can raise their body temperature high enough during the first days of the infection, they will kill the otherwise lethal fungus. In fact, many poikilotherms engage in behavioral fever. They fight pathogens by behavioral increases in body temperature. How do they do this? By elevation seeking, moving toward the light, basking, perhaps hyperactivity—in other words, the same behaviors that might also appear to enhance the transmission of some parasites, especially those transmitted in the food chain. Perhaps those trophically transmitted parasites (such as the cystacanths) have usurped the host's defensive behavior and turned it to their own advantage.

Indeed, a large suite of altered behaviors promotes the fitness of parasitized hosts. Behaviors associated with temperature shifts are important. Even our own metabolic fever, the fever of homeotherms, has behavioral correlates that enhance fitness. When we (or our pets) are feverish, we want to sleep, often to the exclusion of other activities. We say we "feel sick," dogs refuse to eat, normally fastidious cats look a mess. However, it is also possible that the almost mandatory rest imposed by fever is one way to conserve energy that is then devoted to the immune response and to the energetic demands of fever itself.

Defenses are not limited to temperature, but can also include chemicals. Thus, grooming not only mechanically removes ectoparasites, but saliva may be effective against microscopic ones as well. The saliva of some mammals is harmful to some bacteria, which may be why dogs were encouraged to lick human wounds in the Middle Ages. Moreover, the natural world contains medicinal compounds that we have long forgotton; ailing chimpanzees, however, make good use of vegetation that contains natural "worming" components, as well as soils that are analogous to the compounds we take for intestinal disorders. In all of these cases—moving to hot (or cold) locations, grooming, choosing special foods—infected animals behave differently than uninfected ones in ways that offer defenses against parasites.

Parasites can also affect the behavior of uninfected hosts, which may go to great lengths to avoid being parasitized. When we housebreak a puppy or provide a litter box for our cat, we are taking advantage of the fact that many animals are particular about eliminative areas; for instance, many herbivores avoid grazing in areas where they deposit feces—and perhaps parasites. As for ectoparasites, howler monkeys spend up to one-quarter of their metabolic budgets combating blood-feeding flies, and reindeer may migrate to get away from warble flies. If one doesn't move away, joining a group may be advantageous. In the so-called selfish herd, animals on the edges of the herd are at greater risk of attack from either flies or predators than animals in the center are. Thus, Holstein cows will "bunch" when in the presence of face flies, and stickleback fish will form larger shoals in the presence of fish lice. On the other hand, sociality exposes hosts to more directly transmitted pathogens than they would otherwise encounter; in fact, this may contribute to xenophobic behavior in many primates. Increasing human population density probably contributes to the appearance of seemingly new diseases that simply could not be maintained and transmitted in earlier, less crowded times. We now know that even mate selection is heavily influenced by parasites; many organisms will choose mates on the basis of traits that are consistent with good health—choices that not only may provide "good genes" for offspring but may also reduce the probability of contagion.

In summary, parasites can influence behavior in ways that benefit parasites or hosts, or in some cases, neither. Even more intriguing is the realization that the parasite-induced behavioral alterations we know best are those we see. However, parasites can also alter odor, sound, and other stimuli we perceive only dimly. Moreover, given the near ubiquity of parasites, one must wonder if "normal" animals really exist. In the presence of these hidden guests, things are not always as they seem.

[*See also* Sex Ratios; Virulence.]

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HOX GENES

Hox genes are the architects of embryonic development. Their discovery in 1984 fundamentally altered the courses of evolutionary and developmental biology, and led to a molecular reunion of these two fields in the form of evolutionary developmental biology.

Naturalists had long been aware of the potential for development to occasionally generate bizarre mutants, in which one body part developed in place of another. These mutations ranged from development of an extra thumb to formation of entire limbs at ectopic positions in the body. These anatomical substitutions, or transformations, were originally labelled "homeotic" by the English naturalist William Bateson. Geneticists working with the fruit fly, *Drosophila*, also observed spontaneous

mutants in which body parts developed in inappropriate positions, but unlike those oddities found in the gardens of Victorian naturalists, the mutant flies could be bred in the laboratory and isolated using genetics. A collection of homeotic mutants emerged from this approach, displaying such radical alterations to the body plan as formation of a second pair of wings in place of halteres, and development of legs in place of an antennae. [*See* Body Plans.] The mutants were named in accordance with their phenotypes, with the aforementioned four-winged fly being labelled "Bithorax" (because the wing-bearing second thoracic segment developed in place of the haltere-bearing third thoracic segment), and the fly with legs in place of antennae receiving the fitting title of "Antennapedia." A common feature of these homeotic mutants is that they exhibit changes in the *identity* of anatomical structures, as if a developmental fate switch has been thrown at an inappropriate position of the body. Once the switch is triggered, the cells seemingly follow the normal course of development, but in abnormal sites. For example, the antennapedia mutant flies form a morphologically sound leg, with the exquisite details of a fly leg, but this leg is situated in a position normally occupied by an antenna. At a cellular level, the cells that form the leg are the same cells that normally form the antenna, only in the mutant these cells follow the genetic program for leg development. Thus, homeotic transformations are caused by mutations in genes that determine the fate of particular cells during development. Accordingly, these genes were dubbed *homeotic genes*.

Physical mapping of the *Drosophila* homeotic genes revealed that they are physically linked in two complexes, termed the Bithorax and Antennapedia Complexes. The Bithorax Complex (BX-C) contains the *abdominal (abd)A, AbdB and Ultrabithorax (Ubx)* genes, and the Antennapedia Complex (ANT-C) contains the *labial (lab), proboscopedia (pb), Deformed (Dfd), Sex combs reduced (Scr)* and *Antennapedia (Antp)* genes. Collectively these genes act to draw a molecular map of the early embryo by specifying the positional identities of cells in different locations, such that cells in a particular segment of the fly know, based on the genes expressed within, whether they should form antennae, legs, wings, halteres, eyes, mouthparts, or genitalia (for a review of positional information, the reader is directed to the paper by Wolpert). Homeotic genes act cell autonomously, affecting differentiation only in the cells in which they are expressed (nonexpressing neighbors are not affected).

Cloning of the homeotic genes revealed the presence of a highly conserved sequence of 180 base pairs, known as the homeobox, which encodes a 60 amino acid DNA binding region known as the homeodomain. Homeobox genes encode transcription factors, which regulate expression of other genes in the same cell (hence their cell-autonomous activity). The homeobox is found in each of the fly homeotic genes described above. *Hox* genes belong to the larger family of homeobox genes, but the designation "*Hox*" is reserved for the eight linked genes found within the fly homeotic (HOM or *HOX*) cluster, and their homologues in other taxa.

Hox genes have been found in a remarkably wide range of animals. The evolutionary history of the *Hox* cluster is beginning to come to light, thanks to important new work in comparative genomics and comparative developmental biology. True *Hox* gene clusters are found throughout the *triploblasts* (higher animals with three germ layers) including vertebrates, arthropods, annelid worms, nematode worms, echinoderms and molluscs. In diploblasts (basal animals with two germ layers), the situation is somewhat more complex; genes that are quite closely related to the *Hox* genes have been found in the cnidarians (e.g., corals, sea anemones, hydroids, jellyfish) and ctenophores (comb jellyfish), but whether these genes are clustered remains largely unresolved (for a review of this topic, the reader is directed to the paper by Ferrier and Holland). At least some cnidarians appear to have linked *Hox*-like genes, suggesting that the origin of the *Hox* cluster could date back to the origin of eumetazoans, and in the absence of clear data from sponges, an origin at the base of metazoans cannot be excluded. Further work is needed to resolve these early nodes. The emerging picture of *Hox* gene evolution is now sufficiently detailed to allow for the proposal of a hypothetical scenario for *Hox* origins. According to the model, tandem duplications of a single ancestral homeobox gene gave rise to an ancestral cluster known as the Proto*Hox* cluster. This hypothetical ancestral cluster may have consisted of four *Hox*-like genes (a posterior gene, a middle gene, an *Xlox/Hox3* gene and an anterior gene). Subsequent duplication of the Proto*Hox* cluster gave rise to the *Hox* cluster and Para*Hox* cluster. The single *Hox* cluster was then expanded by tandem duplications, and descendents of this single *Hox* cluster can be found in all triploblastic animals. A single cluster has been retained by the invertebrate triploblasts (including *Drosophila* and the cephalochordate amphioxus), but in the vertebrates, partial or complete genome duplications have resulted in the evolution of multiple *Hox* clusters. Mammals, for example, have four *Hox* clusters (designated *HoxA, HoxB HoxC* and *HoxD*), and in teleost fishes, another round of genome duplication has resulted in seven clusters (the eighth cluster was not retained). Each of these clusters contains members of the thirteen *Hox* paralogy groups (though losses of individual genes during evolution means that no single cluster contains all 13 paralogues).

The fact that *Hox* genes are clustered in the genome is of paramount importance, because this genomic or-

ganization is causally linked to their expression patterns and functions during development. Genes situated at the 3′ end of the cluster are expressed in the anterior (head) region of the embryo, 5′ genes are expressed in the posterior (tail) end of the embryo, and genes in the middle of the cluster are expressed in the middle of the embryo. This correspondence between position of genes within the cluster and expression pattern in the embryo is termed spatial colinearity. The timing of expression is also related to the clustering of *Hox* genes. Temporal colinearity refers to the sequential cascade of *Hox* gene transcription, in which 3′ genes are expressed early and 5′ genes are expressed late.

Colinear expression of *Hox* genes during development results in molecular regionalization of the embryonic body, with cells at different positions along the anterior to posterior axis expressing different combinations of *Hox* genes, like a molecular coordinate system. These differences in *Hox* gene expression are translated by the cells to determine their relative positions within the embryo. Ultimately, qualitative and quantitative differences in *Hox* gene expression cause cells at different positions to follow different programs of development. In anatomical terms, this could mean cells at one position in the developing gut will give rise to the stomach and cells at a more posterior position will give rise to the rectum. *Hox* genes are involved in specifying regional identity throughout the body. In vertebrate embryos, for example, the central nervous system, axial skeleton (vertebrae), gut, skin, limb skeleton, muscle, and hair are patterned by *Hox* genes. If the same ancient set of genes is operating to control development of all organ systems in all animals, how has variation been introduced? This question lies at the heart of evolutionary developmental biology.

Introduction of heritable variation occurs during embryonic development. Embryogenesis therefore represents the window of opportunity for modification of developmental programs that can lead to the emergence of morphological novelties. The idea that evolution progresses by the generation of "hopeful monsters" is not new, but is it a viable mode of evolution? Probably not. Experiments in mouse and fly genetics have demonstrated quite clearly that mutations in *Hox* genes can result in radical alterations to the body, by altering the course of development. Although at first glance this may seem like a good way to generate rapid and radical diversification, mutations in *Hox* genes alter every organ system in which that gene operates. This means that a mutation in a single *Hox* gene could, for example, result in an animal with a longer neck, but this alteration is likely to be accompanied by changes to the cerebellum, gastrointestinal tract, urogenital system, limb skeleton and inner ear. Such a radical reorganization of the body is likely to be incompatible with life. However, changes in *Hox* gene expression during development can be altered on a smaller scale, and in a modular pattern. Important work on the genetic regulation of *Hox* genes has revealed that their fine-scale expression patterns in different tissues are controlled by genetic modules, such as enhancers, situated adjacent to the gene. Mutations in these *cis*-regulatory elements can result in localized and often less catastrophic, changes to the body. Evolutionary modifications to the regulators of *Hox* genes, rather than to the *Hox* genes themselves, would allow more localized and subtle changes to the body. This process is known as *regulatory evolution*, and there is increasing evidence that modification of *cis*-regulatory elements has been a real mechanism of evolutionary change.

Boundaries are a key feature of *Hox* gene expression, and refer specifically to the spatial limits of a gene expression domain. *Hox* gene expression domains generally have sharply defined boundaries, and these molecular boundaries result in the specification of precise anatomical boundaries during development. A good example of anatomical boundaries can be seen in the vertebral column, where there are clear transitions between the different types of vertebrae. The transitions between cervical (neck), thoracic (chest), and lumbar (lower back) vertebrae are not graded, but are sharp transitions that occur over the space of one segment. A cursory glance at a vertebrate skeleton shows that on one side of an intervertebral disk lies a cervical vertebra, and on the other side lies a thoracic vertebra. These anatomical boundaries are established during development by boundaries of *Hox* gene expression. A one-segment shift in the position of a *Hox* expression boundary will result in a one-segment shift in vertebral identity. Importantly, these are changes in segment identity, but not segment number. For example, if the *Hox* gene expression boundary between thoracic and cervical vertebrae is shifted anteriorly by one segment, so that the most posterior cervical vertebra now lies within the thoracic *Hox* expression territory, the identity of that cervical vertebra will be posteriorized, and it will now develop as a thoracic vertebra. Although the thoracic region of the spine will be increased by one segment and the cervical region will be decreased by one segment, the total number of vertebral segments remains unchanged. This is an example of a homeotic transformation caused by a change in *Hox* gene expression. If the position of such a *Hox* expression boundary is controlled by a *cis*-regulatory element, then a precise, localized anatomical transformation could be achieved by changing its DNA sequence.

Although *Hox* genes are generally associated with regionalization of the primary body axis of the embryo, they are also involved in development of secondary axes, such as wings, legs, genitalia, and other append-

ages. Initially, the position at which appendages develop on an embryo is controlled by *Hox* gene expression in the trunk. For example, the fly wing develops only on the second thoracic segment and the haltere forms on the third. As described above, if the positional identity of a trunk segment is changed, then this will be accompanied by a concomitant change in the identity of the associated appendage (as in the bithorax mutant). *Hox* genes are also involved in later development of appendages. In the vertebrate limbs, 5′ genes of the *Hox*A and *Hox*D complexes play a crucial developmental role. As in the trunk, *Hox* genes are involved in specifying regional identities of limb structures. Detailed studies of *Hox* gene function in the limbs has also revealed that *Hox* genes control growth by regulating cell proliferation. Involvement of *Hox* genes in the regulation of growth is not exclusive to the limb, but seems to be a general role of *Hox* genes. Indeed, many of the homeotic transformations described above are the results of altered growth programs.

The molecular rebirth of evolutionary developmental biology has resulted in the identification of molecular genetic mechanisms that can account for evolutionary modifications to the body plan. *Hox* genes have been the most intensively studied candidates for these mechanisms, and there are already a number of examples in which evolutionary modifications of animal anatomy are associated with changes in the regulation of *Hox* gene expression. Snakes serve as an interesting example. Snakes lack paired limbs and have hundreds of morphologically similar, thoracic-like (i.e., ribbearing) vertebrae. Snakes evolved from lizardlike ancestors with complete forelimbs and hindlimbs, and a clearly regionalized axial skeleton. A study of *Hox* gene expression in snake embryos showed that the evolutionary respecification of cervical and lumbar vertebrae towards a thoracic identity was associated with an expansion of the *Hox* gene expression domain that controls development of the thorax. Similarly, loss of forelimbs is associated with an anterior shift in the expression boundary of a *Hox* gene that is normally expressed in the limbless body wall of tetrapods. Anterior expansions of *Hox* gene expression domains resulted in posteriorization of the anterior part of the snake trunk, transforming cervical vertebrae into thoracic vertebrae and the forelimb region into limbless body wall. Thus, the snake body plan has evolved by homeosis. Although the precise nature of the regulatory changes responsible for the modulation of gene expression is not yet know, the structure of this snake *Hox* protein is so similar to that of other tetrapods that the same antibody can recognize the protein in mouse, chicken and python embryos. This suggests that the change in *Hox* expression is caused by regulatory evolution and not by evolution of the structural *Hox* gene. Similar transformations in *Hox* expression are responsible for evolution of crustacean appendages. During crustacean evolution, the anterior limbs have been modified into feeding appendages that resemble mouthparts. Comparative studies of *Hox* gene expression in a diverse sample of crustacean embryos revealed that transformation of limbs to mouthparts was associated with retraction of a *Hox* gene expression boundary from the segment that bears the transformed limbs. Thus, by eliminating *Hox* expression from certain limb-bearing segments, the identities of the appendages on those segments were transformed. This is another example of evolution by homeosis. There are also examples of what appear to be homeotic mutants that have evolved by evolutionary changes to the downstream targets of the *Hox* genes. For example, butterflies resemble the *Drosophila* bithorax mutant, in that they have two pairs of wings (rather than one pair of wings and one pair of halteres). However, development of the second pair of wings is not associated with changes in expression of *Ultrabithorax* (*Ubx*) gene, which is responsible for the fly bithorax mutant. Instead, evolution of insect wing number was associated with changes in the Ubx-regulated target genes. Thus, homeotic mutations can arise from changes to the upsteam regulation of, or the downstream response to, *Hox* genes.

Our understanding of *Hox* gene regulation and targets is still at an early stage of development. As the mechanistic details of these genetic pathways are uncovered, our understanding of the role of *Hox* genes in evolution will be improved. Undoubtedly, morphological evolution does not occur solely by homeosis, and as the field develops, we can expect to discover more fine-scale anatomical changes that are the results of evolving *Hox* gene regulation.

[*See also* Homeobox; Homeosis.]

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