20 Evolutionary Developmental Biology

Evolutionary developmental biology, now often known as "evo-devo," is the study of the relation between evolution and development. The relation between evolution and development has been the subject of research for many years, and the chapter begins by looking at some classic ideas. However, the subject has been transformed in recent years as the genes that control development have begun to be identified. This chapter looks at how changes in these developmental genes, such as changes in their spatial or temporal expression in the embryo, are associated with changes in adult morphology. The origin of a set of genes controlling development may have opened up new and more flexible ways in which evolution could occur: life may have become more "evolvable."

20.1 Changes in development, and the genes controlling development, underlie morphological evolution

Morphological evolution is driven by developmental evolution

Morphological structures, such as heads, legs, and tails, are produced in each individual organism by development. The organism begins life as a single cell. The organism grows by cell division, and the various cell types (bone cells, skin cells, and so on) are produced by differentiation within dividing cell lines. When one species evolves into another, with a changed morphological form, the developmental process must have changed too. If the descendant species has longer legs, it is because the developmental process that produces legs has been accelerated, or extended over time. Evolutionary changes in development, and developmental genetics, are the mechanism of all (or almost all) evolutionary change in morphology. We need to understand developmental evolution in order to understand morphological evolution. The same need not be said of molecular or chromosomal evolution: we do not need to study development in order to study molecular and chromosomal evolution. Some other kinds of evolution, such as behavioral evolution, can also have a developmental basis. But this chapter concentrates on the developmental basis of morphological evolution.

Biologists have recognized since the nineteenth century that development is the key to understanding morphological evolution. In the past 10–15 years, a new field of research has grown up. Many genes that control development have now been identified, and molecular techniques can be used to study how those genes have changed between species. The new field is often called by the informal term "evo-devo." In this chapter we shall look briefly at some older theories about developmental change and morphological evolution. We then look in more detail at some examples of modern "evo-devo" research. The ancient and modern research is imperfectly integrated because modern genetics has not yet identified the genes that underlie the structures and organs that were studied in earlier work. However, we can see how modern ideas can be used in an abstract way to explain earlier observations. The aim of all the research, from the nineteenth century to today, is to use a knowledge of development to explain how morphological evolution proceeds.

20.2 The theory of recapitulation is a classic idea (largely discredited) about the relation between development and evolution

Recapitulation is a bold and influential idea that is particularly associated with Ernst Haeckel (Section 1.3.3, p. 12) though many other biologists also supported it in the nineteenth and early twentieth centuries.

According to the theory of recapitulation, the stages of an organism's development correspond to the species' phylogenetic history: in a phrase, "ontogeny recapitulates phylogeny." Each stage in development corresponds to (that is, "recapitulates") an ancestral stage in the evolutionary history of the species. The transitory appearance of structures resembling gill slits in the development of humans, and other mammals, is a

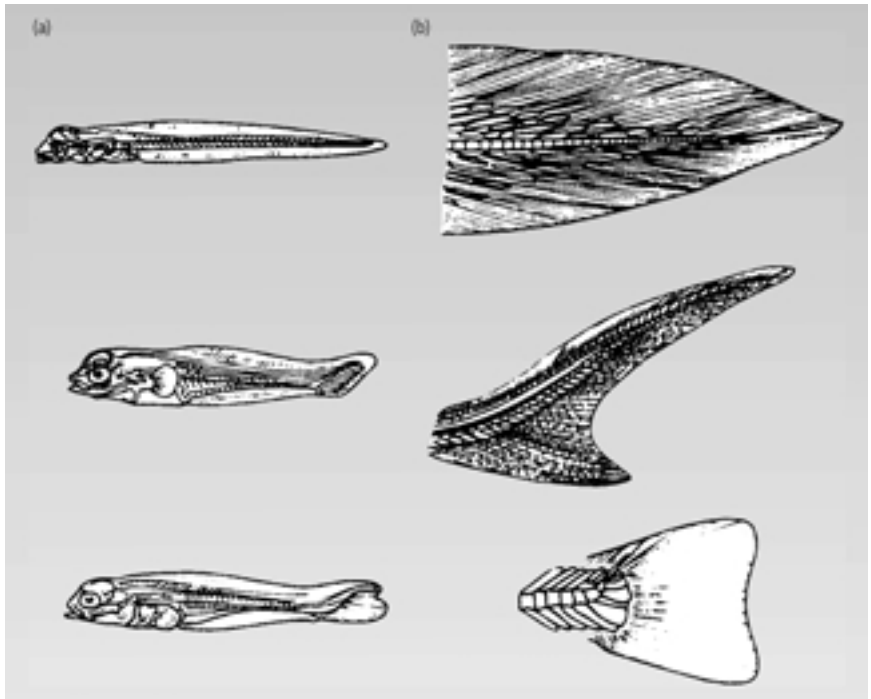

Figure 20.1
Recapitulation, illustrated by fish tails. (a) The development of a modern teleost, the flatfish *Pleuronectes*, passes through (starting at the top) a diphycercal stage, to a stage in which the upper lobe of the tail is larger (heterocercal), to the adult, which has a tail with equal-sized lobes (homocercal). (b) Adult forms in order of evolution of tail form, from top to bottom: lungfish (diphycercal), sturgeon (heterocercal), and salmon (homocercal). Reprinted, by permission of the publisher, from Gould (1977a).

striking example. Mammals evolved from an ancestral fish stage and their embryonic gill slits recapitulate the piscine ancestry.

Another example, often quoted in the nineteenth century, is seen in the tail shapes of fish (Figure 20.1). During the development of an individual, evolutionarily advanced fish species, such as the flatfish *Pleuronectes*, the tail has a diphycercal stage in the larva. It then develops through a heterocercal stage, to the homocercal form of the adult. However, not all fish have homocercal tails in the adult. Indeed fish species can be found with all three kinds of tail in the adult. The lungfish, sturgeon, and salmon in Figure 20.1b are examples. The lungfish is thought to most resemble an early fish, the sturgeon to be a later stage, and the salmon to be the most recently evolved form. Thus evolution has proceeded by adding on successive new stages to the end of development. We can symbolize the diphycercal, heterocercal, and homocercal tails by A, B, and C, respectively. The development of the early fish advanced to stage A and then stopped. Then, in evolution, a new stage was added on to the end: the development of the fish at the second stage was A → B. The final type of development was A → B → C. Gould (1977a) named this mode of evolution *terminal addition* (Figure 20.2a).

Recapitulation results from evolution by terminal addition

When evolution proceeds by terminal addition, recapitulation is the result. An individual at the final evolutionary stage in Figure 20.2a grows up through stages A, B, and C, recapitulating the evolutionary history of the ancestral adult forms. However, evolution does not always proceed by terminal additions. We can distinguish two kinds of exception. One is that new, or modified, characters can be intruded at earlier

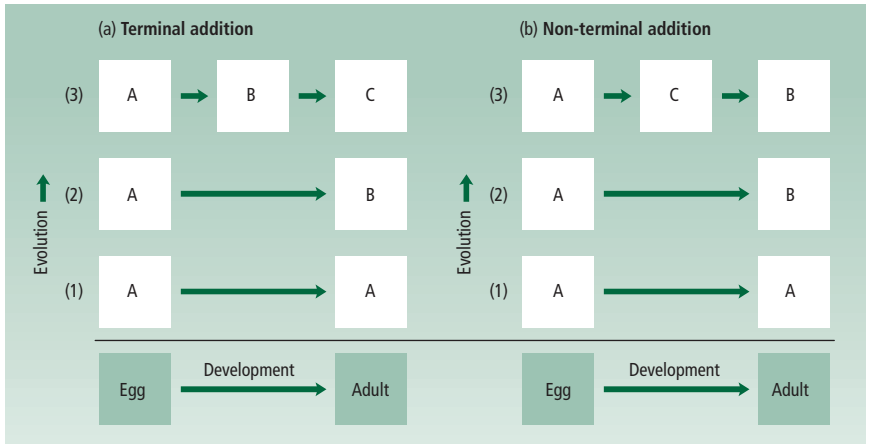

Figure 20.2
(a) Evolution by terminal addition. The stages in an individual's development are symbolized by alphabetic letters. (1), (2), and (3) up the page represent three successive evolutionary stages. With terminal addition, new stages are added only to the end of the life cycle. (b) Evolution by non-terminal addition. A new evolutionary stage has been added in early development, not on to the end of the life cycle in the adult.

developmental stages (Figure 20.2b). Many specialized larval forms are not recapitulated ancestral stages (for example, the zoea of crabs, the Müller's larva of echinoderms, and the caterpillar of Lepidoptera). They probably evolved by modification of the larva, rather than by adding on a new stage in the adult.

The time of maturity may shift to an earlier developmental stage . . .

The second kind of exception arises when the members of a species evolve to reproduce at an earlier developmental stage. We need to distinguish the rate of reproductive development from the rate of somatic development. (The somatic cells make up all the cells in the body except the reproductive cells.) Somatic development proceeds through a series of stages, from egg to adult. If the organism becomes reproductively mature at an earlier stage, then its development will not fully recapitulate its ancestry. Its ancestral adult form has been lost. Reproduction in what was ancestrally a juvenile form is called *pedomorphosis*. Pedomorphosis can arise in two ways (Figure 20.3). One is *neoteny*, where somatic development slows down in absolute time, while reproduction development proceeds at the same rate. The other is *progenesis*, where reproductive development accelerates while somatic development proceeds at a constant rate.

. . . and axolotls are examples

Among modern species, the classic example of neoteny is the Mexican axolotl, *Ambystoma mexicanum*. The axolotl is an aquatic salamander. (Actually, we should say "axolotls" because there are a number of types, and Shaffer's (1984) fine-scale genetic work has shown that the kind of larval reproduction described below has evolved many times, independently, even within what appears to be one species.) Most salamanders have an aquatic larval stage that breathes through gills; the larva later emerges from the water as a metamorphosed terrestrial adult form, with lungs instead of gills. The Mexican axolotl, however, remains in the water all its life and retains its external gills for respiration. It reproduces while it has this juvenile morphology. However, a Mexican axolotl can be made to grow up into a conventional adult salamander by a simple treatment (it can be done, for instance, by injection of thyroid extract). This strongly suggests that the timing of reproduction has moved earlier in development during the axolotl's evolution. Otherwise there would be no reason for it to possess all the unexpressed adaptive information of the terrestrial adult.

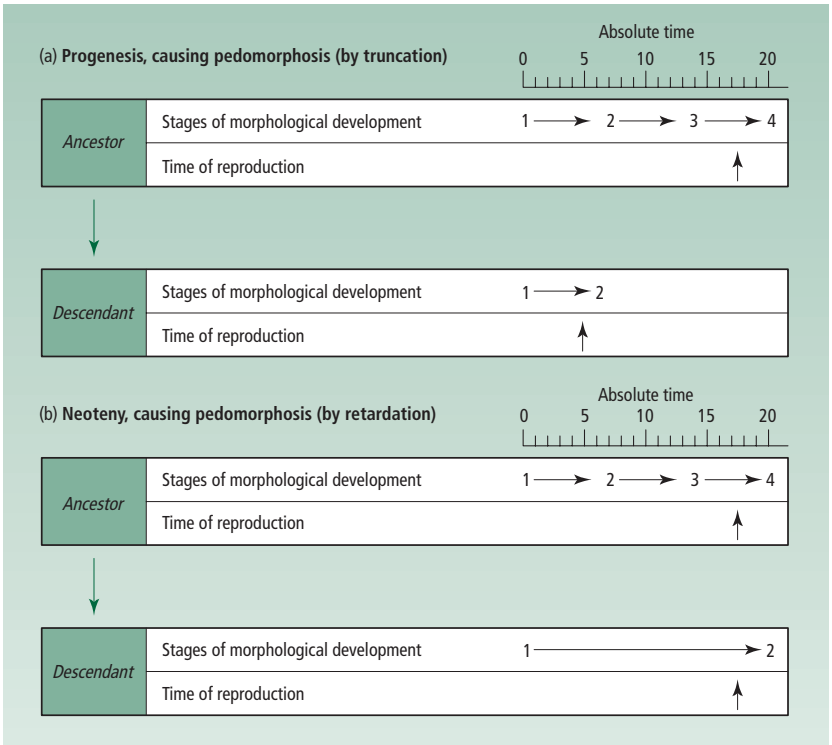

Figure 20.3
Pedomorphosis, in which a descendant species reproduces at a morphological stage that was juvenile in its ancestors, can be caused by (a) progenesis, in which reproduction is earlier in absolute time, or (b) neoteny, in which reproduction is at the same age but somatic development has slowed down.

Pedomorphosis can evolve in two ways

So the Mexican axolotl is pedomorphic — but is it neotenous or progenetic? Its age of breeding (and the body size at which it breeds) is not abnormally early (or small) for a salamander. Its time of reproduction has therefore probably stayed roughly constant, while somatic development has slowed down. The axolotl is an example of neoteny. Humans have also been argued to be neotenous. As adults, we are morphologically similar to the juvenile forms of great apes. This pedomorphosis, if it is real (and there is a serious argument that it is not), would be neotenous rather than progenetic because our age of breeding has not shifted earlier relative to other apes. Our age of first breeding is actually later than other apes. Our somatic development has not simply slowed down while reproductive development has stayed the same. What might have happened was that our somatic development slowed down even more than our reproductive development.

In summary, Haeckel and others initially suggested that evolution almost always proceeds in one mode. Changes are made only in the adult, and new stages are added on to the end of the existing developmental sequence. Through the 1920s, biologists come to accept a broader view. Evolution does often proceed by terminal addition, and recapitulation results. But other developmental stages can also be modified, and the timing of reproductive and somatic development may be altered in any way — some of which result in recapitulation, and others which result in pedomorphosis (Table 20.1).

The changes that we have been considering in the relative rate of somatic and reproductive development are one example of an important general concept: *heterochrony*.

Table 20.1
Categories of heterochrony. In modern work, the term pedomorphosis is sometimes substituted for recapitulation. From Gould (1977a). © 1977 President and Fellows of Harvard College.

Developmental timing			
Somatic features	**Reproductive organs**	**Name of evolutionary result**	**Morphological process**
Accelerated	Unchanged	Acceleration	Recapitulation (by acceleration)
Unchanged	Accelerated	Progenesis	Pedomorphosis (by truncation)
Retarded	Unchanged	Neoteny	Pedomorphosis (by retardation)
Unchanged	Retarded	Hypermorphosis	Recapitulation (by prolongation)

Developmental change can be by heterochrony

Heterochrony refers to all cases in which the timing or rate of one developmental process in the body changes during evolution relative to the rate of another developmental process. In progenesis, neoteny, and so on (Table 20.1) the rate of reproductive development is sped up or slowed down relative to the rate of somatic development.

Heterochrony is a more general concept, however. It also refers to changes in the development of one somatic cell line relative to another. Consider, for example, a *D'Arcy Thompson transformation* (Figure 20.4). D'Arcy Thompson (1942) found that related species superficially looking very different could in some cases be represented as simple Cartesian transformations of one another. We met the most thoroughly worked out modern example in an earlier chapter (Raup's analysis of snail shell shapes: Figure 10.9, p. 278). With some simplification, the axes on the fish grids in Figure 20.4 or the snails of Figure 10.10 can be thought of as growth gradients. The evolutionary change between the species would then have been produced by a genetic change in the rates of growth in different parts of the fish's body.

Apparently complex change may have a simple basis

One general point is that evolutionary changes between species may be simpler than we might at first think. If we looked at, for example, *Scarus* and *Pomacanthus* without the grids of Figure 20.4 we might think that an evolutionary change from one into the other would be at least moderately complicated. The interest of D'Arcy Thompson's diagrams is then to show that shape changes could have been produced by simple regulatory changes in growth gradients. The more specific point here is that changes in the growth gradients of different parts of the body are further examples of heterochrony. Evolutionary changes in morphology are often produced by changes in the relative rates of different developmental processes: that is by heterochrony. Heterochrony also explains evolutionary changes in allometry, which we looked at in Section 10.7.3, (p. 279).

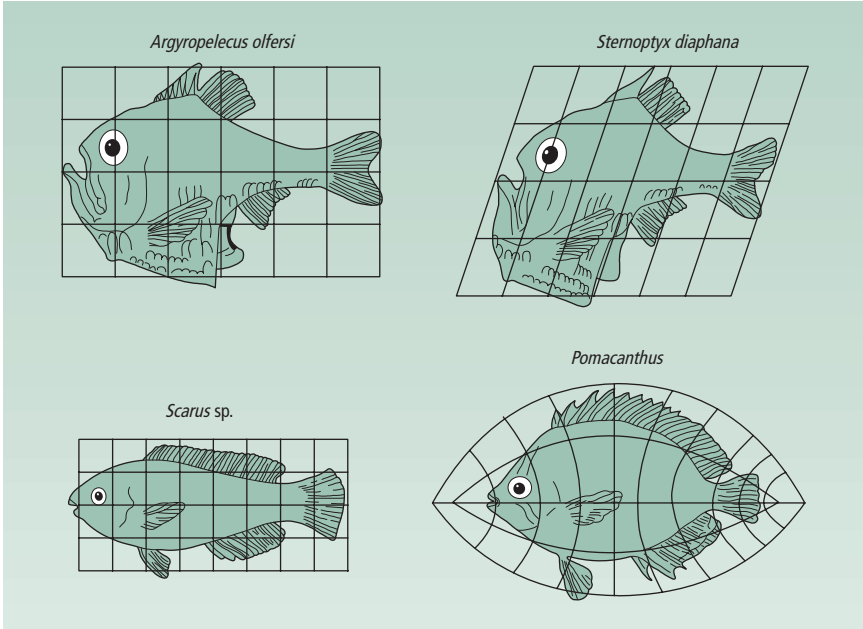

Figure 20.4
A D'Arcy Thompson transformational diagram. The shapes of two species of fish have been plotted on Cartesian grids. *Argyropelecus olfersi* could have evolved from *Sternoptyx diaphana* by changes in growth patterns corresponding to the distortions of the axes, or the direction of evolution could have been in the other direction, or they could have evolved from a common ancestral species. Likewise for *Scarus* and *Pomacanthus*. Reprinted, by permission of the publisher, from Thompson (1942).

20.3 Humans may have evolved from ancestral apes by changes in regulatory genes

Biologists distinguish between regulatory genes and structural genes. Structural genes code for enzymes, building block proteins, and transport and defensive proteins. Regulatory genes code for molecules that regulate the expression of other genes (whether structural or regulatory). The distinction is imperfect, but can be used to make a point about evolution.

Britton and Davidson wrote some influential early papers in which they suggested that reorganizations within the genome's regulatory gene pathways could cause important evolutionary changes (for instance, Britton & Davidson 1971). King & Wilson (1975) then applied this general perspective to a striking example: human evolution. King and Wilson used several techniques to infer that the DNA of humans and chimpanzees is almost identical. Later work has supported their conclusion that only about 1.5% of nucleotide sites differ between human and chimpanzee DNA.[1] And yet, to our eyes, humans and chimpanzees are phenotypically very different. Human bodies have been redesigned for upright walking, human jaws have become shorter and weaker,

Humans and chimps are more similar genetically . . .

[1] See Figure 15.12 (p. 422). As also noted near that figure in Chapter 15, Britton (2002) has recently revised the percent similarity between human and chimpanzee DNA to more like 95%, after allowing for insertions and deletions. About 1.5% of nucleotide sites show substitutions, and another 3.5% of sites differ because of insertions or deletions. However, King and Wilson's essential argument is unaltered.

. . . than might be expected from morphology

and human brains have expanded, and we have acquired the use of language. In human evolution, a large phenotypic change appears to have been produced by a small genetic change. King and Wilson hypothesized that most of the genetic changes of human evolution were in regulatory genes. A small change in gene regulation might achieve a large phenotypic effect. We shall not know what genetic changes occurred in human evolution until we have (and understand) the genome sequences for chimpanzees and some other apes, as well as for human beings. But King and Wilson's hypothesis remains a popular idea about human evolution.

20.4 Many genes that regulate development have been identified recently

A long list of genes that operate during development is now known, and the list is rapidly expanding. The genes fall into two main categories: genes that code for transcription factors and genes that code for signaling proteins (Figure 20.5). Transcription factors are molecules that bind *enhancers*. An enhancer is a stretch of DNA that can switch on a specific gene. Signaling proteins function in the cell's control pathways for switching specific genes on and off. For instance, a receptor protein in the cell membrane might change shape when bound by a hormone. The shape change might trigger further molecular changes in the cell, ultimately leading to the release of a transcription factor that switches on a specific gene. The protein in the cell membrane, or any other problem in the chain of reactions, would be an example of a signaling protein. Almost all the genes discussed in this chapter are transcription factors. The *Hox* genes, for

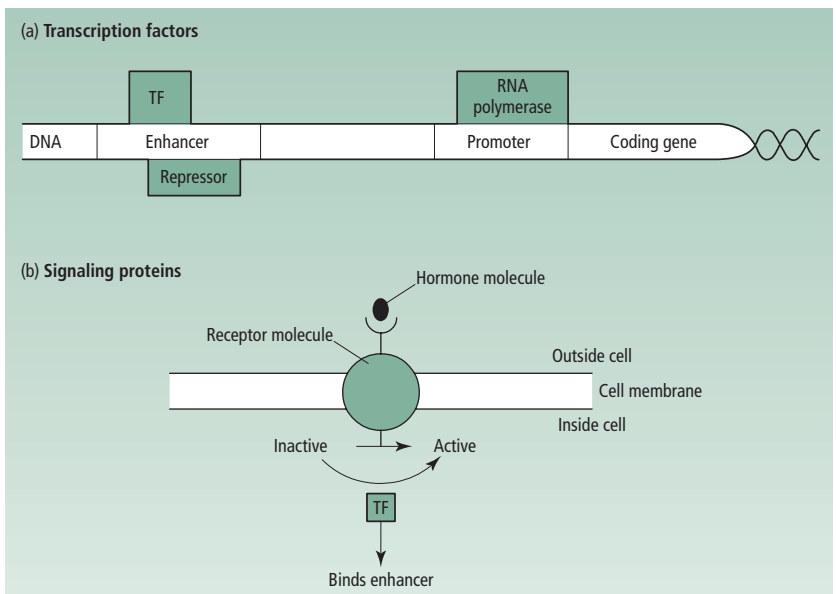

Figure 20.5
There are two main classes of developmentally influential genes. (a) Transcription factors (TF) that bind enhancers, which can switch genes on or off. The state of the enhancer determines whether RNA polymerase binds the promotor. The binding of RNA polymerase to the promotor is the first step in the transcription of a gene. A stretch of DNA may exist between the enhancer and promotor. (b) Signaling proteins. A signaling pathway in the cell may lead from a receptor molecule in the cell membrane, ultimately to a transcription factor which can be active or inactive. When the transcription factor is activated, it can switch a gene on by the process shown in (a). Many proteins may be able to interact with a receptor protein in the control of cellular metabolism: all such molecules are (provided they are proteins) examples of signaling proteins. Also, receptor proteins may be bound by molecules other than those conventionally classified as hormones. From Carroll *et al.* (2001).

example, as well as such genes in fruitflies as *distal-less*, *eyeless*, and *engrailed* all code for transcription factors. However, other developmental genes, such as the genes in fruitflies called *hedgehog*, *notch*, and *wingless*, are signaling proteins, and most of the points of principle that we look at for transcription factors would also apply for signaling proteins.

The genes that regulate development are best understood in two species, the mouse and the fruitfly. However, geneticists have looked for the same genes in other species and their findings have led to an important generalization. All animals seem to use much the same set of genes to control development. For example, the *Hox* genes were first studied in fruitflies. After the genes were cloned it was possible to look for them in other species too, and they were duly found in every other animal taxon. The *Hox* genes have similar functions in all animals. They act as region-specific selector genes. The basic map coordinates of the early embryo are set out by another set of genes. Then, during development, specific sets of genes are switched on to cause the correct structures to develop in each region of the body. The genes for building a head have to be switched on at the top of the body, for example. Different *Hox* genes are expressed in different body regions, and act to switch on other genes that code for appropriate structures. The *Hox* genes mediate between the basic body map information and the genes that code for the structures in each body region.

The *Hox* genes function in the development of all animals

The finding that all animals use much the same set of developmental genes might not have been predicted. The main groups of animals — the Protostoma and Deuterostoma (Figure 18.5, p. 536) — were initially defined by basic differences in how the animals develop. In the protostomes, cleavage in the egg is spiral; in the deuterostomes it is radial. In protostomes the embryonic structure called the blastopore develops into the mouth; in deuterostomes the blastopore develops into the anus. And so on. It might have been expected that these deep differences in development would reflect different genes regulating development. But in fact the same set of genes is at work in both taxa. The genes that regulate development presumably evolved once, when animals with development first originated, and has been conserved ever since.[2]

20.5 Modern developmental genetic discoveries have challenged and clarified the meaning of homology

The eyes of insects and the eyes of vertebrates were, until the early 1990s, considered to be a standard example of "analogous" structures. They perform the same function but have utterly different internal structures, suggesting that they evolved independently from a common ancestor that lacked eyes. Then the laboratory of Walter Gehring in Switzerland began to research genes that are crucial for eye development in fruitflies and mice. One gene, *ey*, was known to be needed in fruitflies; another gene, *Pax6*, was

[2] The remarks here about "all animals" apply most clearly to triploblastic Bilateria: that is, to all animals except sponges, Cnidaria (corals, jellyfish, and sea anemones), and ctenophores (Figure 18.5, p. 536). The developmental genetics of sponges, Cnidaria, and Ctenophores are more uncertain.

A similar gene works in eye development in both mice and flies

needed in mice. The sequences of the two genes turned out to be similar, suggesting that they are really the same (that is, homologous) gene. The *ey* gene could be shown to cause eye development in fruitflies, because if the gene is switched on in inappropriate parts of the body, such as a leg, it induces the development of an "ectopic" eye.[3] Then genetic tricks were used to introduce the fruitfly *ey* gene into mice. These mice grew up with fly-type compound eyes. It seems that the same gene is used in both mice and fruitflies to cause eye development. If the insect and vertebrate eyes have evolved independently, we would hardly expect them to have hit on the same gene to act as the master gene of eye development.

Two interpretations are possible. One is that the common ancestor of fruitflies and mice had eyes. The structure of insect and vertebrate eyes are still so different that they probably evolved independently, but perhaps from a common ancestor that had, rather than lacked, eyes. The eye in that common ancestor might have been a much simpler structure (Section 10.3, p. 261), but there would be an element of homology between the insect and vertebrate eyes. The evolution of eyes in the two taxa would have been easier if they already possessed the developmental genetic machinery for specifying something about eye development.

The homology may be more, or less, specific

Alternatively the homology may be more abstract: *ey/Pax6*, or the ancestral gene from which they evolved, might have specified some activity only in a particular location in the body (the top front of the head). Then the use of the same gene in mice and fruitflies would reflect only the fact that the two animals grow eyes in a similar body region. The common ancestor of mice and fruitflies had a head, and would have had genes to work in the regions of the head. It would be less remarkable if mice and fruitflies have homologous genes for controlling development in a particular region of the head, than if they have homologous genes for developing eyes. At some level, homology must exist between mice and fruitfly eyes; the question is whether the homology is at the level of eyes, or head regions.

In general, structures that are not homologous at one level will be homologous at another, more abstract level. Ultimately, this reflects the fact that all life on Earth traces back to a common ancestor near the origin of life. Consider the wings of birds and bats. As wings, they are not homologous. They evolved independently from a common ancestor that lacked wings. But as forelimbs, they are homologous. Bird wings and bat wings are modified forelimbs, descended from a common ancestor that possessed forelimbs.

Since the Gehring lab's work on eyes, several other structures that had been thought to be analogous rather than homologous in insects and vertebrate have been found to have common genetic control. Some of these structures may turn out to be homologous in a specific sense, others only in an abstract sense. We shall not know which until the actions of the genes concerned are better understood. Meanwhile modern molecular techniques have added a new, genetic layer to our understanding of homology to add to the classic criteria we met in Chapter 15.

[3] An ectopic structure is one in the wrong place. An ectopic pregnancy, for instance, means that gestation is occurring somewhere other than the womb — the most common kind of ectopic pregnancies are in the Fallopian tubes.

20.6 The *Hox* gene complex has expanded at two points in the evolution of animals

Are changes in the developmental genes associated with major evolutionary changes in the history of life? The *Hox* genes are the most hopeful gene set for answering this question at present. More is known for the *Hox* genes about which genes are present in which animal taxa than is known for any of the other genes associated with development. We mainly know about the number of *Hox* genes in different taxa, and can therefore look at when in animal evolution the numbers of *Hox* genes changed. (The work is similar to the work we looked at in Section 19.3, p. 559, about how to test whether major evolutionary events are associated with duplications of genes.)

The number of *Hox* genes increased . . .

Figure 20.6 shows the *Hox* genes of 12 animal groups. It shows that the *Hox* gene complex clearly expanded at two points in the phylogeny. One is near the origin of the triploblastic Bilateria (see Figure 18.5, p. 536, for this taxon). Cnidaria have radial symmetry and only two cell layers. They are simpler than the other animal groups in the figure, which have three-cell layers and bilateral symmetry. Only two *Hox* genes have been found in Cnidaria, against a common set of at least seven *Hox* genes in Bilateria. Probably the number of *Hox* genes went up by about five some time near the origin of the Bilateria.

. . . near the origin of Bilateria . . .

A second major expansion occurred near the origin of the vertebrates. Invertebrates have a single set of up to 13 *Hox* genes. This set is also found, in a single copy, in the closest relative of the vertebrates, the lancelet *Amphioxus* ("cephalochordates" in Figure 20.6). Vertebrates, including humans, have four copies of the 13-gene set. The *Hox* gene set was increased fourfold, perhaps in a series of duplications, during the origin of vertebrates. Some biologists have explained the fourfold increase in the *Hox* genes by Ohno's hypothesis that the genome as a whole was duplicated twice near the origin of the vertebrates. Ohno's hypothesis is not well supported (Section 19.3, p. 561), but even if the genome as a whole was not tetraploidized, the *Hox* gene set itself was. So also were some other sets of genes that operate in development. This increase in gene numbers may have contributed to the evolution of vertebrates.

. . . and the origin of vertebrates

Vertebrates are arguably more complex life forms than invertebrate animals, for one thing they have more cell types. Also, many biologists think that the anatomic complexity of vertebrates is greater than for invertebrates. Complexity is difficult to measure objectively, but if vertebrates are more complex than invertebrates, the increase in the number of *Hox* genes may be part of the explanation. Once life forms had evolved with extra *Hox* genes they may have become able to evolve, in the future, increased complexity. Figure 20.6 also hints at some other periods of *Hox* gene change. For instance, the number of *Hox* genes concerned with the posterior end of the body seems to have expanded in the origin of the deuterostomes (echinoderms plus chordates at the top of the figure; see also Figure 18.5, p. 536).

The accuracy of inferences about when *Hox* gene numbers changed depends on the accuracy of the phylogeny. For example, in the phylogeny of Figure 20.6, *Hox* gene numbers appear to have decreased in the nematodes (represented by the worm *Caenorhabditis elegans*). This may be correct. However, the position of the nematodes in a group with the arthropods is based on recent molecular evidence from a small

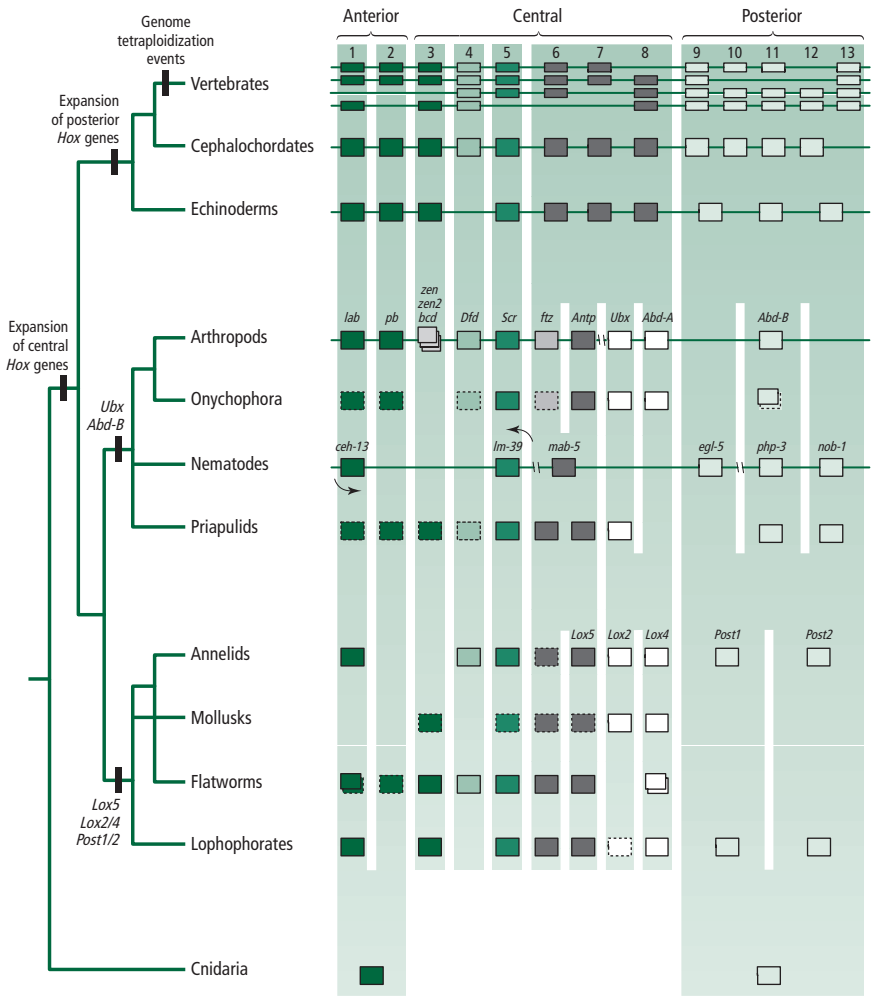

Figure 20.6
History of the *Hox* genes. Modern taxa contain many homologous *Hox* genes, and the distribution of the genes can be used to infer the time when new genes originated, and of a possible tetraploidization near the origin of vertebrates (compare Figure 19.2, p. 561). From Carroll *et al.* (2001).

number of genes. Traditionally nematodes belonged to a branch nearer the base of the tree, between the Cnidaria and the rest of the Bilateria. Then we should not infer that they have lost genes, but that they are an intermediate stage in the early increase from two to seven *Hox* genes. The inferences for these early events are uncertain, and in any case we require a well substantiated phylogeny before we can draw confident conclusions.

20.7 Changes in the embryonic expression of genes are associated with evolutionary changes in morphology

The vertebrae that make up the spine, or backbone, of a mouse differ from head to tail. For instance, the cervical vertebrae in the mouse's neck differ in form from the thoracic

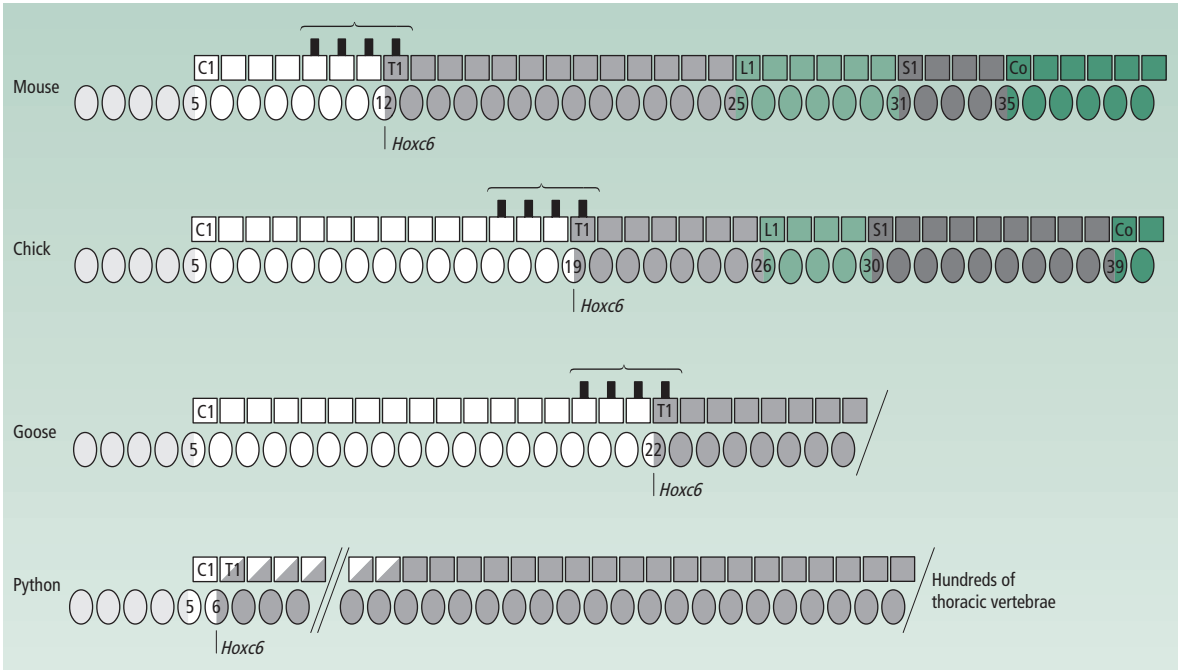

Figure 20.7
Change in gene expression associated with morphological evolution. The form of the vertebrae varies down the spine, with cervical vertebrae (C) in the neck and thoracic vertebrae (T) down the back. The vertebrae change from cervical to thoracic at different positions down the spine in the mouse, the chicken, the goose, and the python. The boundary of *Hoxc6* expression corresponds to the position where the vertebral form changes from cervical to thoracic. A change in the spatial expression of *hoxc6* could have contributed to the evolutionary change in the form of the backbone. Co, coccyx; L, lumbar; S, sacral. Modified from Carroll *et al.* (2001).

Changes in spine morphology . . .

vertebrae down the mouse's back. The cervical and thoracic vertebrae also differ in other vertebrate animals, such as chicken and geese. Geese and chickens have more neck vertebrae than mice do, and the division between cervical and thoracic vertebrae occurs further down the spine. The difference between species appears early in the embryo. The position of the boundary between cervical and thoracic vertebrae is further down the developing goose embryo than in a mouse embryo.

. . . are associated with changes in a *Hox* gene's spatial development

The boundary in the embryo between developing cervical and thoracic vertebrae is associated with the anterior boundary of expression of the *Hoxc6* gene (Figure 20.7). The *Hoxc6* gene is probably part of the control system that switches on the development of thoracic, rather than cervical, vertebrae. Thus, an evolutionary change in the morphology of the spine was probably partly produced, at a genetic level, by a change in the spatial expression of the *Hoxc6* gene in the embryo. Vertebrates develop in an anterior–posterior direction, with the head being specified first. A delay in switching on *hox6c* could cause the cervical–thoracic boundary to be shifted to the posterior, down the spine.

Changes in the timing of *Hox* gene expression can also contribute to morphological evolution. The five-digit limb of tetrapods, for example, has evolved from a fin in fish.

Hox genes are expressed in two phases during the development of fish fins. These phases might, for instance, help to cause an outward growth of bones to form the fin. In tetrapods, the *Hox* genes are also expressed in a third, later phase during limb development. The third phase is associated with the further growth outwards of the limb bones, to form the limb and hands. Thus, part of the mechanism by which fins may have evolved into limbs may have been for certain *Hox* genes to be switched on for a third time in the developing limb. Earlier in the chapter we met the concept of heterochrony (Section 20.2), which was based on classic morphological research. Here we can see a genetic example, in which a change in the timing of a developmental genetic process leads to evolutionary change in morphology.

Changes in arthropod limbs are associated with . . .

Morphological evolution may be caused by a change in which genes a *Hox* gene interacts with. For example, insects differ from some other arthropods in lacking legs on their abdomens. An insect has legs on its thorax and not its abdomen, but myriapods and many crustaceans have abdominal legs. During evolution, leg development came to be switched off in the embryonic insect abdomen. The genetic mechanism, simplified, is that the *Hox* genes *ultrabithorax* (*Ubx*) and *Abd-A* are expressed down the abdomen of insects, crustaceans, and myriapods. They are regional controllers of development. In insects, *Ubx* and *Abd-A* repress the gene *distal-less* (*Dll*); *Dll* is the gene that directs leg development. In myriapods and crustaceans, *Ubx* and *Abd-A* do not repress *Dll*.

. . . changes in *Hox* gene interactions

Two hypotheses can explain events such as the loss of limbs from the insect abdomen. One is a change in a transcription factor such as *Ubx*. In the evolution of insects, *Ubx* may have changed such that it became able to repress the genes, such as *Dll*, controlling limb development. The other hypothesis is that the enhancer of *Dll* may have changed during insect evolution. The enhancer may have ceased to bind *Ubx*. Alternatively, the enhancer may have continued to bind *Ubx*, but has changed its interaction with it such that *Ubx* now switches off limb development in the abdomen rather than switching it on. Some evidence supports the first hypothesis (Levine 2002). Crustacean *Ubx* is unable to repress *Dll* in fruitflies. That result suggests that *Ubx* itself has changed between crustaceans and insects. If *Ubx* were unchanged, crustacean *Ubx* should have the same effect in fruitflies as normal fruitfly *Ubx*.

In summary, we have seen three developmental mechanisms that are thought to have contributed to evolutionary changes in morphology. One is the change in the spatial expression of genes. A second is the change in which genes are switched on or off by transcription factors that have not themselves changed; this is achieved by changes in enhancers. A third is the change in transcription factors, such that they change their interactions with enhancers.

20.8 Evolution of genetic switches enables evolutionary innovation, making the system more "evolvable"

The examples in the previous section illustrate how evolutionary changes in gene regulatory networks can underlie morphological evolution. In the *hoxc6* example, in which the number of cervical vertebrae changed between mice and geese, the change concerned the regulatory relations between the *hoxc6* gene and some higher control

gene. The anterior–posterior coordinates of the animal are probably given by a chemical gradient down the body. These chemicals may bind the enhancer of *hoxc6*, switching it off at some chemical concentrations and on at other concentrations. The *hoxc6* gene is then switched on in a certain region of the body. Morphological change can be produced if the enhancer of *hoxc6* changes such that it is switched on and off at somewhat different concentrations of the chemicals that specify the anterior–posterior axis. In the example of insect abdominal legs, the change was in which other genes were regulated by *Ubx* and *Abd-A*.

Whether the changes in these examples came about by the exact genetic mechanisms suggested here is not important. Several kinds of change in an enhancer, or the molecules that interact positively and negatively with an enhancer, could produce the same general outcome. What does matter, and is of broad interest, is that morphology can be altered by adding or subtracting switches that control existing genes. If a gene can cause, or help to cause, a leg to develop, then new legs can be added to (or old legs subtracted from) the body by switching the gene on or off. The gene may gain, or lose, an enhancer that binds to a transcription factor produced by one of the embryo's regional-specifier genes.

A gene may modify its function by sequence evolution . . .

It is instructive to compare evolutionary change produced by gain or loss of regulatory elements with change produced by sequence change in the gene itself. We have seen many examples in this book of changes in the sequence of a gene. The sequence of a globin gene may change, for example, such that the oxygen-binding attributes of the hemoglobin molecule are altered. This is an obvious way for a molecule to change its function, and much functional change has likely been produced by sequence changes.

. . . but add new functions by evolution of its regulatory relations

The importance of genetic switches may be more in the evolutionary addition of new functions. Brakefield *et al.* (1996) and Keys *et al.* (1999) describe how a five-gene regulatory circuit has come to control the development of "eyespots" on the wings of butterflies. The gene circuit is able to produce borders, or boundaries, and is used in all insects to produce a certain boundary in the structure of the wing. Most insect wings do not have eyespots but some butterfly wings do. The eyespot has a distinct circular shape, with a boundary at the edge. Eyespots probably evolved when this "boundary-producing" gene circuit came to be expressed in a new gene network. In a butterfly eyespot, the boundary-producing genes are controlled by certain spatial-specifier genes within the wing, and they in turn control certain pigment-producing genes. Thus, a pre-existing set of genes came to be expressed in a new circumstance, probably by changes in the enhancers of the genes concerned. The boundary-producing gene circuit had gained a new function.

When a gene adds an enhancer, which switches it on in a new circumstance, it can gain a new function without compromising its existing function. If a molecule, or morphological organ, changes to add a new function, it will usually perform its existing function less well. If a mouth is used for both eating and breathing, it is likely to do each less well than if it did one alone (see Section 10.7.5, p. 284, on trade-offs). A molecule can add a new function by changes in its internal sequence, although this evolutionary process is inherently difficult. However, the molecule is also likely to perform its old function less well as it adds its new function. The difficulty is avoided if the new function is added by a change in gene regulation. The existing, unchanged gene comes to be switched on in new circumstances and the old function need not be compromised at all.

Switching systems may have made life more evolvable

Enhancers, and their associated gene-regulatory relations, have not always existed in the history of life. They evolved in order to improve the precision with which genes were switched on and off. These improvements probably became more important as genomes evolved to be larger, and as life forms (that is, animals and plants) originated with development from egg to differentiated adult. But once genetic switches had originated, they arguably had the effect of making some kinds of evolutionary change easier. It became easier for genes to add new functions. Thus, a greater variety of animals and plants may have been able to evolve. Genetic switches did not evolve in order to promote biodiversity; but they may have done so, as a consequence.

The term evolvability has been used to refer to how probable, or "easy," it is that a species, or life form in general, will evolve into something new. Some species may be inherently more "evolvable" — more likely to evolve innovations and evolve into new, different species. Many suggestions have been made about factors that promote evolvability. Genetic switches are one example. Maybe, after the origin of genetic switches, life became more evolvable than it was before.

20.9 Conclusion

Genetics is increasingly used to study macroevolution

We can finish with some general reflections that apply to both this and the previous chapter. The two chapters have not had space for a full survey of either evolutionary genomics or evo-devo. Instead they have looked at a sample of examples, which are mainly intended to illustrate the promise — and the interest — of the two fields. However, they also illustrate one other general point. Traditionally in evolutionary biology, genetics provided the main methods and materials for studying microevolution. Evolutionary genomics and evo-devo are two ways in which genetics is now being used to answer macroevolutionary questions.

Evolutionary genomics, as we saw in Chapter 19, looks at questions that biologists had paid little attention to previously. The data that have made evolutionary genomics possible hardly existed before about the year 2000. In the case of evo-devo, biologists have always realized that morphological evolution must be driven by changes in development. They had concepts, such as heterochrony, for thinking about the development basis of evolution. The modern developmental genetic work provides a new way of thinking about these long-established problems. The modern work is more concrete than the earlier work, because it builds on a knowledge of individual genes and the developmental processes that they influence.

Maynard Smith & Szathmáry (1995, 1999) have identified a small number — 10 or so — of what they call the "major transitions" in evolution. These are events such as the origin of life, of chromosomes, of cells, of eukaryotic cells, of multicellular life, of the development of sexual reproduction, and of Mendelian inheritance. They are the big breakthroughs that made much of future evolution possible. The major transitions are all changes in the way inheritance occurs, and in the relation of genotype and phenotype. Understanding the major transitions is largely a matter of understanding evolutionary genomics and evo-devo. The advance of these two subjects should give us some insights into the grandest questions of macroevolution.

Summary

1 Morphological change in evolution usually occurs by changes in developmental processes. The identification of genes that influence development is a major area of modern biology, and its methods can be applied to study the relations of development and evolution, a field known as "evo-devo."

2 Heterochrony refers to evolutionary changes in the relative timing and rate of different developmental processes. For instance, the time of reproduction may shift relative to somatic development. Also, shape changes can result from changes in growth gradients, and D'Arcy Thompson's transformational diagrams can be interpreted in terms of heterochrony.

3 Regulatory genes influence the expression of other genes, and evolutionary change can result from changed regulatory relations among genes as well as changes in the sequence of genes.

4 Structures, such as the eyes of insects and vertebrates, that had been thought to be non-homologous, have been found to be developmentally controlled by the same gene. Insect and vertebrate eyes may share an element of homology, but it is uncertain what the level of the homology is.

5 The number of *Hox* genes increased from perhaps two to seven near the origin of the triploblastic Bilateria, and quadrupled from 13 to 52 near the origin of vertebrates. *Hox* genes control spatial differentiation within the body during development, and increases in the number of *Hox* genes may be associated with increases in developmental complexity.

6 Changes in the expression of developmental genes are likely achieved by gains, losses, and changes in the regulatory elements (particularly enhancers) of those genes.

7 Some forms of life may be more evolvable than others: that is, be more likely to undergo innovative evolutionary change. The origin of genetic switches may have made life more evolvable.

Further reading

General developmental biology texts, such as Gilbert (2000) and Wolpert (2002) contain chapters on evolution, as well as developmental biology background. Wilkins (2001), Carroll *et al.* (2001), and Hall (1998) are texts more specifically on evo-devo. *Proceedings of the National Academy of Sciences* (2000), vol. 97 (9), pp. 4424–540 contains the proceedings from a conference on evo-devo. Gerhart & Kirschner (1997) is a stimulating book, more about the evolution of cells, but containing much relevant material for this chapter. Meyerowitz (2002) gives an evo-devo comparison of plants and animals.

Gould's (1977a) book discusses the history of recapitulatory ideas and modern work on heterochrony. Gould (2002b) contains further material. Raff (1996) is a more recent general book, and Levinton's (2001) even broader book also covers the topic. Both Gould and Raff are good on heterochrony, but see also the review article by Klingenberg (1998), the web-page on heterochrony (and on D-Arcy Thompson's transformations) by Horder in www.els.org, and the think-piece by Smith (2001).

Britton & Davidson (1971) is an early work discussing gene regulation and evolution. See also the introductory article by A.C. Wilson (1985), the recent book by Davidson (2001), as well as the general references and some further references below.

Gehring & Ikeo (1999) is a recent paper on the *Pax6* gene and eye homology, and refers to the original papers in the early 1990s. Many authors have discussed what this and similar genetic findings reveals about homology. See Dickinson (1995), Abouheif *et al.* (1997), McGhee (2000), and Mindell & Meyer (2001).

On the origin of *Hox* genes see also the material on duplications in the genomics section of this chapter. Slack *et al.* (1993) discuss a further topic — the "phylotypic stage." They suggest: (i) that all animals are more similar at a certain developmental stage than earlier or later in development; (ii) the stage of maximum similarity is the stage at which *Hox* genes are expressed; and (iii) animals can be taxonomically defined by the possession of the phylotypic stage.

Carroll *et al.* (2001) give references for the examples in which gene expression in development is associated with morphological evolution. On butterfly spots, see also the general review by McMillan *et al.* (2002) and the particular contributions of Beldade *et al.* (2002a, 2002b), the second paper particularly connects with another classic theme, that of developmental constraints on evolution — discussed in this text in Chapter 10.

The general point about switches and evolvability is implicitly discussed in Carroll *et al.* (2001) and more explicitly in Ptashne & Gann (1998). The general concept of evolvability was introduced by Dawkins (1989b). It is also discussed in Gerhart & Kirschner (1997) and Kirschner & Gerhart (1998). Another, related finding concerns heat shock protein 90, which "canalizes" (Section 10.7.3, p. 276) development in animals and plants. The breakdown of canalization by *hsp90* increases the range of genetic variation in a population; *hsp90* could therefore normally reduce evolvability by decreasing variation but could increase evolvability in stressful times. Pigliucci (2002) introduces the topic and refers to the primary sources. Chapter 9 of this text has further material on canalization.

Study and review questions

1 If a descendant species, in its reproductive (adult) form, morphologically resembles a juvenile ancestral stage, what (a) is the descriptive term for this morphological pattern, and (b) are two possible heterochronic processes that could produce it?

2 The eyes of vertebrates and the compound eyes of insects have utterly different structures, and almost certainly evolved independently. And yet a related gene seems to control the development of eyes in both the mouse and fruitfly. How can we reconcile these two observations?

3 (a) What is meant by "evolvability"? (b) How can the evolution of gene regulatory circuits influence the evolvability of a life form?