# 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?

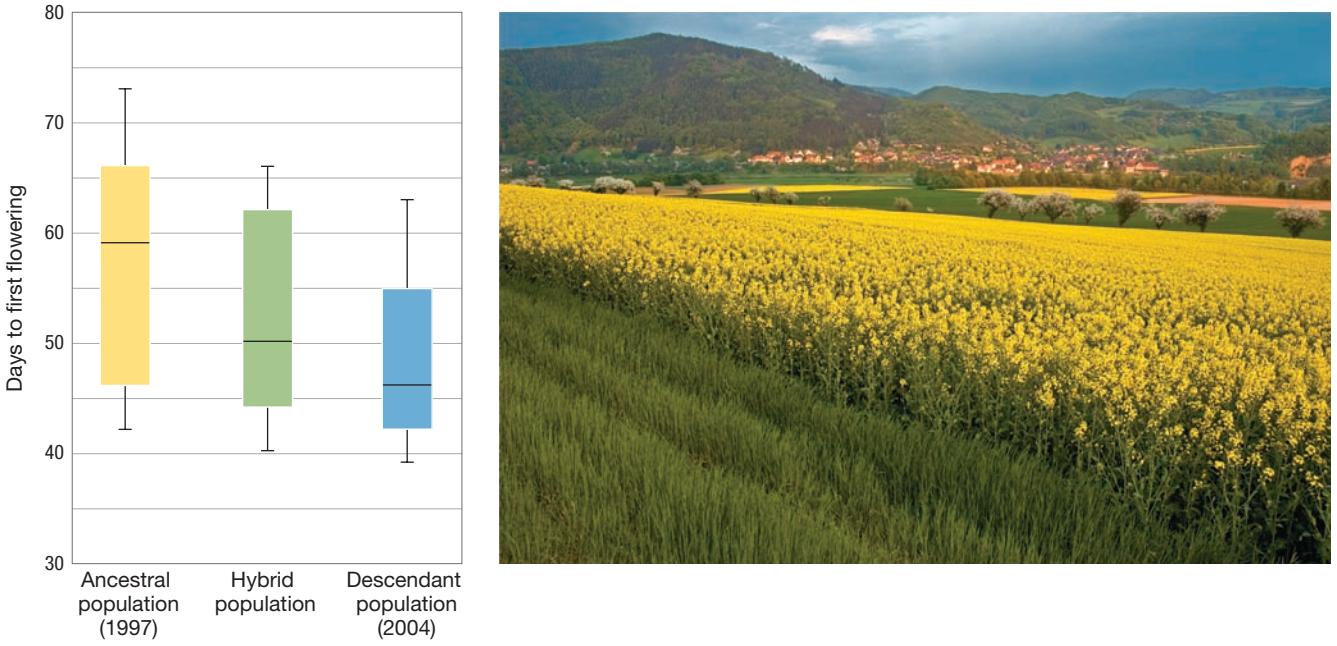



**FIGURE 3.1 A prolonged drought alters flowering time.** Descendant populations of *Brassica rapa* from after the intense 2000–2004 drought flowered much earlier in the season than those from predrought (ancestral) populations. Hybrids—crosses between the ancestral and descendant populations—show intermediate 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,

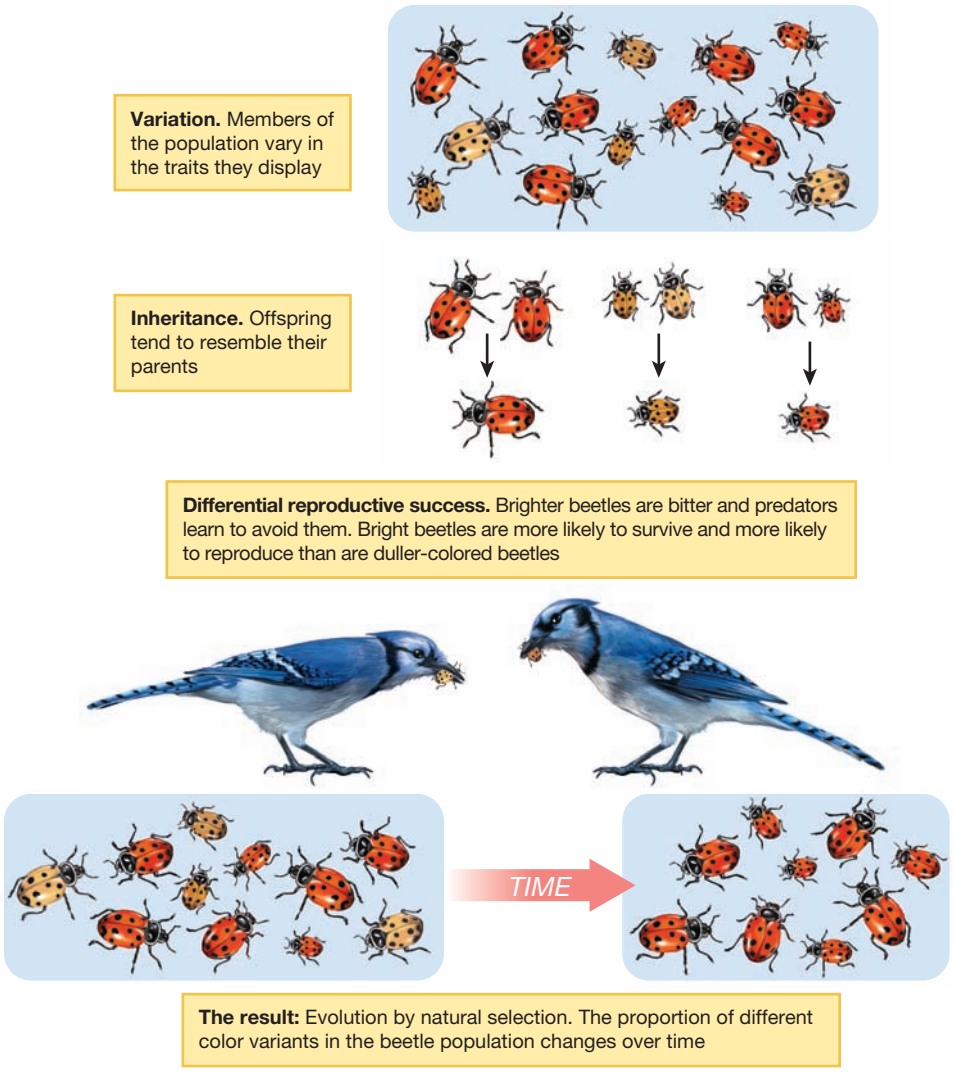



**FIGURE 3.2 The three components of natural selection.** Evolution by natural selection occurs when there is variation, inheritance, and differential reproductive success among individuals in a population.

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,

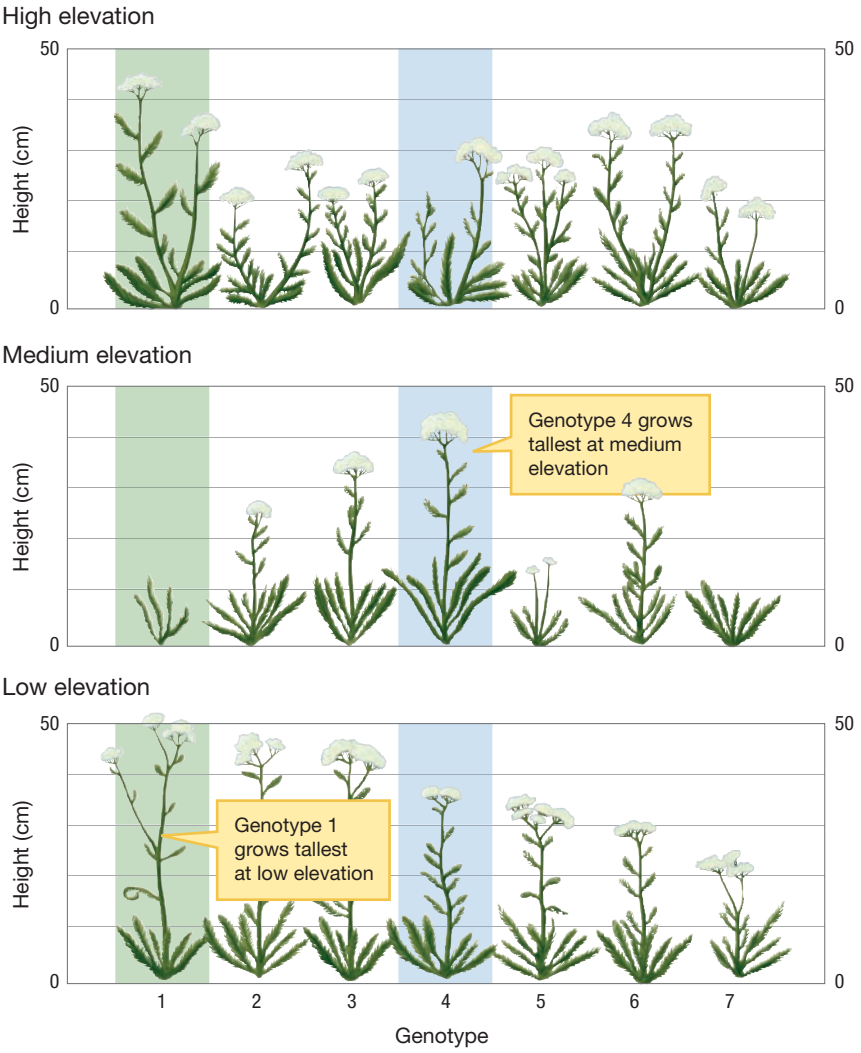



**FIGURE 3.3 Phenotype depends on the effects of both genotype and environment.** Here we see how the height of a yarrow plant (*Achillea millefolium*) depends on its genotype and the altitude at which it is raised, as shown by populations of yarrow plants grown in gardens at three sites that were at 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

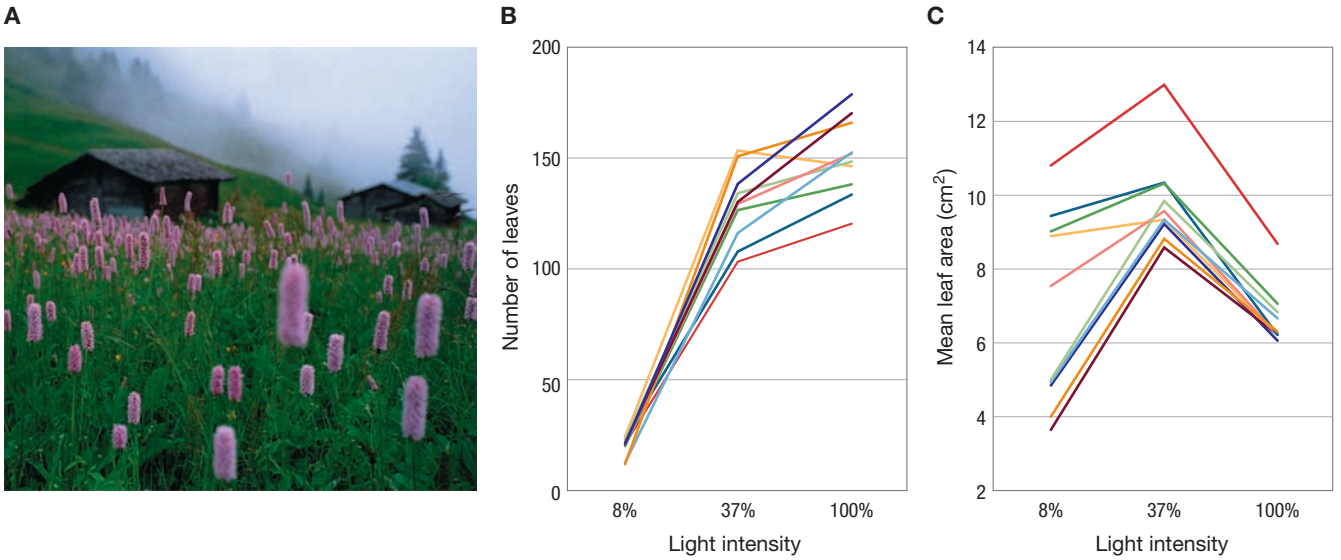



**FIGURE 3.4 Norm of reaction curves.** In the weedy annual plant *Persicaria maculosa* **(A)**, the total number of leaves **(B)** and the mean leaf area **(C)** depend on the light intensity—ranging from full shade to full direct sunlight—that the plant experiences. Each curve for one specific genotype 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**).

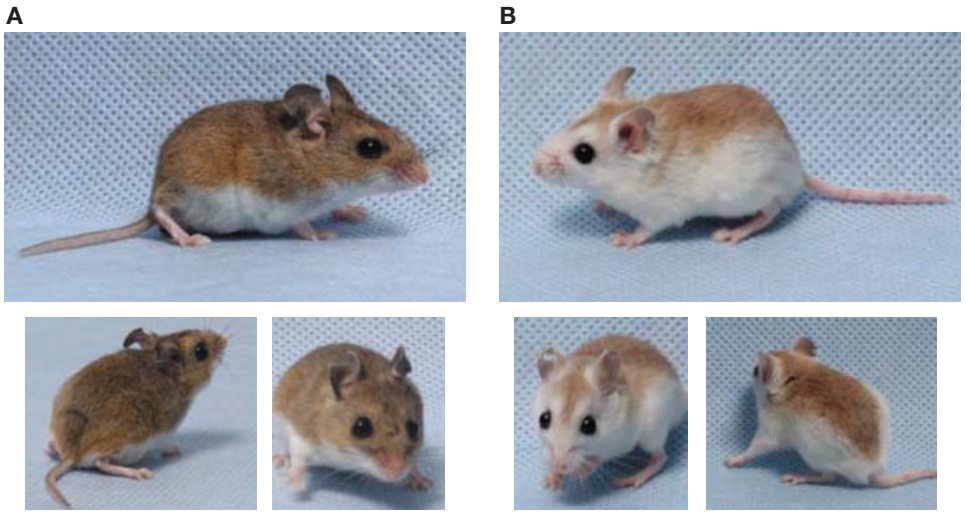



**FIGURE 3.5 Coat color variation in mice.** Two color variants of *Peromyscus polionotus*: **(A)** the darker inland form, and **(B)** the lighter beach-dwelling form.

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.

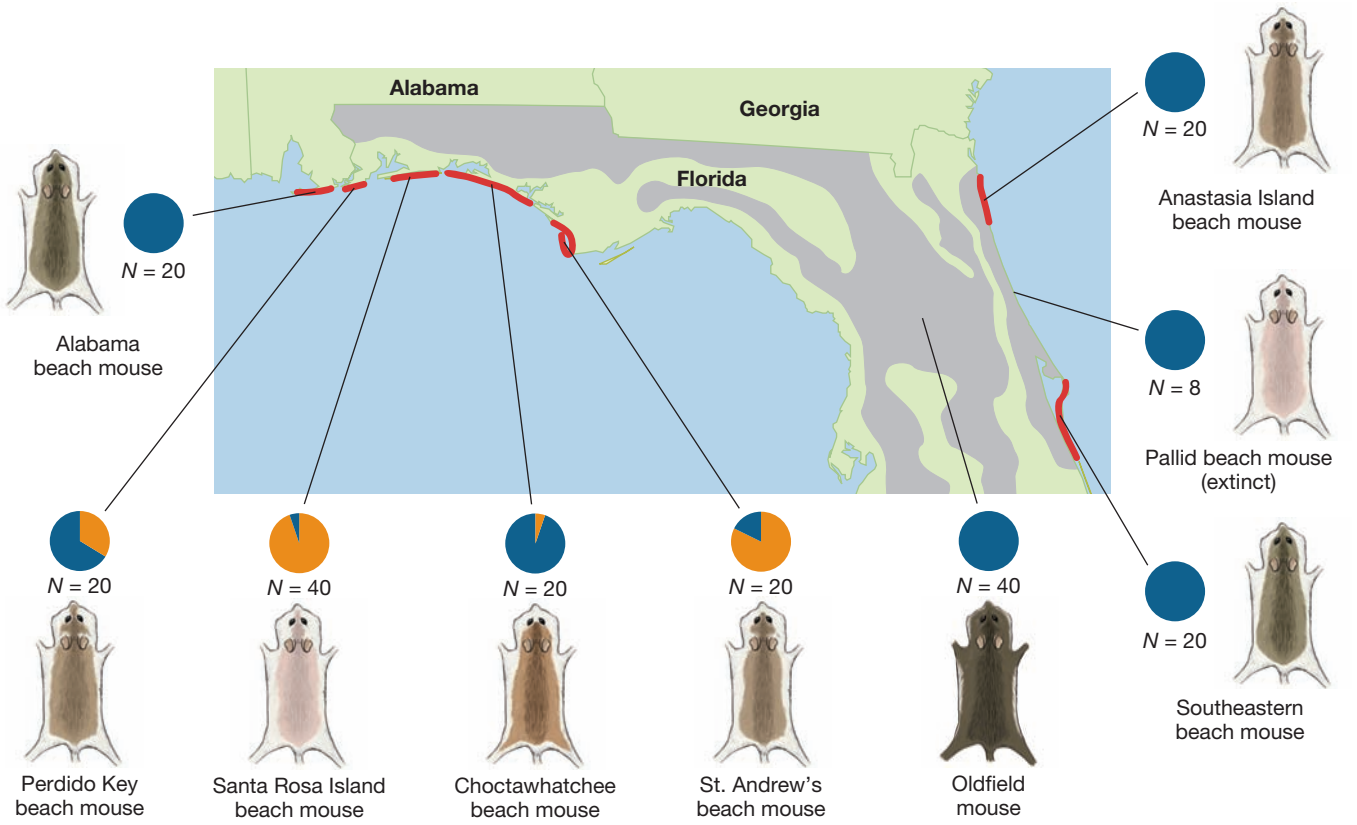



**FIGURE 3.6 Variation in coat color and genotypes at the Mc1R locus.** *Peromyscus polionotus* exhibits extensive coat color variation across localities in Florida. Red areas indicate the distribution of beach populations; gray areas denote the distribution of inland populations. Characteristic phenotypes 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

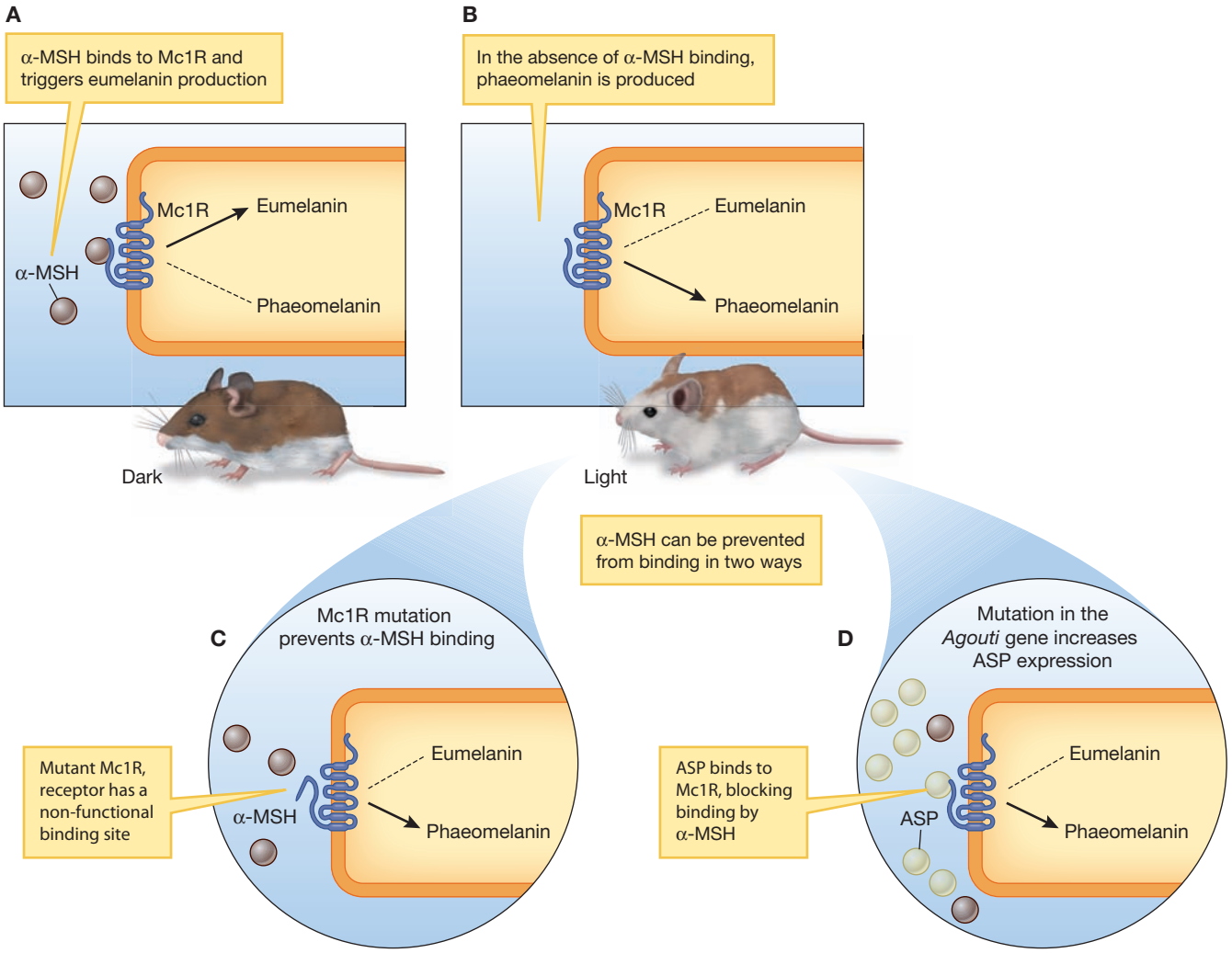



**FIGURE 3.7 Genetics of coat color determination in mice.** The protein Mc1R acts as a genetic switch, determining whether dark eumelanin or light phaeomelanin is produced. **(A)** When the Mc1R receptor binds α-MSH, it triggers eumelanin production. **(B)** When α-MSH is absent, phaeomelanin is produced 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).

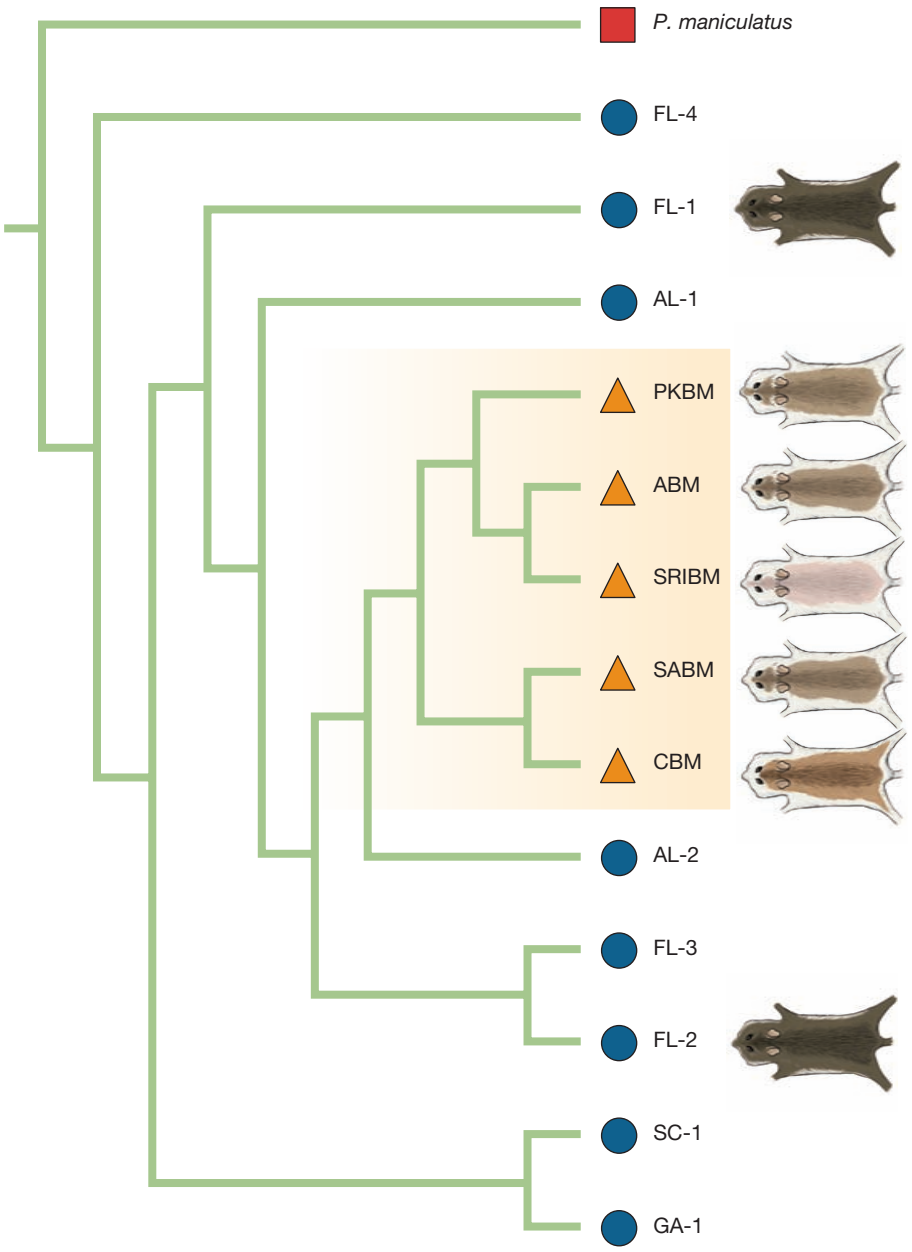



**FIGURE 3.8 Phylogeny of oldfield mouse populations.** Genomic data from the *Mc1R* locus, as well as from other areas in the genome of oldfield mice, allows researchers to reconstruct colonization events for beach mice. All beach populations form a single clade (shaded in orange) and share a derived "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.

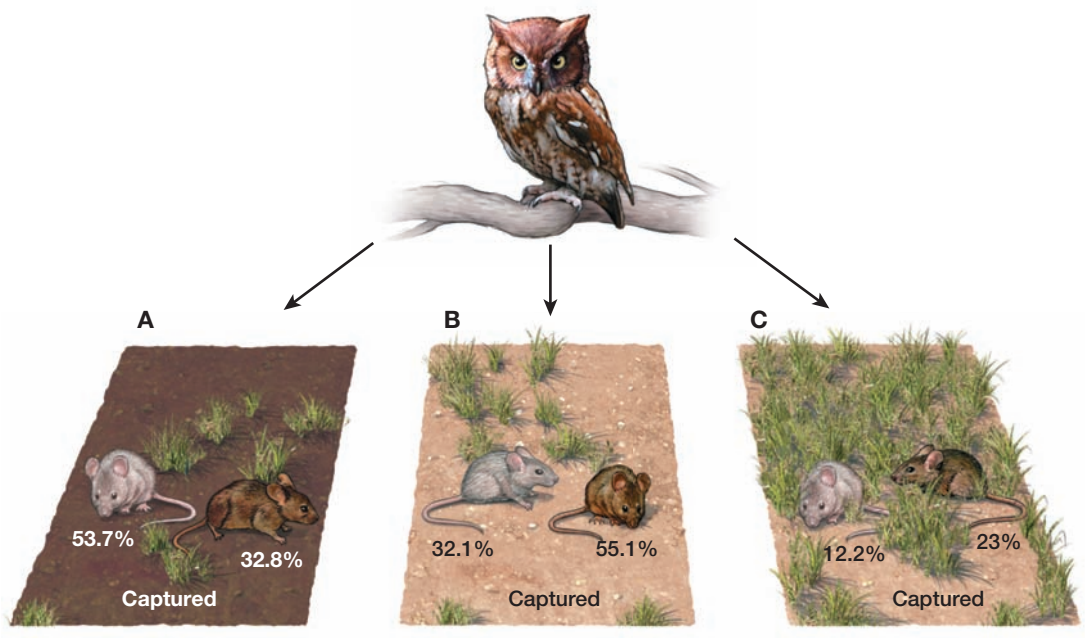
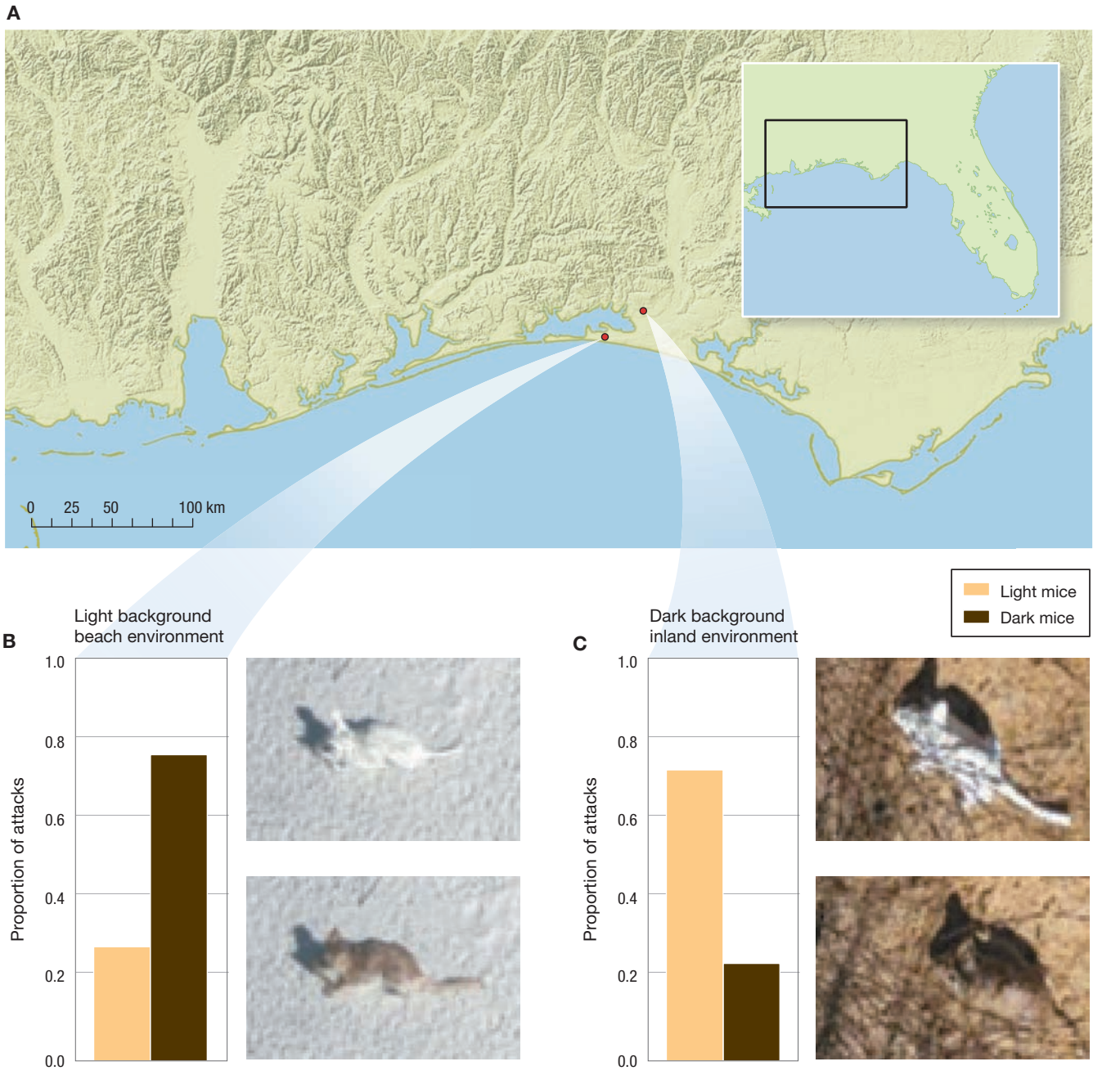



**FIGURE 3.9 Early work on predation, coat color, and fitness in the oldfield mouse.** Mice with light and dark coats were exposed to owl predators in three different environments: dark background with sparse vegetation **(A)**, light background with sparse vegetation **(B)**, and light background with 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

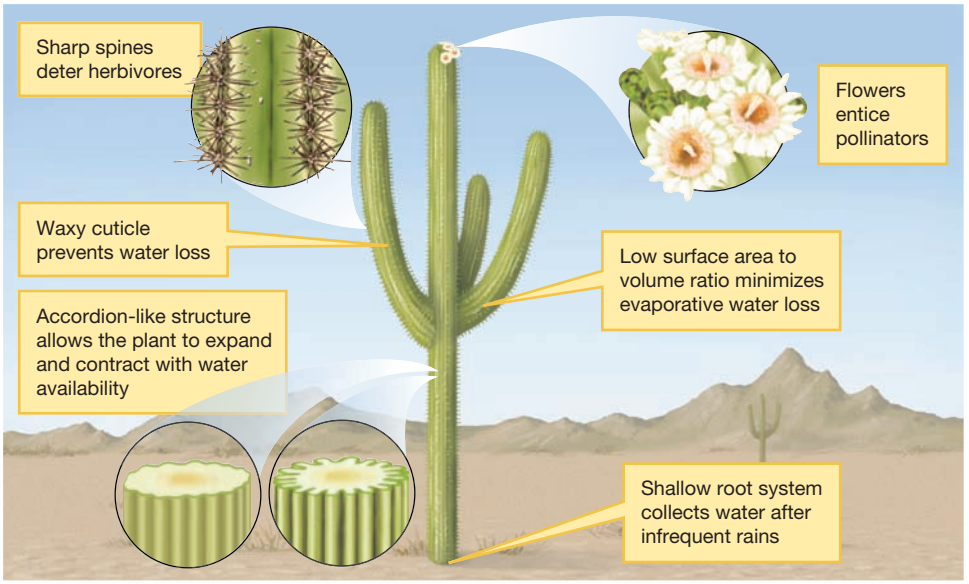



**FIGURE 3.11 Adaptations of a cactus.** A saguaro cactus exhibits adaptations to its abiotic environment (waxy stem coating, shallow root system, low surface area to volume ratio) and to its biotic environment (spines to keep away herbivores, flowers to attract pollinators).

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

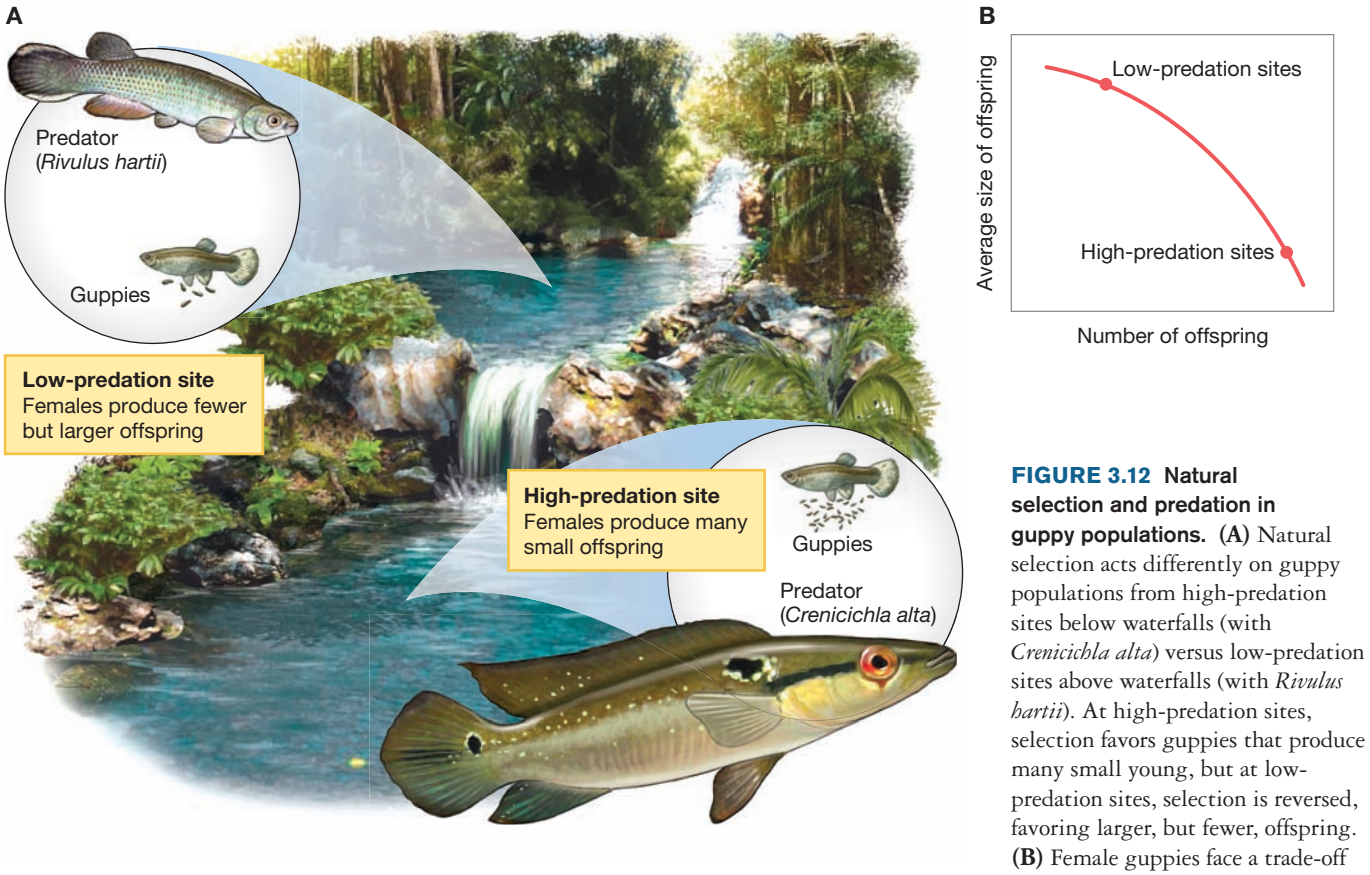



**FIGURE 3.12 Natural selection and predation in guppy populations.** **(A)** Natural selection acts differently on guppy populations from high-predation sites below waterfalls (with *Crenicichla alta*) versus low-predation sites above waterfalls (with *Rivulus hartii*). At high-predation sites, selection 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?

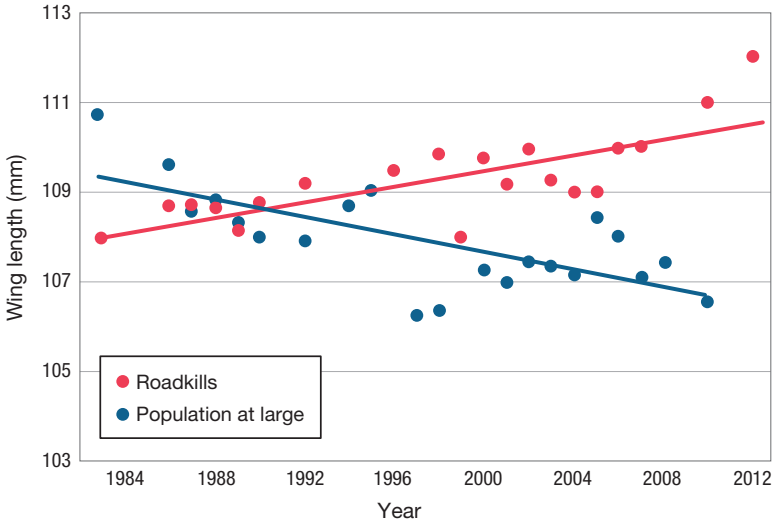



**FIGURE 3.15 Roadkill, natural selection, and wing length in swallows.** Over the course of three decades, the mean wing length of swallows that died via roadkill increased, while the mean wing length of swallows in colonies decreased. The increasing divergence between lines suggests that natural selection 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

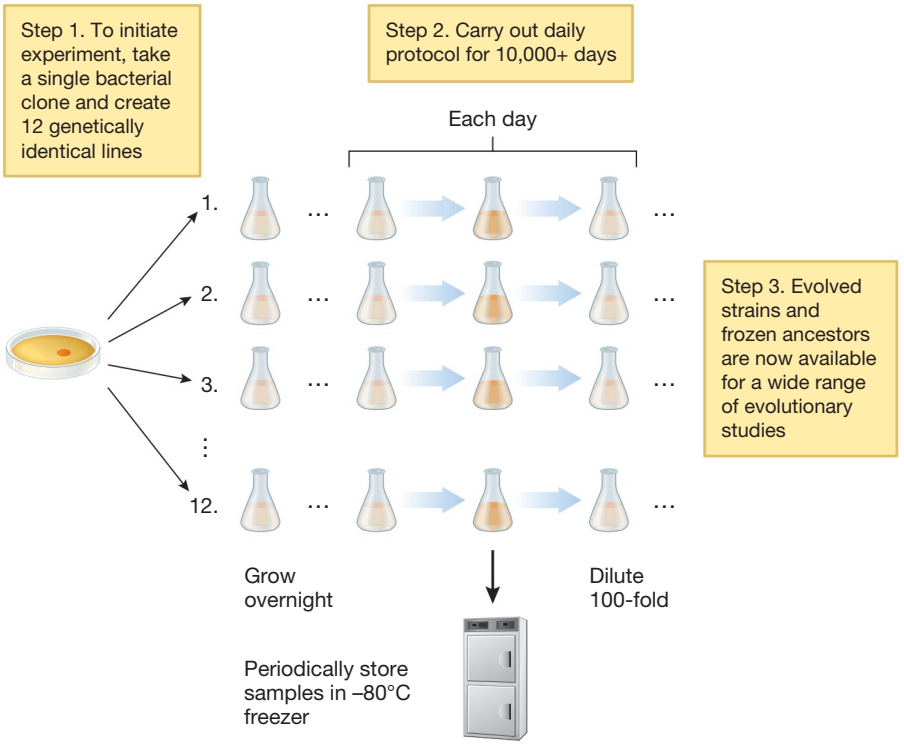



**FIGURE 3.16 Lenski's experimental evolution system.** The basic protocol for the Lenski *E. coli* experiment. Each day, Lenski and his team transferred cells from the 12 lines into fresh growth medium. These cells went through six to seven generations of replication overnight, and the next day the 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?

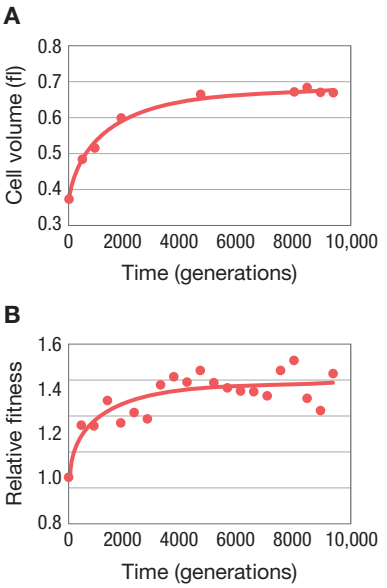



**FIGURE 3.17 Cell size and fitness in one *E. coli* line.** **(A)** Change in average cell volume (fl. = femtoliter) in one of Lenski's 12 long-term lines. **(B)** Change in fitness for the same line, relative to its ancestor. Fitness values greater than one indicate higher fitness than the ancestor.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

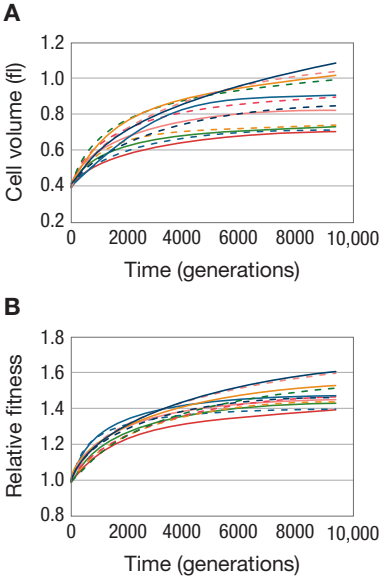



**FIGURE 3.18 Cell size and fitness in 12 *E. coli* lines.** Change in **(A)** cell volume (fl. = femtoliter) and **(B)** relative fitness in each of the 12 lines. Fitness values greater than one indicate higher fitness than the ancestor. From Lenski and Travisano (1994).

## 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%.

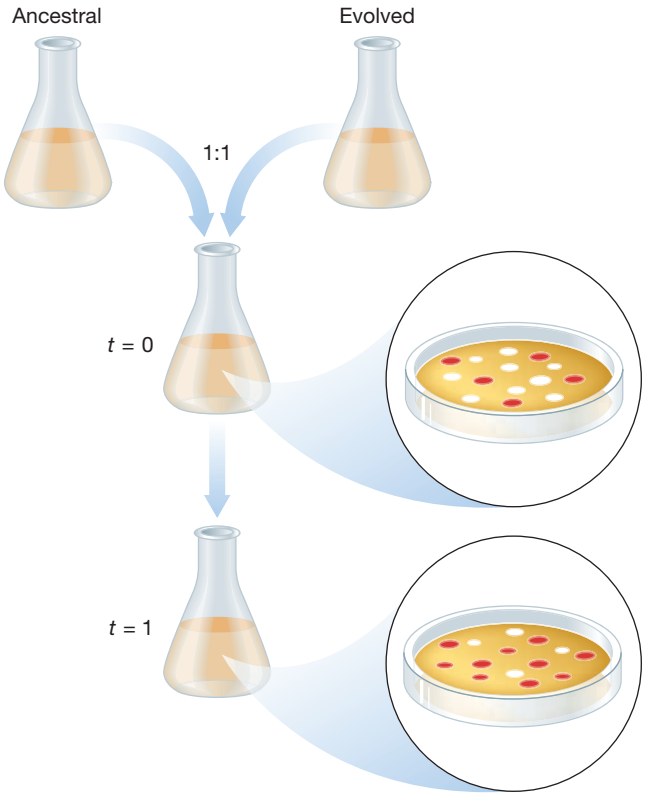



**FIGURE 3.19 Measuring bacterial genotype frequency and fitness in the laboratory.** Ancestral and descendant populations are competed against each other, and fitness is assayed using the neutral $Ara^+$ (white) and $Ara^-$ (red) markers to count colonies. Adapted from Elena and Lenski (2003).

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.

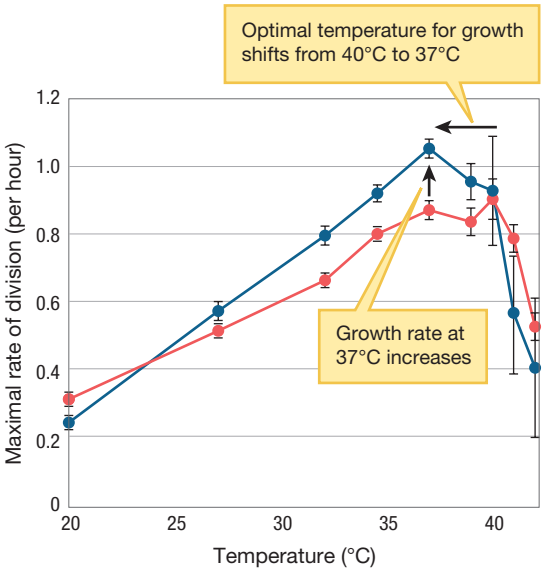



**FIGURE 3.20 Thermal adaptation in *E. coli*.** The red line represents ancestral population, and the blue line represents the population after 20,000 generations at 37°C. Adapted from Cooper et al. (2001).

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.*

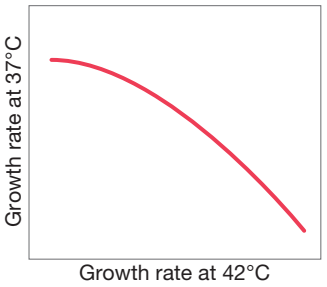



**FIGURE 3.21 Antagonistic pleiotropy.** The antagonistic pleiotropy hypothesis predicts a trade-off between two characteristics. Shown here is a hypothetical trade-off between growth rates at 37°C and 42°C.

**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.

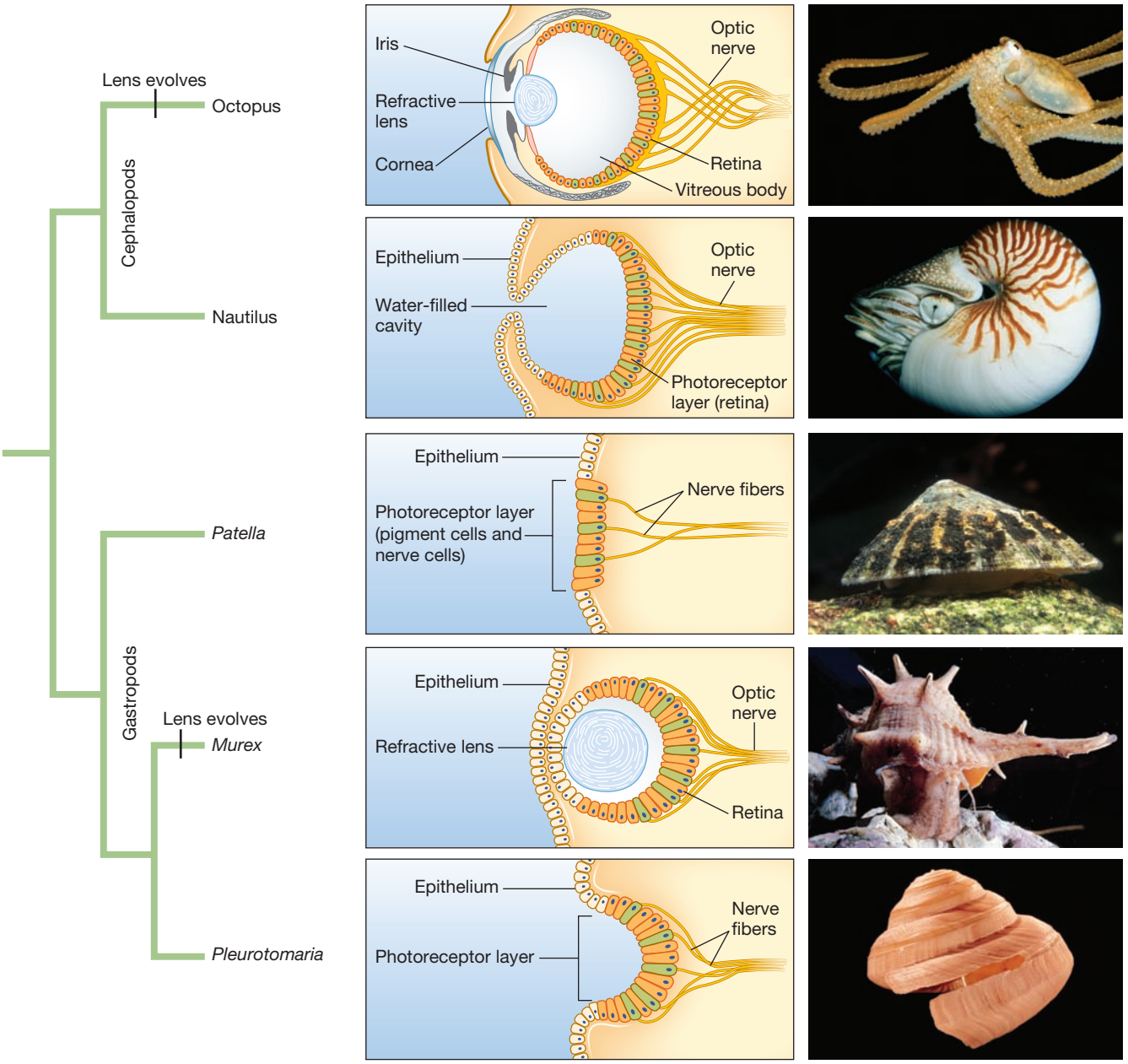



**FIGURE 3.22 The evolutionary history of the mollusk eye.** Taking a phylogenetic perspective on eye morphology in the mollusks, we see that complex eyes with a lens evolved independently in the cephalopods and in the gastropods (Oakley and Pankey 2008). From top to bottom: The octopus eye uses a lens 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

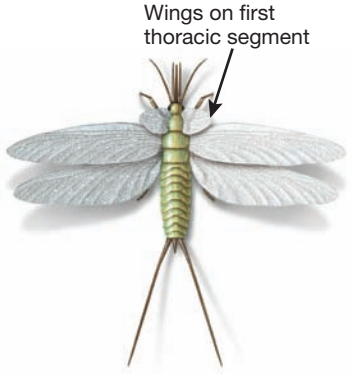



**FIGURE 3.24 Primitive wing development in an extinct insect.** In *Stenodyctya lobata*, an extinct species of insect, small wings developed on the first thoracic segment (arrow). Modern insects only have wings on the second and third segments of the thorax. From Moczek (2011).

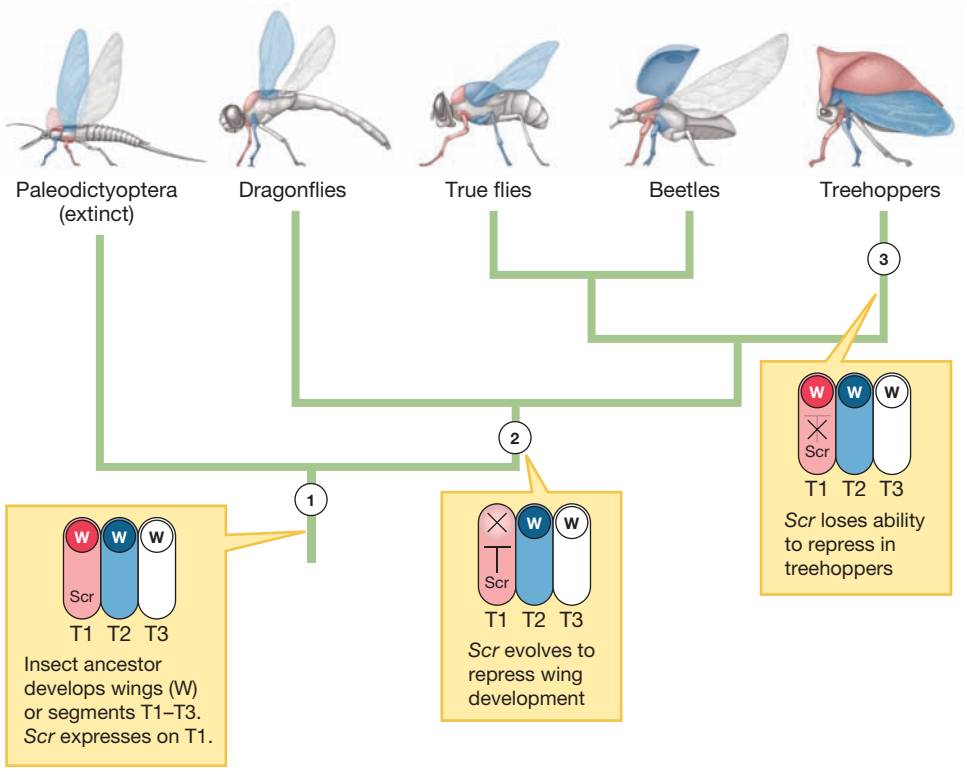



**FIGURE 3.25 Development of wings and helmets.** A phylogenetic view of *Scr* expression and its role in wing and helmet development on the insect thorax. T1 = thoracic section 1 (in pink), T2 = thoracic section 2 (in blue), T3 = thoracic section 3 (in white). Initially, *Scr* had no effect on wing 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**).

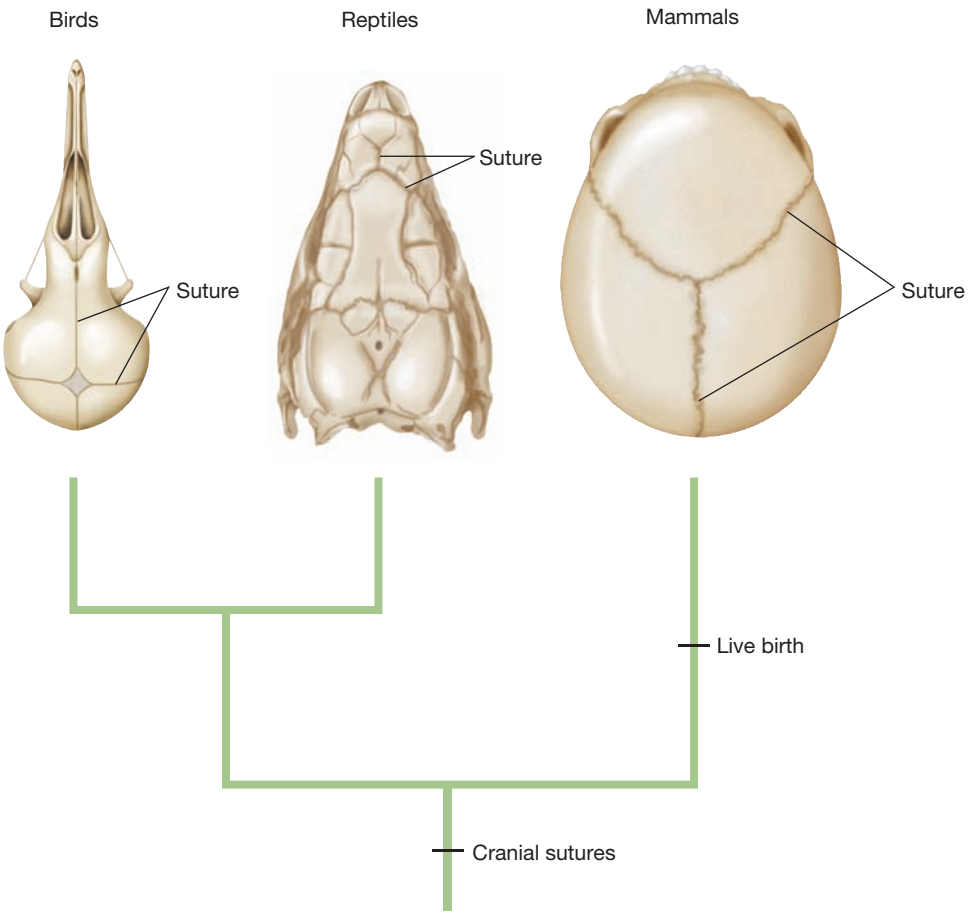



**FIGURE 3.26 Darwin realized that cranial sutures evolved before live birth.** Darwin used phylogenetic reasoning to conclude that skull sutures did not originally evolve to facilitate parturition. Because cranial sutures are present in birds, reptiles, and mammals alike, Darwin reasoned that they evolved 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.

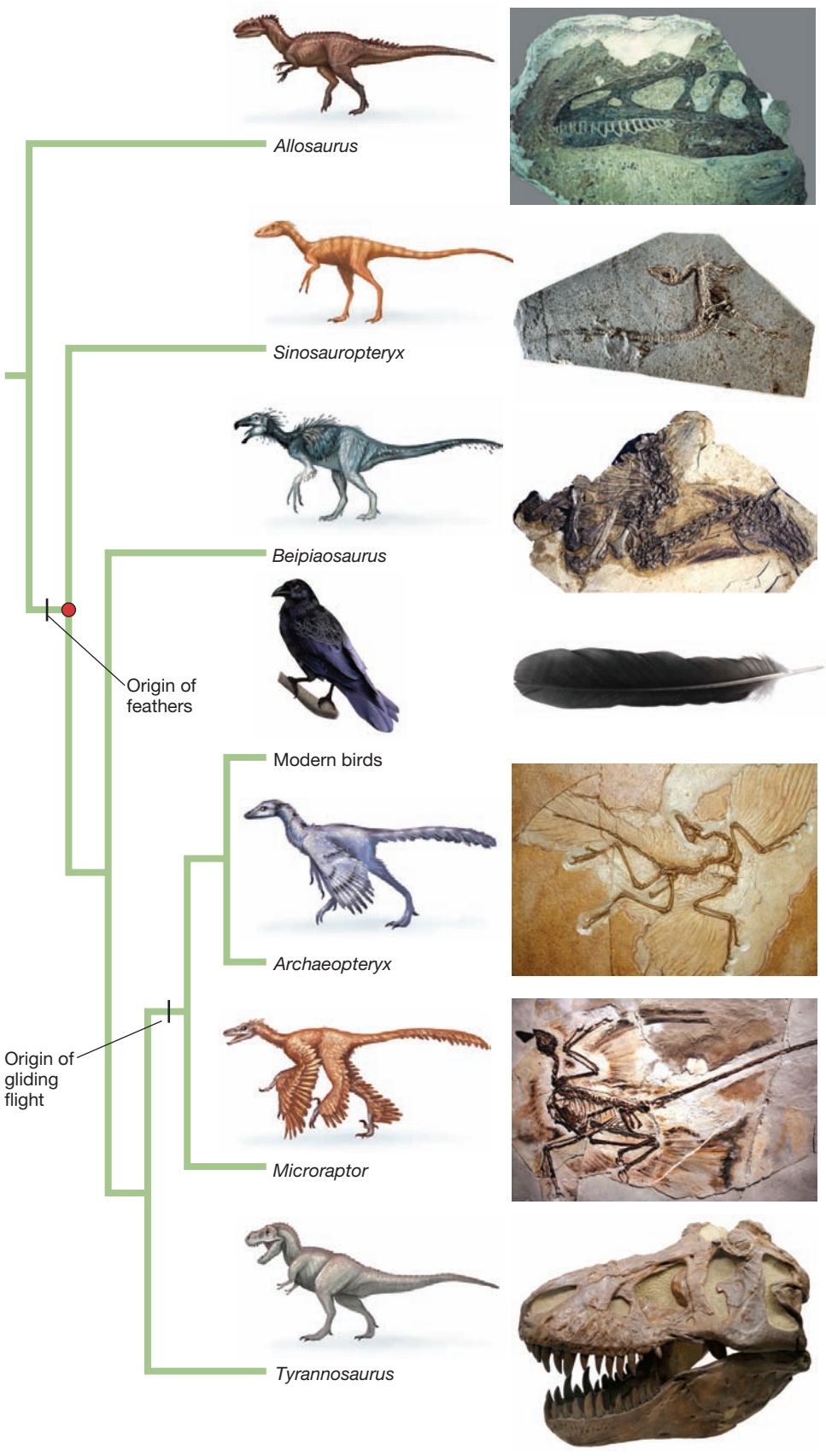



**FIGURE 3.27 The evolutionary origin of feathers.** Phylogenetic reasoning suggests that feathers did not originally evolve for flight. Feathers likely arose in a lineage of theropod dinosaurs. The common ancestor of these feathered dinosaurs (including birds) is marked with a solid red circle. This 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).

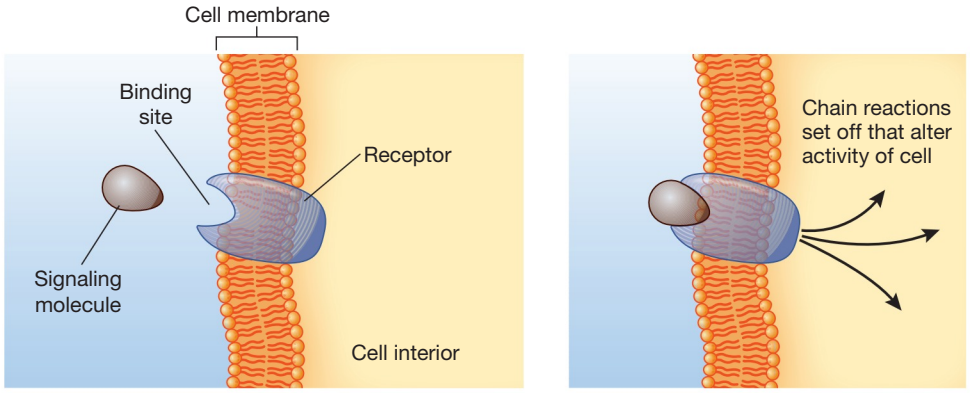



**FIGURE 3.28 Lock-and-key systems.** The lock-and-key mechanism of many hormone–receptor pairs.

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."

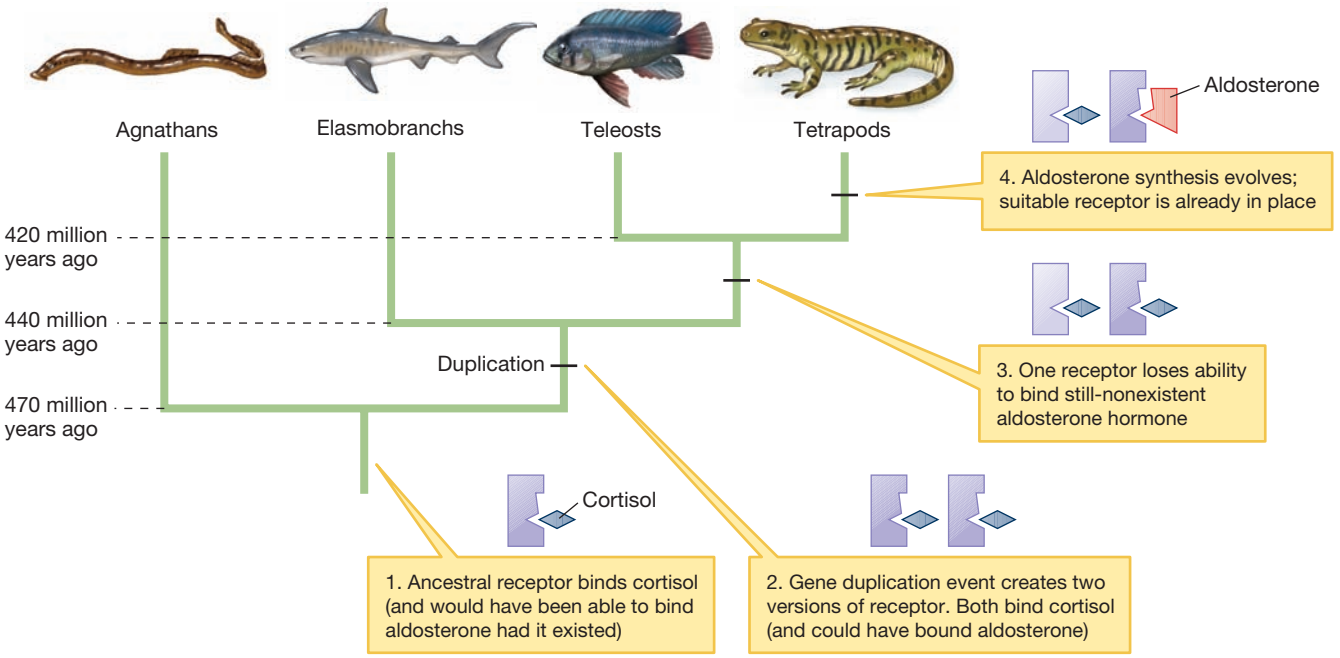



**FIGURE 3.29 Gene duplication and the evolution of the aldosterone receptor.** Neither the aldosterone hormone nor the aldosterone receptor were present in the vertebrate lineage 470 million years ago. (1) A single glucocorticoid receptor bound cortisol—and would have bound aldosterone, had it been 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

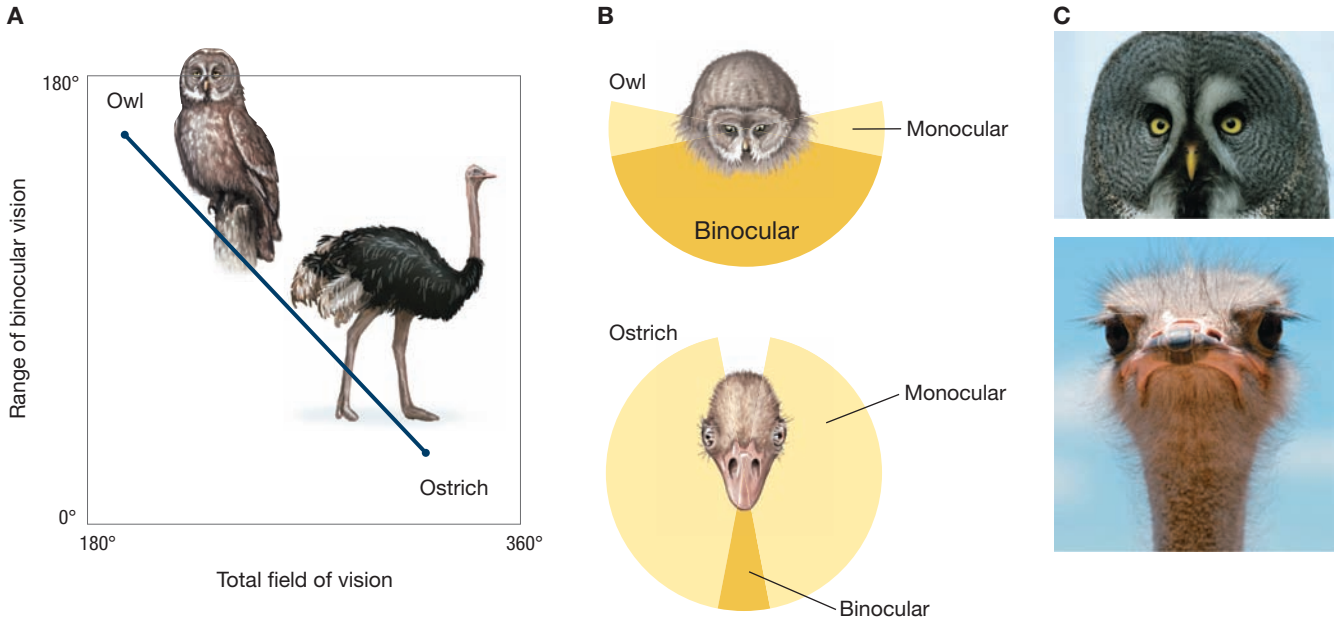



**FIGURE 3.30 Trade-offs in binocular vision.** **(A)** Birds face a trade-off between the total field of vision (*x* axis) and the range of binocular vision (*y* axis). Because of the different challenges they face, the ostrich and the owl have evolved to different points along this trade-off curve. **(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.

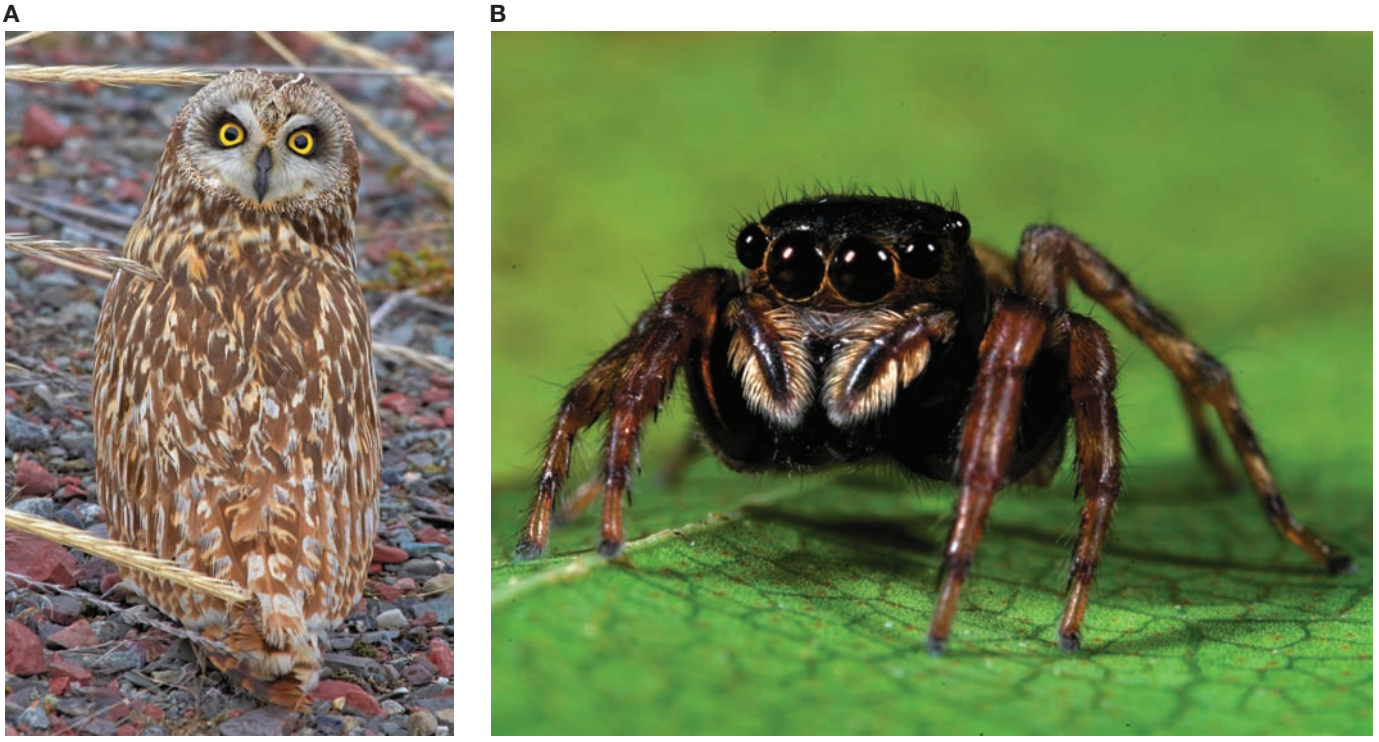



**FIGURE 3.31 Overcoming constraints.** **(A)** A partial solution to the limited field of view: Owls can turn their heads nearly 180° to look behind themselves, as shown by this short-eared owl. **(B)** A different solution: The jumping spider has eight eyes, allowing both stereoscopic forward vision 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$^2$.

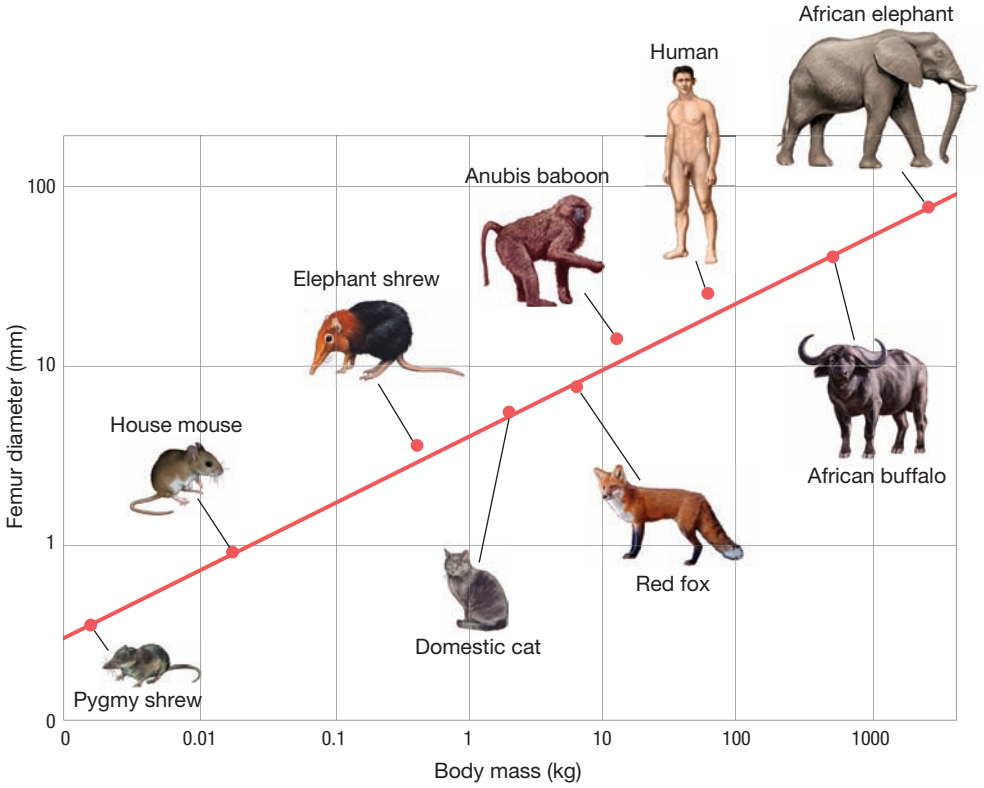



**FIGURE 3.33 Femur size and body mass.** Femur diameter exhibits a tight relationship with body mass for mammals ranging in size from the 3-gram pygmy shrew to the 5000-kilogram elephant. Both the *x* and *y* axes are plotted on a logarithmic scale. Adapted from Alexander et al. (1979).

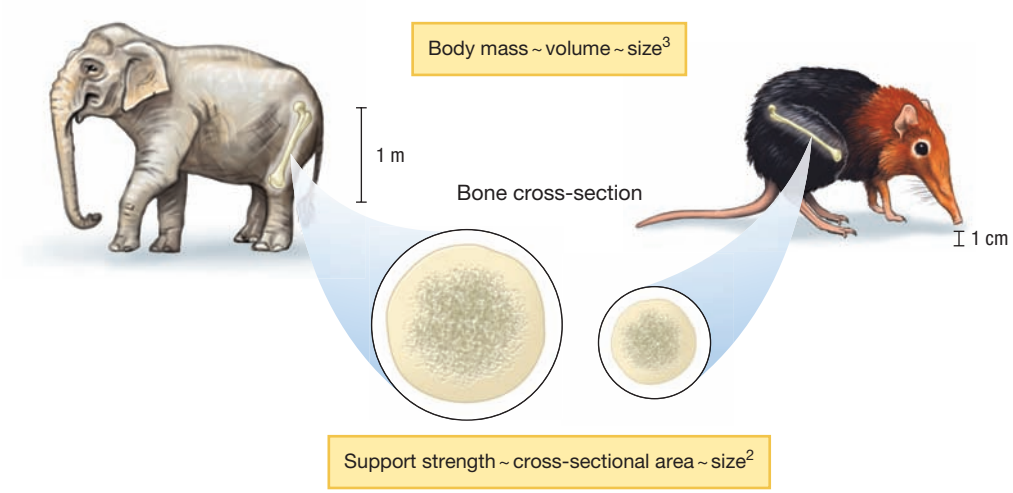



**FIGURE 3.34 Elephants require proportionally thicker legs.** Body mass scales with the third power of size, but support strength scales with the second power of size. As a result, larger animals such as elephants require proportionally thicker legs than small animals such as elephant shrews. This physical 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**).

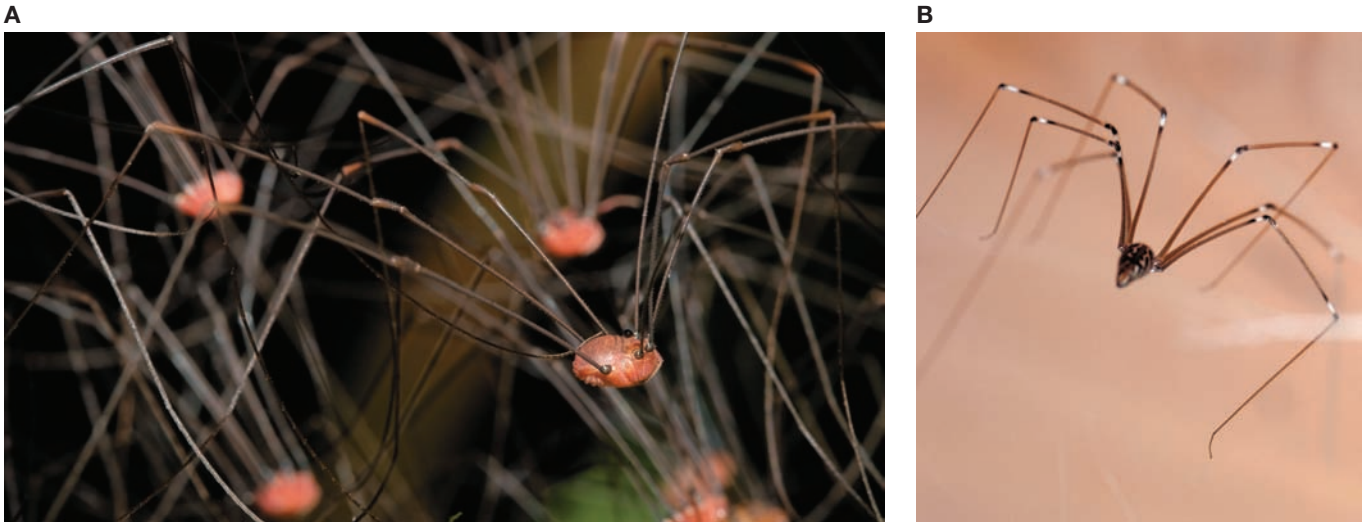



**FIGURE 3.35 Harvestman and cellar spider.** Arachnids show us that the relative dimensions of Dali's elephant—a large body on long, tiny legs—are not impossible in and of themselves. The problem is having these dimensions at the size of an elephant. **(A)** The harvestman (order Opiliones) is not a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

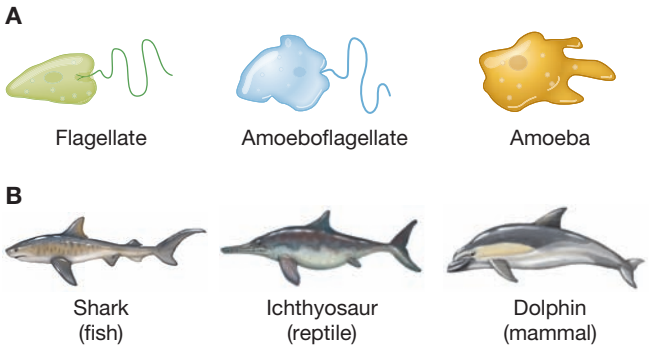
adaptation (p. 78)
antagonistic pleiotropy (p. 89)
coevolution (p. 104)
differential reproductive success (p. 67)
evolutionary arms race (p. 104)
exaptation (p. 79)
gene duplication (p. 97)
gene sharing (p. 97)
inheritance (p. 67)
life history strategy (p. 80)
marker gene (p. 85)
norm of reaction (p. 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?




## SUGGESTED READINGS

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