CHAPTER 9

Four-Winged Fruit Flies

In Darwin's theory, evolution is a product of two factors: natural selection and heritable variation. Natural selection molds populations by preserving favorable variations that are passed on to succeeding generations. Small-scale evolution within a species (such as we see in domestic breeding) makes use of variations already present in a population, but large-scale evolution (such as Darwin envisioned) is impossible unless new variations arise from time to time. Darwin devoted the first two chapters of *The Origin of Species* to establishing the existence of heritable variations in domestic and wild populations, but he did not know how they are inherited or how new ones arise.

It wasn't until the advent of neo-Darwinism and molecular genetics in the twentieth century that many biologists finally felt they understood the mechanism of heredity and the origin of variations. According to modern neo-Darwinism, genes consisting of DNA are the carriers of hereditary information; information encoded in DNA sequences directs the development of the organism; and new variations originate as mutations, or acci-dental changes in the DNA.

Some DNA mutations have no effect, and most others are harmful. Occasionally, however, a mutation comes along that is beneficial—it confers some advantage on an organism, which can then leave more offspring. According to neo-Darwinism, beneficial DNA mutations—though not needed for limited modifications within a species—provide the raw materials necessary for large-scale evolution.

Beneficial mutations are rare, but they do occur. For example, mutations can have biochemical effects that render bacteria resistant to antibiotics or insects resistant to insecticides. But biochemical mutations cannot explain the large-scale changes in organisms that we see in the history of life. Unless a mutation affects morphology—the shape of an organism—it cannot provide raw materials for morphological evolution.

One organism in which morphological mutations have been extensively studied is the fruit fly, *Drosophila melanogaster*. Among the many mutations that are now known in *Drosophila*, some cause the normally two-winged fruit fly to develop a second pair of wings. Since 1978, the four-winged fruit fly has become increasingly popular in textbooks and public presentations as an icon of evolution. (Figure 9-1)

But four-winged fruit flies do not occur spontaneously. They must be carefully bred in the laboratory from three artificially maintained mutant strains. Furthermore, the extra wings lack flight muscles, so the mutant fly is seriously handicapped. Four-winged fruit flies testify to the skill of geneticists, and they help us to understand the role of genes in development, but they provide no evidence that DNA mutations supply the raw materials for morphological evolution.

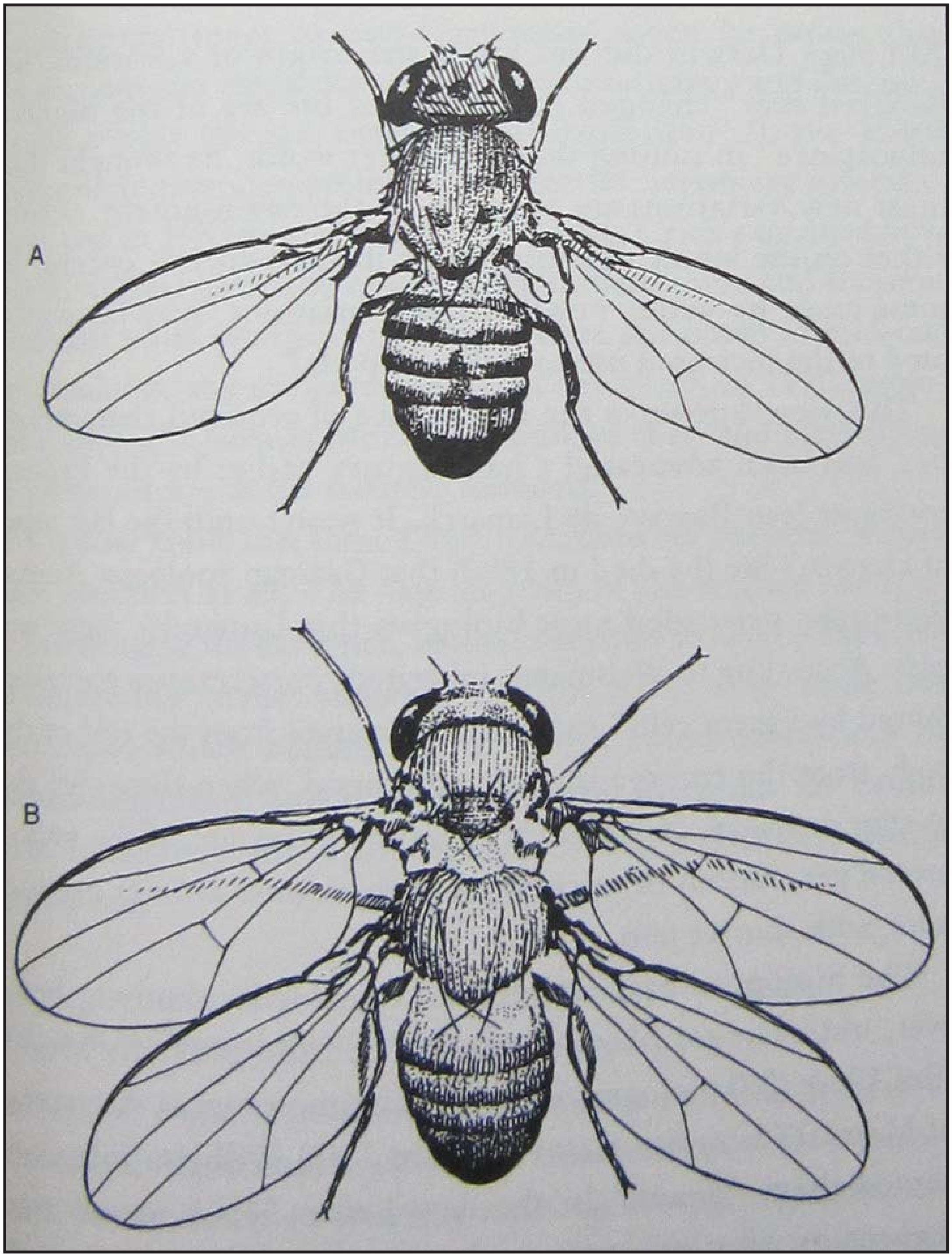

FIG U R E 9-1 Normal and four-winged fruit flies.

(A) A normal or «wild-type» fruit fly, with two wings and two balancers or «halteres» (tiny appendages on either side between the wings and the rear legs). (B) A mutant fly in which the halteres have developed into normal-looking wings.

The origin of variations from Darwin to DNA

Although Darwin did not know the origin of variations, he believed that "changed conditions of life are of the highest importance" in causing them. In other words, he thought that most new variations are induced by the environment, acting either on the whole organism or on its reproductive system. In some cases, he wrote, new heritable variations "may be attributed to the increased use or disuse of parts."

This view, known as the inheritance of acquired characteristics, had been advocated a half century earlier by the French zoologist Jean Baptiste de Lamarck. It wasn't until the last years of Darwin's life (he died in 1882) that German zoologist August Weismann persuaded most biologists that Lamarck's view was false. According to Weismann, inherited characteristics are transmitted by "germ cells" that remain separate from the rest of the body from the embryo through adulthood, when they give rise to eggs or sperm. In a famous experiment, he cut off the tails of several generations of mice to prove that disuse did not produce mice with shorter tails.

The biological basis of heredity remained unknown, however, until Gregor Mendel's theory became generally known after 1900. Cell biologists identified chromosomes as the carriers of Mendel's heredity factors, and in 1909 Wilhelm Johanssen named them "genes." In the days before DNA, genes were regions on chromosomes, and American fruit fly geneticist Thomas Hunt Morgan studied spontaneous changes in individual genes that he called mutations (a term he borrowed from Dutch botanist Hugo DeVries).

By the 1930s many geneticists believed that the sort of mutations Morgan studied were the source of new variations needed for evolution. In 1937 Theodosius Dobzhansky made this a fundamental tenet of neo-Darwinism when he wrote that «mutations and chromosomal changes...constantly and unremittingly supply the raw materials for evolution.» In the 1940s microbiologists showed that DNA carries hereditary information, and in 1953 James Watson and Francis Crick explained how the molecular structure of DNA might determine and transmit heritable traits. Morgan's mutations were attributed to molecular accidents, and the picture seemed complete. In 1970, molecular biologist Jacques Monod announced that «the mechanism of Darwinism is at last securely founded.»

We now know that some DNA mutations are «neutral»—they have no effect at all. The vast majority of the rest are harmful. In the struggle for existence, natural selection would be expected to ignore the former and eliminate the latter. Only those rare mutations which benefit the organism could be favored by natural selection, and thus provide raw materials for evolution. Some mutations that affect biochemical pathways fit this description.

Beneficia1 biochemical mutations

Antibiotics work by poisoning molecules in bacteria. Most cases of medically significant antibiotic resistance are not due to mutations, but to complex enzymes that inactivate the poison, and which bacteria either inherit or acquire from other organisms. Some cases of resistance, however, are due to spontaneous mutations that alter the bacteria's molecules just enough so an antibiotic can no longer poison them. Bacteria lucky enough to have such mutations (like those lucky enough to have inactivating enzymes) can resist an antibiotic and survive to reproduce.

Like antibiotic resistance, most insecticide resistance is due to inactivating enzymes. There are cases, however, in which resistance is due to spontaneous mutations. Like the mutations that confer resistance to antibiotics, these can benefit the organism by enabling it to survive and reproduce despite the presence of the poison.

Since mutations leading to antibiotic and insecticide resistance are clearly beneficial in certain environments, biology textbooks invariably list them as evidence that mutations provide the raw materials for evolution. Many textbooks also list sickle-cell anemia, because the same mutation that causes this crippling genetic disease can also, in a milder form, benefit infants growing up in malaria-ridden areas. In all of these cases, however, the evolution that occurs is trivial. The raw materials for large-scale evolution must be able to contribute to fundamental changes in an organism's shape and structure.

Since biochemical mutations—such as those leading to antibiotic resistance and sickle-cell anemia—do not affect an organism's shape or structure, evolution needs beneficial mutations that affect morphology. Neo-Darwinists know this, of course, and to provide evidence of morphological mutations a growing number of them are using pictures of mutant fruit flies with an extra pair of wings.

The four-winged fruit fly

The bodies of fruit flies consist of segments, three of which are in the thorax (midsection). Normally, the second thoracic segment bears a pair of wings, and the third bears a pair of "halteres," or balancers—tiny appendages that enable the insect to maintain its balance in flight. (Figure 9-la) In 1915 geneticist

Calvin Bridges (working in Thomas Hunt Morgan's laboratory) discovered a mutant fruit fly in which the third thoracic segment looked a bit like the second, and the halteres were slighdy enlarged and looked like miniature winglets. This spontaneously occurring *«bithorax»* mutant has been maintained as a laboratory stock ever since.

In 1978 California Institute of Technology geneticist Ed Lewis reported that by breeding flies possessing the *bithorax* mutation with flies possessing another mutation, «*postbithorax*,» he was able to produce a fruit fly in which the halteres were even more enlarged, and looked almost like a second pair of wings. He subsequently found that if flies combining these two mutations were bred with flies possessing a third, *«anterobithorax»* the triple-mutant offspring had an extra pair of wings that looked like the fly's normal wings. (Figure 9-1, B)

Lewis had to use three mutations because no single mutation affected the entire segment. Each fruit fly segment is divided into an anterior (forward) compartment and a posterior (rearward) compartment. The *postbithorax* mutation induced the posterior compartment of the third thoracic segment to produce the rear half of a wing, while the combination of *anterobithorax* and *bithorax* mutations caused the anterior compartment to produce the forward half of a wing. Only a fly possessing all three mutations bears four normal-looking wings. (Figure 9-2)

Of course, Lewis's goal was not to produce sideshow freaks, but to understand the molecular interactions involved in fruit fly development. It turns out that all three mutations in the four-winged fruit fly affect a single large gene, «*Ultrabithorax*.» The mutations do not affect the protein produced by the gene, but only where the protein is produced. Every cell in the fruit fly's body receives the same genes from the fertilized egg; but as the

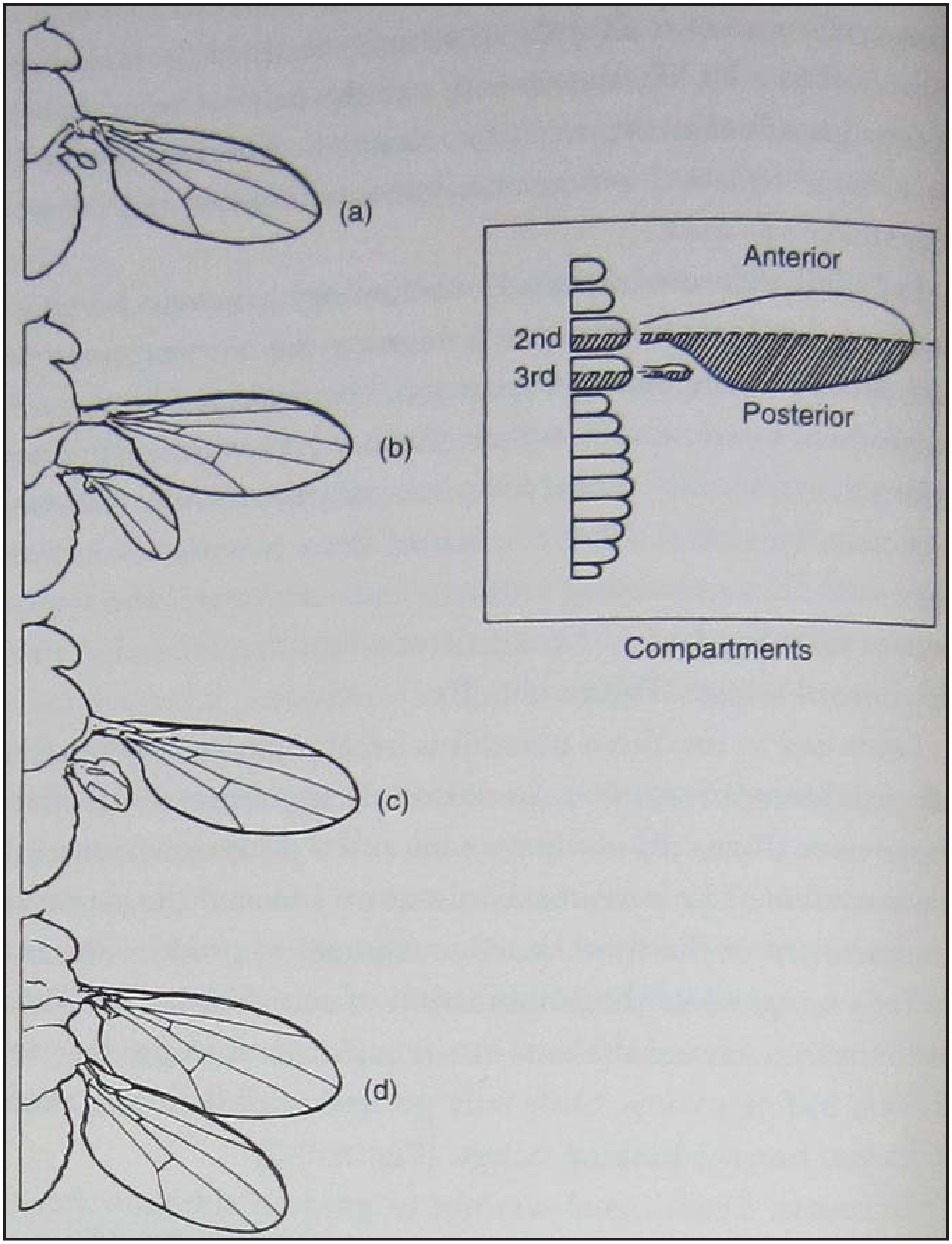

FIGURE 9-2 Steps in the construction of a four-winged fruit fly.

The box at the upper right shows how each segment is divided into an anterior and posterior compartment, (a) Normal fly; (b) *bithorax* mutant (c) *post-bithorax* mutant; (d) triple mutant (*anterobithorax*, *bithorax*, and *postbithorax*). The *anterobithorax* mutation enhances the effect of *bithorax*.

embryo develops, specific genes are turned on only in those cells where they are needed. This process depends on «regulatory sequences» associated with each gene. Such sequences act like switches, allowing genes to be turned on or off in different parts of the embryo.

In a normal fruit fly, the *Ultrabithorax* gene is turned on in the third thoracic segment, and the segment produces halteres rather than wings. The *anterobithorax*, *bithorax*, and *postbithorax* mutations each turn the gene off to some degree: The first two turn it off in the anterior compartment, and the third turns it off in the posterior compartment. When all three mutations are present, the gene is completely turned off in the third thoracic segment, which then produces a pair of normal-looking wings instead of halteres.

By deciphering the genetic interactions involved in turning off *Ultrabithorax*, Lewis was able to shed considerable light on the molecular biology of fruit fly development, and his research earned him a Nobel Prize in 1995. But how much light do four-winged fruit flies shed on evolution?

Four-winged fruit flies and evolution

According to Peter Raven and George Johnson's 1999 textbook, *Biology*, «all evolution begins with alterations in the genetic message... Genetic change through mutation and recombination [the re-arrangement of existing genes] provides the raw materials for evolution.» The same page features a photo of a four-winged fruit fly, which is described as «a mutant because of changes in *Ultrabithorax*, a gene regulating a critical stage of development; it possesses two thoracic segments and thus two sets of wings.»

The textbook does not explicitly claim that the four-winged fruit fly shows us evolution in action, but it uses the fly in its discussion of evolution to imply that genetic mutations are the origin of new variations. The textbook fails to explain, however, that three separate mutations had to be artificially combined in one fly to produce a second set of normal-looking wings. Such a combination is exceedingly unlikely to occur in nature.

Even more seriously, the textbook fails to point out that the second pair of wings is non-functional. Biologists have known since the 1950s that the extra wings on *bithorax* mutants lack flight muscles. The hapless insect is thus disabled, and the disability increases with the size of the mutant appendages. In aerodynamic terms, a triple-mutant four-winged fruit fly is like an airplane with an extra pair of full-sized wings dangling loosely from its fuselage. It may be able to get off the ground, but its flying ability is seriously impaired. Because of this, four-winged males have difficulty mating, and unless the line is carefully maintained in a laboratory it quickly dies out.

So four-winged fruit flies are not raw materials for evolution. Even neo-Darwinists acknowledge this. Ernst Mayr wrote in 1963 that major mutations such as *bithorax* "are such evident freaks that these monsters can be designated only as 'hopeless.' They are so utterly unbalanced that they would not have the slightest chance of escaping elimination" through natural selection. In addition, finding a suitable mate for the "hopeless monster" seemed to Mayr to be an insurmountable difficulty. Given this long-standing objection to the evolutionary significance of such monsters, the recent popularity of four-winged fruit flies is puzzling. Perhaps, like pictures of peppered moths on tree trunks, they are just too "visual" to resist.

Adding to the confusion, textbook accounts typically leave the reader with the impression that the extra wings represent a *gain* of structures. But four-winged fruit flies have actually *lost* structures which they need for flying. Their balancers are gone, and instead of being replaced with something new have been replaced with copies of structures already present in another segment. Although pictures of four-winged fruit flies give the impression that mutations have added something new, the exact opposite is closer to the truth.

Someone attempting to salvage these mutants as evidence for neo-Darwinism might point out that even a loss of structures can have evolutionary significance. And indeed it can. Evolutionary biologists believe that two-winged flies evolved from four-winged flies. It is conceivable that ancestral four-winged flies acquired genetic mutations which reduced one pair of wings to tiny rudiments, and these became halteres. Perhaps *bithorax* is showing us mutations back to the ancestral state—in other words, evolution in reverse. This scenario is plausible, but once again the evidence points in the wrong direction.

Evolution in reverse?

In support of the view that two-winged flies evolved from four-winged flies, a 1998 booklet published by the National Academy of Sciences points out that «geneticists have found that the number of wings in flies can be changed through mutations in a single gene.» Although this statement is technically true, it is quite misleading—and not just because three separate mutations are necessary and the extra wings are nonfunctional.

What really changes the number of wings in a fly is a complex genetic network. A four-winged fly does not become a two-winged fly because mutations knock out some hypothetical «wing gene,» but because the fly acquires a whole network of developmental controls that transform one set of wings into functional halteres.

The *Ultrabithorax* gene itself is large and complex. It consists of about a hundred thousand DNA subunits, most of which are involved in regulating when and where the gene is turned on in the embryo. And *Ultrabithorax* does not function alone. In 1998 Scott Weatherbee and a team of developmental biologists reported that *Ultrabithorax* affects haltere development "by independently regulating selected genes that act at different levels of the wing patterning hierarchy." It is this entire hierarchy, and not just one gene, that had to evolve in order to convert wings into halteres. According to Weatherbee and his colleagues, "the evolution of the haltere progressed through the accumulation of a complex network of [*Ultrabithorax*]-regulated interactions." Biologists do not understand how fruit flies acquired this complex network, but it certainly could not have originated from just a few mutations in a single gene.

What the four-winged fruit fly shows us is that mutations can shut down a complex network of interactions. But there's nothing surprising about this; we know that a single mutation can shut down an entire embryo and kill it outright. Damaging a complex regulatory network with mutations doesn't explain how the network originated, any more than killing an embryo with a lethal mutation explains how flies evolved. Yet it is precisely the origin of the network that we need to understand if we are to explain how four-winged flies evolved into two-winged flies.

So the four-winged fruit fly is a useful window on the genetics of development, but it provides no evidence that mutanons supply the raw materials for morphological evolution. It does not even show us evolution in reverse. As evidence for evolution, the four-winged fruit fly is no better than a two-headed calf in a circus sideshow.

Why, then, has it become popular to feature the four-winged fruit fly in textbooks and public presentations defending Darwin's theory? Could it be concealing a deeper problem with the evidence for neo-Darwinism?

Are DNA mutations the raw materials for evolution?

According to biology textbooks, DNA mutations are unquestionably the source of new variations for evolution. For example, the 1998 edition of Cede Starr and Ralph Taggart's *Biology: The Unity and Diversity of Life* tells students that «every so often, a new mutation bestows an advantage on the individual... beneficial mutations, and neutral ones, have been accumulating in different lineages for billions of years. Through all that time, they have been the raw material for evolutionary change—the basis for the staggering range of biological diversity, past and present.» Burton Guttmans 1999 textbook, Biology, declares that «*mutation is ultimately the source of all genetic variation and therefore the foundation for evolution*.» (emphasis in original)

Yet the evidence cited in these textbooks falls far short of supporting these sweeping claims. To be sure, biochemical mutations lead to antibiotic and insecticide resistance, and human beings carrying the sickle-cell trait are more likely to survive malaria as infants. But only beneficial morphological mutations can provide raw materials for morphological evolution, and evidence for such mutations is surprisingly thin. As we have seen, four-winged fruit flies do not provide the missing evidence, despite their current popularity.

If textbook-writers have no good examples of beneficial morphological mutations, it's not because biologists haven't been looking for them. About the time that Lewis was studying *Ultrabithorax*, German geneticists Christiane Nüsslein-Volhard and Eric Wieschaus were using a technique called «saturation mutagenesis» to search for every possible mutation involved in fruit fly development. They discovered dozens of mutations that affect development at various stages and produce a variety of malformations. Their Herculean efforts earned them a Nobel prize (which they shared with Lewis), but they did not turn up a single morphological mutation that would benefit a fly in the wild.

Saturation mutagenesis has also been used in a tiny worm studied by many developmental biologists, and is currently being applied to zebrafish. So far, no morphological mutations that would be beneficial in nature have been found in these animals, either.

Since direct evidence has been so hard to come by, neo-Darwinists usually cite indirect evidence. Genetic differences between two organisms are taken to indicate that their morphological differences are due to changes in genes. But without direct evidence, neo-Darwinists can only assume that genetic differences are the cause of morphological differences. As we saw in the chapter on homology, there are many cases in which similarities and differences in genes are not correlated with similarities and differences in morphology. Obviously, it is reasonable to question the neo-Darwinian claim that genetic mutations are the raw materials for large-scale evolution.

But people who question the claim are likely to encounter considerable resistance from defenders of neo-Darwinism. If they persevere in their questioning, however, they will find that they are not alone, and that the problem is bigger than they imagined. According to many biologists in the past, and many non-American biologists in the present, genes are not as important as neo-Darwinists make them out to be.

Beyond the gene

Like fruit flies, human beings begin life as a single fertilized egg cell. As the egg divides, it bequeaths a full set of genes to each of its progeny. Eventually, the fertilized egg divides into several hundred types of cells: A skin cell is different from a muscle cell, which in turn is different from a nerve cell, and so on. Yet with a few exceptions, all these cell types contain the same genes as the fertilized egg.

The presence of identical genes in cells that are radically different from each other is known as "genomic equivalence." For a neo-Darwinist, genomic equivalence is a paradox: If genes control development, and the genes in every cell are the same, why are the cells so different?

According to the standard explanation, cells differ because the genes are differentially turned on or off. Cells in one part of the embryo turn on some genes, while cells in another part turn on others. This certainly happens, as we saw in the case of *Ultrabithorax*. But it doesn't resolve the paradox, because it means that genes are being turned on or off by factors outside themselves. In other words, control rests with something beyond the genes—something "epigenetic." This does not imply that mystical forces are at work, but only that genes are being regulated by cellular factors outside the DNA.

Many biologists during the first half of the twentieth century investigated epigenetic factors in their attempts to understand embryo development, but the factors proved elusive. As the neo-Darwinian synthesis of Mendelian genetics with Darwinian evolution rose to prominence between the two World Wars, biologists studying epigenesis were increasingly marginalized. According to historian Jan Sapp, American geneticists such as Thomas Hunt Morgan took "an operational approach to their work, defining heredity and the gene in terms of the experimental operations by which they might be demonstrated." They thereby opted for "rapid production of results based on studies which could be carried out easily by established procedures."

At the same time, the neo-Darwinian synthesis of genetics and evolution was becoming increasingly popular, and neo-Darwinists welcomed the gene-centered emphasis in American research. Biologists who continued the difficult search for epigenetic factors were unable to match the flood of data being turned out by genetics labs. Furthermore, as Sapp put it, their ideas "seemed to threaten the significance of the merger of Mendelian genetics and selection theory and therefore had to be denied." The operational success and doctrinal aggressiveness of American neo-Darwinists enabled them to establish a near-monopoly over academic jobs, research funding, and scientific journals that persists to this day.

But neo-Darwinian genetics never resolved the paradox of genomic equivalence. In fact, the paradox recently deepened with the discovery that developmental genes such as *Ultrabithorax* are similar in many different animals—including flies and humans. If our developmental genes are similar to those of other animals, why don't we give birth to fruit flies instead of human beings?

The paradox of genomic equivalence has been largely ignored by gene-centered American biologists, but less so by Europeans. In March 1999 I attended a conference on "Genes and Development" in Basel, Switzerland. About fifty European biologists and philosophers of science were present, all of them critical of the neo-Darwinian doctrine that genes control embryo development.

One of the speakers began her talk with some jokes about the obligatory confessions of faith in Darwinism that are expected of speakers at scientific conferences. She went on to explain that DNA sequences do not even uniquely determine the sequence of amino acids in proteins, much less the larger features of cells or embryos. During the question-and-answer session that followed, a participant pointed out that most biologists already know this. She asked: "Then why don't they say so publicly?" The participant responded that it would "reduce their chances of getting money."

Later, at lunch, the lecturer told me about an experience she had had a few months earlier at a conference in Germany. There she had made some remarks critical of neo-Darwinian evolution, after which a prominent American biologist and textbook-writer had taken her aside. He had told her that she would be wise not to criticize neo-Darwinism if she ever found herself speaking to an American audience, because they would write her off as a creationist—even though she's not. She laughed as she told me the story; obviously, she was more amused than intimidated.

I was amused, too—but also saddened. It seems that scientists in Germany, like scientists in communist China, have more freedom to criticize Darwinism than scientists in America. Yet we are constantly told that scientists welcome critical thinking, and that America treasures freedom of speech. Except, apparently, when it comes to Darwinian evolution.