in two populations because it was inherited from a recent common ancestor. Evolutionary biologists aim to use synapomorphies in phylogenetic reconstruction, as synapomorphies provide useful information about the evolutionary relationships among populations. (Chapter 4)
synonymous mutation A base pair substitution that does not change the amino acid that a codon normally produces. Also known as a silent mutation. (Chapter 6)
systematics The scientific study of classifying organisms. (Chapter 2)

taxon A group of related organisms. (Chapter 4)
trade-off A situation in which constraints prevent simultaneously optimizing two different characters or two different aspects of a character. (Chapter 3)
traits Any observable characteristics of organisms, such as anatomical features, developmental or embryological processes, behavioral patterns, or genetic sequences. (Chapter 4)
***trans* regulatory elements** DNA sequences that modify the expression or activity of genes that are not nearby on the chromosome, often by coding for transcription factors. (Chapter 6)
transcription The process of copying a DNA sequence into a complementary messenger RNA (mRNA). (Chapter 6)
transcription factors Proteins that bind to DNA and influence gene expression. (Chapter 10)
transduction Horizontal gene transfer that occurs when a bacteriophage packages host DNA into its capsule. If that DNA is injected into a new host, it can be incorporated into the genome. (Chapter 10)
transformation Horizontal gene transfer that occurs when a bacterial cell picks up free DNA from the environment and incorporates this DNA into its genome. (Chapter 10)
transformational process A process of change in which the properties of a group change because every member of that group changes. (Chapter 2)
transition A mutation in which a purine (adenine or guanine) is replaced by a purine or a pyrimidine (cytosine or thymine) is replaced by a pyrimidine. *See also* transversion. (Chapter 6)
transitional hominins Hominin species intermediate in time between the archaic hominins and the premodern hominins, including *Homo habilis* and *Homo rudolfensis*. (Chapter 19)
translation The process by which an amino acid chain is synthesized by a ribosome on the basis of the template provided by a messenger RNA. (Chapter 6)
translocation A mutation in which a section of a chromosome is moved to a nonhomologous chromosome. (Chapter 6)
transmission genetics The study of the mechanisms by which genes are passed from parents to offspring. (Chapter 6)
transposable element A self-replicating genetic unit that can move or copy itself within a genome. (Chapter 10)
transversion A mutation in which a purine is replaced by a pyrimidine or vice versa. *See also* transition. (Chapter 6)
tree of life A phylogenetic tree that depicts the evolutionary relationships among all living things. (Chapter 1)
twofold cost of sex The observation that—with all else equal—an asexual lineage introduced into a population of sexually reproducing organisms would initially double in representation in each generation. (Chapter 16)

underdominance A form of frequency-independent selection in which the heterozygote genotype has a lower fitness than either corresponding homozygote genotype. (Chapter 7)
uniformitarianism Charles Lyell's theory that the very same geological processes that we observe today have operated over vast stretches of time and explain the geology of the past and the present. (Chapter 2)
unrooted tree A phylogenetic tree in which the root, and thus the direction of time, is unspecified. (Chapter 4)

variation In evolutionary biology, genetic variation is one of the components of the process of natural selection. (Chapter 3)
variational process A process of change in which the properties of an ensemble change, not because the individual elements change, but because of some sorting process. In evolutionary biology, the sorting process is natural selection. (Chapter 2)
vesicle A small, fluid-filled compartment surrounded by a lipid membrane. (Chapter 11)
vestigial traits Traits that have no known current function but that appear to have had a function in the evolutionary past. (Chapter 4)
vicariance model A form of allopatric speciation in which a population splits into two comparably sized subpopulations separated by geographic barriers. (Chapter 14)
virulence factors Specialized genes that assist bacteria in exploiting eukaryotic hosts. (Chapter 10)

Wright's *F*-statistic A statistical measure of the degree of homozygosity in a population. (Chapter 7)
Wright–Fisher model A population genetic model of evolutionary change in small populations with non-overlapping generations. (Chapter 8)

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Chapter 1

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Chapter 2

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