



# 19

## Development and Evolution

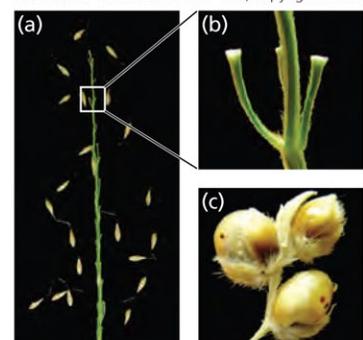
**A** key innovation in human history was the domestication of crops, particularly high-calorie cereals such as sorghum, rice, corn, and wheat. The wild relatives of these cereals share a trait called seed shattering. An abscission layer forms between the seed coat and the pedicel, or seed base, that lets mature seeds readily fall off the plant (right). Shattering facilitates seed dispersal, but it is problematic in domesticated crops because fallen seeds are hard to harvest. By gathering seeds that remained on the stalk, early farmers presumably selected for the non-shattering phenotypes now seen in all major cereal crops.

Zhongwei Lin and colleagues (2012) uncovered the genetic basis for the non-shattering phenotype in sorghum, the world's fifth most important cereal. Mutations in the *Shattering1* (*Sh1*) gene in domesticated sorghum are associated with failure to form an abscission layer during seed development. Mutations in *Sh1* are also associated with non-shattering phenotypes in domesticated corn and rice.

Charles Darwin would have been pleased, for Lin's study combines research in two areas that were of fundamental importance to his understanding of evolution and the evidence for it: artificial selection in domesticated plants and developmental biology. "Development," Darwin wrote (1872, p. 386), "is one of the most important subjects in the whole round of natural history."

Above, women harvesting sorghum in Burkina Faso. Below, due to differences in development, vigorous shaking scatters the seeds of wild sorghum (a and b), but leaves the seeds of domesticated sorghum firmly attached (c). From Lin et al. (2012).

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How are developmental biology and evolution related? Developmental biology is the study of the processes by which an organism grows from zygote to reproductive adult. Evolutionary biology is the study of changes in populations across generations. As with non-shattering cereals, evolutionary changes in form and function are rooted in corresponding changes in development. While evolutionary biologists are concerned with why such changes occur, developmental biology tells us how these changes happen. Darwin recognized that for a complete understanding of evolution, one needs to take account of both the “why” and the “how,” and hence, of the “important subject” of developmental biology.

In Darwin’s day, studies of development went hand in hand with evolution, as when Alexander Kowalevsky (1866) first described the larval stage of the sea squirt as having clear chordate affinities, something that is far less clear when examining their adults. Darwin himself (1851a,b; 1854a,b) undertook extensive studies of barnacles, inspired in part by Burmeister’s description (1834) of their larval and metamorphic stages as allying them with the arthropods rather than the mollusks. If the intimate connection between development and evolution was so clear to Darwin and others 150 years ago, why is evolutionary developmental biology (or *evo-devo*) even considered a separate subject, and not completely integrated into the study of evolution? The answer seems to be historical. Although Darwin recognized the importance of development in understanding evolution, development was largely ignored by the architects of the 20th-century codification of evolutionary biology known as the modern evolutionary synthesis.

In Section 19.1 of this chapter, we consider why development was thus ignored. In Section 19.2 we look at how the two fields of study came back together. The third and fourth sections cover recent trends in *evo-devo*. Finally, in the last section, we look ahead to the future of evolutionary developmental biology.

## 19.1 The Divorce and Reconciliation of Development and Evolution

Unaware of the work of his contemporary, Gregor Mendel, Darwin developed his evolutionary theory without a clear understanding of inheritance. In the early 20th century, after both Darwin and Mendel had died, Mendel’s work was re-discovered by geneticists. The modern evolutionary synthesis thus arose from the unification of Darwinian natural selection with Mendelian genetics. This unification allowed Ronald A. Fisher, J. B. S. Haldane, Sewell Wright, and others to lay the foundations of population genetics (see Mayr 1982). The term *modern synthesis* was coined by Julian Huxley to denote the compilation during the late 1930s and early 1940s, in the context of the new evolutionary genetic framework, of advances in the understanding of variation in natural populations, paleontology, and speciation. Notably missing from the synthesis was developmental biology.

### The Divorce

According to Ernst Mayr (1982), another key figure in the modern synthesis, the reasons for development’s omission from the modern synthesis were practical. Because the genetic and molecular mechanisms underlying development were so poorly understood at the time, a direct connection to evolutionary genetics could not be drawn. The belief was that development and genetics needed to be “properly separated” in order to make progress in both fields (Mayr 1982, p. 893).

A second explanation for the separation was the rejection of many Darwinian tenets by prominent developmental biologists and morphologists of the late 19th and early 20th centuries, sometimes characterized as *saltationists* and *structuralists*. For example, Darwin and the architects of the synthesis held that most evolutionary change was gradual, via almost imperceptible steps from one generation to the next. This conception followed one of Darwin's favorite adages, derived from Linnaeus (1751) and repeated often in *On the Origin of Species*: Nature does not make leaps. By contrast, "saltationists" believed that major evolutionary changes, such as the origin of new species, were the result of mutations of large effect.

Another fundamental Darwinian idea was that natural selection is the primary mechanism of change; mutation and variation merely provide the raw material for natural selection to act. Variations, in this view, are ever present, small in scale, and unbiased toward certain adaptations. "Structuralists," by contrast, proposed that physical and mathematical principles directed growth and form along defined pathways that could account for most of life's diversity (Thompson 1917).

Less well recognized was the comparative zoologist Libbie Hyman, whose comprehensive studies on the invertebrates during this period fluidly combined adult and developmental features into an encyclopedic understanding of adaptations and relationships (i.e., Hyman 1940). Nevertheless, most developmental and evolutionary biologists pursued their disciplines separately; it was only the advent of molecular biology in the late 20th century that began to heal the rift.

## The Reconciliation

In the 1940s and 50s, the identification of DNA as the genetic material finally revealed the machinery of variation. The subsequent cracking of the genetic code tied the information content of DNA to the amino acid content of proteins.

Using the correspondence of DNA to proteins, Marie Claire King and Allan Wilson (1975) revisited a question controversial since Darwin's day: How closely related are humans and chimpanzees (Figure 19.1)? Comparing amino acid sequences, they estimated the genetic similarity at 99%. Charles Sibley and Jon Ahlquist (1987) used a different method to reach the same conclusion. More recently, our close kinship to chimps has been confirmed by genome sequencing.

A second set of findings from molecular biology related to how genes are turned on and off. Francois Jacob and Jacques Monod (1961) elucidated the mechanism by which certain proteins regulate gene activity in bacteria. In 1963, Ed Lewis explicitly drew the connection from Jacob and Monod's findings to developmental biology, describing how **gene regulation** could orchestrate developmental processes in insects and thus explain for the first time how a single genome could produce a diversity of cells throughout a multicellular body.

The time was ripe for a reconciliation between evolution and development. In 1971, Roy Britten and Eric Davidson interpreted the new discoveries of gene regulation in an evolutionary and developmental context (p. 129): "It is clear ... that alterations in the [regulatory] genes ... could cause enormous changes in the developmental process and that this would be a potent source of evolutionary change." This proposal challenged the notion of gradualistic evolution at the heart of the modern synthesis by suggesting that relatively modest changes at the DNA level in regulatory genes could have profound impacts on development, and hence evolution. Given that human and chimp DNA is 99% similar, was it possible that relatively modest genetic changes in regulatory genes could lead to profound evolutionary changes after all?

Due to inadequate understanding of the mechanisms of development and to disagreements over theory, developmental biology was left out of the modern evolutionary synthesis.



**Figure 19.1 Humans and chimpanzees are 99% similar genetically** Debbie Cox has devoted her career to chimpanzee conservation. Despite being close relatives, humans and chimps differ in important ways, both in appearance and behavior. What is the nature of our 1% genetic difference, and how can it account for our many different physical and behavioral features? Evo-devo approaches will assist in our ability to one day answer these questions.

Stephen Jay Gould (1977) drew on the discoveries of gene regulation to suggest that much evolutionary change could be attributed to alterations in the relative timing of developmental events (known as **heterochrony**), another example of modest changes in gene regulation leading to dramatic morphological change.

Previously, David Wake (1966) had discussed how evolutionary patterns in salamanders could be explained by their developmental trajectories. His student Pere Alberch postulated developmental rules that, along with selection, determine the direction of evolution. Alberch (1981) proposed that the evolution of large size in tree-dwelling salamanders—compare **Figure 19.2a versus b**—depended on a developmental modification in the relative length and shape of the distal finger and toe bones in smaller ancestral species (Figure 19.2c). This developmental change, and the associated origin of webbing between the digits, allowed for increased suction efficiency, which let larger salamanders climb trees without falling. A modest change in the rules of development—the relative growth of fingers and toes versus other body parts—could facilitate a macroevolutionary change. Alberch was offering a second-generation structuralist view of evolution in the context of a modern understanding of developmental mechanisms.

These works, while groundbreaking, initially did not have a strong impact either on developmental biology, which remained focused on a few well-studied organisms like fruit flies, roundworms, thale cress, chickens, and house mice, or on evolutionary biology, where the modern synthesis was still the dominant paradigm. Both fields continued to expand in scope throughout the 20th century, but they had distinct journals, different jargon, and limited integration.

And then came a discovery that suddenly had developmental biologists not only discussing evolution, but proposing bold hypotheses regarding major evolutionary issues such as the origin of animal phyla. Species from widely divergent branches on the animal phylogeny, fruit flies and house mice, appeared to build their segmented bodies in similar ways. The field of modern evo-devo was born.

## 19.2 Hox Genes and the Birth of Evo-Devo

As the tools of developmental biology became more sophisticated in the last decades of the 20th century, Britten and Davidson's hypothesis—that alterations in regulatory genes could cause enormous changes in development—found increasing support. It had long been known that mutations in embryonically active genes in the fruit fly *Drosophila melanogaster* could dramatically alter the formation of larval and adult structures. The most famous class of these mutations was discovered in the late 19th century by William Bateson (1894), often remembered for his saltationist views and resistance to the modern synthesis. In 1915, Calvin Bridges uncovered the genetic basis for one of Bateson's phenotypes. To Bateson, and later Ed Lewis (1978), “homeotic mutations” showed that evolution could make leaps. As we will see, they were partially correct but for the wrong reasons.

When **homeotic genes** are mutated, appendages appear in the wrong places. A mutant *bithorax* gene yields flies with four wings instead of the normal two, and a mutant *Antennapedia* gene yields flies with legs in place of antennae (**Figure 19.3, next page**). Lewis and others confirmed that homeotic genes were clustered in two locations in the genome: the *bithorax* complex (BX-C) and the *Antennapedia* complex (ANT-C). However, in the absence of DNA sequences and functional molecular studies, the nature of these clustered genes remained a mystery.

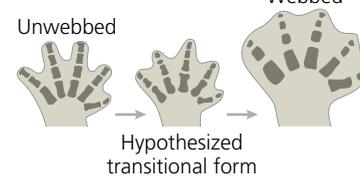
(a) Giant palm salamander  
(*Bolitoglossa dofleini*)



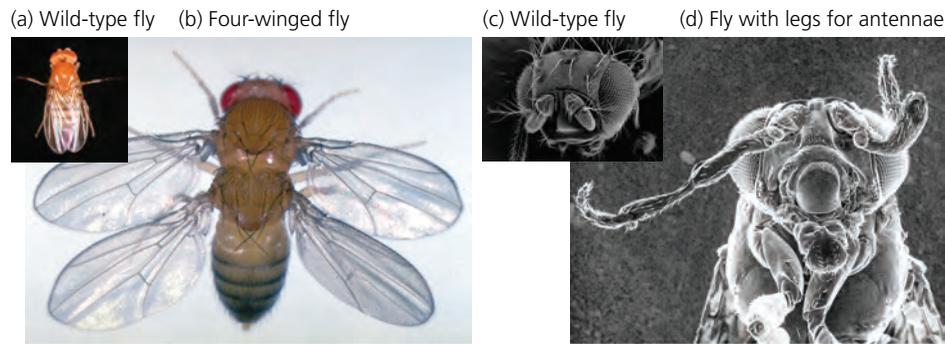
(b) Atoyac salamander  
(*Bolitoglossa oaxacensis*)



(c) Hypothesized evolutionary scenario



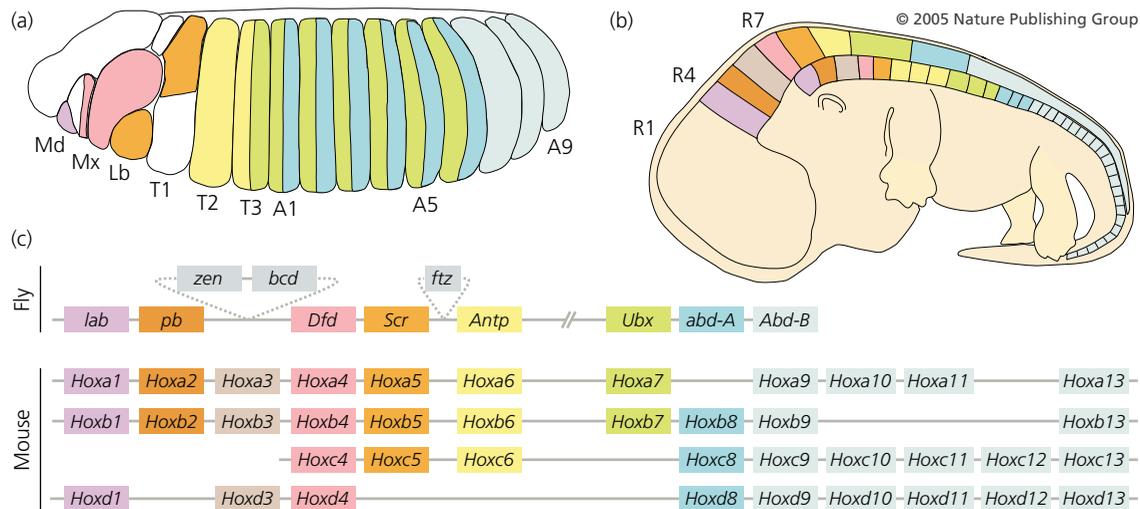
**Figure 19.2 Insights on salamander evolution** The arboreal giant palm salamander (a), the largest of the *Bolitoglossa* salamanders (~12 cm snout-vent length) has more extensive webbing between the fingers and toes than the Atoyac salamander (b), a small-bodied ground form (~5 cm snout-vent length) exemplifying the presumed ancestral condition. (c) A hypothesized transitional species had small body size and webbing. *B. dofleini* juveniles have hands and feet that resemble the hypothesized intermediate. (b) by David Wake; (c) after Alberch (1981).



**Figure 19.3 Homeotic mutants in *Drosophila*** (a) Normal two-winged fly with wings on the second thoracic segment (T2); the third segment (T3) has balancer organs. (b) Four-winged *Ultrabithorax* (*Ubx*) mutant with identity of T3 transformed into T2. (c) A normal fly with small antennae. (d) *Antennapedia* (*Antp*) mutant with legs instead of antennae.

In 1983 that all changed. Scientists in Switzerland and the United States realized that the products of genes from both ANT-C and BX-C had a common stretch of amino acids, suggesting that they were evolutionarily related (McGinnis et al. 1984; Scott and Weiner 1984). Soon thereafter, the Switzerland group found multiple copies of this same amino acid sequence, the **homeodomain**, in a beetle, earthworm, frog, chicken, mouse, and human. Functional studies showed that the homeodomain directly interacted with DNA. The homeodomain proteins were identified as **transcription factors**—they regulate the transcription of other genes. Here were genuine developmental regulatory genes—the “Hox” genes—that were conserved in sequence across distantly related animals.

This finding surprised biologists. Most had assumed, based on the gradualism of the modern synthesis, that “the search for homologous genes is... futile except in... close relatives” (Mayr, 1963, p. 609). A bigger shock came when the mouse and fly Hox genes were found not only to be clustered on the chromosomes of both animals and expressed along the anterior–posterior body axis of both animals, but also expressed in spatial patterns that mirror the arrangement of the clustered genes on the chromosome (Gaunt 1988). As shown in **Figure 19.4a**, the



**Figure 19.4 Hox genes in flies and mice** (a) Fruit fly (*D. melanogaster*) and (b) mouse (*M. musculus*) embryos showing Hox gene expression on the anterior–posterior axis. (c) Hox gene arrangements. Flies have a split cluster—the Antennapedia complex (left of split) and the bithorax Complex (right) are separated by 9 million nucleotides. Mammals have four clusters, though to derive from two whole-cluster duplications followed by gene losses. The embryonic expression domains are less discrete than shown. Colors suggest orthology of fly and mouse genes for which strong evidence is, in some cases, lacking. Modified from Pearson et al. (2005).

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fruit fly *Deformed* (*Dfd*) gene is near one end of the cluster and is expressed in the developing head of the embryo, whereas the next gene, *Sex combs reduced* (*Scr*), is expressed just posterior to *Dfd*, and so on. This correspondence between genomic order of Hox gene loci and their spatial locations of expression along the body axis is known as **spatial colinearity**.

Subsequent genetic studies showed that mutations in mouse Hox genes (see Figure 19.4b) also cause homeotic transformations of body parts, for example by adding extra neck vertebrae (Kessel et al. 1990). It looked as though a Rosetta stone of animal development and evolution had been found, showing a common developmental mechanism underlying the divergent bodies of flies and mice.

In the wake of these discoveries, students of development in the 1990s saw numerous false-color images of mouse and fly embryos like those in Figure 19.4. The similarities were striking, but was the evidence sufficient to suggest a specific evolutionary path? Are the anterior Hox genes of flies and mice really direct descendants of a single gene in their last common ancestor? What would that imply about the ancestor, which lived over 530 million years ago? Can we infer what it looked like, where its Hox genes were expressed, and what functions they had?

Flies and mosquitoes have essentially the same number of Hox genes. The *Deformed* (*Dfd*) gene occurs in the same position in the mosquito Hox cluster as in the fly. We can infer that the last common ancestor of flies and mosquitoes likely had one *Dfd* gene that was inherited faithfully by both species. Genes in different species derived from a common ancestor's gene are said to be **orthologous**.

In vertebrates there are four Hox clusters—Hoxa, Hoxb, Hoxc, and Hoxd—that contain most of the same genes in the same order. We can infer that *Hoxa4*, *Hoxb4*, *Hoxc4*, and *Hoxd4* arose from gene duplications in an ancestral vertebrate. Genes within a species that arise via duplication are said to be **paralogous**.

Can we also infer that the *Hox4* genes in vertebrates and the *Dfd* genes in insects are orthologous? Orthology determinations are difficult in Hox genes. They are based on just a few dozen shared amino acid sequences (e.g., Monteiro and Ferrier 2006), leaving us low statistical confidence in evolutionary relationships. Despite the color coding in illustrations like Figure 19.4, we do not really know in many cases whether specific fly and mouse Hox genes are true orthologs. We may never know, although data on a wider variety of animals could help.

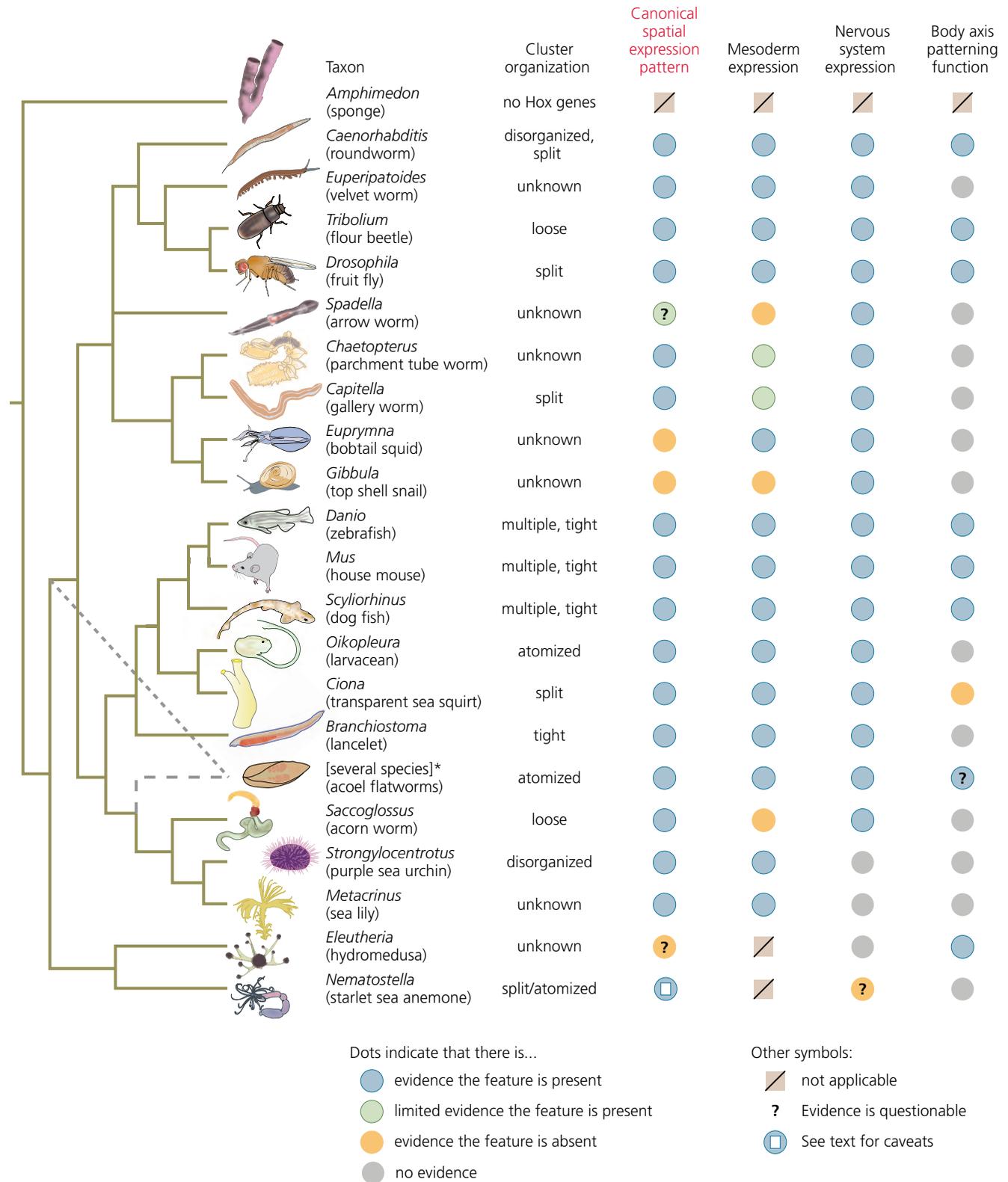
The Hox genes were not the only genes found in both flies and mice. Other classes of genes were involved in the development of mouse and fly eyes and mouse and fly hearts. Previously, these organs in mice and flies had been thought to have evolved independently, due to their anatomical differences and differing embryonic origins. However, the discovery of similar developmental mechanisms forced reexamination of such ideas. In 1993, Slack and colleagues proposed that all animals would show Hox gene spatial colinearity, and that this would be the defining feature of animals. It seemed that developmental biology was poised to radically alter views on how animals and other complex creatures evolved.

### Hox Paradox: The More Things Changed, the More They Stayed the Same?

There is a nagging problem with this view of conservation. If evolution is about change, does it not appear that the Hox gene story in Figure 19.4 implies sameness? From such conservation, how can we comprehend the diversity of life?

One resolution to the paradox comes when we look at the details more closely (Figure 19.5). Since the initial Hox discoveries, the gene expression patterns,

The discovery that homologous transcription factors influence fundamental aspects of development in insects and vertebrates sparked renewed interest among developmental biologists in evolution, and among evolutionary biologists in development.



**Figure 19.5 Diversity in Hox gene expression and function** The phylogeny shown here is a consensus from the recent literature. The affinities of acoel flatworms are disputed; we show two alternative placements (dashed lines). \*The information on acoel flatworm Hox genes is assembled from data on several species.

functions, and genomic arrangements of Hox genes have been elucidated in many more phyla. Figure 19.5 shows a conservative arrangement of the still-debated animal phylogeny, with representative groups that have well-studied Hox genes and/or occupy key phylogenetic positions, and is designed to highlight diversity.

The most striking feature of the mouse–fly comparison (Figure 19.4) was the parallel between Hox gene expression along the anterior–posterior body axis of and the order of the genes on the chromosome—the spatial colinearity. What is the connection between chromosomal position and spatial gene expression?

Let us hypothesize that chromosomal order is somehow the mechanistic key to spatial colinearity of Hox gene expression. How would we test this conjecture? The tools of modern evo–devo offer us two main approaches:

1. Manipulate the genomes of well-studied, amenable organisms;
2. Look for natural experiments by making comparisons between organisms that may show variation for the developmental phenomenon in question.

In the case of spatial colinearity, approach 1 can involve generating chromosomal rearrangements in Hox gene clusters to see if the body segments along the anterior–posterior axis are scrambled or maintained. Such experiments have been carried out extensively in vertebrates, roundworms, and insects, and proper development along the anterior–posterior axis tends to be faithfully maintained despite disruptions in the chromosomal order of genes (Ferrier 2007, 2011).

Approach 2 involves identifying species that show variation in Hox gene clustering compared to the tightly clustered, ordered Hox genes in vertebrates. How widespread is spatial colinearity among animals as a whole? Recall that the two fruit fly Hox complexes, ANT-C and BX-C, are in separate locations in the genome. In fact, the *Cluster organization* column in Figure 19.5 shows that tight clustering of Hox genes seems rare outside the vertebrates and lancelets. More common is a loose clustering, in which the Hox genes are gathered on a single part of one chromosome, but with non-Hox genes interspersed, as in many insects and an acorn worm. Split clusters, with different Hox genes widely dispersed in the genome into two or more sub-clusters, are found in a sea squirt, a gallery (segmented) worm, a nematode, and fruit flies; different fruit fly species have different split points. The purple sea urchin has a disorganized cluster, where more anterior Hox genes apparently have changed positions in the cluster with more posterior ones. And a planktonic larvacean and an acoel flatworm have atomized clusters, where the Hox genes are not linked at all. Nevertheless, in almost all cases, the spatial order of gene expression along the body axis is similar to that of the mouse. This pattern of Hox gene expression is sometimes called the **canonical spatial expression pattern** (see first column of symbols in Figure 19.5).

These comparative data are consistent with a scenario in which ancestral Hox genes were clustered, and the ordered clustering has been lost multiple times. An alternative scenario, given the lack of a single Hox cluster in sponges and anemones, is that different Hox genes arose early in animal evolution in dispersed genomic locations and clustering was a later event that linked these genes (Duboule 2007). More comparative data are needed to decide between these alternatives.

Timothy Dubuc and colleagues (2012) published data on Hox genes for a relative of sea anemones, the coral *Acropora digitifera*. In *A. digitifera*, several Hox genes are tightly clustered together in one location of the genome, a finding that lends support to the ancestral cluster hypothesis. However, the coral and anemone Hox genes are particularly difficult to assign to specific orthology groups, so

Among the mysteries to arise from early studies of Hox genes was spatial colinearity—a parallel between the order of Hox genes on the chromosome and their expression along the anterior–posterior body axis.

Experiments and comparative studies suggest that spatial colinearity may have been ancestral, but is not essential to proper Hox gene function. However, the spatial pattern of Hox gene expression is highly conserved.

it is not yet possible to distinguish between a single ancestral cluster and tandem gene duplications in different lineages, yielding independent mini-clusters.

Despite all this diversity in the degree of clustering, almost all species examined show evidence of the canonical spatial expression pattern of their Hox genes. Thus, a paradoxical conclusion from Figure 19.5 is that a vertebrate-like spatial expression of Hox genes does not actually depend upon the order of the Hox genes along chromosomes. But what about Hox gene function? It is important to note that a gene expression pattern (i.e., the production of messenger RNA) is not always directly indicative of expression of the protein, which actually performs the cellular function of regulating transcription of other genes. As a result, we cannot confidently ascribe a developmental function to a Hox gene simply based upon when and where the gene is transcribed. In fact, even confirming the presence of the protein does not, in itself, confirm a predicted function there.

As such, additional studies are required to establish gene function. Functional studies could involve expressing a protein at inappropriate times or locations in the embryo or interfering with normal function through mutations or other manipulations. Such functional studies in insects, nematodes, and vertebrates typically confirm that Hox gene expression patterns correspond to regions of Hox protein function; nevertheless, there is one striking counterexample.

Sea squirts are invertebrate relatives of the vertebrates. They have a nonfeeding, tadpole stage with a clear anterior–posterior axis, a brain, and a notochord (Figure 19.6a). During a dramatic metamorphosis, the tail and notochord and many other tadpole organs are resorbed, and the adult emerges as a sessile filter feeder that superficially resembles a sponge more than a vertebrate (Figure 19.6b).

The sea squirt *Ciona intestinalis* has a split Hox cluster in which the canonical spatial expression pattern is more or less maintained. Thus the prediction was that sea squirt Hox genes would function as in other animals: Disruptions in Hox gene function would cause defects along the main body axis. Ikuta and colleagues (2010) tested this prediction in *C. intestinalis* using a technique called RNA interference (RNAi), where an injected double-stranded RNA molecule, targeted at a specific messenger RNA (mRNA), results in the failure of that mRNA to be translated into protein. Ikuta and colleagues blocked production of Hox proteins in *C. intestinalis* embryos, but saw only minor phenotypic changes and none of the major impacts expected of Hox genes. Controls showed that the RNAi technique reduced protein levels. Therefore, as far as the scientists could tell, Hox genes have no body-axis function in sea squirt embryonic development, despite being expressed in the canonical spatial pattern. Is it possible that the canonical spatial pattern of Hox gene expression is what has been conserved across animals, while the functions are—like the genomic positions—more evolutionarily labile?

Another clue comes from the echinoderms, a phylum of animals with odd, five-parted (“pentamer”) body structures that includes sea stars, sea urchins, sea cucumbers, and sea lilies. Echinoderm embryos generally develop into a bilaterally symmetric larva. Then, after a period of growth and development, they undergo a dramatic metamorphosis to the pentamer adult. Surprisingly, some purple sea urchin (*Strongylocentrotus purpuratus*) Hox genes are not expressed at all during larval development, and others are expressed in the canonical spatial pattern in only a single body cavity (Arenas-Mena et al. 2000) that does not seem to have a key function in the initial morphogenesis of either the larval or adult body axis. *Metacrinus rotundus*, a sea lily, has almost the same pattern of expression in the homologous body cavity (Hara et al. 2006). In living echinoderms, as in the



**Figure 19.6 An invertebrate chordate** (a) Nonfeeding tunicate larva, with readily identifiable chordate features such as a head, tail, and notochord. Photo by Daniel Clemens, Napa Valley College. (b) Filter-feeding adult tunicate (*Ciona intestinalis*), with yellow-fringed incurrent (top) and excurrent (left) siphons.

sea squirts, we see maintenance of the canonical spatial expression pattern despite the lack of apparent function in patterning along the primary body axes (see Mooi and David 2008 for a discussion of extinct echinoderms).

In some animals, Hox genes are also expressed in other locations in a pattern parallel to the canonical one, such as the urogenital system, gut, and limbs of some vertebrates, and the dorsal–ventral axis in an anemone (see Ryan et al. 2007).

Finally, the comparative data on pre-bilaterian Hox genes discussed earlier (Dubuc et al. 2012) indicates that after the Cnidaria (anemones, jellyfish, coral) split off from the line leading to the bilateral animals, and again after the bilateral animals diversified, the Hox gene sets must have expanded substantially. Thus, the evolutionary expansion of Hox clusters by gene duplication must have resulted in further subdivisions of the body into specific Hox gene expression domains. Again, this observation indicates a primacy of the canonical spatial expression patterns, perhaps independent of specific functions of the genes themselves.

With such diversity in Hox gene clustering, expression, and function, can we make any predictions about Hox function in early animals? Figure 19.5 reveals two things almost all animal Hox genes have in common: They show the canonical spatial expression pattern and are expressed in the nervous system. The most parsimonious hypothesis for the common ancestor of the bilateral animals (including beetles, snails, urchins, and humans) is that its Hox genes were expressed in the canonical spatial pattern in the nervous system (Samadi and Steiner 2010).

Is there a way to test this hypothesis of a nervous system origin of Hox gene expression in our deep animal ancestors? What did these ancestors look like? Did they have segments, a heart, limbs, eyes? If the ancestral function of Hox genes was in nervous system development, how can we account for the various functions that have evolved in different animal lineages since that time? And how can we explain the strikingly similar segmental patterns of Hox gene expression in vertebrates, segmented worms, and arthropods? For answers, we need to go beyond Hox genes and consider broader issues in evo-devo that have been discussed for years, but have found substantial experimental support only recently.

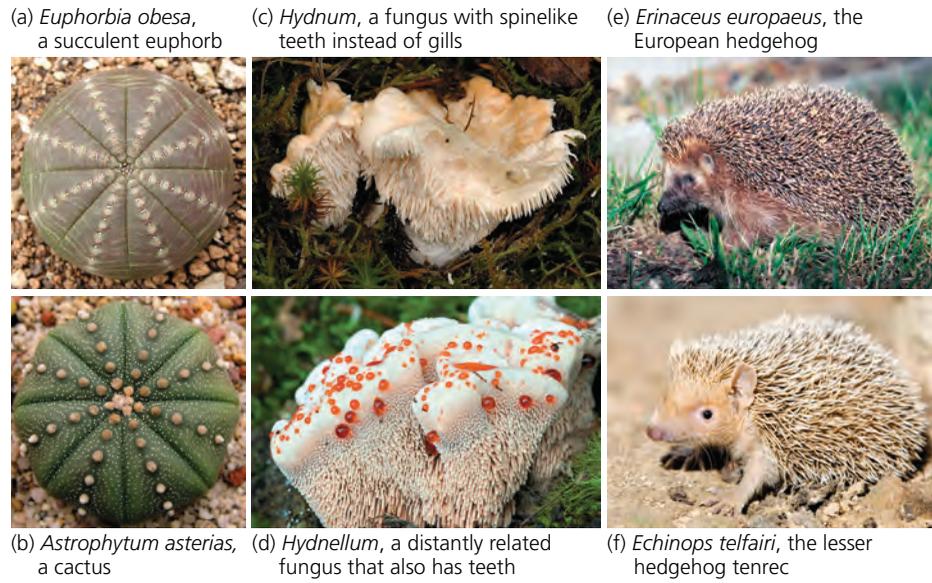
The canonical spatial expression pattern of Hox genes is most strongly conserved in the nervous system, suggesting that directing the nervous system's development was their ancestral function.

## 19.3 Post Hox: Evo-Devo 2.0

### Homology and Homoplasy: The Eternal Recurrence

Organisms show curious similarities in structure, despite differences in function. The forelimbs of a mole and a bat have the same arrangement of bones, even though one serves as a shovel and the other as a wing (see Chapter 2). Darwin provided the first meaningful explanation for such similarities: common descent. Just as a child resembles her brother more than a randomly chosen classmate, a human resembles a chimp more than a lemur. Traits shared because they were present in, and inherited from, a common ancestor are called **homologous**.

However, similarity can also arise independently. Consider the similarities, shown in **Figure 19.7**, between New World cacti and African euphorbs, between tooth fungi from the genera *Hydnellum* and *Hydnum*, and between hedgehogs and Malagasi hedgehog tenrecs. These are plants, fungi, and mammals from distantly related families. Their similarities are due to independent evolution, not common descent. Another term for similar features in two organisms that were not present in, and inherited from, their most recent common ancestor is **homoplasy**.



**Figure 19.7 Homoplasy in plants, fungi, and animals** (a, b) Distantly related plants with similar growth forms. (c, d) Fungi with independently derived “tooth fungus” phenotypes, showing spinelike teeth instead of gills. (e, f) Mammals with similar morphologies. The lesser hedgehog tenrec shown in (f) is found only on Madagascar. Other tenrecs occur on Madagascar and in Africa. Different species of tenrecs resemble hedgehogs, shrews, and otters. Nevertheless, they belong to the order Afrosoricida, and are all more closely related to armadillos and elephants than they are to true hedgehogs, shrews, or otters.

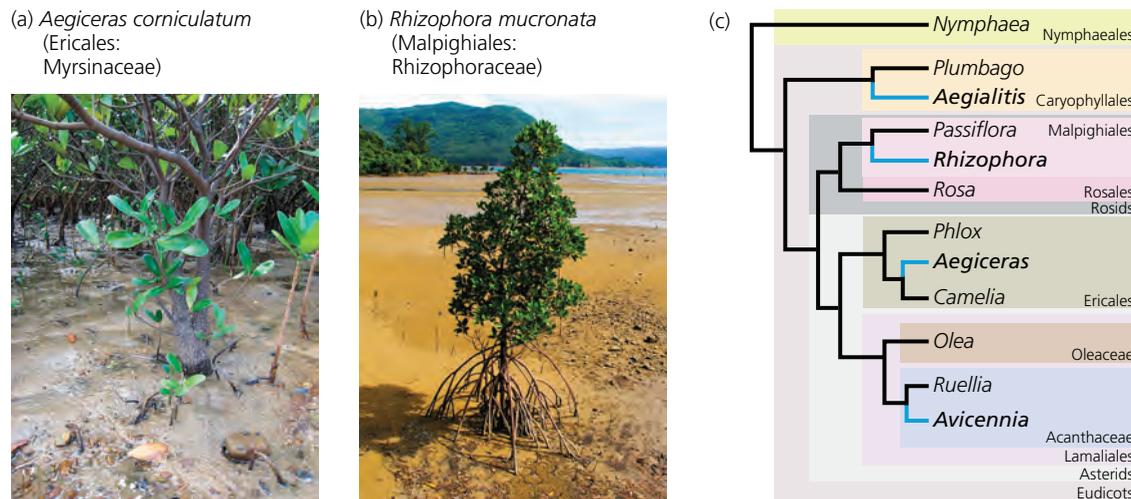
What does this have to do with developmental biology? Previously, we defined development as the processes by which an organism grows through its life cycle to produce the reproductive stage(s) and all of the stages in between. Let us here consider a related, yet slightly different and more technical definition. Every organism has a genotype, essentially the same in all cells in the body. Development is the process by which that genotype, in coordination with the environment, produces an organism’s phenotypes through the life cycle (Figure 19.8).



**Figure 19.8 One conception of development** Development is the process by which genes are expressed in an environmental context to yield phenotypes. Genotype is inherited, but phenotype is the target of selection.

In the examples of homoplasy shown in Figure 19.7, the phenotypes are strikingly similar. The standard explanation is that similar selection pressures in different taxa caused similar evolutionary changes to arise independently. But what if the developmental processes that build those phenotypes are also the same?

One such example can be seen in the independent evolution of mangroves in distantly related families of flowering plants (Figure 19.9). Figure 19.9a and b show



**Figure 19.9 Parallel evolution of developmental physiology in mangroves** Mangroves, a shoreline-adapted growth form, occur in distantly related plant families, including (a) *Aegialitis* and (b) *Rhizophora*. The phylogeny in (c) shows a sample of mangrove genera in bold, and a few of their non-mangrove relatives. Background colors show taxonomic groups. Lineages indicated by blue lines show reproductive vivipary.

two such independently evolved mangroves from different plant families. Figure 19.9c shows a phylogenetic hypothesis for how these and other mangrove genera (blue branches on the phylogeny) are related to several non-mangrove genera (black branches). “Mangrove” describes a growth form (not a clade) of shoreline plants whose roots are often submerged in their brackish or saltwater habitats. Living in such an environment is challenging; mangroves need to rid their leaves of salt, and their seeds must either root quickly when dropped or remain buoyant until reaching a suitable spot to root. Both of these characteristics depend upon the plant hormone abscisic acid (ABA). High levels of ABA confer salt tolerance to leaves. Low levels of ABA in embryos cause them to start developing while the seed is still attached to the plant. This trait, known as vivipary, confers buoyancy and allows quick rooting.

Elizabeth Farnsworth and Jill Farrant (1998) examined ABA regulation in leaves and embryos of four independently evolved mangrove groups (including the two pictured in Figure 19.9) as well as in closely related non-mangroves. Every mangrove tested had high levels of ABA in leaves, and reduced levels of ABA in their embryos, compared to related non-mangroves. Independently evolved mangroves thus not only look superficially similar and exist in similar habitats, but their underlying developmental physiology is similar. This class of homoplasy, where similarity results from the same underlying developmental mechanism, is known as **parallel evolution**. This is contrasted with **convergence**, or similarity resulting from a different underlying developmental mechanism (Hodin 2000).

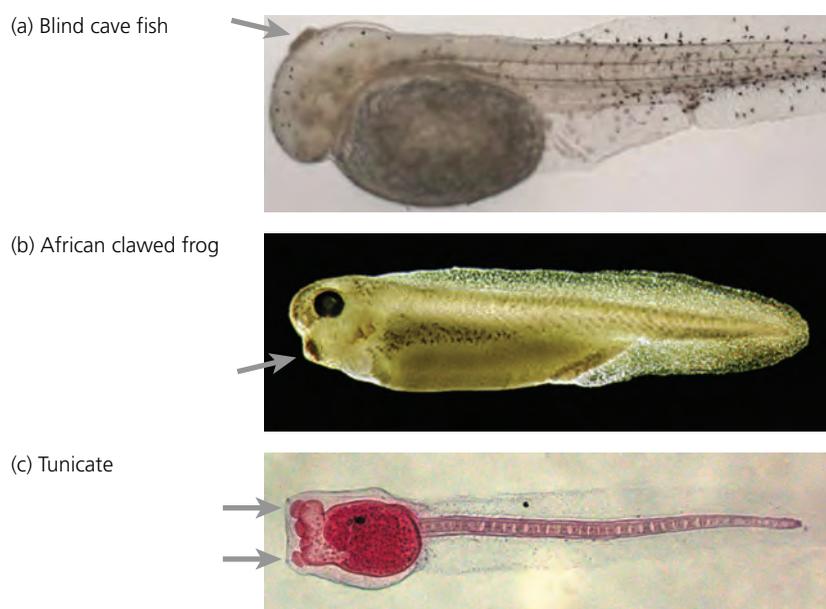
It is important to recognize that the contrasting concepts of homology versus homoplasy and parallel versus convergent evolution can be applied at hierarchical levels ranging from genes to behavior. For example, one can identify parallel evolution at the level of amino acids, as in the independent origin of alanine-rich antifreeze proteins in arctic and antarctic fish, or at the level of colonial behavior, as in the independent origins of eusociality in different insect groups. It is thus crucial to specify the hierarchical level being discussed. For example, bat wings and pterodactyl wings are homologous as limbs, but homoplasious as flying limbs. Since this is a chapter on evo-devo, we are considering parallel and convergent evolution at the level of the developmental mechanism.

## Parallel Evolution

As modern developmental biology is applied to comparative questions, many examples of parallel evolution are being uncovered. For example, independently evolved larval skeletons in two classes of echinoderms (sea urchins and brittle stars) involve a parallel embryonic activation of genes responsible for formation of the adult skeleton (Koga et al. 2010). Even more striking is the parallel evolution of juvenile attachment structures in three distant groups of chordates: sea squirts, frogs, and fish (Pottin et al. 2010). These attachment structures, shown in **Figure 19.10**, are clearly not homologous (Hall 2012). They have completely different embryonic origins, their morphologies are quite different, they reside in different locations on the respective larvae, and attachment organs are rare among chordates. Nevertheless, the formation of attachment organs in sea squirts, frogs, and fish involves the activation of related genes (*Bmp* and *Otx*). Moreover, the frog and fish adhesive organs receive neural inputs from the same part of the brain—the trigeminal ganglion—which processes a wide range of sensory information.

When evolution follows such similar trajectories using similar mechanisms, this is often seen as evidence for **developmental constraints**, defined as a bias

Similar traits that evolved independently may arise via the same or different developmental mechanisms.



**Figure 19.10** Parallel evolution of attachment organs in tadpole-like larvae of distantly related chordates (a) Blind cave fish larva, *Astyanax mexicanus* (subphylum Vertebrata, class Actinopterygii), and its dorsal attachment organ. From Protas and Jeffery (2012). (b) African clawed frog tadpole, *Xenopus laevis* (subphylum Vertebrata, class Amphibia), and its ventral attachment organ. Photo by Edgar Buhl, Bristol University. (c) Tunicate larva (subphylum Urochordata [=Tunicata], class Ascidiacea), and its anterior attachment organs. Photo by Daniel Clemens, Napa Valley College.

in the production of phenotypic variation due to developmental factors (Maynard Smith et al. 1985). Three things are important to keep in mind about constraints:

1. The term *bias* is crucial; *constraint* often conjures up notions of prohibition, whereas bias merely indicates likelihood and directionality of variation. For this reason, Wallace Arthur (2004) advocates the term **developmental bias**.
2. This notion of biased development challenges the concept espoused in the modern synthesis (following Darwin) that variation has no directionality and that all directional evolution is due to selection.
3. It is often difficult in practice to distinguish developmental constraint from strong directional selection.

We explore developmental constraints/biases, and the evidence for their existence, in the next section of this chapter.

Recent findings of unexpected levels of parallel evolution, such as the one illustrated in Figure 19.10, have spawned a robust discussion in the evo-devo community. If independently evolved phenotypes have similar underlying developmental mechanisms, does this imply that the developmental processes are in some sense homologous? If so, does this so-called deep homology (Shubin et al. 2009) suggest that parallel evolution is really a hybrid between homology (at the level of the developmental mechanism) and homoplasy (at the level of the phenotype)?

One could, of course, argue that because all organisms use proteins to perform cellular functions, any homoplasy has a deep homology in the use of proteins. This extreme example illustrates the need to be clear about the hierarchical level at which we are defining homology or homoplasy. In the case of developmental mechanisms, genes are known to interact in networks of cross regulation, and some of these networks may be billions of years old. But we must be cautious in ascribing homologous functions to these networks. A screwdriver can be used to turn a screw, split glued boards along their seam, and open soda bottles. If individuals in Cameroon and Paraguay use screwdrivers to open bottles, that does not necessarily imply a meaningful homology in the mechanism of bottle opening. Screwdrivers might just have been seized independently as the best tool at hand.

In a surprising number of cases, the homologies among developmental mechanisms appear to be much deeper than the homologies among the similar traits whose development they control.

### Equal Variation under (Darwin's) Law?

In Darwin's time, natural selection was criticized as merely a negative mechanism. St. George Mivart (1872), for example, argued that because selection simply removes the unfit, it cannot explain the origin of more-fit individuals. Mivart noted the numerous examples of mimicry in insects. These include striking cases of masquerade in which insects look like fresh or decaying leaves or twigs and sometimes even act like vegetation—as when stick insects sway in a breeze. Mivart objected that small, imperceptible evolutionary changes, due to culling of less well-camouflaged individuals, could never add up to these striking resemblances.

In response, Darwin (1872), reasoned as follows:

Assuming that an insect originally happened to resemble in some degree a dead twig or a decayed leaf, and that it varied slightly in many ways, then all the variations which rendered the insect at all more like any such object, and thus favoured its escape, would be preserved, whilst other variations ... if they rendered the insect at all less like the imitated object ... would be eliminated.

The key to Darwin's argument, as Darwin himself noted in an 1862 letter to Charles Lyell, is that variation is always present and is unbiased in direction. When the mean phenotype shifts toward closer mimicry, the population still shows variation in all directions—including even better mimicry.

But is variation really of equal probability in all directions and almost always present? In the case of insect mimicry, perhaps the variants are not of equal probability in all directions as Darwin supposed. What if certain types of variants are more likely to arise than others? What if insects are, for some reason, more likely to resemble a twig than a leaf? If variations are thus biased, then we must modify the Darwinian—and modern synthesis—view of natural selection as the predominant creative process of evolution, and accordingly elevate the prominence of internal processes such as developmental bias as an explanation for life's diversity. Can modern evo-devo help settle this age-old debate?

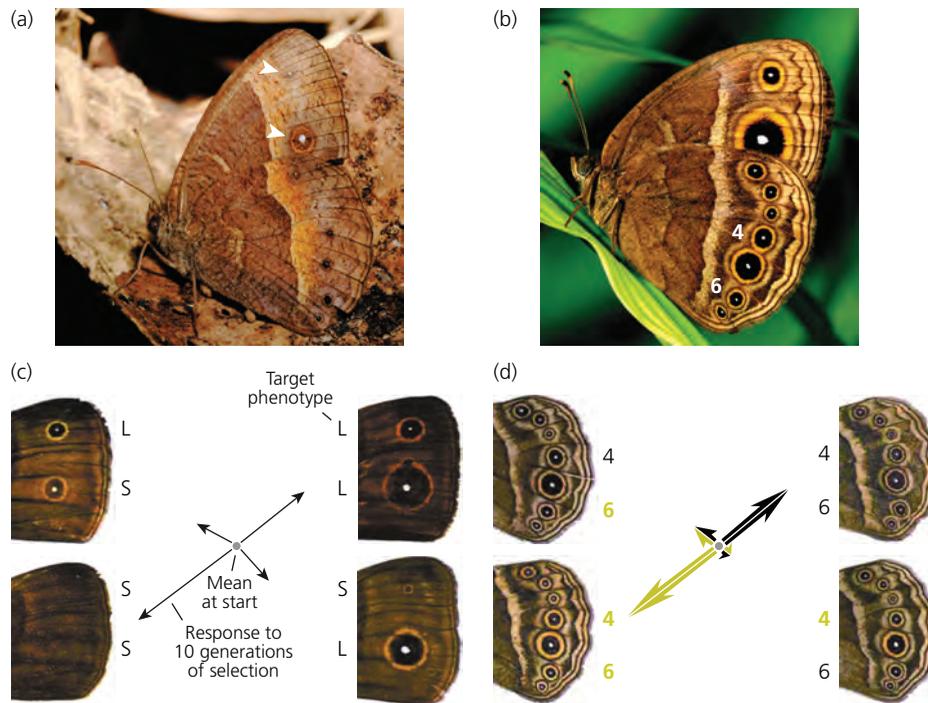
Many butterfly wings have striking patterns, known as eyespots, that distract bird predators by promoting sublethal attacks at the spots rather than at the body (Olofsson et al. 2010). They may also be sexually selected. One particularly well-studied species is the squinting bush brown butterfly from southern Africa, *Bicyclus anynana*. In 2008, Cerisse Allen, working with Paul Brakefield and colleagues, reported on experiments designed to test whether characters such as eyespot size and color could respond to selection in all directions, as hypothesized by Darwin.

Allen and colleagues selected on both eyespot size and eyespot color. As shown in **Figure 19.11a and b**, *B. anynana* has two forewing eyespots. Using the dry season morph of *B. anynana* (Figure 19.11a), the scientists imposed on lab populations 10 generations of artificial selection for four distinct spot size phenotypes:

1. Larger anterior and posterior eyespots (upper right in Figure 19.11c)
2. Smaller anterior and posterior eyespots (lower left in Figure 19.11c)
3. Larger anterior and smaller posterior eyespots (upper left in Figure 19.11c)
4. Smaller anterior and larger posterior eyespots (lower right in Figure 19.11c)

As is clear from the images, 10 generations were sufficient to independently alter both the anterior and posterior eyespot size, even though their sizes are normally correlated across *Bicyclus* species.

Allen and colleagues then tried selecting on hindwing eyespot color. This time they used the wet season form, which is more brightly colored (Figure 19.11b).



**Figure 19.11 Constraint in butterfly wing eyespot color, but not size** (a and b) Dry season and wet season morphs of the butterfly *Bicyclus anynana*. Photos by Antónia Monteiro, Yale University. (c) Allen and colleagues (2008) found that lab populations respond to selection for all combinations of larger and smaller forewing eyespot size. (d) In contrast, lab populations responded to selection for only two combinations of enhanced color in hindwing eyespots: enhanced black or enhanced yellow in both eyespot 4 and eyespot 6. But the populations did not respond to selection for enhancement of different colors in the two eyespots, an indication of a constraint on color variation. Photos in (c and d) from Allen et al. (2008).

*B. anynana* eyespots have three concentric colors: a gold ring surrounding a black ring with a white center. The scientists again imposed artificial selection for 10 generations, and separately on eyespots 4 and 6 (indicated on the hindwing image in Figure 19.11b), because these eyespots are approximately the same size. The researchers attempted to select for the following four eyespot color phenotypes:

1. More black color in eyespots 4 and 6 (upper right in Figure 19.11d)
2. More gold color in eyespots 4 and 6 (lower left in Figure 19.11d)
3. More black in eyespot 4 and more gold in 6 (upper left in Figure 19.11d)
4. More gold in eyespot 4 and more black in 6 (lower right in Figure 19.11d)

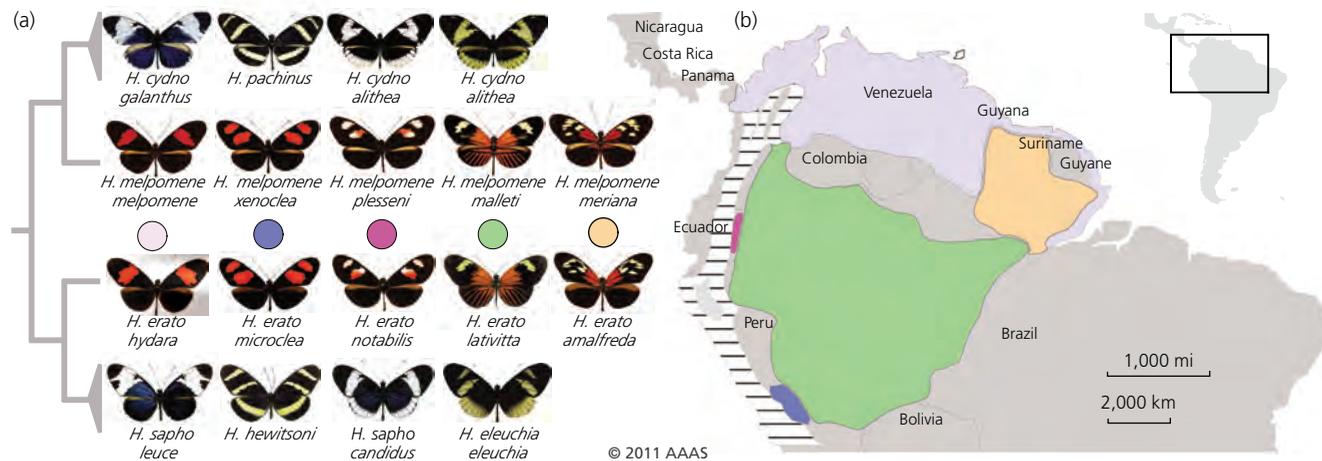
The butterfly populations responded to selection for enhanced gold or black color in both eyespots simultaneously, but not to selection for different color enhancements on the two eyespots. This result, in contrast to the eyespot size selection experiment, provides evidence for some constraint or bias, where certain types of variants are much more common than others.

What is the mechanism underlying this biased pattern? The explanation may be related to developmental timing. Classic and modern experiments on the determinants of butterfly wing eyespot patterns indicate that the size of the eyespot is determined in forming wing tissue in the late larval (caterpillar) stage, whereas color is determined later, during the chrysalis stage (French and Brakefield 1995; Beldade et al. 2002, Monteiro et al. 2006). Antónia Monteiro (personal communication) speculates that by the time color is determined in the chrysalis, a convenient set of positional molecular signals that can differentiate the eyespots from each other may no longer be available.

### Parallel Evolution and Biased Evolutionary Trajectories

Another dramatic example of wing-pattern variation in butterflies involves mimicry in the genus *Heliconius*. In Central and South America, *Heliconius erato* and *Heliconius melpomene* exist in “mimicry rings.” Each species has more than a dozen

Artificial selection experiments demonstrate that evolutionary change proceeds more readily in some directions than others. One explanation for this phenomenon is developmental bias—the notion that alterations in a developmental pathway can more easily produce some alterations in phenotype than others.



**Figure 19.12** Co-mimicry in multiple *Heliconius* butterfly morphs (a) Morphs of *H. melpomene* and *H. erato* resemble each other across species more than they resemble other morphs in their own species. *H. melpomene* and *H. erato* are not sisters; the closest relatives of each (*H. cydno* and *H. sapho*, for example) look different from *H. melpomene* and *H. erato*.

Co-mimics have overlapping ranges, shown by colored dots in (a) and map (b). The entire range of the two species is shown in gray. Many morphs are not shown. Hatched areas have no populations of either species. From Reed et al. (2011).

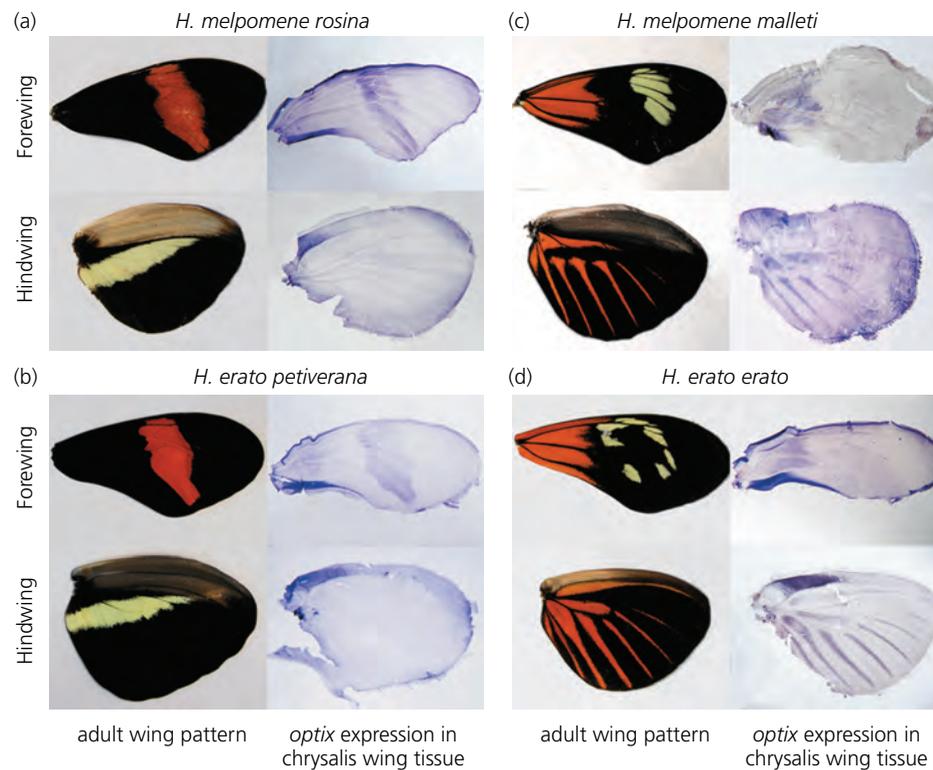
Reed RD, et al. 2011. *Optix* drives the repeated convergent evolution of butterfly wing pattern mimicry. 2011. *Science*, 333: 1137-41. Reprinted with permission of the AAAS.

genetically determined color morphs (Figure 19.12). Each morph is restricted to a particular locale, and each has an almost perfect co-mimic in the other species. The paired morphs are considered “co-mimics,” rather than mimic–model pairs, because both species are unpalatable and both apparently benefit from their mutual resemblance. Note that *H. erato* and *H. melpomene* are not sister species (Figure 19.12a) and cannot hybridize. Thus *H. melpomene* morphs not only resemble their *H. erato* co-mimics more than they resemble closer relatives, they resemble their co-mimics more than they resemble other members of their own species.

How is the diversity of phenotypes maintained within each species? Different color morphs within each species hybridize, but offspring with intermediate phenotypes are quickly selected against by bird predators. Still, bird predation seems an insufficient explanation for the observed geographic diversity.

Recent evidence has provided clues to the mystery. The genetic determinants of wing-color pattern in *Heliconius* largely map to loci of single genes or tightly linked gene clusters: one each for black, yellow/cream, and red color patterns (Counterman et al. 2010; Joron et al. 2011). What are these genetic loci, and how does variation in each regulate so much diversity in wing patterns while at the same time promoting stable co-mimicry across the geographic ranges?

Robert Reed, Ricardo Papa, Owen McMillan, and colleagues (2011) made a remarkable discovery that provides the beginning of an answer. Variation in the expression pattern of a single homeobox transcription factor, called *optix*, accounts for variation in red color pattern in both *H. melpomene* and *H. erato* across their geographic ranges. Depending on the *optix* allele a butterfly carries, *optix* is expressed in different places on the wings during chrysalis development, the stage when color is determined. One can conceive of the expression of *optix* at this stage as similar to how an artist might make a pencil sketch on a canvas before executing a painting. In this way, the locations of *optix* transcription in the forming wing tissue of the chrysalis (as indicated by blue color in the right half of each of the image pairs in Figure 19.13) precisely matches the locations of red coloration in the adult wing (left half of each image pair). By contrast, *optix* expression zones



**Figure 19.13** *Optix* expression patterns in chrysalis wing tissue predict where red color will form in the adult wing in both *H. melpomene* and *H. erato* (a) *H. m. rosina* and (b) its co-mimic *H. e. petiverana*. (c) *H. m. malleti* and (d) *H. e. erato*, a morph with a similar red color pattern. In each panel, the right side shows *optix* mRNA expression patterns revealed by in situ hybridization, a technique using a tagged RNA molecule synthesized to complement the nucleotide sequence of—and bind specifically to—*optix* mRNA. Wherever blue color is present in chrysalis wing tissue, the *optix* mRNA is also present. Note that the blue patterns in every right-side image match the red-colored regions in the corresponding adult wings (but not the black, yellow, or cream). From Reed et al. (2011).

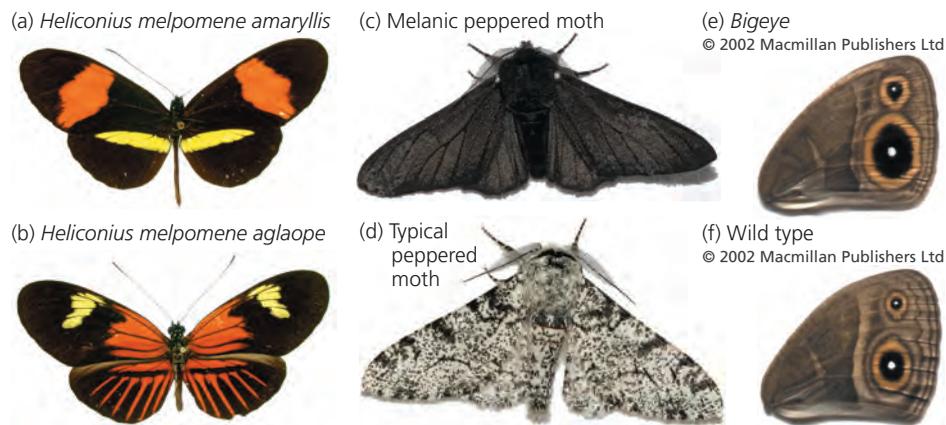
in the chrysalis wing tissue do not predict the adult wing's black-, yellow-, or cream-colored regions; these map to different genetic loci (see below).

The correspondence of *optix* expression in pupal wing tissues and red color pattern in adult wings—in both species and across color morphs—seems an astonishing example of adaptive parallel evolution. But is it possible that all of the different color pattern alleles for *optix* were already present in the last common ancestor of *H. melpomene* and *H. erato*? If so, then the evolution of mimicry simply would have involved fixation of the same color pattern alleles in co-occurring populations of the two species, inherited in both lineages from their common ancestor. To distinguish between these “parallel evolution” and “ancient alleles” scenarios, Heather Hines and colleagues (2011) undertook a phylogenetic analysis of *optix* alleles from many populations in both species. The ancient alleles scenario would predict that co-mimics have similar or identical *optix* alleles. The parallel evolution scenario would predict unique *optix* alleles arising independently in the co-mimic pairs. Hines and colleagues' comparative analyses of *optix* sequences corresponding to the rayed wing-pattern phenotypes (for example, the rear wing patterns seen in Figure 19.13c and d) within and between species is inconsistent with the ancient alleles scenario. This is genuine parallel evolution.

The black color phenotype in *Heliconius* has been mapped to the *WntA* locus (Martin et al. 2012). Wnt proteins are secreted molecules with multiple functions in cell–cell signaling and developmental patterning, and they are active in many forms of cancer. Interestingly, the only other diffusible signaling molecule with a known function in animal color patterning comes from another *Wnt* gene called *wingless*, which is involved in butterfly wing patterning (Martin and Reed 2010) and also patterns of black spots on fruit fly wings (Werner et al. 2010).

The genetic identity of the third major *Heliconius* locus—the determinant of yellow- and cream-colored patterns—has not yet been identified, but it maps to

**Parallel evolution is the independent appearance of similar phenotypes via similar alterations of the same developmental mechanism.**



**Figure 19.14 Moths and butterflies with pattern variation mapping to the same locus**

The gene or genes underlying cream and white color patterns in *Heliconius*—as in (a) and (b)—map to a single locus. The same locus may be responsible for melanism in *Biston betularia* (c vs. d), and the Bigeye mutant in *Bicyclus anynana* (e vs. f). (a, b) from Joron et al. (2006); (e, f) from Beldade and Brakefield (2002).

(e and f) Reprinted by permission of Macmillan Publishers Ltd: *Nature Reviews Genetics* 3: 442–452, copyright 2002.

the same genomic location as two wing-color pattern polymorphisms under active investigation: the *Bigeye* mutant in the squinting bush brown butterfly, *Bicyclus anynana*; and the *carbonaria* locus underlying industrial melanism in the famous British peppered moth, *Biston betularia* (Figure 19.14; Van't Hoff et al. 2011).

Taken together, what can we conclude from these findings on the evolutionary genetics of butterfly wing patterns? Allen's *Bicyclus* selection experiments indicate that some aspects of wing patterning show a bias in variation, contradicting Darwin's supposition of variation as “almost always present, enough to allow any amount of selected change.” Furthermore, the data with *Heliconius* also points to some bias in evolutionary trajectories, because the same developmental-genetic mechanisms seem to underlie a startling array of butterfly wing-pattern variation, both within and among species.

Is it appropriate to think of these biases, or constraints, as evolutionary limitations? The spectacular examples of *Heliconius* mimicry certainly seem to suggest the opposite. Perhaps biases in developmental-genetic mechanisms actually favor the rapid and repeated evolution of pattern variations, making the course of adaptations in co-mimics more efficient and effective. In this conception, developmental bias or constraint is not a limitation at all, but instead an evolutionary opportunity for organisms to respond nimbly and rapidly to selection.

### Pleiotropy and Developmental Trade-Offs

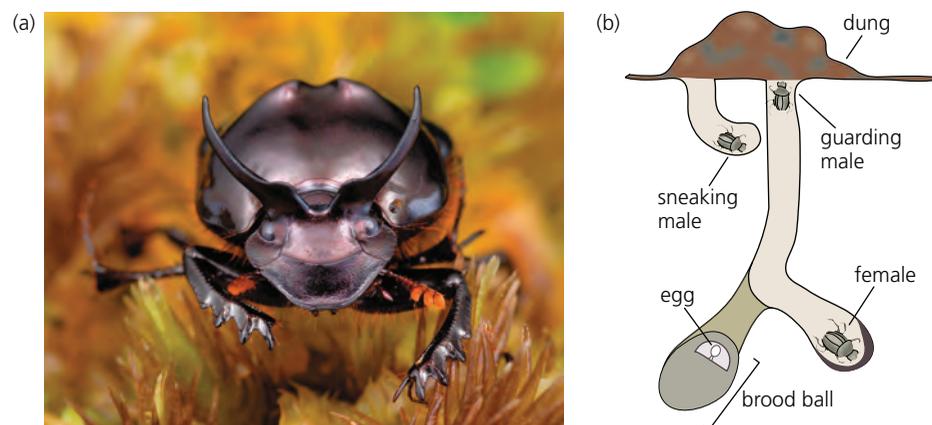
Another observation that follows from these and many other studies in evo-devo is that the same genes seem to function repeatedly at different times and places during development. This phenomenon is known as **pleiotropy**. Thus the *optix* gene has important functions in multiple tissues in insects including eye development and wing patterning. Hox genes pattern the main vertebrate body axis and also the limb proximal–distal axis. A plant homeobox gene called *KNOX* is involved in both primary and secondary leaf (or leaflet) patterning during multiple independent origins of compound leaf development in the orders Brassicales and Asterales. There are hundreds of similar examples in the literature.

An implication of such findings is that evolution involves reuse and repurposing of ancestral gene networks. A gene network can be thought of as a “food web” of genes with a complex, hierarchical series of interacting components, including feedbacks among levels of the hierarchy. In a simple food web, plants are eaten by grazers, which are eaten by predators, which eventually die and decay, feeding back as nutrients for the plants. In a gene network, environmental changes cause hormone release, which binds to transcription factors

to activate a variety of genes, which carry out cellular functions that feed back to activate or repress release of the hormone. Different gene networks have evolved to carry out specific functions, such as setting up boundaries between regions of an embryo, or causing cells to move a certain way, or initiating an abscission layer in plant tissues. Such networks are modular and pleiotropic in the sense that they are used again and again within and among organisms across evolutionary time.

An assumption regarding pleiotropy is that selection for one function might limit or constrain selection on an alternative function. One of the big questions in evo-devo is, to what extent does pleiotropy in genes that belong to networks, or in entire gene networks themselves, limit potential variation? We do not yet know the answer, but it has significant implications for Darwin's concept of variation as "almost always present."

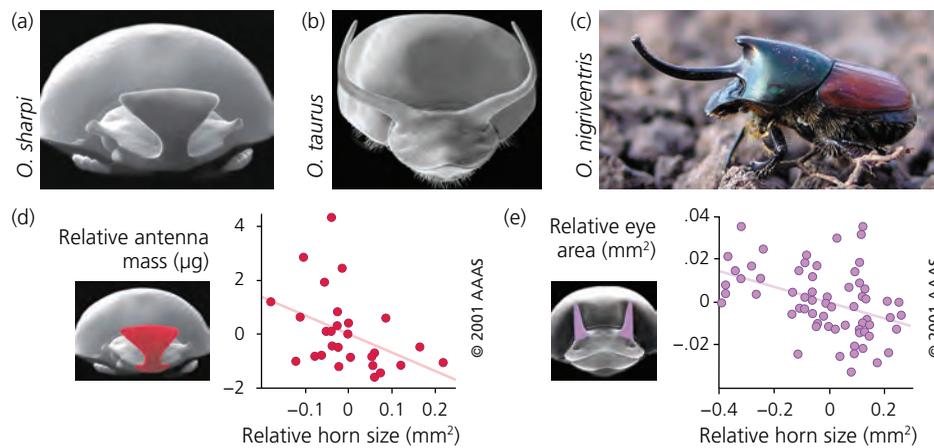
A concept related to pleiotropy is a **trade-off**, where one feature of an organism can be promoted only at the expense of another. This concept was discussed earlier in the book (see Chapter 10), but here we recast the issue as a question in evo-devo. Why are certain features of organisms traded off against others, while other features appear able to vary independently?



**Figure 19.15** Alternate reproductive strategies in dung beetles (a) An *Onthophagus* dung beetle. (b) A female dung beetle digs a tunnel below a dung pile where she will lay her eggs, provisioning each with a ball of dung ("brood ball"). Her long-horned mate ("guarding male") guards the tunnel entrance, but small-horned males ("sneaking male") may dig side tunnels and surreptitiously mate with her. Redrawn from Emlen (2000).

Many groups of dung beetles are characterized by horns on the head or thorax of males, females, or both. The best-studied horned dung beetles are in the genus *Onthophagus* (Figure 19.15a). In these beetles, the female buries dung balls to feed her larvae, and her large-horned mate uses his horns to guard the entrance to her tunnel, thus preventing other males from entering to mate with the female below. In many *Onthophagus* species, large-bodied males have relatively large horns, whereas small-bodied males have relatively small horns. Body size is determined by larval nutrition, so the size of horns in males is phenotypically plastic. A single genotype can produce either small or large horns, depending on its growth environment. This would seem to be disadvantageous to smaller males, but Doug Emlen (1997) discovered that hornless small males use an alternative mating strategy (Figure 19.15b): They dig their own tunnels to surreptitiously enter the burrow. The lack of horns allows the smaller males to dig tunnels that horned males cannot, because the horns would get in the way.

The consequences of large horns, and potential advantages of small horns, do not end there. Emlen (2001) identified trade-offs with not only horn size but also horn location in different *Onthophagus* species. For example, antenna size is negatively correlated with horn size, but only in species with horns projecting from the front of the head, not from the rear of the head or the thorax. A species



**Figure 19.16 Developmental trade-offs in dung beetles**

(a, b, and c) Different species of *Onthophagus* have horns in different places. (d) In *O. sharpi*, the horn (colored red) is adjacent to the antennae, and the sizes of these structures are negatively correlated. (e) In this *Onthophagus* from Ecuador, eyes are adjacent to the paired horns (lavender), and horn size trades off with eye size. Photos by Doug Emlen; (d and e) from Emlen (2001).

(d, e) Emlen, D. J. 2001. "Costs and the Diversification of Exaggerated Animal Structures." *Science* 291: 1534–1536. Reprinted with permission of the AAAS.

with a horn on the front of its head, *O. sharpi*, appears in Figure 19.16a. One with horns on the back of its head, *O. taurus*, appears in Figure 19.16b. One with a horn projecting from the front of its thorax, *O. nigriiventris*, appears in Figure 19.16c. Figure 19.16d documents the trade-off, in *O. sharpi*, between horn size and antenna size.

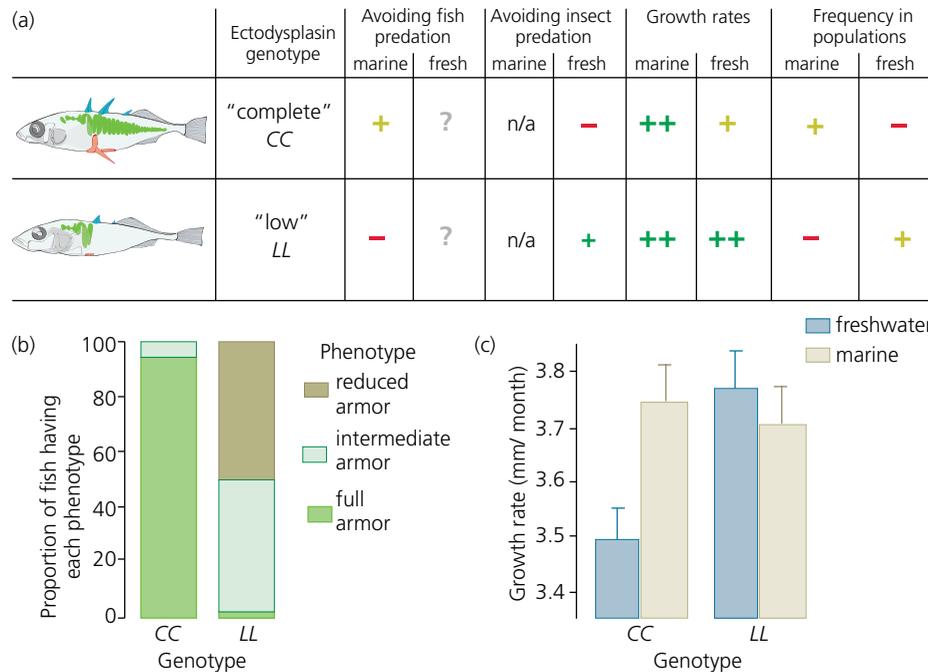
Emlen found other trade-offs as well. Eye size is negatively correlated with horn size, but only in species with horns projecting from the rear of the head (Figure 19.16e). Nocturnal species (for which large eyes aid vision under low light) tend not to have horns at the rear of the head. Finally, wing size is negatively correlated with horn size, but only in species with horns on the thorax.

In all of these cases, the horn size trade-off is with nearby structures: Eyes are at the rear of the head, antennae are at the front of the head, and wings emerge from the thorax. Are these merely correlations, or is there some mechanistic cause? To address this question, Emlen (1996) artificially selected *O. acuminatus* males, which have horns near their eyes at the rear of the head, for longer or shorter horns over seven generations. The resulting beetles showed a negative correlation with eye size. The increased horn size group had reduced eye size, while the decreased horn size group had increased eye size. Similar results are seen by giving unselected beetles juvenile hormone (JH) treatments during pupal development, the stage at which the size of these organs is determined. JH treatment results in increased horn size and decreased eye size at a given body size. JH levels are, likewise, known to differ between small and large males.

Taken together, these results suggest that some short-range signal or factor is involved in determining the size of organs in these beetles and that competition for a limited supply of this factor could explain the trade-offs. What could this factor be? One candidate is insulin-like growth factor, which may determine organ size in insects, is directly related to nutrition, and is regulated by insect juvenile hormone (Wu and Brown 2006; Emlen et al. 2012). In this conception, the growing horn tissue expresses high levels of insulin receptor, which acts as a sink, locally depleting circulating insulin and leaving less available for nearby tissues. The fact that these events occur during pupal development, when the organism is immobile, may explain how such processes of local depletion could occur.

However, not all beetle horn trade-offs are with nearby structures. Surgical removal of the developing genitalia results in increased horn size in adults, and gonad size also shows a negative correlation with horn size both within and among species (Moczek and Nijhout 2004). Therefore, we still await a definitive

Developmental trade-offs may arise because different body parts compete with each other.



**Figure 19.17 Pleiotropy in *Ectodysplasin* alleles in three-spine sticklebacks** (a) Summary comparing the armored and reduced armor morphs, and their advantages and disadvantages in marine and freshwater habitats. The two *Ectodysplasin* alleles (*C*—complete; *L*—low) show partial dominance, but we present only data for homozygotes. Question marks indicate that the optimal phenotype depends on variable environmental factors, such as water clarity. (b) In the homozygous offspring of heterozygotes, *Ectodysplasin* genotype is closely related to phenotype (Barrett et al. 2009). (c) Reared in marine conditions, *CC* and *LL* fish grow at approximately the same rate. But in freshwater, *LL* fish grow faster. From Barrett et al. (2009).

mechanism to explain these trade-offs in beetles, if indeed there is a singular mechanism. In any case, the comparative results indicate that resource allocation trade-offs bias developmental as well as evolutionary trajectories.

Earlier, we introduced the concept of pleiotropy as related to trade-offs. Pleiotropy refers to multiple functions for the same gene within a single organism. The evolutionary implication is that there is a limitation to how specialized a gene can be for one function when it simultaneously has to perform another function. Such a situation appears in the threespine stickleback, *Gasterosteus aculeatus*.

Threespine stickleback populations are essentially one of two types. Fish in marine populations have life cycles similar to those of salmon: They live most of their lives in salt water, but swim into freshwater lakes and streams to reproduce. However, over the last several thousand years, various populations have come to spend their entire lives in freshwater. A notable feature of *G. aculeatus* from marine populations is their body armor: skeletal plates that offer protection from predation by other fish (Figure 19.17a, upper row). In most freshwater populations, by contrast, the major predators are aquatic insects like dragonfly larvae, which rely on agility rather than crushing strength to capture juvenile sticklebacks (Marchinko 2009). Freshwater *G. aculeatus* populations have reduced body armor and instead can grow faster to their adult stage, where they are no longer subject to dragonfly predation (Figure 19.17a, lower row). These reduced-armor freshwater fish also show increased burst swimming speeds (Bergstrom 2002).

The connection to pleiotropy comes from evidence suggesting that changes in a single gene, *Ectodysplasin*, can account for both the reduction in body armor and the increase in growth rates in freshwater populations (Figure 19.17b, c; Barrett et al. 2008, 2009). This scenario suggests that body armor loss in freshwater populations is not merely a passive process akin to what Darwin and Wallace referred to as loss of features from “disuse.” Instead, the multiple functions of *Ectodysplasin* seem to actively promote body armor loss in freshwater populations via an automatic and pleiotropic growth advantage.

Developmental trade-offs may also arise because of pleiotropy—the involvement of a gene in the development of traits.

In sum, we have seen evidence—ranging from selection experiments to comparative biology to genome analyses—that Darwin’s postulate that variation is ever-present and omnidirectional was overstated. The implications of this evidence are significant, since it suggests that internal, developmental features of organisms guide evolution hand in hand with selection. Although the evidence does not topple the Darwinian pillar of the primacy of natural selection, it seems to validate a substantial modification of the concept. Next, we will evaluate whether findings in evo-devo may likewise call for restructuring a second Darwinian pillar: that evolution occurs only in small steps.

### Nature Sometimes Makes Leaps

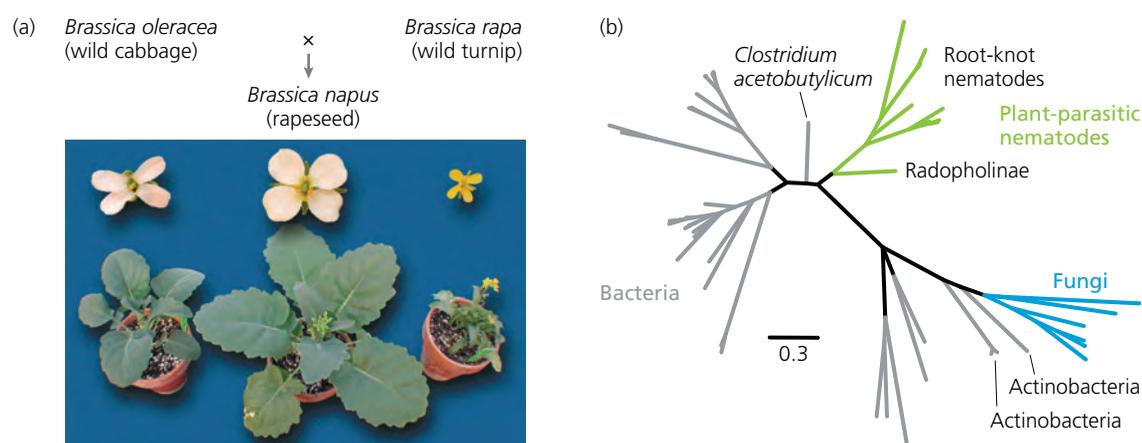
In *The Origin of Species* (1872, p. 156), Darwin wrote: “Natural selection acts only by taking advantage of slight successive variations; she can never take a great and sudden leap.” This notion of gradual change was a hallmark of Darwinism and arguably the main organizing principle of the evolutionary synthesis in the early 20th century. Has this paradigm of gradual, continuous change held up?

The opposing concept of leaps in (“saltational”) evolution has an uneven history in evolution and development. Most proponents of saltational evolution in the late 19th and early 20th centuries set themselves in opposition to Darwinian evolution. For example, Richard Goldschmidt’s name evokes, in the minds of most evolutionary biologists, the concept of “hopeful monsters,” where major mutational changes explain the origin of species, while microevolutionary changes below the species level are irrelevant to the evolution of life’s diversity. Less well known is that Goldschmidt also believed that small changes in early development might propagate through ontogeny to yield large effects on the adult phenotype (Goldschmidt 1940), a notion not far outside the current orthodoxy.

William Bateson (1894, pp. 410–411), whom we met when discussing homeotic mutations, made a simple yet elegant point in the debate on continuous versus discontinuous evolution. Bateson noted that the antennae of long-horned beetles typically have 11 segments, and asked how 12-segmented antennae could arise via gradual acquisition of a new joint. “With evidence that transitions of this nature may be discontinuously effected,” he noted, “the difficulty is removed.”

Bateson thus demonstrated that evolution can proceed in leaps, in this case via the addition of segments, whether in the antennal segments of long-horned beetles or in the body-segment numbers of centipedes—which curiously are always an odd number, so must proceed in leaps of two. Another example is the direction of spiraling of shells (dextral versus sinistral): There are only two options, so any transition between the two is a leap. Therefore the question for us is not Does nature proceed in leaps? because it clearly does; the questions are, How big are the leaps? How often do they occur? and How do they occur?

Perhaps the most straightforward example of evolution by leaps is cross-species hybridization in plants, where pollen from one species fertilizes the ovum of another, yielding a potential third species. Rapeseed (*Brassica napus*) offers an example. Rapeseed is the third most important oil crop in the world; canola is one variety. Rapeseed originated from a hybridization between wild cabbage (*B. oleracea*) and wild turnip (*B. rapa*) around the Middle Ages in Europe (Gupta and Pratap 2007). This event has been intentionally replicated many times by biologists. **Figure 19.18a** shows an individual from one such newly synthesized *B. napus* lineage, posed between plants from each of the parental species. Newly synthesized *B. napus* lineages show considerable genetic and phenotypic variation (Pires



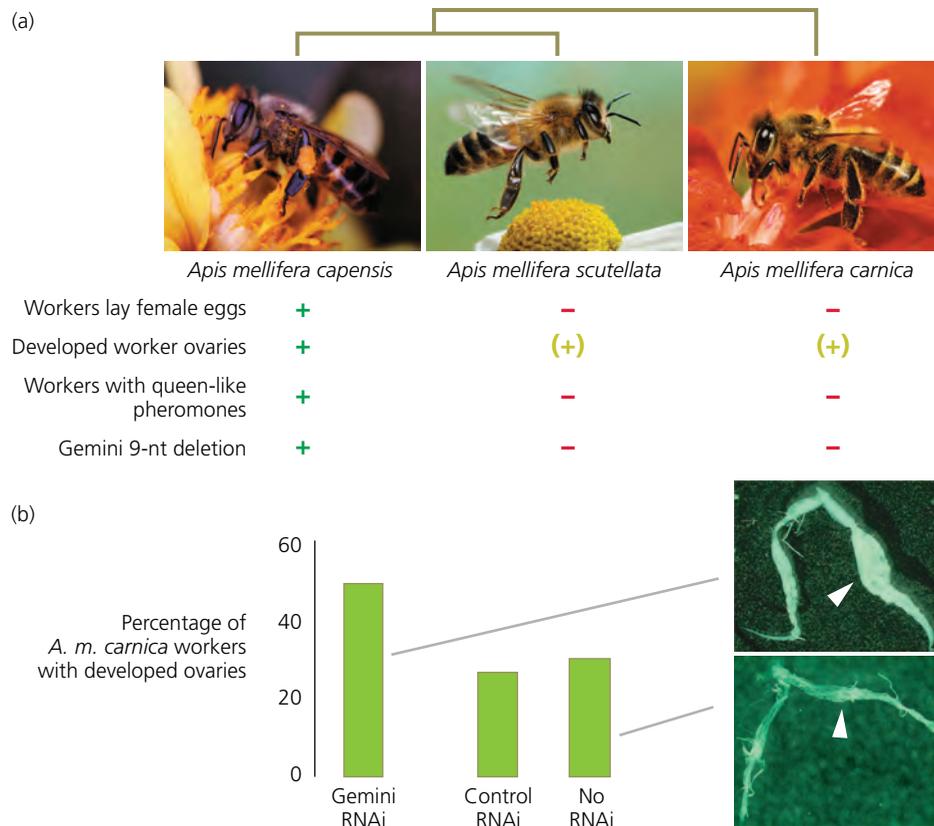
**Figure 19.18 Evolutionary leaps** (a) A newly synthesized rapeseed, the offspring of a wild cabbage and a wild turnip. Note the vigorous growth of the hybrid versus the parents. Photo by J. Chris Pires. (b) Unrooted phylogeny of hemicellulose-digesting xylanase genes in a variety of organisms. The xylanases of nematode worms that parasitize plants (green) branch from within the bacterial genes and are not closely related to the xylanases of the other eukaryotes known to make the enzyme, the fungi (blue). From Danchin et al. (2010).

et al. 2004; Gaeta et al. 2007). Warren Albertin and colleagues (2006, 2007) re-hybridized *B. oleracea* and *B. rapa* and examined the expression of over 1,600 stem and root proteins in the *B. napus* hybrid offspring. Many of the proteins showed non-additive effects, such as quantitative expression outside the range of both parents, and none of the proteins showed mis-expression or other obvious defects in gene regulation. These results suggest that hybrids with unique features can form stably in a single generation.

A second mechanism for evolutionary leaps is horizontal gene transfer, in which foreign DNA integrates stably into a new genome. Horizontal gene transfer is common in microbes, and the evidence for its importance in other kinds of organisms is growing (see Dunning Hotopp 2011). Striking examples are found in plant-parasitic roundworms, whose genomes encode cellulases and other cell-wall-degrading enzymes that aid the worms in exploiting their hosts. Phylogenetic analyses reveal that the genes for several of these enzymes, including the xylanases depicted in Figure 19.18b, came from bacteria (Danchin et al. 2010). Because horizontal gene transfers are all-or-none phenomena—the foreign gene is either integrated into the genome or not—they represent evolutionary jumps.

The movement of genetic elements within genomes can also cause evolutionary leaps. Transposable elements, or transposons, are widespread across organisms and can increase rates of evolution by elevating mutation rates. Increased mutation rates can be harmful, and eukaryotic and prokaryotic genomes have mechanisms to suppress mobility of transposons. Nevertheless, genome sequencing has revealed that such immobilized transposons can subsequently perform important cellular functions. In one example, a transposon specific to tetrapods and our lobe-finned fish ancestors is found in multiple places in the genome and has acquired key functions, including regulation of a homeobox gene involved in neural development (Bejerano et al. 2006). In a follow-up study, Lindblad-Toh and colleagues (2011) analyzed noncoding DNA in 29 mammals with fully sequenced genomes, focusing on those sequences that showed a signature for positive selection. They determined that about 20% of such sequences are immobilized transposons. Lowe and colleagues (2007) found that such sequences showed preferential association with developmental regulatory genes.

Via a variety of mechanisms, evolutionary change sometimes proceeds in discontinuous bursts.



**Figure 19.19 A polymorphism in the gemini gene alters life history in honeybee**

(a) The cape honeybee (*Apis mellifera capensis*) from South Africa's Cape of Good Hope, its neighbor and closest relative (*A. m. scutellata*), and the eastern European honeybee (*A. m. carnica*). The cape honeybee's unique traits are associated with a 9-nucleotide deletion in the gemini gene. (b) Results of feeding recently emerged *A. m. carnica* workers sugar water with: an RNA molecule designed to mimic the gemini deletion (gemini RNAi), an RNA with a scrambled sequence (control RNAi), or just sugar water (no RNAi). Images at right show a maturing ovary with active oogenesis (arrowhead pointing to bulge, top) compared to a non-maturing ovary with no active oogenesis (arrowhead, bottom). From Jarosch et al. (2011).

An additional challenge to Darwin's concept of evolution by slow steps is the finding that single alleles can have major life-history and population-level effects. *Optix* in *Heliconius* butterflies and *Ectodysplasin* in sticklebacks are two examples. A third involves the cape honeybee (*Apis mellifera capensis*; **Figure 19.19a**) from South Africa. In most honeybee colonies, a single queen reproduces. If the queen dies, the workers can lay eggs; but because the eggs are unfertilized, they yield only male (drone) offspring; the workers cannot re-queen the colony. This is due to bees' haplodiploid sex determination. Fertilized diploid eggs are female; unfertilized haploid eggs are male. The cape honeybee is an exception. Its female workers can asexually produce female eggs and hence re-queen a colony. When such a reproductively active *A. m. capensis* worker enters a colony of the ecologically dominant African honeybee (*A. m. scutellata*; **Figure 19.19a**), she starts laying female-determined eggs, and the local African honeybee workers treat her like a queen. The result is chaos: The cape honeybee's daughters themselves become pseudo-queens, and the colony collapses in disarray. Beekeepers have unwittingly spread this *A. m. capensis* social parasitism throughout southern Africa.

The genetic basis for these differences in reproductive life history is found in a gene orthologous to the *Drosophila* gene *gemini*, which encodes a CP2 family transcription factor involved in genital development and egg production (Jarosch et al. 2011). The *gemini* allele in *A. m. capensis* has a 9-nucleotide deletion, which results in a change in the protein products. All other African honeybee races and European honeybees so far examined have those nine nucleotides intact. The deletion seems to give honeybee workers more developed ovaries, the ability to give birth to queens, and a queen-like cuticle pheromone profile. Antje Jarosch and colleagues (2011) tested this hypothesis by feeding an RNA molecule

designed to mimic the effects of the deletion to European honeybees (*A. m. carnica*). Compared to controls, treated bees showed increased ovary development (Figure 19.19b). We thus have a case in which a 9-nucleotide deletion apparently changed the life history of the subspecies in which it arose, spread through wild populations, and altered their dynamics, producing ecological disruption.

The reason a single gene—*geminin*—is thought to have such a wide range of effects on bee reproduction, physiology, and behavior is that it (like the Hox genes and *optix*) encodes a transcription factor, which itself interacts with and regulates numerous other genes. Several other classes of genes also can have effects on multiple other genes and are additional candidates for evolution in leaps. Among them are morphogenetic hormones, which are known to orchestrate animal and plant life histories. We discussed abscisic acid (ABA) in mangroves earlier in the chapter. Other cases include alterations in thyroid hormone metabolism or expression underlying the evolution of alternate life histories in salamanders, frogs, and sea urchins; changes in ecdysteroid cellular responses underlying the evolution of larval reproduction in flies; and changes in juvenile hormone metabolism underlying many aspects of insect evolution, such as seasonal morphs in butterflies, horn morphology and mating strategies in dung beetles, and worker and soldier caste difference in ants. Such hormones are likely targets for evolution because small changes in their timing or mode of action can have profound effects on the timing of life cycles (heterochrony) and their morphological and behavioral outcomes (reviewed in Heyland et al. 2005).

In sum, while we cannot say for certain how frequent are jumps in evolution, we can be confident that they are neither impossible nor necessarily rare.

### The “5 Percent of a Wing Problem” and the Evo-Devo Solution

Although we have shown how findings in evo-devo have led to revision of some tenets of the modern synthesis, most work in the field has confirmed the basic concepts of descent with modification and the mechanisms of evolution. Indeed, some of the most important discoveries in evo-devo have helped address a persistent mystery in evolution, one that Stephen J. Gould (2002, p. 1220) called the “5 percent of a wing” problem: “How can evolution ever make a wing in Darwin’s gradualist and adaptationist mode if five percent of a wing can’t possibly provide any benefit for flight?” As with so many other puzzles in evolution, Darwin himself (1872, p. 148) offered a key suggestion: “Bear in mind the probability of conversion from one function to another.” Gould and Elizabeth Vrba (1982) coined the term **exaptation** to describe such conversions.

The proposed solution regarding wings is that the original small “proto-wings” were either adaptive for another function or even nonadaptive, but were not for flying. Their usefulness in flight came later as an exaptation. Joel Kingsolver and Mimi Koehl (1985) tested the hypothesis that the proto-wings of insects functioned in thermoregulation by building physical models on which they could vary the size of the wings at will. They found that even the slightest increase in wing size improves a model insect’s ability to regulate its body temperature. Once these proto-wings get big enough, Kingsolver and Koehl’s experimental models suggest that they begin to provide an aerodynamic function.

Although plausible, such conclusions remain tentative. We cannot go back in time and recreate the evolutionary history of wings. Nevertheless, the tools of evo-devo have allowed researchers to rigorously test some exaptation hypotheses, thus lending support to Darwin’s solution to the 5 percent of a wing problem.

Among the mechanisms for evolutionary leaps is mutation in the genes encoding transcription factors.

Novel structures sometimes evolve via change in function.

We can, for example, identify exaptations in protein function with some confidence. If 120 of 125 amino acids in two proteins are identical, likely the two are evolutionarily related—by either orthology or paralogy. With phylogenetic analysis and parsimony arguments, we can assess whether the function of a given protein has changed, and in what direction. An example appears in **Figure 19.20**.

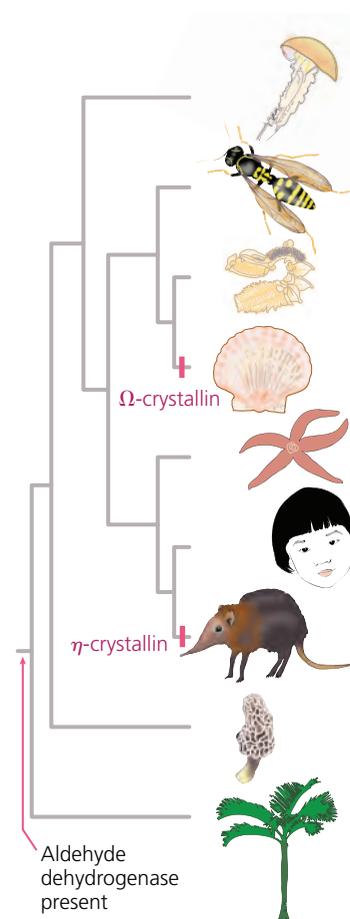
Lenses are a common feature of complex eyes, including the independently evolved eyes of cephalopods and vertebrates. In both groups, the lenses are made of long-lived cells that lack a nucleus and most other organelles and whose contents are transparent and stable. The major structural proteins of lens cells are called crystallins. Many of these crystallins are well-known functional enzymes involved in basic metabolism; they are merely enriched in the lens (Wistow and Piatgorsky 1987). So, for example, the major crystallin in bird lenses is also a functioning urea cycle enzyme in the liver, and the major crystallin in elephant shrews is also an alcohol detoxifying enzyme—aldehyde dehydrogenase.

Neither of these enzyme functions, which are ancient and hence predate the evolution of eyes, are relevant when the enzymes are expressed at such high levels in the lens. These genes were presumably exapted due to their solubility at high concentrations, optical transparency, and longevity. In some taxa, the enzyme crystallin genes have duplicated. One daughter gene specializes in the lens function (and has lost enzyme activity) while the other gene continues to perform the original enzymatic function. In this sense exaptation, followed by gene duplication and functional divergence, may be a common mechanism by which new protein functions are acquired in evolution.

Although the lenses of vertebrates and invertebrates evolved independently, the major crystallin in both scallops and elephant shrews is an aldehyde dehydrogenase (Figure 19.20; Graham et al. 1996; Piatgorsky et al. 2000). Is this just happenstance? Probably not. The total set of possible proteins that fulfill all of the functional requirements for lens crystallin function (solubility, transparency, longevity) is a fraction of the total diversity of proteins available. It would therefore be expected for evolution to repeat itself now and again.

This finding raises a recurring issue in research on development and evolution. If we see a similarity between two organisms in some aspect of development, how can we decide if that similarity is due to homology or homoplasy? This choice was straightforward for aldehyde dehydrogenase crystallins. The original function was clear, and the vast phylogenetic separation of shrews and scallops makes their independent origins all but certain. But what about other cases? Comparisons of vertebrates and invertebrates (often mouse and fly) reveal that similar genes are involved in heart development, appendage development, anterior–posterior and dorsal–ventral axis development, and eye development. These are basic processes found in many groups of animals. How can we determine their evolutionary histories, and hence judge between homology and homoplasy?

Eric Davidson (2001, pp. 189–190) notes that although the heads, hearts, appendages, and eyes in vertebrates and invertebrates look superficially similar, their anatomy and underlying developmental processes are quite different. Nonetheless, “Over and over the same transcriptional regulators are found to be used for what appear at least externally to be similar purposes.” We saw this in our discussion of the Hox paradox. It is perhaps the most unexpected finding of modern evo-devo. Anatomical structures that are classical examples of homoplasy—such as the octopus and the human eye—in fact use similar regulatory genes during their development.



**Figure 19.20 Ancestral and derived functions of proteins**

Aldehyde dehydrogenases are ancient enzymes that catalyze the oxidation of aldehydes in archaea, bacteria, and eukaryotes; their sequence similarities make it clear that they are evolutionarily related. Related (but not orthologous) aldehyde dehydrogenases have been independently recruited as major structural proteins—lens crystallins—in two divergent groups of animals: scallops and elephant shrews. In vertebrates, aldehyde dehydrogenases are enriched in the eyes, which has probably predisposed them for co-option as a lens crystallin in elephant shrews.

Davidson's resolution to this quandary involves exaptation (emphasis added):

However they are structured, brains must deploy neuronal differentiation programs, hearts need certain kinds of contractile cells; eyes need photo-receptor cells... So a possible solution to our paradox is that the regulatory genes which we find [for example in insect and vertebrate hearts] originally ran the differentiation gene batteries required for [heart] functions, and since these genes were expressed in the right place *they could be coopted during evolution to produce successively more elaborate pattern formation functions, differently in each clade.*

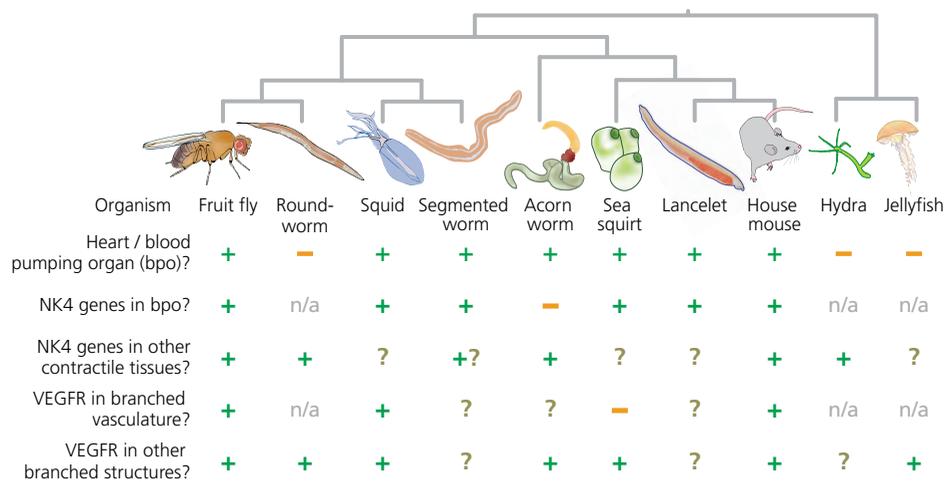
When Davidson speculates about the original functions of the regulatory genes in question, he is imagining their functions in the last common ancestor of flies and vertebrates, an organism that must have lived more than half a billion years ago. One possibility is that this ancient ancestor had a rudimentary heart, and the development of that ancestral heart was regulated by the regulatory genes in question. This first scenario suggests that despite the anatomical differences and different embryonic origins, vertebrate and insect hearts are in fact homologous; we can call this the "ancient heart" scenario.

Davidson suggests an alternative possibility, one that does not require overturning the classical concept that insect and vertebrate hearts evolved independently. While we do not know if the insect-vertebrate ancestor had a heart, we can predict that it had structures that undergo rhythmic, pulsatile contractions such as gut peristalsis; such functionality is found not only in the diverse descendants of this ancestor, but also in the rhythmically contractile structures in more ancient animal lineages such as jellyfish, sea anemones, and possibly even sponges.

Therefore, Davidson's alternative scenario suggests that the original function of these regulatory genes in the insect-vertebrate ancestor was a basic function in rhythmic contractility. Then, independently in vertebrates and insects, hearts evolved and came under the control of the same generic regulator of contractility. In other words, just as in the scallop and elephant shrew lens, the similar gene regulation of insect and vertebrate hearts would have evolved in parallel.

To distinguish the ancient heart versus parallel evolution scenarios, we need to look more deeply at vertebrate and insect hearts and the genes known to control their development. A key homeobox-class transcriptional regulator underlying vertebrate heart cell specification is *Nkx-2.5*, a gene in the NK4 class. This gene is expressed in mesoderm that gives rise to the heart as well as associated gut cells. *Nkx-2.5* mutants in mice actually start forming a normal heart, but defects arise later in the heart tube. Remarkably, one of the NK4 family genes in *Drosophila* fruit flies, *tinman*, is also involved in fruit fly heart formation, though in *tinman* mutant flies, the heart does not form at all (see Olson 2006 for a review).

Involvement of NK4 genes in heart development is not the only such similarity. Like most transcriptional regulators, NK4 genes are part of regulatory gene networks, which in the case of *Nkx-2.5* in vertebrates include genes for proteins such as two transcription factors known as MEF2 and GATA and a signaling molecule from the BMP family. Orthologs of these genes function in insect heart development as well. Furthermore, hearts are often associated more broadly with branched vascular structures, and vascular endothelial growth factor receptor (VEGFR) is used in vascular development in both insects and vertebrates. Indeed, family members of some of these same genes (NK4, MEF2, VEGFR) are expressed during the development of the heart, or heart-like organs, and the associated vasculature, in other animal groups as well. These groups include squids,



**Figure 19.21 NK4 and VEGFR: ancient heart or parallel evolution?** Diagram shows relationships among animals with and without hearts or blood pumping organs (bpo), plus the correlation of NK4 and VEGFR genes with bpo and associated vasculature, and their underlying developmental processes. In all examined cases, NK4 expression is associated broadly with contractile structures, and VEGFR expression with diverse branching structures, from neuronal arborizations to cellular filopodial extensions.

lancelets, and annelids (Figure 19.21; Yoshida et al. 2010)—all of which share the same ancestor that insects share with vertebrates.

All of the animals just mentioned have hearts or blood-pumping organs: rhythmically contractile structures that drive blood circulation in circulatory systems that are open (insect) or closed (lancelets, squid, annelid, vertebrate). Davidson’s parallel evolution scenario suggests that the commonality is the ancient regulation of rhythmic contractility. Can we imagine evidence in modern organisms that would allow us to confirm or refute the parallel evolution scenario?

The roundworm *Caenorhabditis elegans* is another descendant of the same insect-vertebrate ancestor. It has no heart, but it does have an NK4 gene that functions in the development of the pharynx—a rhythmically contractile structure involved in digestion (Okkema et al. 1997). The hemichordate acorn worm has a heart-like contractile cardiac vesicle; its NK4 gene is not expressed there, but is again associated with pharynx development (Lowe et al. 2006). Still, if we want to infer the original function of NK4 in the insect-vertebrate (“bilaterian”) ancestor, it would be best to have data on groups that diverged before the bilaterian ancestor appeared. Such data exist for the cnidarian *Hydra magnipapillata*. *Hydra* has no heart, but once again, its NK4-class gene is expressed in a contractile pharynx-like structure near the base of the stalk (Shimizu and Fujisawa 2003).

What about VEGFR? Its expression is also known from a jellyfish *Podocoryne carnea*. Jellyfish VEGFR is expressed in the branches of the digestive system (which also serves as a kind of vascular system) that extend into the tentacles. In roundworms, VEGFR is involved in chemosensory neuron branching. This unexpected finding has prompted a search for additional functions of VEGFR in other animals, and VEGFR orthologs have been found to be involved in neuronal path finding in vertebrates, in the branched circulatory system connecting individuals in a colonial sea squirt, and in tubular extensions in specialized “border cells” in fruit fly oocytes (reviewed in Ponnambalam and Alberghina 2011).

Data on more phyla with and without hearts would be useful, but based on available information (Figure 19.21) it seems likely that NK4 genes originally were involved in development of a rhythmically contractile structure, like the pharynx, and later exapted for the development of hearts and heart-like pumping organs. The lack of expression of NK4 in the simple hemichordate heart-like organ could be explained by loss of NK4 from heart-like development in hemichordates, or it could indicate the independent exaptation of NK4 genes for heart

development in protostomes and deuterostomes. Data on NK4 and its network from a greater diversity of animals are needed. In particular, examining comb jellies (phylum Ctenophora) would be edifying. Like cnidarians, comb jellies evolved before the bilaterian ancestor. However, unlike cnidarians, comb jellies have more extensive mesoderm-like structures, and bilaterian hearts and blood pumps are mesodermal. As for VEGFR, the common denominator of all the invertebrate and vertebrate data to date is that this signaling system is specialized for formation of highly branched structures, whether neuronal, gastric, or vascular.

Thus the emerging details of the development and evolution of animal hearts is consistent with Davidson's parallel evolution scenario for similar gene regulation underlying dissimilar structures.

Can such findings help clarify the 5 percent of a wing problem? Remember that a transcription factor merely regulates the transcription of other genes. It is like the foreman of a construction crew. The foreman does not build anything, but coordinates the work of the carpenters, plumbers, and electricians. If we need a new building, we just contact the foreman—who brings along the whole team. If the parallel evolution scenario is correct, then in the course of evolution organisms did not have to completely reinvent the process of forming a fluid-pumping organ or a highly branched structure in every case. They simply may have recruited the NK gene, which brought along a gene network for constructing the pumping organ, and the VEGFR gene, which brought along a network for constructing branched structures. The exaptation of preexisting gene regulatory networks is an efficient way to evolve a complex structure.

These examples remind us that when discussing homology and homoplasy, we have to be clear about the hierarchical level. The comparative data on NK4 genes do not indicate that the roundworm pharynx is homologous to the vertebrate heart. They merely suggest that homologous transcription factors (and likely their associated gene regulatory networks) are used in the processes that underlie the formation of two similar, though nonhomologous fluid-pumping organs.

Evolutionary changes in the function of regulatory gene networks is one explanation for the deeper homology of developmental mechanisms versus the structures they control.

## 19.4 Hox Redux: Homology or Homoplasy?

Recall that we left our earlier discussion of Hox genes with the observation that the two principal commonalities across most bilaterally symmetric animals were spatial colinearity of Hox gene expression patterns along the main body axis and association of Hox genes with the nervous system. We also noted that in several different animal groups (sea anemones, lancelets, vertebrates, insects), expansions in the Hox gene cluster resulted in newly evolved genes that maintained spatial colinearity. This pattern suggests that spatial colinearity is a generic outcome of Hox gene clustering, a finding that has gained support in observations of the crystal structures of Hox clusters in different parts of the mouse embryo undergoing active transcription (Noordermeer et al. 2011).

Nevertheless, we described many examples where inverted, split, or even atomized clusters retained the canonical spatial expression pattern. These latter findings suggest that additional buffering mechanisms, unrelated to the clustering per se, have evolved repeatedly across animals to ensure proper spatial expression of these genes. And finally, the surprising absence of any significant function in sea squirt Hox genes indicates that the highly buffered expression patterns of Hox genes may be more fundamental than the axial functions themselves.

Furthermore, the canonical spatial expression patterns of Hox genes are found in three cases that do not involve anterior–posterior axial patterning: in a coelomic compartment in sea urchins and sea lilies (Echinodermata); along the dorsal–ventral axis in sea anemones (Cnidaria); and in vertebrates along the limb axis, in the urogenital system, and in the gut. The vertebrate and echinoderm examples seem to be exaptations and suggest that Hox genes are ideal candidates to co-opt for regionalization along an alternate axis or an internal structure.

We are now equipped to address one of the hypotheses of Hox gene evolution: Does the strikingly similar expression and even function of Hox genes in conferring segmental identity in arthropods and chordates suggest that segmentation itself is homologous in these two groups, and thus that the common ancestor of the Bilateria was segmented? Instead of relying on broad, cross-phyllum comparisons, we can address this question by looking at more closely related groups.

Although the definition of segmentation is disputed, a common one is “internal and external repetition of body structures and organs along the main body axis.” Velvet worms are now accepted as the closest living relatives to the arthropods; but unlike arthropods, velvet worms are not segmented according to this definition. Their morphology suggests two alternative scenarios. Either the arthropod–velvet worm ancestor was not segmented, and segmentation arose during the early evolution of arthropods, or the arthropod–velvet worm ancestor was segmented, and segmentation was lost in the velvet worm lineage.

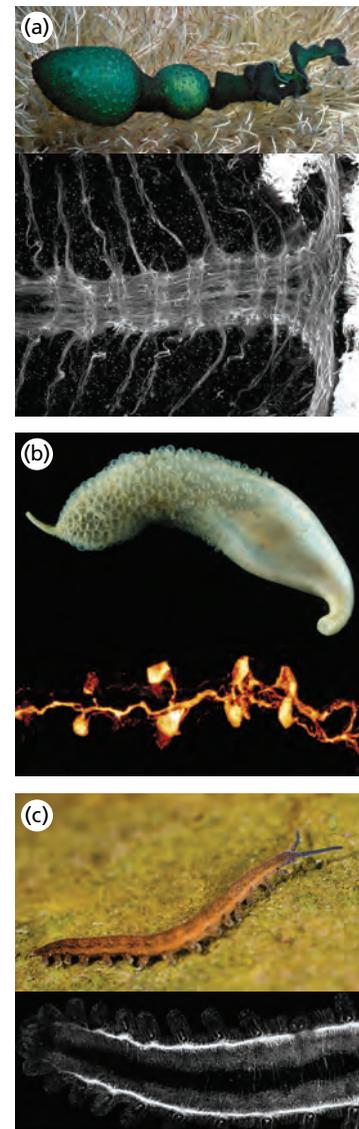
Often when a character is lost it leaves a remnant, some trace of its existence—like the hindlimb bones in a whale. We now have evidence of two cases where segmentation was lost in animals. Two groups of unsegmented worms—spoon worms (echiurans) and peanut worms (sipunculans)—appear to be derived from within the phylum of segmented annelid worms (Struck et al. 2007). If so, these two lineages must have lost segmentation sometime in their evolutionary history. Indeed, though they show no external signs of segmentation, the embryonic nervous systems of spoon worms and peanut worms are still segmented (Hessling 2002, 2003; Kristof et al. 2008): a clear vestige of their segmental past (Figure 19.22a, b).

What about velvet worms? Unlike spoon and peanut worms, velvet worms show virtually no indication of segmentation in their developing nervous system, musculature, or other internal structures (Figure 19.22c; Mayer and Whittington 2009; Whittington and Mayer 2011). This evidence supports the notion that the arthropod–velvet worm ancestor was not segmented, and thus that segmentation arose independently in arthropods and vertebrates, as well as annelids.

What are the implications for Hox gene evolution? If segmentation in arthropods and chordates is an example of homoplasy, then the similar function of Hox genes in regulating segmental identity in vertebrates and insects is an example of parallel evolution, perhaps exapted from a regionalized expression of Hox genes in the central nervous system of some worm-like bilaterian ancestor.

## 19.5 The Future of Evo-Devo

Recent decades have been exciting for evo-devo. We have gained profound insights into evolution through the application of developmental biology approaches and techniques. In particular, insights into evolution have come from studying the development of an ever-widening range of organisms. Less common



**Figure 19.22 Vestigial nervous system segmentation** In (a) spoon worms (echiurans) and (b) peanut worms (sipunculans), close examination of the nervous systems reveals vestiges of segmentation. Not so with (c) velvet worms (onychophora), which are also unsegmented but have repetitive appendages along their body. Examination of their nervous system reveals no clear vestiges of segmentation. Nervous system photo (a) by Rene Hessling; see Hessling (2002, 2003). Nervous system photos (b) and (c) from Kristof et al. (2008) and Mayer and Whittington (2009).

has been the adoption of explicit comparative approaches, where evolutionary questions are framed and studied with carefully chosen taxa and independent contrasts, so that the generality of the conclusions can be assessed.

Thus far only two of the great multicellular taxa, animals and plants, have been studied to any appreciable degree. Although some single-celled organisms can be said to undergo a type of development during their life cycle, each appearance of multicellularity clearly involves the unique origin of a higher level of developmental complexity. But multicellularity, and hence complex developmental processes, arose only once each in plants and animals, so we are at risk of over-concentrating on provincial aspects of these two developmental systems. For full appreciation of how development shapes evolution, we need to explore the other great multicellular taxa—kelp, fungi, red algae, and green algae—some of which, themselves, show multiple origins of multicellularity and hence complex developmental processes. Because these poorly studied multicellular groups are important in global ecosystems, we have additional impetus to expand our horizons to include these taxa.

The integration of fields should extend beyond development and evolution. Although much of evo-devo focuses on gene function, few practitioners are well trained in biochemistry. To fully understand developmental biases in evolution, we need to better incorporate biochemistry. Furthermore, the rapid explosion in sequence information has spawned great advances in systems biology and network modeling. Ultimately, our understanding of organic evolution will have to synthesize all of these approaches. As such, evo-devo is really only a signpost on the road to a fully integrated biology. We thus welcome the day when we can drop the *devo* and fulfill Darwin's vision by calling it simply evolution.

## SUMMARY

Darwin and his contemporaries recognized the intimate relationship between evolution and development, but Darwin's writings lacked a satisfying genetic mechanism. The rediscovery of Mendelian genetics in the 20th century led to the modern evolutionary synthesis, which excluded consideration of developmental biology. The explosion in molecular and genetic understanding since the late 20th century has heralded a reconciliation under the auspices of evo-devo.

The discovery of Hox genes across animals brought excitement, but Hox studies have often remained focused on commonalities among animals instead of explaining diversity. Indeed, the Hox gene story is more complex than originally thought, and it still yields interesting evolutionary insights.

Since the initial Hox discoveries, the field of evo-devo has matured and expanded, leading to reconsideration of these pillars of Darwinian thought: the predominance of gradual change in evolution, the near ubiquity of natural selection as the predominant ex-

planation for life's diversity, and the notion that variation is ever present and unbiased. Evo-devo has not overturned these concepts but has elevated additional perspectives, such as mutations of large effect, and the surprising commonality of homoplasy as indicative of biases or constraints in evolution.

Nevertheless, most work in evo-devo has provided additional evidence and details about the functioning of evolution, quite in line with Darwinian thinking. For example, the multiple findings of co-option and exaptation in the origin of new and perhaps novel features of organisms were explicitly predicted by Darwin.

Evo-devo continues to yield surprising insights, such as the counterintuitive findings that expression patterns might be more stable evolutionarily than their canonical functions, as seen in the Hox genes and in insect segmentation.

That evo-devo remains a separate discipline speaks to the need to fully integrate developmental biology into evolutionary thinking.

## QUESTIONS

1. Why did the evolutionary synthesis not include developmental biology? What discoveries initiated the reconciliation of development and evolution?
2. Can evolution proceed in jumps? Give examples to support your answer.
3. What is the canonical Hox gene expression pattern. Is it maintained when the Hox genes are not found in a single cluster? What is the evidence?
4. How did Darwin explain the “5% of a wing problem”? Was his explanation correct? On what evidence?
5. In what sense are the lens crystallins of elephant shrews and scallops homologous? In what sense are they homoplasious? What about the red spots on the wings of *Heliconius melpomene xenoclea* and *H. erao microclea*?
6. Define exaptation and give an example. How do you know the trait you chose involved a change in function? Can you identify exaptations in your own body?
7. Do biases in developmental pathways limit evolutionary possibilities? How can this hypothesis be tested?
8. What is the Hox paradox? Can you suggest a solution?
9. Did the common ancestor of bilateral animals have a heart? Justify your answer by drawing an evolutionary tree and mapping hearts on it.
10. Do you think it would be possible, with artificial selection, to breed fully-armored freshwater sticklebacks that grow fast? Why or why not?
11. Why has it been useful to study Hox genes in many taxa? What has it suggested about their original function?

## EXPLORING THE LITERATURE

12. Which came first, gene expression patterns or the complex structures they regulate? We discussed segmental differentiation along the anterior–posterior body axis, where the evidence suggests that expression patterns came first. But we must beware of hasty conclusions. Segmental gene expression patterns are seen in the unsegmented limbs of velvet worms as well as the segmented limbs of their sister group, the arthropods, suggesting that expression patterns came first. But recent fossil evidence shows that some ancient lobopods—presumed ancestors of velvet worms—had segmented limbs (though not segmented bodies). Thus the segmental gene expression pattern in extant velvet worm non-segmented limbs was probably inherited from a lobopod ancestor that had segmented limbs after all. See: Janssen, R., B. J. Eriksson, et al. 2010. Gene expression patterns in an onychophoran reveal that regionalization predates limb segmentation in pan-arthropods. *Evolution and Development* 12: 363–372.  
Liu, J., M. Steiner, et al. 2011. An armoured Cambrian lobopodian from China with arthropod-like appendages. *Nature* 470: 526–530.  
For analysis of another example, see:  
Pani, A. M., E. E. Mullarkey, et al. 2012. Ancient deuterostome origins of vertebrate brain signalling centres. *Nature* 483: 289–294.
13. One might suppose that the major transitions in evolution would have been accompanied by an increase in genetic complexity. For example, the appearance of practically all modern animal phyla in the Cambrian explosion over half a billion years ago was often assumed to have been accompanied by an explosion in genetic complexity. Was it? See: Domazet-Loso, T., J. Brajkovic, and D. Tautz. 2007. A phylostratigraphic approach to uncover the genomic history of major adaptations in metazoan lineages. *Trends in Genetics* 23: 533–539.
14. Although complex social behavior apparently only evolved once in an ancestor of modern ants, the division of labor into multiple worker castes has arisen independently in different ant lineages. An extreme example is the origin of specialized “supersoldier” ants with large bodies and powerful jaws. Not only have two independent origins of supersoldiers followed parallel modifications in hormonal regulation during soldier ant larval and pupal development, but related ants without supersoldiers can be induced to make them if these same hormone regulation pathways are artificially altered in their larvae. See: Rajakumar, R., D. S. Mauro, et al. 2012. Ancestral developmental potential facilitates parallel evolution in ants. *Science* 335: 79–82.
15. For experimental evidence on the involvement of a Hox gene in the evolution of tetrapod limbs from fish fins, see: Freitas, R., C. Gómez-Marín, et al. 2012. *Hoxd13* contribution to the evolution of vertebrate appendages. *Developmental Cell* 23: 1219–1229.  
Schneider, I., and N. Shubin. 2012. Making limbs from fins. *Developmental Cell* 23: 1121–1122.
16. Stomata (epidermal pores) in plants are a possible example of an evolutionary module—a quasi-separable entity within a multicellular organism that is a potential target for evolutionary change. For a review of the diversity, origin, and loss of stomata in land plants, the developmental-genetic control of stomata in thale cress (*Arabidopsis*), and comparative data on how variants in stomata are generated, see: Vátén, A., and D. C. Bergmann. 2012. Mechanisms of stomatal development: An evolutionary view. *EvoDevo* 3: 11.

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