FEATURE ARTICLE

Homeobox Genes, Fossils, and the Origin of Species

JEFFREY H. SCHWARTZ*

Ever since Darwin there has been a history of debate on the tempo and mode of evolution. Is speciation a gradual process involving the accumulation of minute variations extant within a species, or is it rapid, the result of major organismal reorganization? Does one define a species on the basis of genes, morphology, or geographic or reproductive isolation? In this communication I present a model of evolutionary change that is based on the Mendelian inheritance of mutations in regulatory genes and the fact that most nonlethal mutations arise in the recessive state. Since the new recessive allele will spread through many generations without expression until there is a critical mass of heterozygotes capable of producing homozygotes for the mutation, the novel feature thus produced will appear abruptly in the population and in more than one individual. This picture of punctuation is consistent with the fossil record, which typically fails to provide evidence of smoothly transitional states of morphological change. Given that the first of their kind in the fossil record are organisms in which their novel characteristics are often more fully expressed or complex than in their descendants, it would seem that, after the mutation involving a regulatory gene is introduced, the general tendency is for its effects to become diminished. Among the implications for speciation is that this process does not depend on either reproductive isolation or genetic incompatibility. Rather, barring effects on reproductive organs or behavior, homozygotes for a novelty should be able to breed with heterozygotes and homozygotes for the wild state of the original population. This, in turn, suggests that the species barrier between individuals is probably a matter of mate recognition. ***Anat Rec (New Anat): 257:15–31, 1999.***

KEY WORDS: homeobox gene; development; Mendelism; Darwinism; evolution; punctuation; gradualism; speciation

When people think about human evolution, they usually think about early species of hominid, like *Australopithecus afarensis*, the species the specimen Lucy represented, or much more recent species, such as *Homo erectus* or *H. neanderthalensis*. But human evolution actually begins hundreds of millions of years earlier, with the origin of cellular life, and the subsequent emergence of increasingly more diverse and complex clades of multicellular organisms. For if these evolutionary events had not occurred, there would never have been occasion to investigate the origin of our own group and to muse about the greatness of being human. But the history of many of these earlier evolutionary events, which we know had to have preceded the emergence of hominids, is not as clear cut as that of our own evolutionary group.

We think we know that, somehow, humans and apes are related. And we think the best hypothesis at present is that one, or something like one, of the currently known 10- to 14-million-year-old African or Eurasian fossils could have been the ancestor from which the large-bodied hominoids ultimately diverged. And we even think we have a few good candidates to serve as the potential ancestors of the larger group of anthropoid primates. In fact, in spite of the normally patchy fossil record of land mammals, the evolutionary history of primates and the evolutionary relationship of primates to other animals seems fairly well understood. But we can hardly say as much for the origins of all of the earlier ancestors from which mammals eventually evolved.

You and I are chordates. A chordate is an animal which, for at least some part of its life, has a stiffening cartilaginous rod—a notochord—that lies dorsally along its body's midline longitudinal axis. The primary trunk of a chordate's central nervous system also lies dorsally, parallel with this rod. A chordate's mouth is at the front end and its anus at the opposite end of its body. A chordate is also a bilaterally symmetrical animal, having a right and a left side and paired anatomical structures, such as gill slits and trunk musculature. You and I belong to a

Dr. Schwartz, a professor of physical anthropology at the University of Pittsburgh and research associate at the American Museum of Natural History, has spent his career struggling with theoretical and methodological issues in systematics and phylogenetic reconstruction. A long-time proponent of incorporating developmental biology into evolutionary theory, he has most recently been studying the history of evolutionary thought and the debates between paleontologists, geneticists, and developmental and comparative anatomists. This pursuit has led to the formulation of a new theory about evolution and the origin of species, the entire background to which will be published in *Sudden origins: fossils, genes, and the emergence of species* (John Wiley & Sons, April, 1999). The essence of this theory and its theoretical implications are explored here.

***Correspondence to: Jeffrey H. Schwartz, Department of Anthropology, University of Pittsburgh, Pittsburgh, PA 15260. Fax: 412-648-7535; E-mail: jhs+@pitt.edu**

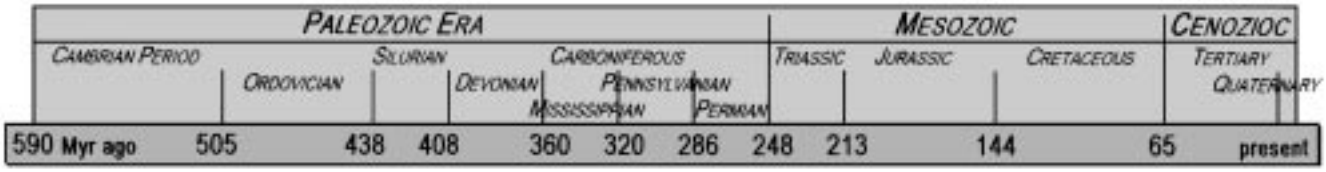

Figure 1. Geological time scale of eras and periods relevant to the evolution of chordates. The scale is in millions of years (Myr).

subclass of chordates, Vertebrata. Vertebrates are animals in which the notochord becomes segmented early in development into separate, often bony vertebrae. Vertebrate brains are encased in a protective, usually bony, shell. Vertebrates have paired appendages: two behind the head region, and two closer to the anus. Most vertebrates have teeth, which are typically replaced by sets of new teeth.

Nobody knows for certain where chordates came from, who their last common ancestor was. As far as the fossil record goes, for millions of years, there were no chordates.[4] Then, suddenly, in the Cambrian (Fig. 1), they appear in the fossil record. Equally as suddenly, after some millions of years, at the Ordovician-Silurian boundary, vertebrates emerged, replete with paired appendages, numerous vertebrae, and dermal plates which in some taxa covered not only the head, but also the entire body. After some additional millions of years, early in the Silurian, vertebrates with teeth intrude upon the fossil record. These vertebrates didn't merely have some teeth. They had complete sets of teeth, which not only filled up the entire lengths of the jaws, but which also lasted a lifetime through the continual replacement of generation after generation of teeth.

Where is the trail of fossil intermediates, of missing links leading from invertebrates to chordates to vertebrates? The typical paleontologist's answer has been, simply, that these fossils haven't been found yet. They were there, of course. But the vicissitudes of preservation and fossilization and the uncertainty of discovery by paleontologists have—hopefully only temporarily—thwarted the filling in of these annoying gaps in the fossil record. Otherwise, the fossil record would provide us with the full picture of the evolutionary transition of one form into another. This expectation was first articulated most fully well over a century ago by Charles Darwin, who based his speculations on the realization that all life forms are related by virtue of descent from a common ancestor.

DEBATING DARWINISM

When Darwin was formulating his particular theory of evolution by way of natural selection, he, like his contemporaries and those naturalists of the next few generations, had not a clue about inheritance. But the simple observation that offspring look like their parents in some aspects of anatomy and behavior was not lost on Darwin and other evolutionists. Since there obviously was a continuity from one generation to the next, it must also extend to species and their descendent species. Even in the absence of any real understanding of inheritance, Darwin and other evolutionists could speculate seriously about change and the origin of new species.

Even in the absence of any real understanding of inheritance, Darwin and other evolutionists could speculate seriously about change and the origin of new species.

For Darwin, most aspects of an organism's anatomy or behavior serve a purpose and are important for the survival of the individual.[7] The nature of difference between individuals lies in the degree to which they are better or less well adapted in terms of any given feature. Change is brought about by natural selection choosing, from among the available variation in a population, those variants that would cause the individuals bearing them to be better adapted than those without, and this process would be borne out by the better-adapted individuals producing more offspring than those less well endowed. In this way, organisms become fitted to their particular circumstances, and as these circumstances change, so, too, do individuals, and, consequently, species.

The only illustration Darwin published in *On the Origin of Species* was a connect-the-dots-like diagram depicting his view of evolution: species descended from a common ancestor; gradual change of organisms over time; episodes of diversification and of extinction of species (Fig. 2). Given the simplicity of Darwin's theory of evolution, it was reasonable for paleontologists to believe that they should be able to demonstrate with the hard evidence provided by fossils both the thread of life and the gradual transition of one species into another. In truth, while claims of such demonstrations have been the rule rather than the exception among paleontologists, we are still in the dark about the origin of most major groups of organisms. They appear in the fossil record as Athena did from the head of Zeus—full blown and raring to go.[4] Nevertheless, Darwin's model of evolution, being predicated upon the gradual accumulation of countless infinitesimally minute variations, would demand the existence of insensible series of transitional forms in the fossil record, even if their presence in the rocks cannot readily be documented.

But even in Darwin's day there were alternative ideas about how anatomical novelty might arise. The precocious and outspoken English comparative anatomist Thomas Henry Huxley, who did not fully accept Darwin's theoretical underpinnings but nonetheless championed his intellectual right to advance them, actually promoted abrupt, major organismal reorganiza-

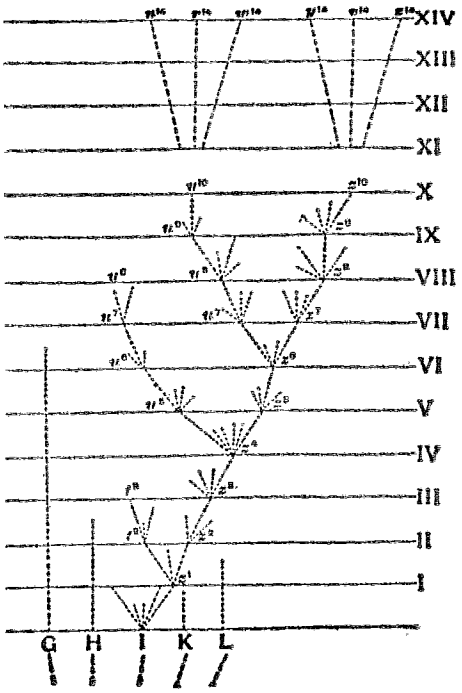

Figure 2. Partial reproduction of the only illustration Darwin published in *On the Origin of Species*.[7] The dotted lines depict the gradual transformation of single lineages over time (G, H, J, K) as well as gradual transformation in conjunction with multiplication of lineages (I), with extinctions scattered throughout. (Reprinted with the permission of Harvard University Press.)

tion as the mode by which new forms came into being.[25] Novelty emerged in a leap-frog or saltatory fashion, not by the tranquil process of gradual evolution. Coincidentally, a saltational model for the introduction of organismal novelty paralleled observations on the fossil record that were actually made by one of the most vocal of opponents of evolution.

In late 18th- and early 19th-century France, the anti-evolutionary, totally biblically informed, yet brilliant comparative anatomist and father of paleontology Georges Cuvier had documented that the extinct plants and animals entombed in the limestones of the region did not form a gradual succession of species throughout time.[6] Rather, with the rare case of a species persisting from one stratum to the next, new species merely replaced those of earlier times. As a believer in the biblical flood, Cuvier explained the demise of each time period's species as the result of such a catastrophic event. New species, of course, were created by a divine being. The occasional species that survived the catastrophe had somehow managed to find refuge in a Garden of Eden. The biblical interpretation aside, however, Cuvier's observations were real. Species did not grade into one another over time. As would be proposed by the saltationists, novelty seemed to appear out of nowhere.

From the late 19th and into the 20th century the debate among evolutionists was phrased in terms of continuous vs. discontinuous variation. Were the features of individuals, and consequently of their species, analogous to the colors of the spectrum, which, when viewed collectively, graded imperceptibly from one into another? If so, then individuals within a species, as well as species themselves, could be arranged along a continuum of variation. Or, to the contrary, no matter how similar they might seem, were characters actually discretely different from one another? If so, then individuals within species, as well as species, did not form an unbroken continuum of slightly differing shades of difference. Rather, individuals and species were distinguishable because their differences were disjunct or discontinuous. The side of the debate in favor of discontinuous variation was promoted by two of the leading figures in the emergent field of genetics: the Englishman William Bateson and the Dutchman Hugo de Vries.

At the beginning of their careers, both Bateson and de Vries were supporters of Darwin. Indeed, Bateson, who trained in physiology and comparative developmental anatomy, sought to document Darwin's suggestion that organismal adaptation was tied to—indeed, that it tracked—change in the environment.[1] After years of field studies, Bateson was forced to admit that he could not find any support for Darwin's suppositions.[1] Upon discovering and then championing the earlier genetic studies of the Austrian monk Gregor Mendel, Bateson became further convinced that Darwin's evolutionary explanations were incorrect.[2] For Darwin, continuous variation was inextricably tied to a notion of blending inheritance: lacking discrete identities, the features of both parents blended in the creation of offspring.[8] Since, however, as Mendel had demonstrated,[34] an individual's features were the result of the differential expression of the discrete units of inheritance that had been contributed by each parent, then the differences between individuals, and thus even between species, could not be continuously variable. If Mendel was right about separate units of inheritance, then, as Bateson saw it, features, and consequently the individuals bearing them and the species these individuals formed, had to be discrete and discontinuously distributed entities.

Although de Vries thought he could reconcile his views on selection with Darwin's, he was too impressed by the spontaneous mutations that occurred in the evening primroses he studied to accept Darwin's gradualism and its underlying premise of continuous variation.[10] In the presentation of his Mutation Theory, he argued that, while natural selection acting on minor individual variation might be well and good at the level of the daily lives of a species' members, this process is not relevant to the origin of species, which he conceived as being an abrupt phenomenon. Speciation, he suggested, is the result of significant genetic change, not, as would be adduced from Darwin's model, the mere shifting of the expression of already present genetic material. De Vries was also skeptical of Darwin's concept of natural selection. Rather than picking and choosing the best or most adaptive features, de Vries envisioned natural selection as a force that eliminated the worst features, leaving behind those that

were of no harm to the individual bearing them.

The rejection of Darwin by an increasing number of population geneticists continued into the 20th century. Among the most vocal of these scientists was the American Thomas Hunt Morgan. As had so many of his colleagues, Morgan initially trained in comparative developmental anatomy. In the early phase of his career at Bryn Mawr College he was convinced that neither Darwinian nor Mendelian explanations were relevant to understanding evolution and the origin of species.[35] Even when he later became his own convert to both doctrines, Morgan refused to embrace natural selection as a viable entity in evolution. As he saw it, new features just happened or chanced to appear, and whether or not they persisted in a species was also a matter of chance. Essentially following de Vries, Morgan's attitude was: If a new feature didn't kill you, you had it. As for Darwin's concept of continuous variation, Morgan rejected this conceit, as well.

THE MELDING OF DARWINISM AND MENDELISM

But in 1915 Morgan, now at Columbia University, and his collaborators in experimental studies on fruit fly population genetics seemingly unearthed the Rosetta stone of genetics and evolution. One day, upon returning to their laboratory, they discovered that there were now eyeless mutants amidst their fruit flies.[36] When they bred these mutants with normal individuals, they could, over many generations, gradually increase the number of eyeless individuals. By such manipulative experimental breeding, Morgan and his group could alter the prevailing character of a population of fruit flies. They were most impressed by their ability to shift a population of fruit flies from one in which the wings were typically a bit longer than the body to one in which the wings were usually a bit shorter than the body. They speculated that, were this process to continue indefinitely, their fruit flies would end up being wingless.

In one fell swoop, these discoveries convinced Morgan[37] that both Mendel and Darwin had been correct. As Mendel had predicted, morphological characters were indeed tied to discrete genetic units, which were inherited according to very consistent rules. A gene could come in a dominant or a recessive state, with the former always being expressed over the latter. Only when two recessive states or alleles were present would an individual develop the feature this allele represented. Mutation introduced the novelty that was necessary for, and which would eventually produce, new species. But in Morgan's eyes, and keeping with Darwin's notion of continuous variation, the mutations and the morphological novelties they produced were by themselves only of minor potential significance. Gradually over time, small mutations and the features they produced would accumulate, following the course natural selection dictated as it culled the most advantageous combinations. This was the process that pushed a species along evolutionarily, allowing it to fluctuate smoothly and continuously as its surroundings changed about it. The case seemed closed: Mendelism and Darwinism were compatible with the notion of low-level variation being the fodder of gradual evolutionary change.

In the wake of Morgan and like-minded fruit fly geneticists came the statistical or mathematical population geneticists, the three most prominent of whom were R. A. Fisher,[14] Sewall Wright,[58] and J. B. S. Haldane.[22] Although the former geneticist differed from the latter two more than they did from one another in terms of the pace of evolutionary change, all three agreed on one thing: as Bateson had demonstrated decades earlier, most nonlethal mutations arise in the recessive state. Beyond that point of unanimity, Fisher believed that a new mutation would have to be converted quickly to the dominant state so that it, through the morphology it produced, could be available for natural selection to act on it. Wright and, to a much greater extent, Haldane were willing for the recessive allele to be passed on over many generations until it was widely spread throughout the population. Then, eventually, as the number of individuals bearing a gene in the recessive state increased, so, too, would the chance of their producing offspring with two recessive alleles. Being the bearers of a pair of recessive alleles, these offspring would also have the character represented by that allele. Fisher was adamant that the process of evolutionary change that brought about species involved the slow and gradual accumulation of minor mutations throughout large populations. Wright and especially Haldane took a lesson from successful animal breeding experiments and maintained that, because a mutation will spread quickly in a small population because of the effect of partial inbreeding, new species will arise rapidly from partially (Wright) or fully separated (Haldane) peripheral populations. At some point, the mutant allele might be converted to the dominant state.

THE SYNTHESIS AND BEYOND

When the founders of the Synthetic Theory of Evolution (or, "the Synthesis") convened in the 1930s and '40s, they were faced with a situation in which genetics had assumed the foreground in evolutionary discussions.[26] The fields of paleontology, comparative anatomy, and embryology—which had been the dominant pursuits of earlier evolutionists—were dismissed by geneticists following in Morgan's footsteps as poor cousins that were at best tangential, and certainly not central, to the development of evolutionary theory. Genetics became the language of evolution. Consequently, when naturalists, such as the ornithologist Ernst Mayr, discussed the question of how species might form, they naturally did so in the context of inheritance: how to disrupt genetic continuity within a species so that natural selection could act on the characteristics, and through them the genes, of different populations of individuals.[33]

Faced with alternative suggestions as to how slowly or quickly speciation could occur, and whether it would involve many or few individuals, Mayr and his colleagues opted for slowly and many. They had no time whatsoever for the alternative theory that had been proposed by the German developmental geneticist Richard Goldschmidt. Goldschmidt maintained that the genetics of speciation is totally different from the population genetics of everyday life: Rather than resulting

from the gradual accumulation of minor mutations, the novelties that distinguish a new species derive from an abrupt, almost catastrophic genetic reorganization that occurs within the space of a single generation.[17,18] Mayr,[33] the geneticist Theodosius Dobzhansky,[11] and the vertebrate paleontologist George Gaylord Simpson[50] all went out of their way to ridicule and discredit Goldschmidt. Unfortunately, these attacks were made even more possible not only because Goldschmidt had been rather abrasive about his contemporaries' narrow-mindedness, but also because he had proposed that the unsuspecting bearer of such a major genetic revolution should be called a hopeful monster. To make matters worse, Goldschmidt could not deal adequately in his speculations with the problem of ensuring that there would be more than one hopeful monster, of each sex, at any point in time—which would, of course, be necessary in order for the potential new species to become established.

Goldschmidt's genetical theory of the origin of species did, however, find favor with the German paleontologist Otto Schindewolf.[44,45] By the time of the Synthesis, many prominent paleontologists, led especially by the American G. G. Simpson, were convinced that the fossil records of various groups, particularly of the horse, demonstrated the cumulative process of evolutionary change. Schindewolf, however, looked at the same fossil records, including that of the horse, and saw otherwise. New species, especially the apparent ancestors of new and different groups of organisms, emerged suddenly. Even phases of species change within the new group occurred abruptly, following a stepwise pattern rather than a smooth curve or gradient. The picture of evolutionary change as portrayed in the fossil record was totally consistent, Schindewolf noted, with the expectations of Goldschmidt's theory of major genetic reorganization. But while Goldschmidt received the brunt of the brutal attacks of his detractors, Schindewolf remained relatively unscathed. However, Schindewolf's interpretations of the fossil record also went unappreciated.

As far as the founders of the Synthesis were concerned, evolution occurs by the gradual accumulation of minor mutations. New species could arise by this straightforward process. But this would result in the transformation of a species, not the diversification of life. Following Mayr's[33] concept of a species—being an aggregate of individuals between which there is no genetic or other barrier to their reproducing and producing offspring that, in turn, can successfully reproduce—more than one species could arise if a barrier to gene flow, such as a geographical barrier, were to be imposed between subgroups of the original species. The separated populations would then proceed gradually to accumulate their genetic and morphological differences. In addition, according to Mayr,[33] speciation could only occur when there was a vacant niche into which the new form could expand and become adapted. Following Darwin, Mayr saw species as being perfectly fitted to their environmental circumstances.

The application of Mayr's species definition to fossil rather than living organisms was, of course, more tenuous. But since the members of a species are supposed to be adapted to a particular econiche, there should be something morphological that reflects their adaptation and makes them different from even their closest relatives.[50] With any luck, one or more of these adaptive features would be preserved in the fossilized anatomy of extinct organisms. With the melding of Darwinism and Mendelism complete, the evolution sciences, paleontology included, could then go about the business of trying to figure out the details of the past and the present.

But the notion of gradual transformational evolution was challenged again in the early 1970s, this time not by geneticists, but by paleontologists. Two Columbia University graduate students in invertebrate paleontology, Niles Eldredge and Stephen Jay Gould, proposed the model of punctuated equilibria,[13] which was an extension of Eldredge's[12] argument that allopatric speciation (speciation through the separation of subpopulations) via peripheral isolates was a more accurate depiction of evolution as portrayed in the fossil record than phyletic gradualism (the gradual change of entire populations). By punctuated equilibria, Eldredge and Gould meant to convey the image of, on the one hand, nothing much happening during the lives of species once they emerged on the scene, but, on the other, of a process of speciation that was relatively so abrupt that species appeared almost to pop into existence.

Eldredge and Gould's evidence derived from their respective studies of trilobites and snails, which, being preserved in large numbers, could provide a reasonably intact record of their evolutionary pasts. From afar, the rapid rise of a species gave the impression of there being a gap. In detail, as seen in these fossil invertebrates, the gap appeared to be filled by a fine trail of links between the old and new species. In short, as viewed through the eyes of punctuated equilibria, rather than being inconvenient potholes in the path of a gradual picture of evolutionary change involving large numbers of individuals, the gaps in the fossil record are a reflection of a very rapid process of speciation involving but a small peripheral fraction of the original population.

For the most part, neither geneticists nor morphologists found anything acceptable about the model of punctuated equilibria. Even when Eldredge and Gould responded to five years of accumulated criticism of their proposal,[21] there was still a great wall of resistance to punctuated equilibria, especially among population geneticists. The latter maintained that there was absolutely no need to invoke an unprovable hypothesis—one based on supposedly negative evidence (the gaps), to boot—when simple population genetics could easily explain how a species could be changed over a long period of time by the introduction of minor mutations and the action of natural selection.[5] Even Eldredge and Gould's suggestion[21] that population geneticists had been looking at the wrong genetic level—studying the structural genes rather than the underlying genes that regulate the activity of these structural genes—fell largely on deaf ears.

Although the 1980s saw an increase in the number of paleontologists who embraced rapid morphological change and speciation as an alternative to gradual evolution, the model of punctuated equilibria continued to run up

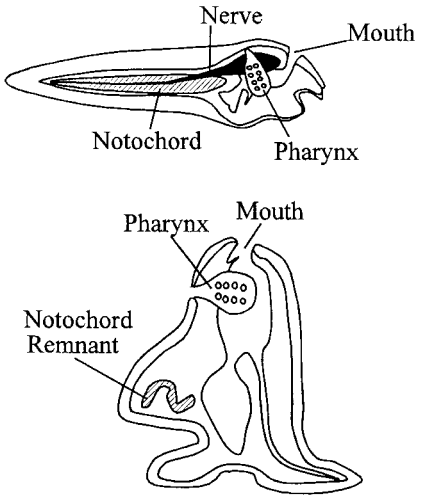

Figure 3. Schematic representation of a larval, free-swimming tunicate (above) and a sessile adult (below). In the larva, the central nerve and notochord lie dorsally, the mouth and the anus (not drawn) are at opposite ends of the trunk, and there are paired structures, such as pharyngeal gill slits. In the adult, the notochord and tail no longer exist, and the body essentially bends in half, bringing the mouth and anus (not drawn) into close proximity. (Drawing by T. D. Smith, ©J. H. Schwartz.[46])

against the objection that natural selection acting on minute variation was all that was necessary to explain evolutionary change. Occasionally, punctuated equilibria and Goldschmidt's hopeful monsters were mentioned in the same anti-punctuational article. The kind of large scale genetic alteration that seemed necessary to provoke the almost explosive production of a species was simply incompatible with current views in population genetics. But while these debates were going on, another sort of geneticist—one ultimately interested in development—was busy at work.

COPING WITH DEVELOPMENT

Almost from its beginnings, the discipline of biology has had its share of embryologists, those scientists who seek to understand an organism by way of studying its growth and development. When embryology was applied to evolution, it was believed that an organism's evolutionary past was revealed through its course of development. The great German comparative anatomist, embryologist, and all around evolutionist Ernst Haeckel thought that one could see in the embryonic phases of an organism a recapitulation of the adult stages of its ancestors.[23] Other comparative anatomists, such Thomas Huxley, more sensibly interpreted the ontogenetic stages shared by different organisms as being what they are: the early phases of development, from which the particular characteristics of a given species later become differentiated as the individual matures.[25] From the latter part of the 19th and well into the 20th century, there was great interest in how the study of similarities between the embryos and fetuses of different organisms might shed light on their evolution.

As with the history of evolutionary studies in general, so, too, did humans as subjects of inquiry figure centrally in the co-development of evolutionary and embryological ideas. An easily reached realization was that, in contrast to most other animals, human adults retain many features that are characteristic of the fetus or child. For instance, a human adult has a relatively large head and brain, a relatively small face, and is essentially hairless, just like a neonate. One embryologist, Louis Bolk, went so far as to proclaim that humans are actually reproductively viable fetuses.[3]

Bolk's musing aside, many embryologists took seriously the fact that the rates at which an individual grows physically and matures sexually are not necessarily the same.[9] The only constant is that, upon reaching reproductive maturity, somatic change becomes significantly decelerated. In the case of humans, the rate of physical change is so prolonged that, in many ways, it appears to be curtailed while still in its juvenile phase at about the time an individual becomes reproductively mature. When all the possible combinations of prolonging or accelerating the rates of physical vs. sexual maturation are taken into consideration, there is an abundance of possible avenues through which major organismal change can be introduced. It did not take very sophisticated laboratory studies to demonstrate that different temperatures could easily and quickly alter an organism's course of development[15,24] (also see reviews in references 9, 20).

The implications of altering developmental timing were not lost on Walter Garstang, who, in the 1920s, proposed a simple and elegant explanation for the origin of chordates from an invertebrate ancestor.[16] Garstang's invertebrate of choice came from among the tunicates, a group of marine organisms that includes sea squirts (Fig. 3). An adult tunicate lacks a notochord and paired structures, and its anus and mouth are closely approximated. In addition, as adults, many species of tunicate are forever fixed to a substrate, as are barnacles. The larvae of some tunicate species, however, are free swimming. And some of these free-swimming larvae even look like primitive chordates, including being bilaterally symmetrical. They have a mouth at one end and an anus at the other end of the body. They have a dorsal nerve trunk and a cartilaginous notochord just beneath and parallel with it. And they also have paired trunk structures, such as muscle cells and pharyngeal gill slits.

To make a chordate, all that needed to occur was for the larval tunicate state to persist until the individual reached sexual maturity. A mere shift in developmental timing—the acceleration of reproductive maturation, perhaps—and the result would be a primitive chordate whose emergence would not have been heralded by a trail of successively intermediate ancestors. And since it seemed that a potential source of significant developmental alteration could be an organism's own surroundings, there was also the possibility that more than one individual with chordate features would emerge at the same time. Although Garstang's hypothesis remains a popular, and essentially singular, explanation for chordate origins,[4] its broader evolutionary implications have not been appreciated by many evolutionary biologists: alterations in development can produce profound differences, and lead to new species, essentially in an instant. But for all the insight developmental biology might provide into probing the question of how evolutionary change might occur, it was accorded little recognition in the formulation of the Synthesis.

Fortunately, the field of developmental biology did not curl up and die, and, in the 1980s, the door to understanding development at its most fundamental level was finally opened with

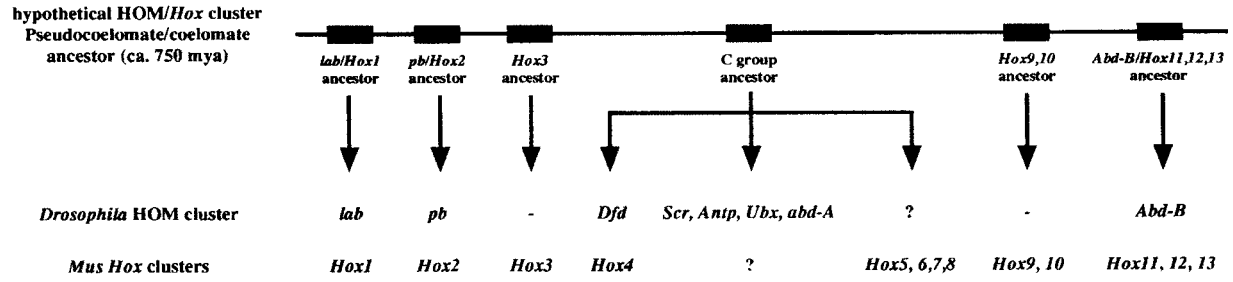

Figure 4. Homeobox genes of the HOM/*Hox* cluster in the fruit fly (*Drosophila*, above) and the mouse (*Mus*, below). These two disparately related organisms share various homeobox genes, one of which appears to have duplicated in the mammal. If we assume that these animals have similar genes because of retention from a common ancestor, the genes are identified as orthologous; if not, they are considered paralogous. Counterparts of some homeobox genes remain unidentified in these animals. (Adapted from Purugganan[41] with permission.)

the discovery of a class of regulatory genes, referred to as homeobox genes, that are arranged in clusters on separate chromosomes.

DEVELOPMENT AND REGULATORY GENES

With each new study it is becoming eminently clear that most homeobox genes are shared by all animals, from unsegmented worms, to fruit flies, starfish, tunicates, zebrafish, chickens, mice, and humans (Fig. 4). They control an organism's development by way of sending signals from one gene to another in the form of the proteins they produce. For example, as determined in the fruit fly, the oocyte, which eventually gives rise to the ovum, receives the messages that determine what will be the head and tail and up, down, right, and left sides of a potential offspring by a back and forth of proteins produced by homeobox genes in this cell and the cells of the ovary around it.[19] The body plan of this bilaterally symmetrical animal is thus determined even before the ovum from which it will develop becomes differentiated. Subsequently, the organism obtains its specific features through the process of certain homeobox genes turning on and off at specific times and in different regions of its developing body.

A radially symmetrical animal starts off life as a bilaterally symmetrical animal, with head and tail, up and down, and right and left sides predetermined. But then, three particular regulatory genes—*distal-less*, *engrailed*, and *orthodenticle*—are activated simultaneously throughout the embryo in a radial pattern.[31] The result is a starfish or a sea urchin. In fully committed bilaterally symmetrical animals—arthropods and chordates alike—these three genes participate in aspects of differentiation along the anteroposterior axis. The *distal-less* gene is involved in organizing the position of the appendage or limb bud along the anteroposterior axis; *engrailed* contributes to the growth of the central nervous system; and *orthodenticle* is essential for the proper differentiation of individual structures of the head region.

Tunicates have the homeobox gene for producing teeth, but this gene is active only in vertebrates.[52] Fruit flies, frogs, mice, and humans have the same homeobox genes for making an eye,[32] yet the eyes of fruit flies have multiple nondeformable lenses, while those of frogs, mice, and humans possess only a single, deformable lens, and the protein in the frog lens is different from that in the mammals. Tetrapods employ the same homeobox genes in limb development as fish do in fin development.[51] In fish, these genes are activated only along the posterior side of the developing fin bud, whereas, in tetrapods, they are turned on along the posterior side as well as across the anterior end of the elongating limb bud (Fig. 5). In addition, in tetrapods, the terminal homeobox gene of this set (*Hoxd-13*) contains a short DNA sequence that codes for a repeat of the amino acid alanine. In mice and humans, representing mammals, there are many more alanine residues encoded in this stretch than in the chicken, representing birds.[38] Muragaki et al.[38] suggested a possible correlation between differences in number of alanine repeats and number of bones in wrists and forefeet and ankles and hindfeet, the mammals having more and the bird fewer alanine residues and bones. The potential role of the polyalanine stretch in the sequence of events leading to the formation of number of foot and wrist and ankle bones is further indicated by a recent report on the development of synpolydactyly in a laboratory colony of mice that resulted from a spontaneous mutation of the *Hoxd-13* gene.[27] In parallel with the etiology of this syn-

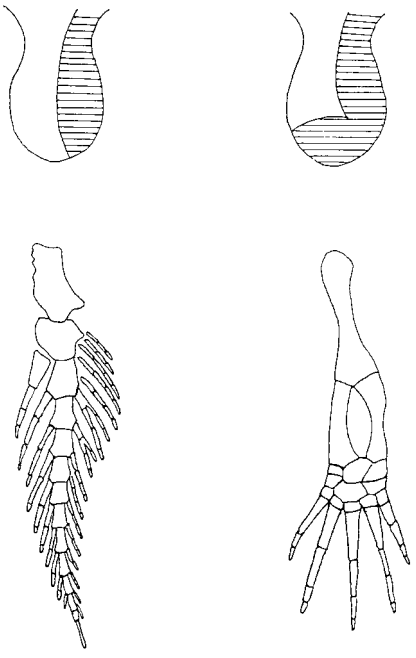

Figure 5. Schematic representation of homeobox gene activity in the developing fish fin (left) and tetrapod (right) limb buds. *Hoxd* genes are active only along the posterior side of the fin bud, whereas these genes are active both along the posterior and anterior aspects of the limb bud. The skeletal structures of an idealized fin of a coelacanth-like fish (left) and forelimb of a typical tetrapod (right) are represented below. (Drawing by T. D. Smith after reference 49, ©J. H. Schwartz.[46])

drome in humans, the mutation increased the length of the polyalanine stretch of the affected mice. Whether the latter causes an activation or deactivation of another gene or genes that, in turn, is part of the developmental cascade that results in synpolydactyly, remains unclear[27] (also, B. R. Olsen, personal communication.

The importance of homeobox genes for understanding evolution has certainly not been lost on the developmental geneticists studying and identifying them, or on those evolutionary biologists engaged in comparative anatomical studies. And for good reason. It is mind-boggling to entertain the possibility that, for all intents and purposes, the difference between a fruit fly and a human might have as much (or even more) to do with the turning on and off of homeobox genes that both animals share as would the acquisition in various ancestral vertebrates and mammals of a small number of mutations that duplicated, added slightly to, or deleted slightly from, already present homeobox genes—in short, that fundamental differences between organisms could result simply from altering the course of development through activating or deactivating certain homeobox genes, changing the timing of interactions of homeobox genes, or both.

When Darwin proposed his theory of evolution by way of natural selection, perhaps the most troubling thing for him was that he was going against church doctrine. According to the Bible, a divine creator placed all living creatures on this earth. As Darwin saw it, all life was connected by way of a common origin. In retrospect, it appears that he was correct, but for the wrong reason. Life is less connected by a trail of transformation from one state to another than by a commonality of regulatory genes. Differences between organisms seem to derive less from the addition of totally new regulatory genes than by the novel combination of existing regulatory genes.

Basically, genes do not evolve specifically for the creation of a particular organism, whether it be a human or a worm, or the parts of an organism. There are only particular combinations of genes that lead to the development of particular organisms with particular characteristics, which also explains why similar shapes recur time and time again in unrelated groups of organisms. After the emergence of a new species by mutation affecting regulatory genes, it might very well be that something we call *natural selection* somehow participates in the fine tuning of a species by picking and choosing from the variation between individuals that derives from differences at the level of structural genes. However, given the possible profound effect of a mutation involving a regulatory gene, the situation may be more as Hugo de Vries envisioned it: If the new feature doesn't kill you, you have it.

It is mind-boggling to entertain the possibility that, for all intents and purposes, fundamental differences between organisms could result simply from altering the course of development through activating or deactivating certain homeobox genes, changing the timing of interactions of homeobox genes, or both.

Clearly, the potential regulatory genes have for enacting what we call *evolutionary change* would seem to be almost unfathomable. But are we at the same place de Vries was when he proposed his mutation theory, or Richard Goldschmidt his hopeful monsters? Must we discard a model that has enjoyed so much success in explaining evolution because we cannot integrate it with those tantalizing pieces of a puzzle that suggest something punctuational? Must we reject the notion of punctuation because it is not clear how to incorporate it into popular evolutionary views?

The answer to all of these questions is no. Their resolution lies in understanding how a mutation in a regulatory gene—either a mutation that simply turns a gene on or off, or one that duplicates it or changes it slightly—fits within the generally accepted Darwinian-Mendelian framework of evolution. And, it turns out, there is a very simple way in which these seemingly incompatible schools of evolutionary thought can come together without invoking special pleading or unknown causes. In fact, many of the pieces of the answer have been right in front of us for decades.

HOMEOBOX GENES, MENDELISM, AND THE ORIGIN OF EVOLUTIONARY NOVELTY

The most important thing to realize when discussing regulatory genes, including homeobox genes, is that they are genes. As such, homeobox and other regulatory genes will be inherited in the same Mendelian fashion as any other kind of gene, such as the structural genes that code for eye color, wherein the allele for blue eye color is recessive and masked by those for dark eye colors. The Mendelian behavior of homeobox genes has clearly been demonstrated by experiments performed on mice in order to learn about the *Rx* homeobox gene, the activation of which is essential to the formation of the eye and its bony orbit.[32] In this particular study, the *Rx* gene, which is normally represented in the dominant state, was experimentally mutated to the recessive state. When heterozygotes for the mutant recessive were bred, offspring that were homozygous for the dominant allele were normal in eye and surrounding cranial development. So, too, were offspring that were heterozygous for the mutant recessive allele. But those offspring that were homozygous for the mutant recessive allele developed neither an eye nor a bony socket for it.

Although the *Rx* gene is now in the dominant state, it probably arose in the recessive state, as has been known to be the case with regard to most nonlethal mutations since William Bateson's studies early in this century.[2] As such, the first individual bearing the *Rx* allele (or the allele that would affect an already present *Rx* gene)

would have been heterozygous for the condition and would not have expressed this mutation phenotypically.[2,34,36] This mutation would have had the same probability of being passed on to offspring as any other recessive allele. Through the process of heterozygosis over some number of generations, this recessive allele could then spread silently throughout the population.[14,22,36,58] Eventually, there would be a sufficient number of heterozygotes for the recessive mutation that their random mating could produce offspring that were homozygous for this allele.

Following Mendel's prediction,[34] the heterozygotes will produce offspring in the phenotypic ratio of 3 (for the dominant) to 1 (a homozygote for the recessive). But, even though homozygotes would be produced in fewer numbers (q^2) than heterozygotes (2pq), with a number of heterozygotes bearing the mutation, there would still exist the opportunity for them to produce more than one homozygote in a single generation and for heterozygotes of subsequent generations to continue to add to the population of homozygotes.[22,58] Since, as Wright and especially Haldane espoused, a potential breeding population is smaller than the species to which it belongs, the spread of the new recessive allele would be relatively more rapid than if it were transmitted throughout a large population, such as the species itself.[22,58] Clearly, the process leading up to a significant number of individuals inheriting the mutation would not be instantaneous, whereas the ultimate expression of the mutation would. The case of the spontaneous mutation of the *Hoxd-13* gene that resulted in the expansion of the polyalanine stretch and synpolydactyly in a laboratory colony of mice demonstrates this model perfectly.[27] The mutation arose in the recessive state and had to have spread silently throughout the colony until the number of heterozygotes was sufficient for the chance production of a number of homozygotes for the trait. Only when the trait was found expressed in homozygotes did the researchers know that a mutation affecting a homeobox gene, which they then proceeded to identify, had occurred.

With the formation of homozygotes for the recessive allele, the morphology it represents—whether anatomical, physiological, or behavioral—would appear abruptly in the population, without a trail of transformational steps leading up to its full expression. Certainly, this was the case, as discussed above, with regard to the presence or absence of eyes and bony orbits in one experimental colony of mice and the development of synpolydactyly in another. In the former case, either the offspring of heterozygotes for the experimentally mutated *Rx* allele had eyes and bony orbits or they did not.[32] There was no in-between state. With heterozygotes for the mutation interbreeding, they, as well as the homozygotes for the mutation, will continue to add to the number of homozygotes.

The implications of this for understanding evolution are significant. For example, since the difference between the chordate-like larvae of one species of tunicate and the non-chordate-like larvae of another is due to the activation in the former of the *Manx* homeobox gene, which both species share,[53] it is likely that a version of Garstang's[16] vision of chordate origins occurred through the simple mechanism of a mutation that kept the *Manx* gene active beyond the larval phase of development. There would not have been a series of now missing links. The emergence of chordates would have been as abrupt as Garstang had suggested.

Similar examples of silent vs. active homeobox genes are not difficult to find. Take, for instance, the *Dlx* gene, the expression of which is necessary for tooth development to occur.[52] Given that this gene exists in tunicates, which are both toothless and jawless, but is not active in tunicates, it is reasonable to conclude that when this homeobox gene was activated in the first homozygotes for the mutation, they developed full sets of continually replacing teeth—just as we see in the fossil record with the emergence of the first toothed vertebrates and as we still see today in extant fish and reptiles.[4] As for the presence of wrist and ankle bones and fore- and hindfeet in tetrapods, the major difference between a fish and a tetrapod is that the *Hoxd* homeobox genes (*Hoxd-11, Hoxd-12,* and *Hoxd-13*) that are active along the posterior side of the enlarging fin bud are active not only along the posterior side of the growing tetrapod limb bud, but also across its anterior face.[49,51] In addition, the tetrapod's *Hoxd-13* gene contains a short molecular insertion that codes for alanine. Consequently, the emergence of tetrapody apparently resulted from two mutations: one which activated the *Hoxd-11-Hoxd-13* genes in a region in which they are silent in other vertebrates, and another involving a short molecular addition to an existing homeobox gene. Studies on mice and humans demonstrate that, when a mutation in the *Hoxd-13* gene increases the number of alanine residues, there is also an increase in number and in size of bones of the wrist, ankle, fore- and hindfoot.[27,38,59] The polyalanine stretch in birds, as represented by chickens, is considerably shorter, and the number of ankle and hindfoot and especially wrist and forefoot bones notably less than in mammals such as humans and mice.[38] As for the vertebrate fossil record, the first tetrapods, such as the Upper Devonian *Acanthostega* and *Ichthyostega*, possessed more digits in fore- and hindfoot, and more bones per digit as well as per wrist and ankle, than any subsequent tetrapod[4] (Fig. 6).

Additional examples from the fossil record demonstrate not only that novel features appear abruptly, but also that they are typically more fully expressed than in descendent forms. For example, among the earliest fish, the agnathans, many were often covered from mouth to anus in dermal plates.[4] Modern fish have small scales. The jaws of the earliest toothed fish were fully occupied along their lengths by teeth and they developed a lifetime supply of replacing teeth to boot.[4] Most mammals, however, have considerably fewer teeth in their jaws than fish and reptiles and at most develop only two functional generations of teeth. Modern birds, of course, are an extreme example of structural reduction in that they do not develop any teeth, although it has been demonstrated experimentally that their oral epithelial cells retain the potential to do so.[29] And, as cited above, the earliest tetrapods had many bones in their limbs' extremities, while all subsequent tetrapods have fewer bones—sometimes, as in birds, saurischian dinosaurs, sloths, and horses, consid-

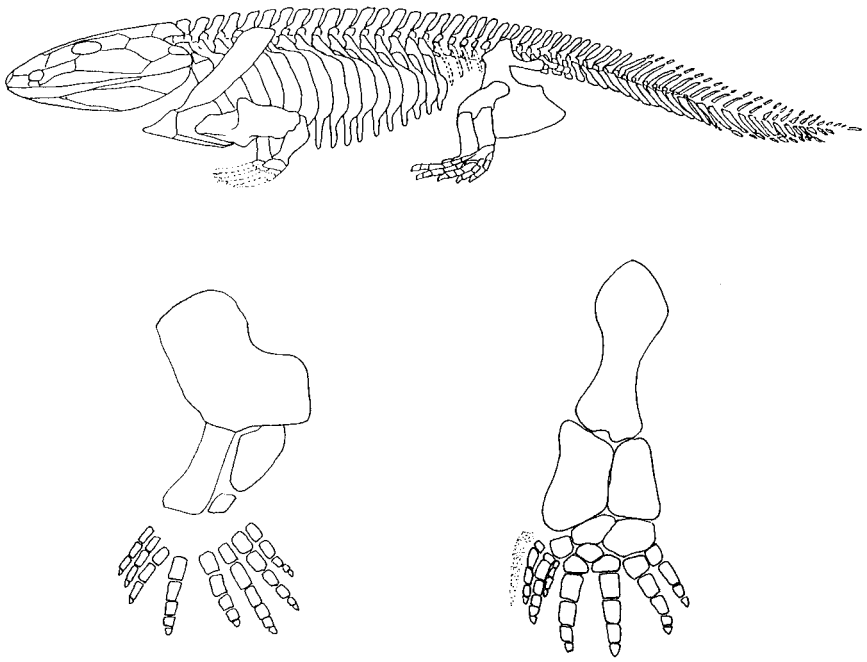

Figure 6. Reconstruction of skeleton of *Ichthyostega* (above) and fore- and hindlimbs of *Acanthostega* (left and right, respectively, below), two of the earliest known tetrapods. *Acanthostega* had at least eight digits on the fore foot and it and *Ichthyostega* had at least seven digits on the hind foot. The most a living vertebrate has is five digits per foot. (Drawing by T. D. Smith after reference 4, ©J. H. Schwartz.[46])

erably fewer bones.[4] Snakes, which lack extremities altogether, provide an extreme example of structural reduction.

I do not mean to suggest that earlier forms always have more of a structure or are more structurally complex than more recent forms. Clearly, vertebral column elongation occurred many times after the emergence of vertebrates, as is noted, for example, in the extinct plesiosaurs, mosasaurs, and ornithischian and saurischian dinosaurs, as well as in cetaceans.[4] Tooth number also secondarily increased in various mammals, such as in cetaceans, dugongs, and phalangeroid marsupials (e.g. wallabies). But it certainly does appear to be the case that when the regulatory gene implicated in the development of a particular structure is first activated, its effects are expressed to the fullest potential. Subsequently, structural complexity often diminishes in descendent taxa because that is the only route available for manipulating structural genes short of another mutation affecting a regulatory gene that might cause an additional increase in structural size, number, or complexity.

If there ever are instances of change that approach the expectations of a model of gradual evolution, they would most likely be found subsequent to the establishment of the basic novelty itself. For instance, even Schindewolf[45] thought that, after the major event in the evolution of the horse in which digital number was reduced to three, further digital reduction occurred as a series of less profound stepwise changes within the group. These changes within the horse clade were, nonetheless, stepwise rather than smoothly transformational because, even as the pair of lateral digits was becoming smaller, and the single central digit longer, there were always three recognizable phalanges in each lateral digit. When the true horse emerged with only a single functional pedal digit, it simply lacked the lateral digits in their entirety, three phalanges and all.

THE ROLE OF THE ENVIRONMENT: EXAMPLES OF ADAPTATION OR EVOLUTION?

The proposed model of evolution by way of regulatory gene inheritance and activity takes into account the claims of population geneticists (e.g. reference 5) who see no reason to invoke any mechanism other than a Mendelian one for accommodating any model of evolutionary change. Certainly, while a mutation is in the recessive state, its spread, even if accelerated by the constraints of a relatively small population,[22,58] will not be fulfilled in the space of a single generation, in contrast to the expectation of de Vries's mutation theory[10] and Goldschmidt's notion of hopeful monsters.[17,18] It will take some amount of time for the recessive allele to spread within a population[14] by the simple mechanism of Mendelian inheritance. But once homozygotes are produced, the feature dictated by the recessive allele will appear as if out of nowhere.[34] At some point, as we know must happen, the recessive allele might be converted to the dominant state. However, just as Fisher, Wright, and Haldane were in the dark as to how this process takes place, we still are today.

The proposed model of evolution by way of homeobox gene inheritance and activity takes into account the claims of population geneticists who see no reason to invoke any mechanism other than a Mendelian one for accommodating any model of evolutionary change.

But while it was de Vries's and Goldschmidt's insistence on the instantaneous expression of a mutation—one so profound that it would create a new species of plant or animal—that led to the dismissal of their theories, the overall picture of evolutionary change that they embraced was based on observations of some substance. Plant breeders had long been well aware that spontaneous mutation could oc-

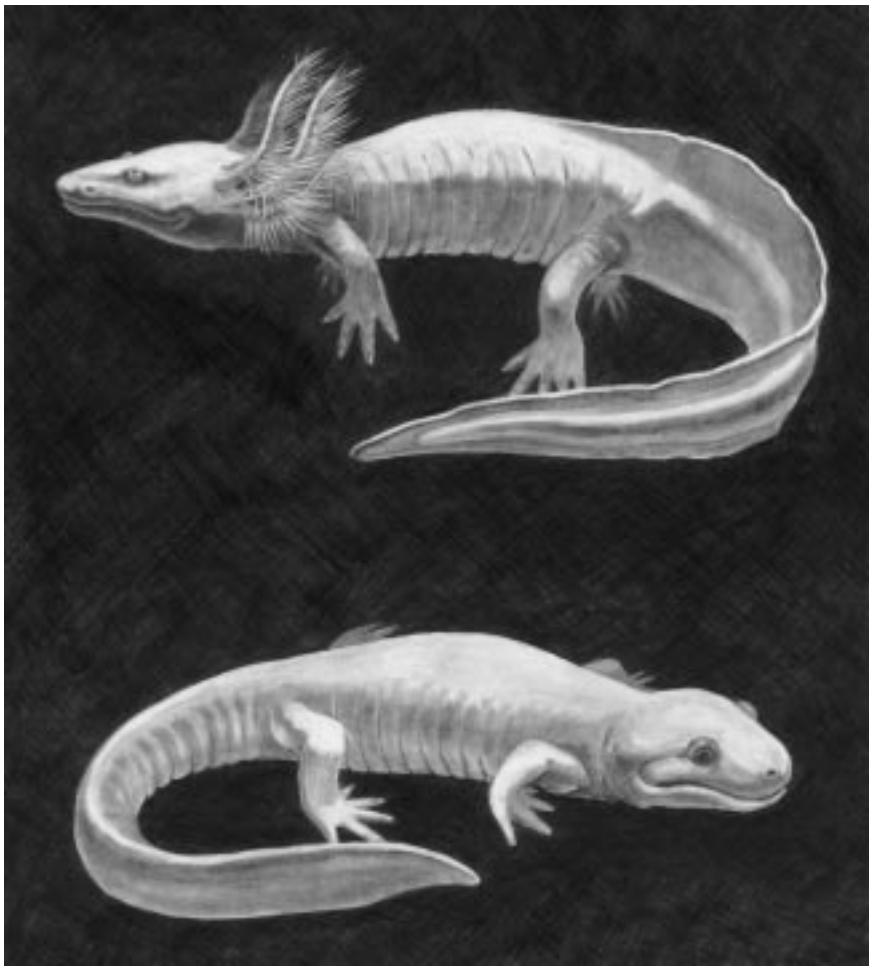

Figure 7. A larval axolotl (above) and adult tiger salamander (below). Under unaltered environmental conditions, the salamander metamorphoses from the larval to the adult state—losing, for instance, the externalized gills—coincident with the time at which it will be reproductively mature. Under conditions less favorable to prolonged somatic development, sexual maturation is accelerated and the animal becomes reproductively viable while still in the larval, axolotl phase. Another amphibian, the mud puppy, which looks similar to the axolotl, never metamorphoses away from the larval state, regardless of its environmental circumstances. (Drawing by T. D. Smith, ©J. H. Schwartz.[46])

cur in more than one individual and produce a new species in the space of a single generation.[10] As we now know, polyploidy in plants, giving rise to new kinds, results when the second meiotic division fails to take place. The problem, however, is that a similar phenomenon producing abrupt change in a number of individuals had not been observed in animals—hence the interest among evolutionary biologists in animals, such as various amphibians (Fig. 7) and insects, whose somatic development could be altered from one generation to the next by the surrounding environment (see discussion in reference 20). Under unfavorable environmental conditions, sexual maturation in these organisms is accelerated, with the result that the individual becomes reproductively viable while still in its larval state (producing paedomorphosis by progenesis). When environmental circumstances are favorable to longer periods of development, the individual matures reproductively in synchrony with the somatic shift from the larval to the adult state.

The leap of evolutionary faith among developmental biologists in terms of the rise of new species involves freezing an organism in a physical state other than the one into which it would otherwise have developed. If an organism, such as the tiger salamander, can be reproductively viable either as a larval axolotl or a physically adult amphibian (Fig. 7), then a permanent interference with the mechanism of somatic development could, it would seem, explain why the mud puppy never leaves the larval state and how Garstang's larval tunicate became the first chordate. Since the environmental trigger that can produce an axolotl vs. a tiger salamander affects more than one individual, evolution via heterochronic manipulation would seem to be a theoretically viable possibility. There is, however, a difference between an organism that is able to respond within certain limits to different stimuli and one that is committed to a particular developmental pathway.

In the first instance, the organism does not become a different organism. Although individuals of some generations may represent themselves as reproductively viable larva, they are still the same organism whose available gene and gene product interactions allow it to generate reproductive hormones either in early or later phases of somatic development (e.g. see references 9, 15, 24). In the latter case, the developmental pathway, however responsive to external cues, channels the organism in a particular way because of the regulatory genes that are activated or deactivated at certain times and in various combinations.

Take, for example, the case of the mud puppy. All mud puppies stay in the larval state, replete with large, frilly externalized gills, regardless of changes in the conditions of the environmental conditions from one breeding season to the next. Clearly, the mechanism available to the tiger salamander that allows for both accelerated and unaccelerated sexual development has been permanently altered in mud puppies, which would suggest that their physical state is due to changes affecting homeobox or other regulatory gene function rather than their environmental circumstances. Thus, while I think that we should take seriously the potential evolutionary roles of differential rates of development, whether they are at base heterochronic[9,20] or epigenetic,[30] it is imperative that we distinguish between the levels at which they might be significant.

It is of intrinsic interest to evolutionary biologists that some organisms,

such as the tiger salamander, can respond to local conditions and switch generationally between being a reproductively viable axolotl or adult. From this example, we see the extent to which certain aspects of development are responsive within a prescribed set of regulatory parameters. In the persistence of paedomorphosis, however—as in the mud puppy (a progenetic amphibian that stays in the larval state because the rate of reproductive maturation is apparently accelerated) or *Homo sapiens* (a neotenic mammal that exhibits delayed onset of adult growth rates)—we should expect that, since the phenomena are permanent (humans in the Arctic or Gobi Desert do not accelerate sexual maturation to become something akin to Bolk's reproducing fetuses), it was mutation that affected the underlying regulatory mechanisms. Similarly, mutation would have caused, for example, Garstang's tunicate to remain in the larval, chordate-like state, the first echinoderm to cease being a bilaterally symmetrical animal, early jawed fish to develop teeth, and the first tetrapods to have numerous digits and digital bones. As mutations, they most likely arose in the recessive state and were transmitted in Mendelian fashion, eventually becoming expressed in homozygotes.

But the quest for environment-change mechanisms continues. A recent study on the heat-shock gene *Hsp90* in fruit flies[43] claims to have demonstrated for the first time "an explicit molecular mechanism that assists the process of evolutionary change in response to the environment" (p. 341) and provides an avenue for the abrupt appearance of a morphological novelty. The unmutated *Hsp 90* gene, via the protein Hsp90, stabilizes cell-cycle and developmental regulators that are crucial for normal development and physiology. The mutated form of *Hsp90, Hsp83* (which had originally emerged spontaneously, but which was also experimentally induced by providing food containing an inhibitor of *Hsp90*), permits the development, depending on whether the structure is bilateral, of a variety of asymmetrical or linear anomalies of fruit fly anatomy: eye (e.g. facet color, number, size, or absence), wing (e.g. size, shape, venation, marginal deformation), bristles (morphology, duplication, distribution), abdomen (e.g. disorganized), thorax (e.g. disc eversion, duplication), haltere (transformation), and leg (deformation, transformation). Since the homozygous state for the mutation is lethal, the mutation has been maintained in laboratory colonies via heterozygotes, which are fertile and among which the severity of these abnormalities varies. Because anomalous heterozygotes were bred successfully with morphologically normal laboratory colony as well as captured wild strains of fruit flies, the case was made that the latter fruit flies harbored polymorphisms similar to those of the mutants that were mediated by *Hsp90*. Selective breeding of individuals with anomalies of eye and wing increased their numbers, but did not replicate the morphologies throughout the populations. Instead, the mutants diverged phenotypically over successive generations, with various variations becoming differentially expressed in different lineages. Other experiments demonstrated that the anomalies of heterozygotes could be enhanced at high, low, or both high and low temperatures.

It is of intrinsic interest to evolutionary biologists that some organisms, such as the tiger salamander, can respond to local conditions and switch generationally between being a reproductively viable axolotl or adult.

There is great potential in studying the mediating roles of proteins on the regulation of development, and this study clearly points out how important, for instance, the *Hsp90* gene is for the maintenance of normal development. But the inhibition of regulatory control through spontaneous or experimentally induced mutation of *Hsp90* does not produce developmentally sound individuals. Instead, the anomalies produced (many of which involve substantial deformation of structure) are asymmetrically expressed if involving bilateral structures, or disorganized if affecting linear structures. Consequently, rather than providing evidence for the introduction of evolutionary novelty that would have the potential to be sustained in wild populations, this study has underscored just how critical the proper orchestration of development is. Inadvertently, it has perhaps also elucidated the possible explanation for the differing patterns of wing venation that Goldschmidt[17,18] observed in gypsy and nun moths: namely, slight differences among individuals in the level of developmental control by *Hsp90*.

Although the expression of these anomalies can be affected by differing temperatures, this is not evidence of evolutionary change. Here, I think, the interpretation has been influenced by the language used to discuss evolution. It may be true, as in this study, that extremes of temperature could alter the severity or penetrance of an anomaly, but this is not proof of the environment's provoking evolutionary change. It demonstrates how the environment—in this case temperature—caused a response within the bounds of an established—in this case, mutant—developmental regime. The anomalies became either more or less severe. They did not emerge anew. The problem is that we have become accustomed to thinking of changing frequencies of *expressed variation*, particularly in a scenario that somehow involves the environment, as being synonymous with *evolutionary change*. It is not, of course. The introduction of novelty constitutes evolutionary change. The manipulation of a novelty once it is established (or of its range of variation within the population) reflects the response of individuals to their immediate circumstances within the limits of established genetic variation. At least for the sake of argument, then, it would be more appropriate to view the changes in intensity of the expressed anomalies in these experimental fruit flies as analogous to adaptations once the novelty or novelties have been introduced.

Were the results of this experimental study to have been representative

of evolutionary change, it would be of further interest because the inheritance of these anomalies is not, as its authors testify, a case of simple Mendelian inheritance. The anomalies appear to be polygenic in origin, being based on multiple alleles—which explains the range of variation within each category of anomaly expressed among individuals, but does not easily lend itself to a model of evolutionary change based on simple models of inheritance. In addition, although such data were not provided, it would appear that the mutation affecting the *Hsp90* gene arises in the dominant state inasmuch as heterozygotes for it, when crossed with normal, wild-type individuals, produce affected offspring. If so, as Fisher[14] pointed out, in the wild, selection would eliminate the mutated dominant allele prior to its being able to get a foothold in a natural population. But, whether fully dominant or recessive, only in the laboratory has this mutation been kept alive and its spread within lineages encouraged.

At present, then, we might do well to recall the admonitions of the young Thomas Hunt Morgan. Prior to his melding Darwinism and Mendelism to produce the model of evolutionary change that has dominated the life sciences, Morgan[35] did not agree with the title of Darwin's[7] book, *On the Origin of Species by Means of Natural Selection*. Rather than producing novelty of the sort that distinguishes species, Morgan argued that, by picking and choosing from among available variations within a population, natural selection's role was in the manipulation of adaptation. Had he thought of it, Morgan would perhaps have retitled Darwin's opus as *On the Origin of Adaptation by Means of Natural Selection*.[46] Indeed, the language that is used to explain evolution continues to conflate shifts in variation within populations with evolutionary change, extrapolating from the former process how the other, grander process would take place. As I have presented here, it is not necessary to do so. Although we must always be cognizant of the hierarchy of developmental levels, we can model evolutionary change on the basis of mutations in regulatory genes and simple Mendelian inheritance.

IMPLICATIONS OF A MODEL OF EVOLUTION BASED ON HOMEOBOX GENES, MUTATION, AND MENDELIAN INHERITANCE

There are various consequences of the model of evolution that I am proposing. First is the realization that, since most mutations arise in the recessive state, they will not be immediately available for any form of selection to act on them. Although this might seem obvious to us all when forced to think about it, it is actually not the way in which the language of evolution often portrays mutation and selection. For instance, G. C. Williams,[57] one of sociobiology's grandparents, writes as if a mutation is immediately accessible for selection to act on it. In a world in which evolutionary change depends on a pool of infinite variability that is constantly being fed by an unending supply of minor mutations, it might be reasonable to expect that the effect of a mutation should be immediately introduced into the population through the vehicle of its initial bearer so that natural selection can scrutinize it. In reality, however, it will take generations before the mutation will surface phenotypically in the population. By then, of course, many individuals, both heterozygotes and homozygotes, will possess this allele, with the trait being expressed in the homozygotes. The characterization of mutation introducing variation that is quickly acted upon by selection is not, however, unique to Williams. Indeed, this image pervades the literature of and after the Synthesis, as can be easily verified by reading, for instance, the works of Ernst Mayr (e.g. reference 33).

The language that is used to explain evolution continues to conflate shifts in variation within populations with evolutionary change, extrapolating from the former process how the other, grander process would take place.

Even after the advent of the first homozygotes for the mutation, breeding between heterozygotes carrying the mutation will continue to produce more homozygotes, just as breeding between heterozygotes for the mutation and homozygotes for the wild state will continue to produce more heterozygotes for the mutation, which, in turn, will continue to feed the production of homozygotes. In this way, even though there will be more than one homozygote for the mutation in the first generation, the number of homozygotes will increase purely by the ongoing process of heterozygosis and the chance that heterozygotes will produce homozygotes. Of course, mating between homozygotes will also increase their representation in the population. From a broader evolutionary perspective, it might seem as if one species was grading into another. In reality, however, the novelty that stems from the mutation will have emerged abruptly and in full expression in some number of individuals. If there is a place for a perception of gradualism in the overall picture, it would be relevant only in terms of the rate of increase over time in the number of homozygotes for the mutation. But this spread would not, of course, constitute evolution, which is heralded by the unexpected introduction of novelty.

The model I am proposing also forces us to realize that the homozygotes expressing a novelty are not necessarily reproductively incompatible with members of the parent species. Since a homozygote for the dominant wild (unmutated) allele can produce offspring with a heterozygote bearing one wild and one recessive (mutated) allele, and heterozygote-heterozygote crosses produce viable offspring, then, theoretically at least, any of these individuals could breed successfully with a homozygote for the recessive (mutated) allele.

We know—as Bateson[2] and Morgan et al.[36] first laid out in Mendelian genealogies—that this occurs in less profound genetic circumstances, such as with eye color, where individuals with blue eyes (homozygotes for the recessive) breed with heterozygotes and homozygotes for dark eye color

without consequence to their offspring. Beginning with Bateson's[2] studies, the production of fertile offspring without regard to homozygosity or heterozygosity has also been demonstrated to apply to more noteworthy cases of physical difference between individuals (e.g. brachy- and polydactyly)—cases that might be regarded as more anomalous or significant than mere differences in eye color. Given these less spectacular examples, why should we not then also expect that, in spite of the potentially profound effect that mutation affecting a regulatory gene might have, homozygotes for the mutation could produce viable offspring with any other individual from their original population?

The answer is simple: This should occur—unless, of course, the novelty emergent in the homozygotes directly affects the reproductive organs in some way, as Goldschmidt had required for his hopeful monsters to be reproductively isolated from their parent species,[17,18] sperm protein recognition by the ovum,[39,54] or zygote viability or fertility.[39,56] When the latter circumstances directly involving reproduction are not of concern, a question that then arises is: What constrains some members of a species from mating with other individuals with which they could produce reproductively viable offspring?

RECOGNIZING SPECIES

The solution to this question appears to lie in the species mate recognition concept of Hugh Paterson.[40] In contrast to Mayr's[33] biological species definition, wherein something external to the organism governs reproductive access of individuals to one another, the species mate recognition concept proposes that there is something inherent in an organism—whether morphological, physiological, behavioral—that determines with what other organism it can or will attempt to mate. Although Paterson saw the species mate recognition concept as being less applicable to plants, bivalves, and organisms of similarly constrained mobility than to more actively mobile sexually reproductive animals, the model may actually be very broadly applicable.

In the case of sessile or essentially immobile marine organisms, for instance, males and females (defined primarily by whether they produce sperm or ova[47]) cast their gametes into their aqueous surroundings. As was demonstrated recently in abalone,[54] either sperm and ova are compatible—that is, the receptors on the egg envelope recognize the protein of the sperm shell—or they are not. Thus, while many species of abalone might be spewing out gametes, only those sperm and ova that recognize each other biochemically will produce fertile offspring. As determined in *Drosophila*,[56] a homeobox gene lies at the base of gametic compatibility or incompatibility. Certainly, gametophyte (i.e. pollen-megaspore) recognition in plants should be analogous to sperm-ovum recognition in animals.

But especially for organisms that are mobile, and where the sexes have the opportunity to cross paths, genetic incompatibility need not be the only avenue available for keeping species apart. Indeed, as argued above, it might be genetically possible for two individuals to produce offspring successfully, but the fact of the matter just might be that, for whatever reasons, they do not recognize or respond to each other as potential mates. Consequently, while homozygotes for the recessive allele could interbreed with individuals of their parent population and produce reproductively viable offspring (unless the mutation affected the reproductive organs or gametic or zygotic function), the novelty that emerges with homozygosity may preclude its bearers from either recognizing or responding to related heterozygotes as potential mates, or vice versa. Certainly, both possibilities of non-recognition may apply simultaneously.

Among examples of factors leading to non-recognition are differences in social, feeding, or reproductive behavior, in color, pelage, size, or other morphology, and/or in pheromonal or other physiological chemistry. If we restrict ourselves intellectually to the typical Darwinian idea of a feature evolving gradually so as to become adapted or fitted to a specific role or function, then we are faced with the dilemma of having to explain the evolution of genes for preference of a trait through mate recognition (or the rejection of a trait because of non-recognition). But in light of the model of evolution proposed here, the adaptationist constraint is unnecessary. Mate recognition response need not be cast solely as a preference for one trait over another. Rather, it may simply be a response to, or perception of, greater vs. lesser degrees of similarity in, for example, feeding or positional behavior, smell, or color. Clearly, the first organisms with teeth, eyes, digited feet, or lungs would have exploited aspects of their environment in ways that were either unavailable to, or broached differently by, their unchanged kin. Individuals may have a proclivity toward mating at random, but their circles of potential mates may be circumscribed by degrees of perceived similarity in morphological, behavioral, or physiological attributes. Taken from this perspective, then, the notion of a species may truly be unto the beholder: either unto the bearer of the mutation and its effects, or unto the bearer's potential mate.

Another realization of the model of evolution I am proposing is that speciation need not occur through geographic isolation leading to the accumulation of genetic change and, ultimately, reproductive isolation, which is still invoked as the preeminent mechanism leading to the rise of new species.[39] Rather, aside from alterations affecting reproductive organs, gametes, or zygotes that would interfere directly with the production or viability of offspring, speciation can result from the introduction of a morphological, physiological, or behavioral novelty that causes some individuals not to mate with one another, in spite of the fact that they could still do so, while permitting others to do so. The reason that we do not observe most organisms, including those we identify as sister species, attempting to breed with one another is not necessarily because they cannot do so successfully (and thus they provide evidence of genetic and reproductive isolation). More likely, it is because they just do not.

The process of speciation (if, indeed, we should continue to refer to it as such) may, therefore, result simply from the silent spread of a mutation affecting a regulatory gene or genes followed by the abrupt expression of the effects of the mutation. It would not have to involve any subsequent

genetic change of note that would lead, for instance, to gametic incompatibility or any other expression of reproductive failure (for example, see references 11, 39). Furthermore, given the way in which the mutation were to spread throughout the population prior to being expressed, one can hardly think of the novelty emergent in homozygotes as having been selected for. Perhaps something (e.g. temperature, light, radiation, chemistry) in the external or internal environment of the organism might provoke the mutation, but that does not mean that the resultant phenotype is directly correlated with the source of the mutation. In fact, since it will take some number of generations for the mutation—which may not directly produce a morphology as much as contribute to a cascade of developmental effects leading to novelty—to spread through the population prior to its being expressed, it seems unlikely that the resultant morphology can be considered a specific adaptation to a specific environmental situation. Rather, it might more realistically be the case, again to paraphrase de Vries, that if a novelty does not kill its possessors, it will remain in the population. Once a novelty persists, however, selection might then act upon slight variations of its expression among the individuals of the species.

In the context of rethinking speciation, we can make some sense of hybrid zones: those areas in which two seemingly distinct species, whose members differ in morphology or behavior, or both, freely intermingle and breed. To some population biologists, a hybrid zone represents an early phase of the process of speciation. To others, the existence of hybrid zones demonstrates the impossibility of defining species on the basis of morphology. To the latter scientists, only with the enactment of genetic isolation, or at least geographic separation, can a species be delineated. Indeed, the fact that various organisms, such as zebras and horses, which normally do not interbreed, can do so successfully under artificially induced situations, also adds to the distrust of morphology in matters of determining species.

But hybrid zones actually demonstrate the fundamental aspects both of inheritance and of the species mate recognition system. In cases of hybridization, such as those documented for various species of baboon,[28] individuals at the poles of the clinal distribution act and look distinctly differently, particularly in the latter in aspects of hair length and coloration, body size, and skull size and shape. We do not know if the different populations had been geographically separated from one another at an earlier time and have since come into proximity, or whether they never did venture far from their original place of differentiation. But it is clear that whatever genetic differences underlie the physical differences, they do not prevent the interbreeding of individuals that we humans can distinguish easily from one another. Obviously, these individuals recognize each other as potential mates and that's all that matters.

The process of speciation (if, indeed, we should continue to refer to it as such) may result simply from the silent spread of a mutation affecting a regulatory gene or genes followed by the abrupt expression of the effects of the mutation.

More recently scientists have discovered that the rusty crayfish, which taxonomists had put in its own species, was expanding its range from Kentucky into Indiana, where it was hybridizing with the local blue crayfish, which had also been placed in its own species.[42] The hybrids are not only aggressive, they are also producing offspring which, in turn, are driving out the original blue crayfish. Since the rusty and blue crayfish were not only phenotypically different, but also geographically separated, it is understandable how a taxonomist would be satisfied that each population represented a different species.

One would predict that the phenotypic difference between the blue and rusty crayfish arose by the spread of a mutant recessive allele. One would also predict that additional genetic difference could have arisen over the period during which the two populations had been geographically separated. Nevertheless, and contrary to expectation, the two morphs of crayfish can obviously successfully interbreed. Although this might perhaps be disturbing to taxonomists, it is totally comprehensible in the context of basic Mendelian principles of inheritance and the species mate recognition concept. In this case, neither phenotypic difference nor geographic (and, consequently, temporal) separation were sufficiently significant to prevent individuals of both species from recognizing each other as mates and, in turn, producing reproductively viable hybrids.

The foregoing discussion is also relevant to one of the most heated debates in paleoanthropology: namely, could Neanderthals and *Homo sapiens* interbreed? The basis of the dispute is whether the profound and profuse morphological differences that exist between theses hominids truly warrant recognizing two species, *H. sapiens* and *H. neanderthalensis*. Clearly, however, this is not the way in which to approach the question. It is not simply a matter of whether Neanderthals and modern humans *could* have successfully interbred. Rather, it is a question of whether they *would* have interbred.

If the situation were as simple as the current debate makes it seem, and the issue truly only about the existence of maximally two species—instead of there being great taxonomic diversity, with some number of species comprising with *H. neanderthalensis* a clade apart from that which subsumes *H. sapiens* and its closest relatives, as the situation really appears to be[48,55]—I expect that there would have been a good chance that they could have interbred. But, given the degree of morphological difference between the two hominids, and recognizing that this reflects only the fossilizable elements of their biology, I would also predict that, if they truly were sister taxa, Neanderthals and modern humans would not have interbred. Perhaps there are some among us who believe

that the morphological differences would not have deterred them from attempting to mate with a Neanderthal. But this presumes that the Neanderthal would also have recognized the human as a potential mate.

As for these different hominids really being distinct species, the most that we can do as systematists is to offer this as an hypothesis. If we do suggest that two groups are probably distinguishable as species, and that they are sister taxa, but we later find reason to relegate them to the same species, our hypothesis of relatedness has not been falsified. At present, it seems to me that, since morphology results from both the underlying genetic information and the specifics of a particular developmental history, it is still a reasonable arbiter in making taxonomic and phylogenetic decisions. For systematists, the task at hand is to try to distinguish between differences among individuals that are structural gene derivatives and those that are governed by regulatory genes. Clearly, the former case represents the noise of individual variation, whereas the latter provides insight into the advent of clades and the differentiation of species within clades. For all evolutionary biologists, the time has come to resynthesize the Synthesis.

ACKNOWLEDGMENTS

The author thanks T. Gill, A. Lumsden, B. R. Olsen, M. Paalman, and an anonymous reviewer for their very insightful comments.

LITERATURE CITED

1 Bateson W. 1894. Materials for the study of variation, treated with especial regard to discontinuity in the origin of species. New York: Macmillan.

2 Bateson W. 1909. Mendel's principles of heredity. Cambridge: Cambridge University Press.

3 Bolk L. 1926. La récapitulation ontogenetique comme phénomène harmonique. Arch Anat Histol Embryol 5:85–98.

4 Carroll RL. 1988. Vertebrate paleontology and evolution. New York: W.H. Freeman.

5 Charlesworth B, Lande R, Slatkin M. 1982. A neo-Darwinian commentary on macroevolution. Evolution 36:474–498.

6 Cuvier G. 1812. Recherches sur les ossements fossiles de quadripèdes; ou l'on rétablit les caractères de plusiers espèces d'animaux que les révolutions du globe paroissent avoir détruites. Paris: Deterville.

7 Darwin C. 1859. On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life. London: John Murray. (Reprinted 1976, Cambridge, MA: Harvard University Press.)

8 Darwin C. 1868. The variation of animals and plants under domestication. London: John Murray.

9 de Beer G. 1930. Embryology and evolution. Oxford: Clarendon.

10 de Vries H. 1906. Species and varieties: their origin by mutation, 2nd edition. Chicago: Open Court.

11 Dobzhansky T. 1941. Genetics and the origin of species, 2nd edition. New York: Columbia University Press.

12 Eldredge N. 1971. The allopatric model and phylogeny in Paleozoic invertebrates. Evolution 25:156–167.

13 Eldredge N, Gould SJ. 1972. Punctuated equilibria: an alternative to phyletic gradualism. In: Schopf TJM, editor. Models in paleobiology. San Francisco: Freeman, Cooper; p 82–115.

14 Fisher RA. 1930. The genetical theory of natural selection. Oxford: Oxford University Press.

15 Ford EB, Huxley J. 1927. Mendelian genes and rates of development in *Gammarus chevreauxi*. Br J Exp Biol 5:112–134.

16 Garstang W. 1928. The morphology of the Tunicate and its bearings on the phylogeny of the Chordata. Q J Microscop Sci 72:51–187.

17 Goldschmidt R. 1934. *Lymantria*. Bibliotheca Genetica 11:1–180.

18 Goldschmidt R. 1940. The material basis of evolution. (Facsimile reprinted 1982, New Haven, CT: Yale University Press.)

19 González-Reyes A, Elliott H, St Johnston D. 1995. Polarization of both major body axes in *Drosophila* by *gurken-torpedo* signalling. Nature 375:654–658.

20 Gould SJ. 1977. Ontogeny and phylogeny. Cambridge, MA: Belknap Press of Harvard University Press.

21 Gould SJ, Eldredge N. 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3:115–151.

22 Haldane JBS. 1932. The causes of evolution. New York: Harper and Brothers.

23 Haeckel E. 1874. The evolution of man: a popular exposition of the principal points of human ontogeny and phylogeny, 1st edition. New York: HL Fowle. (Third edition, translated into English, 1876.)

24 Huxley J. 1923. Time relations in amphibian metamorphosis, with some general considerations. Sci Prog 17:606–618.

25 Huxley TH. 1863. Man's place in nature. New York: D. Appleton.

26 Jepsen GL. 1949. Forward. In: Jepsen GL, Simpson GG, Mayr E, editors. Genetics, paleontology, and evolution. Princeton: Princeton University Press. (Reprinted 1963, New York: Atheneum.)

27 Johnson KR, Hope OS, Donahue LR, Ward-Bailey P, Bronson RT, Davisson MT. 1998. A new spontaneous mouse mutation of *Hoxd13* with a polyalanine expansion and phenotype similar to human synpolydactyly. Hum Mol Gen 7:1033–1038.

28 Jolly CJ. 1993. Species, subspecies, and baboon systematics. In: Kimbel WH, Martin L, editors. Species, species concepts, and primate evolution. New York: Plenum; p 67–107.

29 Kollar E, Fisher C. 1980. Tooth induction in chick epithelium: expression of quiescent genes for enamel synthesis. Science 207:993–995.

30 Løvtrup S. 1974. Epigenetics: a treatise on theoretical biology. New York: John Wiley and Sons.

31 Lowe CJ, Wray GA. 1997. Radical alterations in the roles of homeobox genes during echinoderm evolution. Nature 389: 718–721.

32 Mathers P, Grinberg A, Mahon K, Jamrich M. 1997. The *Rx* homeobox gene is essential for vertebrate eye development. Nature 387:604–607.

33 Mayr E. 1942. Systematics and the origin of species. New York: Columbia University Press.

34 Mendel G. 1866. Experiments in plant hybridisation. (Reprinted 1965, English translation and footnotes by W. Bateson, Cambridge, MA: Harvard University Press.)

35 Morgan TH. 1903. Evolution and adaptation. New York: Macmillan. (Reprinted 1908.)

36 Morgan TH, Sturtevant AH, Muller HJ, Bridges CB. 1915. The mechanism of Mendelian heredity. New York: Henry Holt. (Revised and reprinted 1926.)

37 Morgan TH. 1916. A critique of the theory of evolution. Princeton: Princeton University Press.

38 Muragaki Y, Mundlos S, Upton J, Olsen BR. 1996. Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. Science 272:548–551.

39 Nei M, Zhang J. 1998. Molecular origin of species. Science 282:1428–1429.

40 Paterson H. 1985. The recognition concept of species. In: Vrba E, editor. Species and speciation. Transvaal Museum Monograph no. 4. Pretoria: Transvaal Museum; p 21–29.

41 Purugganan MD. 1998. The molecular evolution of development. BioEssays 20: 700–711.

42 Roush W. 1997. Hybrids consummate species invasion. Science 277:316–317.

43 Rutherford SL, Lindquist S. 1998. Hsp90 as a capacitor for morphological evolution. Nature 396:336–342.

44 Schindewolf O. 1936. Palaeontologie, Entwicklungslehre und Genetik. Berlin: Bontrager.

45 Schindewolf O. 1950. Basic questions in paleontology: geologic time, organic evolution, and biological systematics. (Reprinted 1993, English translation by J. Schaefer, Chicago: University of Chicago Press.)

46 Schwartz JH. 1999. Sudden origins: fossils, genes, and the emergence of species. New York: John Wiley and Sons.

47 Schwartz JH. in prep. The evolution of sex. New York: John Wiley and Sons.

48 Schwartz JH, Tattersall I. 1996. Toward distinguishing *Homo neanderthalensis* from *Homo sapiens*, and vice versa. Anthropologie 43:129–138.

49 Shubin N, Tabin C, Carroll S. 1997. Fossils, genes and the evolution of animal limbs. Nature 388:639–648.

50 Simpson GG. 1944. Tempo and mode in evolution. New York: Columbia University Press.

51 Sordino P, van der Hoeven F, Duboule D. 1995. Hox gene expression in teleost fins and the origin of vertebrate digits. Nature 375:678–681.

52 Stock DW, Ellies DL, Zhiyong Z, Ekker M, Ruddle FH, Weiss KM. 1996. The evolution of the vertebrate *Dlx* gene family. Proc Natl Acad Sci USA 93:10858–10863.

53 Swalla B, Jeffery W. 1996. Requirement of the *Manx* gene for expression of chordate features in a tailless ascidian larva. Science 274:1205–1208.

54 Swanson WJ, Vacquier VD. 1998. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. Science 281:710–712.

55 Tattersall I, Schwartz JH. 1998. Morphology, paleoanthropology, and Neanderthals. Anat Rec (New Anat) 253:113–117.

56 Ting C-T, Tsaur S-C, Wu M-L, Wu C-I. A rapidly evolving homeobox at the site of a hybrid sterility gene. Science 282:1501–1504.

57 Williams GC. 1966. Adaptation and natural selection: a critique of some current evolutionary thought. Princeton: Princeton University Press.

58 Wright S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proc Sixth Int Congr Genet 1:356–366.

59 Zákány J, Duboule D. 1996. Synpolydactyly in mice with a targeted deficiency in the *HoxD* complex. Nature 384:69–71.