Perspectives on Animal Behavior

Second Edition

2001

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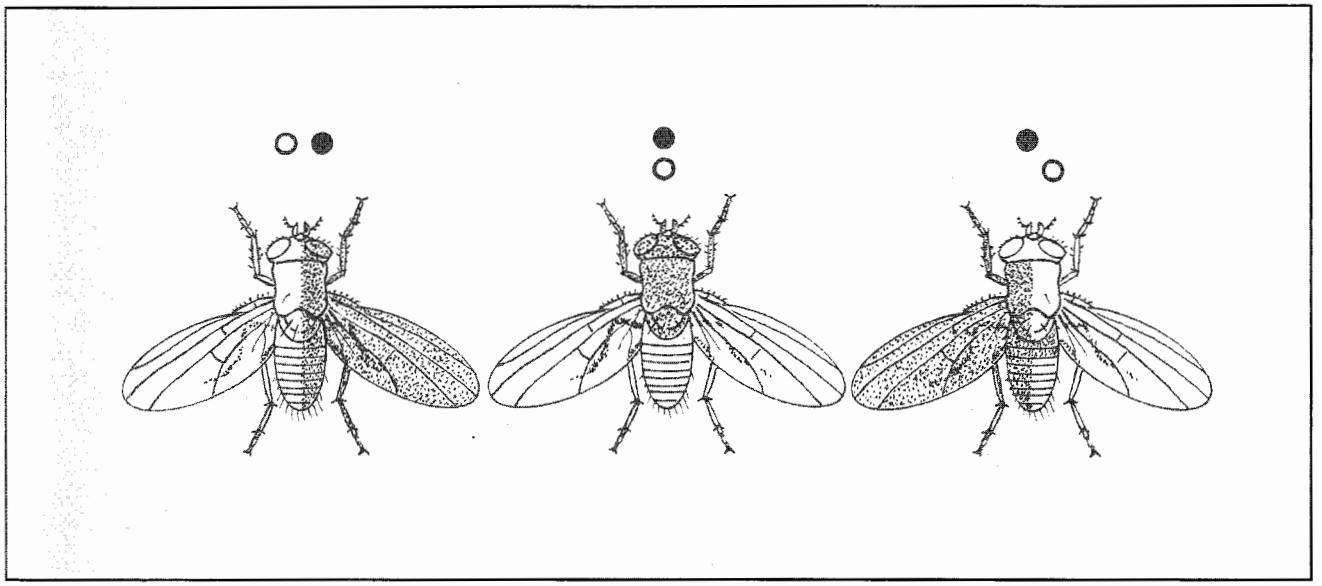

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Genetic Analysis of Behavior

Oh what a tangled web we'd weave! And yet, a spider with its minuscule brain fashions a lacy masterpiece (Figure 3.1). Furthermore, it must create this orb without parental guidance on its first attempt because, right after hatching, it floats away from its parent on a gossamer thread. How can it create such a complex and delicate thing on its first try? It is obvious that its behavior is strongly influenced by genes.

THE RELATIONSHIP BETWEEN GENES AND BEHAVIOR

Does that mean that the spider inherited a gene containing a crash course in web weaving? The answer is no, and the reason is that genes do not contain instructions for performing specific behaviors. Nor are genes blueprints for behavior or even for the circuitry of the nervous system.

So, then, if a gene is not an instruction manual or a blueprint, what does it mean to say that a behavior has a genetic basis? It simply means that the probability that a behavior will be performed is due to the presence of a particular form of a gene. Put another way, if the animal has a certain form of a gene it will be able to perform a behavior, but if it lacks that form it may be unable to perform the behavior, the expression of the behavior may be altered, or the frequency of the behavior may change. Within a population, then, we will find that variation in one or more genes often underlies some of the variation in behavior.

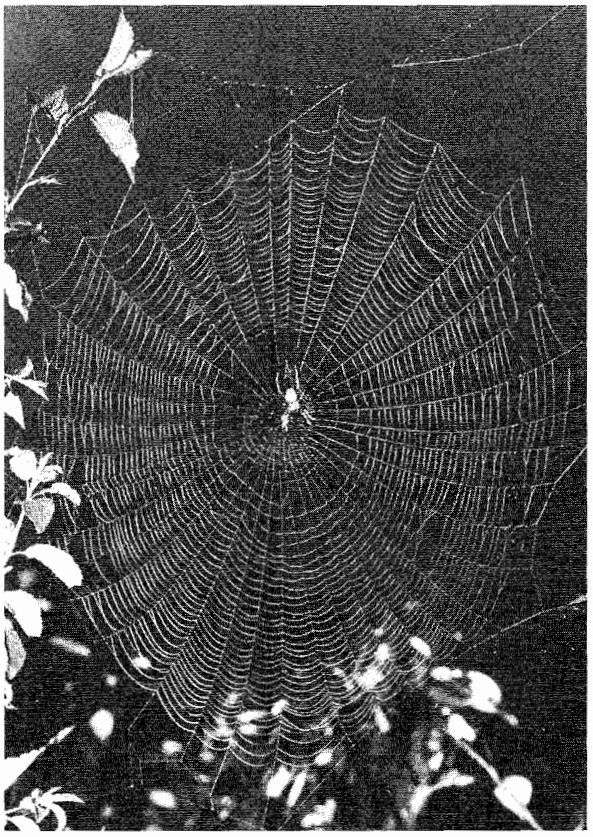


FIGURE 3.1 A spider's web. The ability to construct a web is a behavior that is strongly influenced by genes.

BASICS OF GENE ACTION

What do genes really do, then? How do they work? As you may already know, genes simply direct the synthesis of proteins. Each protein is specified by a different gene, or if the protein consists of more than one chain of amino acids, a gene specifies one of those chains. The protein may be structural and be used as a building block of the organism, or it may be regulatory. The protein produced by a regulatory gene may modify the activity of other genes, or it may be an enzyme that facilitates chemical reactions. In other words, genes code for specific proteins, and the proteins affect the composition and organization of the animal in ways that influence how it behaves. An animal's sensory receptors detect stimuli and send information to the nervous system, where it is interpreted and analyzed. The nervous system may then initiate a response by effectors (muscles and glands) that results in behavior. The structure and function of all these cells—receptors, nerves, muscles, and glands—require information from the genes. Alterations in genes change the proteins they code for, with the result that anatomy or physiology may be altered in a manner that changes behavior. We will look at a few examples of the links between genes and behavior later in this chapter.

To understand the relationship between genes and proteins, it is helpful to know a little biochemistry. Genes are made up of DNA (deoxyribonucleic acid). The structure of DNA is somewhat like a long ladder, twisted about itself like a spiral staircase. The DNA ladder is composed of two long strings of smaller molecules called nucleotides. Each nucleotide chain makes up one side of the ladder and half of each rung. A nucleotide consists of a phosphate, a nitrogenous base, and a sugar called deoxyribose. There are only four different nitrogenous bases used in DNA: adenine (A), thymine (T), cytosine (C), and guanine (G). Although DNA has only four different nucleotides, a DNA molecule is very long and has thousands of nucleotides. When forming a rung of the ladder, adenine must pair with thymine and cytosine must pair with guanine (Figure 3.2). The specificity of these base pairs is important, not only for the accurate production of new DNA molecules, but also for converting the information in the gene into a protein.

The instructions for each protein are written as the sequence of bases in the DNA molecule. The first step is to transcribe the information in DNA into messenger RNA (ribonucleic acid) (mRNA). The DNA unzips for part of its length so that an mRNA molecule can be formed. The mRNA has a structure similar to DNA except for three differences: It is single-stranded, its sugar is ribose, and the base uracil substitutes for thymine. Since adenine must pair with uracil and cytosine must pair with guanine, the sequence of bases on RNA is specified by the sequence of bases on DNA. Now you see why the specificity of base pairing is important in getting the information in a gene translated into a protein. Because the DNA molecule is so long, there is a multitude of ways in which the four bases can be ordered. Therefore, with only four bases, DNA can direct the synthesis of a myriad of different proteins.

The next step is to translate the order of bases on the mRNA molecule into a protein. Proteins are long chains of amino acids. Each different protein has a unique order of amino acids. A group of three bases on the mRNA molecule is translated, three bases at a time, into a protein. The order of bases on DNA specifies the sequence of bases in mRNA, which can be translated into only one array of amino acids. Thus, the information in the gene is its sequence of bases that codes for a specific protein. The "chain of command" can be summarized as follows:

Sequence of bases in DNA → Sequence of bases in mRNA → Sequence of amino acids in a protein

EXAMPLES OF LINKS BETWEEN GENES AND BEHAVIORS

A fundamental question in behavior genetics is how genes interact with one another and the environment to produce an organism's behavior. Certain genes code for proteins that bring about a relatively constant and per-

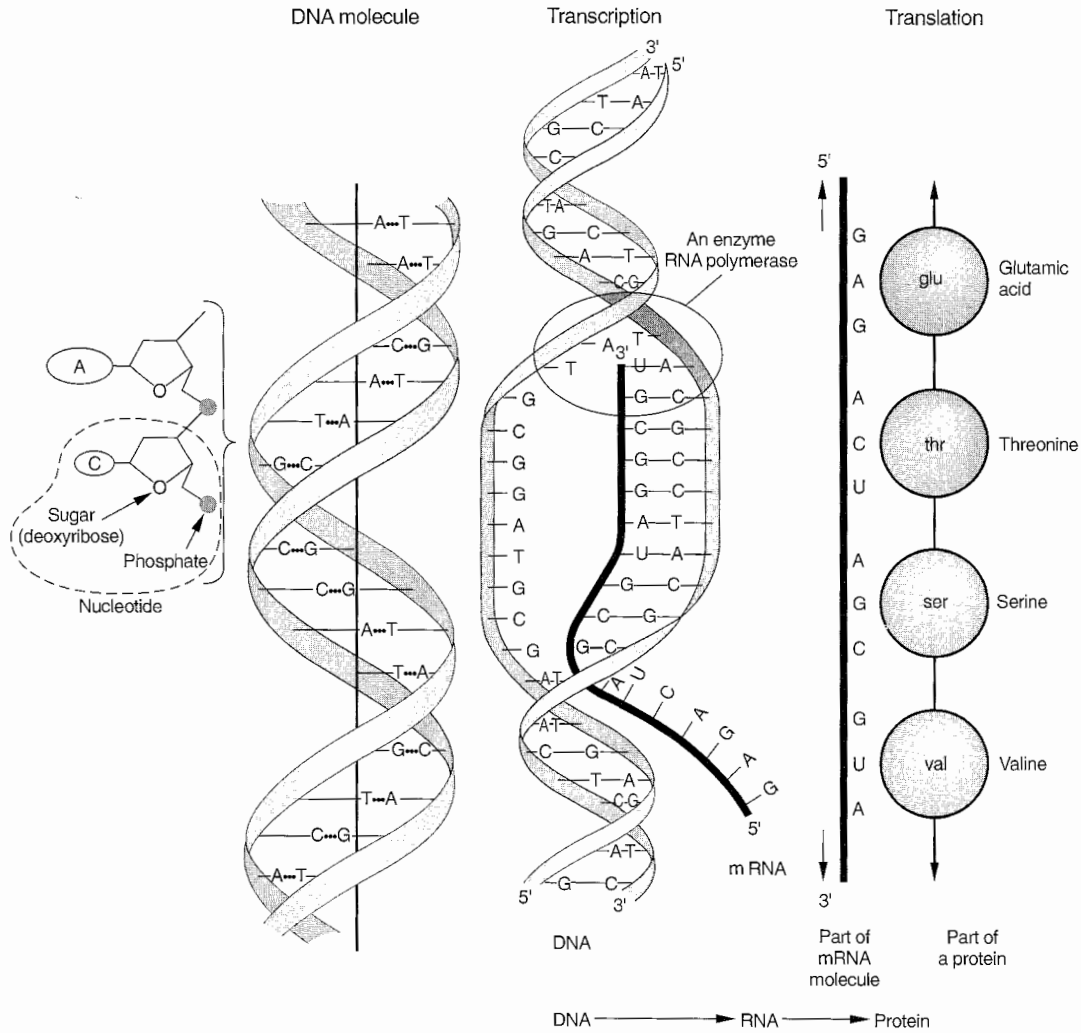


FIGURE 3.2 A diagrammatic representation of the biochemistry of gene expression. A gene is a region of a DNA molecule that has the information needed to make a specific protein. A DNA molecule is made up of nucleotides. A nucleotide, shown on the extreme left, is a nitrogenous base, a sugar (deoxyribose), and a phosphate. In the ladderlike DNA molecule, the two uprights are composed of alternating sugar and phosphate groups and the rungs are paired nitrogenous bases. The pairing of bases is specific—adenine with thymine and cytosine with guanine. During transcription, the synthesis of mRNA, the sequence of bases of the DNA molecule is converted to the complementary sequence of bases of the mRNA molecule. Each unit of three bases on the mRNA molecule signifies a particular amino acid. The message of messenger RNA is, therefore, its sequence of bases that determines the order and kinds of amino acids in the protein product.

manent change in a structure or function. Consider, for example, the *Shaker* gene in the fruit fly, *Drosophila melanogaster*. A particular mutation—that is, a specific change in the sequence of bases in the DNA—in the *Shaker* gene results in flies that shake violently under anesthesia. It turns out that the mutation changes a protein used in the formation of channels in the membranes of nerve cells that allow potassium ions to pass through. Potassium channels are critical to normal nerve cell function, and so the mutation causes a nerve cell defect and abnormal behavior (Kaplan and Trout 1969).

It should be kept in mind that the possession of "a gene for some behavior" in the sense in which we have just described the relationship is a necessary but not always sufficient condition for the expression of that behavior. An animal may have all the genes that are needed to perform a behavior and still not perform it because an essential gene is not active. Although all the cells of an animal's body have the same genes, some of them are turned off in the normal process of things and do not produce a protein. If these are then turned on, however, their proteins are produced, and in some cases

they will modify a structure or function in a way that will alter behavior. Thus, an organism's behavior may *change* as specific genes are turned on or off.

So, then, what factors might regulate gene activity? Important among the answers to this question are steroid hormones. One example of hormonal regulation of gene activity may be familiar to you—puberty. Puberty is the time when, if you are male, your voice deepened and you had to start shaving or when, if you are female, your breasts developed and you started menstruating. These physical changes were probably accompanied by behavioral changes, such as beginning to associate with members of the opposite sex. Studies show that human males have the most interest in sex between the ages of 15 and 25 years, when the level of their steroid sex hormones, the androgens, is peaking (Davidson, Camargo, and Smith 1979). The physiological and behavioral changes experienced at puberty are triggered by the steroid sex hormones that your body began to produce. Only certain body parts change at puberty because a steroid hormone affects the protein production of only the cells that have receptors that can bind to it. In other words, the steroid sex hormones that bring on the anatomical, physiological, and behavioral changes that signify sexual maturity in vertebrates work by stimulating the activity of specific genes, but only in those cells that have receptors that can bind to the hormones.

How can changes in gene activity influence something as complicated as sexual behavior? Although we do not know all the answers, some specific links between gene activity and sexual behavior are known in nonhuman mammals. Receptors for steroid sex hormones are most concentrated in brain regions known to be essential to the control of sexual behavior, including the preoptic area, hypothalamus, and amygdala. Both testosterone and estrogen, two important sex steroids, are known to turn on specific genes in certain brain regions (Sagrillo et al. 1996). We know that gene activity is essential to the performance of certain sexual behaviors because those actions are blocked if chemical inhibitors of RNA or protein synthesis are administered to female rats (Meisel and Pfaff 1984). Changes in gene activity in nerve cells in these brain areas could, in turn, alter the level of certain neurotransmitters, chemicals used for communication among neurons. For instance, changes in gene activity could cause the level of the neurotransmitter dopamine to rise, and elevated dopamine concentration is known to stimulate sexual behavior in a male rat (Bitran et al. 1988). In addition, the activity of certain genes stimulates the formation of new connections between nerve cells. The new connections could then affect sexual behavior by increasing appropriate sensory input or by linking to memories of previous sexual encounters (R. J. Nelson 1995).

Nerve activity can also turn on specific genes, leading to changes in behavior. We see this, for example, when a male zebra finch (*Taeniopygia guttata*) or canary (*Serinus canaria*) is first exposed to the song of its own species. Young males learn to sing their species song by imitating the songs of adult males. It is important that they learn the correct song because, like other songbirds, these birds sing to defend territories and to attract mates.

But what changes in the nervous system underlie a male songbird's learning his species song? Claudio Mello, David Clayton, and their colleagues have uncovered some answers to this question. They suspected that changes, if they occurred, would be found in the bird's forebrain because this region is important in auditory processing. They further guessed that if gene activity changed, it would most likely be the activity of one of the so-called immediate-early genes, which code for proteins that regulate the activity of other genes. These other genes then produce proteins that are thought to be important in the growth of nerve cells and to affect nerve cell activity. In this way, immediate-early genes are involved in the formation of long-term memories. *Zenk* is one of these genes. If *zenk* were turned on by exposure to the song, the levels of zenk mRNA would be expected to rise. So, Mello and his colleagues (1992) played a 45-minute tape recording to young male zebra finches or canaries. The recording was either of the song of its own species, one of another species, or simply bursts of sound. They then measured the level of zenk mRNA in the birds' forebrains. It turns out that *zenk* activity is increased greatly when males hear the song of their own species and much less so in response to the song of another species. Exposure to simple bursts of sound did not increase *zenk* activity above that found in birds that did not experience any auditory stimulation (Figure 3.3). The results of this experiment are consistent with the idea that *zenk* is activated when a male songbird learns the song of its species.

One function of bird song is to defend territories, and it would be a waste of energy to continuously defend borders that are not contested. It should not be surprising, then, that male songbirds can discriminate among the songs of various singers. Such discrimination requires a male to learn the characteristics of songs of specific males, its neighbors for instance. Mello and his colleagues (1995) hypothesized that *zenk* activity underlies the *formation* of long-term memories. Therefore, they predicted that *zenk* activity would increase in response to the songs of unfamiliar males but would not increase in response to the songs of familiar males. To test this hypothesis, they measured the levels of zenk mRNA in auditory forebrain regions of the brains of male zebra finches following repeated exposure to the song of the same zebra finch. As expected, zenk mRNA increased during the first 30

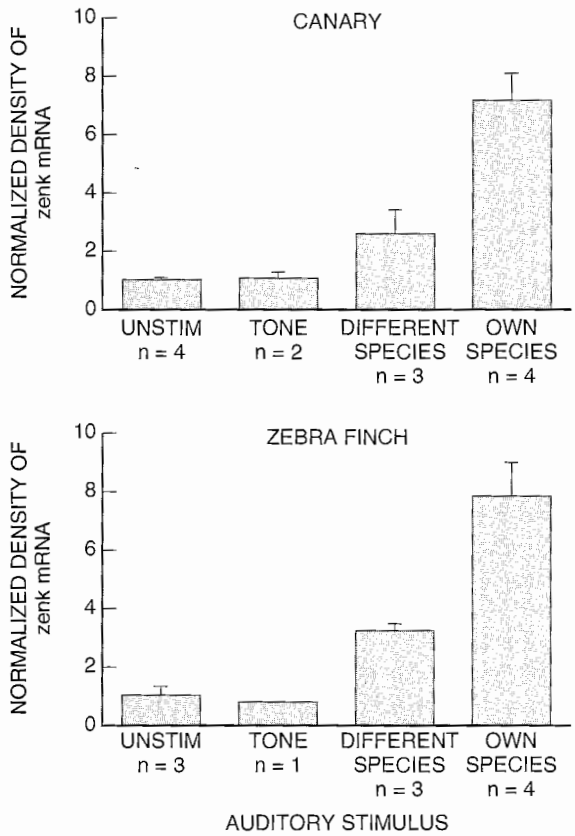


FIGURE 3.3 ***Zenk*** **gene activity in the forebrains of male songbirds following exposure to songs of their own species, songs of another species, bursts of tones that are not song, or no auditory stimulation. The density of zenk mRNA, which reflects gene activity, increases following exposure to birdsong. The increase following exposure to the song of the listener's own species is significantly greater than that following the song of another species. Zenk activity is not a simple response to noise because it is not greater following non-song tones than when the bird is not stimulated by any sound. (Data from Mello et al. 1992.)**

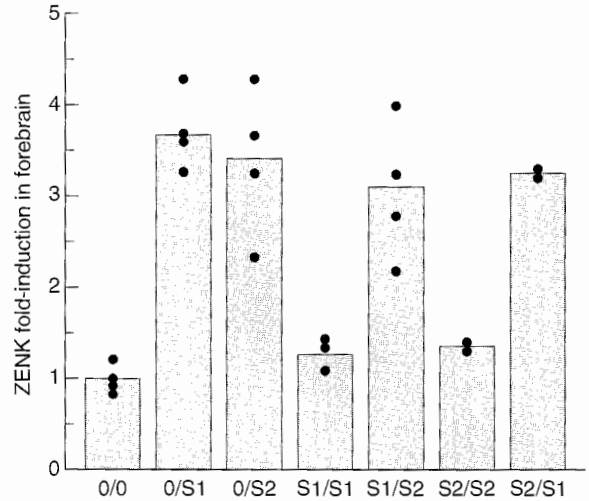


FIGURE 3.4 ***Zenk*** **activity in auditory regions of the forebrain of a male zebra finch drops following repeated exposure to the song of one male, but it can be reactivated by a new song. The birds were exposed to repeated song stimulus for 2.5 hours, which was immediately followed by a second "test" stimulus lasting 30 minutes. The levels of zenk mRNA in the birds' forebrains were then measured. Each dot represents the amount of zenk mRNA in a single bird. The bars represent the mean zenk mRNA level for the experimental group. These results are consistent with the hypothesis that *zenk* activity underlies the formation of long-term memories. We would not expect *zenk* to be activated after the memories have been formed. The 0 represents silence. S1 and S2 indicate specific songs of two different individuals from another aviary. (Data from Mello et al. 1995.)**

minutes. However, in spite of continued stimulation by the same song, zenk mRNA fell to baseline levels. Furthermore, when the same male's song was played after a full day of silence, there was no increase in *zenk* activity. Nonetheless, *zenk* activity did increase following exposure to the species song of another, unfamiliar zebra finch individual (Figure 3.4).

Similar changes in *zenk* activity have been shown in freely ranging song sparrows. When the species' song was played through a loudspeaker within a male's territory, he approached the speaker, searched for the intruder, and began actively singing in defense of his territory. The activity of *zenk* in several brain auditory structures was higher in the males whose territories had been challenged in this way than in unstimulated controls. Thus, natural behaviors in the field affect gene activity in much the same way they do in the laboratory (Jarvis et al. 1997).

We see, then, that the relationship between genes and behavior need not be static. Instead, the environment can continuously alter gene activity and, therefore, modify an organism's behavior.

GOALS OF BEHAVIOR GENETICS

When we observe an animal in nature, its actions are generally a result of many genes interacting with one another and the environment. Also, in most cases, an animal's behavior has been modified by its previous experiences.

We might wonder, then, how we can ever understand the relationship between genes and behavior. One goal of behavior genetics is to determine the strength of the genetic influence on a particular behavior. This goal is usually met through selection experiments or crossing experiments. (When studying human behavior, studying the behavior of twins is a way to see the influ-

ence of genes on certain behaviors.) The results of such experiments can shed light on the heritability of the behavior. In simple terms, heritability is a statistical measure that suggests how strongly a behavior is influenced by genes. Variation in behavior is caused by differences in both genes and environment. Since heritability is a statistical measure, we must measure the differences in the behavior of a sufficiently large number of individuals. Then we must determine how much of the observed variation is due to genetic differences among the individuals and how much is caused by differences in their environments. The heritability of a particular trait in a specific population is the ratio of the variation caused by genetic differences to the total amount of variability in the trait in that population. Heritability can vary from 0 to 1. A value of 0.5 indicates that 50% of the variability in the population studied is due to genetic differences. After a genetic influence on the behavior has been demonstrated, it is often of interest to identify the genes involved in producing the behavior in question. This usually involves mapping the genes, that is, identifying their locations along a specific chromosome. A second goal may be to determine the mechanisms by which the genes influence the behavior.

EXPERIMENTAL METHODS THAT DEMONSTRATE THE INFLUENCE OF GENES ON BEHAVIOR

As we have seen, the observed variation in behavior among individuals results from differences in genes and in environments. Therefore, when we are interested in exploring the influence of genes on behavior, it is important to rule out environmental effects by raising the animals in the same environment. If environmental conditions are identical for all animals, then observed behavioral differences are due to genetic differences.

INBREEDING

Inbred lines, strains created by mating close family members with one another, are useful in behavioral genetics because they provide a way to hold constant the genetic input. By using inbred strains, therefore, it is possible to separate the effects of genes from those of the environment. To show the effects of genes, the behavior of members of two inbred strains is compared in the same environment. In this case, any observed difference in behavior must be caused by a difference in the genes of the strain. However, the influence of the environment on behavior can also be shown by using inbred strains. If members of the same inbred strain, individuals who are almost genetically identical, behave differently when they are raised under different conditions, then the variation must be caused by environmental effects.

Before considering how inbreeding reduces genetic diversity, we should think about why a population of individuals is dissimilar. Usually a genetically controlled characteristic or trait can be expressed in more than one way. For example, coat color in mice might be black or brown. In other words, this gene for coat color can be expressed in two different ways; one produces intense black pigment granules, and the other produces chocolate brown pigment granules. These alternative forms of a gene are called alleles. Sometimes, as in the eye color of fruit flies, there are many possible alleles. The wild type, or most common eye color of fruit flies, is red, but other alleles of this gene can result in white or vermilion eyes. Furthermore, most of the animal species used in genetic studies are diploid, meaning that an individual possesses two alleles of each gene, one from each parent. If the two alleles of a gene are identical, the individual is said to be homozygous for the trait. However, an individual may inherit different alleles for a gene from its mother and father. Such an individual is heterozygous for the gene. The genetic diversity among unrelated individuals results from the particular alleles that they possess and from heterozygosity.

Inbreeding reduces genetic diversity because it creates a population that is homozygous for almost all of its genes. How does it do this? In the laboratory, an inbred strain is usually created by mating brothers with sisters for many generations. If the original parents of an inbred line lacked certain alleles found in the general population, those alleles would also be missing in their offspring. So when close family members mate, the alleles present in the parents will be retained and will increase in frequency relative to the general population; those not present in the founders will be lost. In addition, each parent passes on only one of its two alleles for each gene to the offspring. Therefore, if a parent is heterozygous for a trait, one of its alleles will be missing in the offspring unless the other parent supplies it. With each successive generation of inbreeding, genetic variability is lost. Each generation of brother–sister matings reduces heterozygosity by 25%. After 20 generations of sibling matings, all individuals are expected to have identical alleles for 98% of their genes. As a result, no matter which member of each chromosome pair is transmitted from parent to offspring, the same alleles will be passed on. Inbred lines, therefore, are virtually genetically identical (Plomin, DeFries, and McClearn 1980).

Comparisons of Inbred Strains to Show the Role of Genes

As previously mentioned, inbred lines can be used to demonstrate that differences in genetic makeup may

result in differences in behavior. According to the scientific method, when you want to explore the effect of one variable, you hold the others constant. Inbred lines provide a way to do this. Inbreeding minimizes genetic diversity within strains but maintains diversity between strains and may even enhance it. If individuals of different inbred strains are raised in the same environment, any difference in their behavior is due to a difference in their genes. So the comparison of inbred strains raised under the same conditions is a way to demonstrate that a particular behavior has a genetic basis.

Comparisons of the behavior of different inbred strains have been a popular way to show that genes play a role in behavior in a wide variety of animals. The work of Ádám Miklósi and his colleagues (Miklósi, Csányi, and Gerlai 1997) on the antipredator behavior of paradise fish (*Macropodus opercularis*) larvae is an example. Paradise fish live in densely vegetated, shallow marshes and rice fields in Southeast Asia, along with several predator fish species. Avoiding predation is crucial to survival, and so one might suspect it has a genetic basis. Having raised strains of paradise fish that had been inbred for over 30 generations, Miklósi could investigate this possibility. He crafted model predators from plastic centrifuge tubes. Since eyes are known to be an important cue in predator recognition in many species, some models were created with black eyespots. The larvae of two inbred strains (S and P) of paradise fish were placed individually into an experimental tank, and their responses to model predators were observed for three minutes. There are two common antipredator responses: fleeing, in which the larva suddenly darts by slapping with its caudal fin, and backing, in which the larva swims backward with its body curved. Larvae of strain P were significantly more likely to show antipredator responses, both fleeing and backing, than were the larvae of strain S (Figure 3.5).

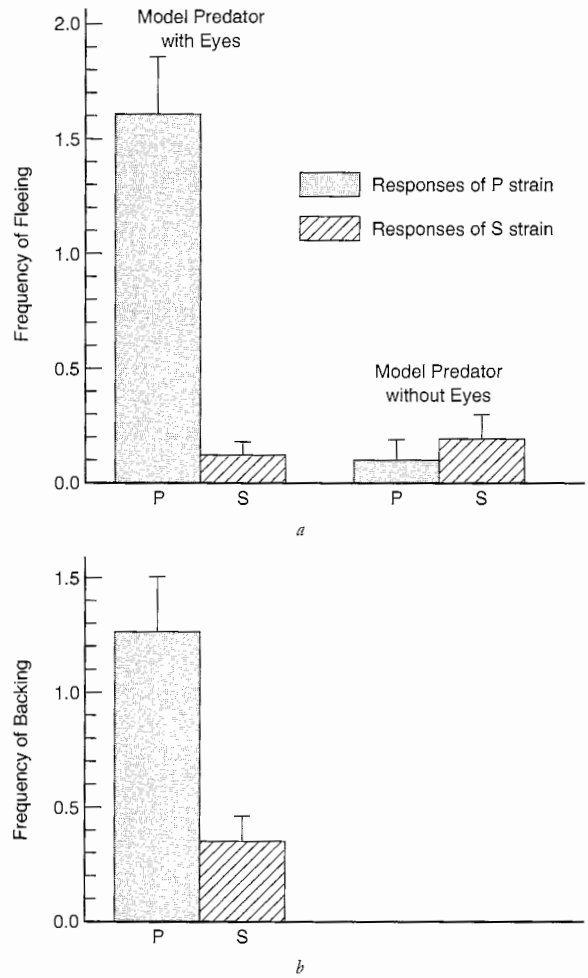


FIGURE 3.5 The antipredator responses of larvae of two inbred strains of paradise fish when presented with model predators. Fleeing consists of darting by slapping the caudal fin. Backing involves swimming backward with a curved body in a direction perpendicular to the body axes of the larva. Larvae of strain P showed a significantly higher frequency of both fleeing and backing than did larvae of strain S, indicating genetic differences in antipredator behavior between the strains. (Data modified from Miklósi, Csányi, and Gerlai 1997.)

Comparisons of Inbred Strains to Show the Role of Environment

Because individuals of inbred strains are identical in 98% of their genes, such strains, as we mentioned, can provide a way to hold the genetic input constant while varying the environment. Thus, if differences in behavior are found, they must be due to the environment.

Some of the environmental effects on behavior take place very early in life. Eliminating the effects of early learning is tricky, but it can be done. The simplest way is by a reciprocal cross, one in which males of inbred strain A are mated with females of inbred strain B and males of strain B are mated with females of strain A. Since the individuals of each strain are homozygous for almost every gene, the hybrid offspring of both of these crosses have the same genotype even though their mothers are from different strains. So if the behavior of the hybrid offspring of reciprocal crosses differs, it must be an effect of the parental environment. If the young are raised solely by their mothers, as they often are in the laboratory, the difference must be an effect of the maternal environment.

Theoretically, it is even possible to determine whether the maternal environment had its greatest influence on the offspring before or after birth. Cross-fostering, transferring the offspring shortly after birth to a mother of a different strain, is a technique for detecting maternal influences that occur after birth. If offspring that were transferred to a foster mother immediately after birth behave more like individuals of the foster mother's strain than like those of their own strain, postnatal maternal influences are implicated.

A cross-fostering experiment involving two different species of voles helped in separating the influences of genes and parental environment in the expression of a particular behavior. Prairie voles, *Microtus ochrogaster*, show more parental care than do meadow voles, *M. pennsylvanicus*. For example, prairie vole females spend more time in the nest with their young, contacting their offspring by huddling over them and nursing them. Male prairie voles also show more parental care than do male meadow voles. In contrast to meadow vole males, who nest separately and rarely enter the female's nest, prairie vole males share a nest with the female and frequently groom and huddle over the young.

To determine whether the species difference in parental care was due to genes or early experience, Betty McGuire (1988) fostered meadow vole pups to prairie vole parents. As a control, she fostered meadow vole pups to other meadow vole parents. When the foster pups became adults and had their own families, she measured the amount of parental care they gave to their second litters. The meadow vole pups that were raised by prairie voles gave more care to their own offspring than did those pups that had been fostered to other meadow vole parents. Cross-fostered females, for example, spent more time huddling over and nursing their young. The early experience of male voles influenced their parental behavior in much the same way as it affected the females' behavior. Male meadow vole pups that were fostered to parents of their own species behaved as meadow vole males usually do, in that they rarely entered the nest with the young. However, four of the eight meadow vole males raised by prairie vole parents nested with their mates and spent time in contact with the young. This cross-fostering experiment shows that the experience a vole has with its own mother can influence the way it treats its own offspring. However, not all behaviors were modified by early experience. Nonsocial behaviors such as food caching and tunnel building and overall activity level were unaffected by the species of the foster parents. Thus, using the technique of cross-fostering, it is possible to determine whether a particular behavior is influenced by the parental environment.

ARTIFICIAL SELECTION

Artificial selection is another means of demonstrating that a behavior has a genetic basis. It differs from natural selection in that an experimenter, not "nature," decides which individuals will breed and leave offspring. The rationale for artificial selection is that if the frequency of a trait in a population can be altered by choosing the appropriate breeders, it must have a genetic basis. Usually the first step is to test individuals of a genetically variable population for a particular behavior trait. Those individuals who show the desired attribute are mated with one another, and those who lack the trait are prevented from breeding. If the character has a genetic basis, the alleles responsible for it will increase in frequency in the population because only those possessing them are producing offspring. As a result, the behavior becomes more common or exaggerated with each successive generation. When it proves possible to select for a trait, it is concluded that genetic inheritance is important to the expression of that trait. Furthermore, the environment is held constant, so it is reasonable to conclude that any change in the frequency of a behavior is due to a change in the frequency of alleles.

Humans often use artificial selection to create breeds of animals with traits that they consider useful. For example, even if you are not a dog lover, you have probably noticed that different breeds of dogs have different personalities. All dogs belong to the same species, but various behavior traits and hunting skills have been selected for in different breeds (strains). Terriers, for example, are fighters. Aggressiveness was selected for so they could be used to attack small game. The beagle, on the other hand, was developed as a scent hound. Since beagles usually work in packs to sniff out game, they must be more tolerant with companions. Therefore, their aggressiveness was reduced by selective breeding. Shetland sheep dogs were bred for their ability to learn to herd sheep. Spaniels, used for hunting birds, are "people dogs." They are more affectionate than aggressive. These behavioral differences among breeds of dogs were brought about by people who placed a premium on certain traits and arranged matings between individual dogs that showed the desired behavior. In other words, the frequency of particular behavior patterns present in all breeds has been modified through artificial selection; new behaviors were not created (Scott and Fuller 1965).

Artificial selection for nesting behavior in house mice (*Mus domesticus*) has not only demonstrated a genetic basis for the behavior but has also shed some light on how natural selection might work on the trait in wild populations. House mice usually live in fields, where they build nests of grasses and other soft plant material. In the laboratory, both male and female house mice will use cotton as nesting material, which makes it easy to quantify the size of the nest constructed. Carol Lynch (1980) noticed that some mice built larger nests than others did (Figure 3.6). These differences could be due to genetic or environmental factors or both. Lynch suspected that there was some genetic basis in nesting behavior. To separate the genetic and environmental influences, she began to selectively mate mice, based on the size of the nest they built, and she raised all mice under the same environmental conditions. She began with a population of house mice that gathered between 13 and 18 grams of cotton over a four-day period to

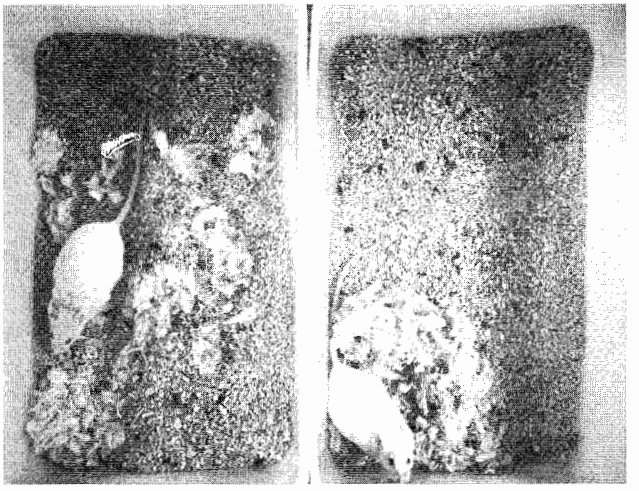


FIGURE 3.6 Individual differences in the size of nests built by house mice in the laboratory. The genetic basis of these differences in nest-building behavior has been demonstrated by artificial selection experiments. Whereas the mouse on the left was selected to build a small nest, the one on the right was selected to build a large nest.

build their nests. Then, she selectively mated mice to create lines of high and low nest-building behavior. She mated males and females that built large nests to create high lines and males and females that built small nests to create low lines. In addition, she created control lines by randomly mating males and females from each generation. After 15 generations of artificial selection, the mice of the high nest-building line used an average of 40 grams of cotton for their nests. In contrast, the mice in the low nest-building line used an average of only 5 grams of cotton in nest construction. The mice of the control line built nests about the same size as those built by the mice in the initial population—15 grams of cotton (Figure 3.7). Selection for high and low nesting behavior was continued for over 40 generations, at which time mice from the high nest-building line collected more than 40 times the amount of cotton than did the mice of the low line.

The results of this experiment confirm that there is, indeed, a genetic basis to nest building in house mice. Thus, natural selection could have a similar influence on nest building if nest size influences fitness (the number of offspring successfully raised). House mice do, in fact, build larger nests in the north than in the south (Lynch 1992). This suggests that large nests may be a factor that helps mice in cold environments raise more offspring. In the laboratory, Lynch bred groups of mice from both lines at 22°C or at 4°C and counted the number of offspring that survived to 40 days of age. Pup survival in both lines was reduced at the lower temperature. Nonetheless, mice from the lines that built larger nests raised more pups that lived to be 40 days old at both environmental temperatures. Thus, nest building is an important component of fitness, and its genetic basis allows it to be shaped by natural selection (Bult and Lynch 1997).

It is interesting to note, however, that selection can lead to the same observed behavior—constructing large or small nests—by favoring different sets of genes. Notice in Figure 3.7 that two high lines and two low strains were created through artificial selection. Crosses between mice of different high strains (or between mice of different low strains) revealed that there were still genetic differences between strains that expressed the behavior in similar ways. This suggests that natural selection can follow different paths in different popula-

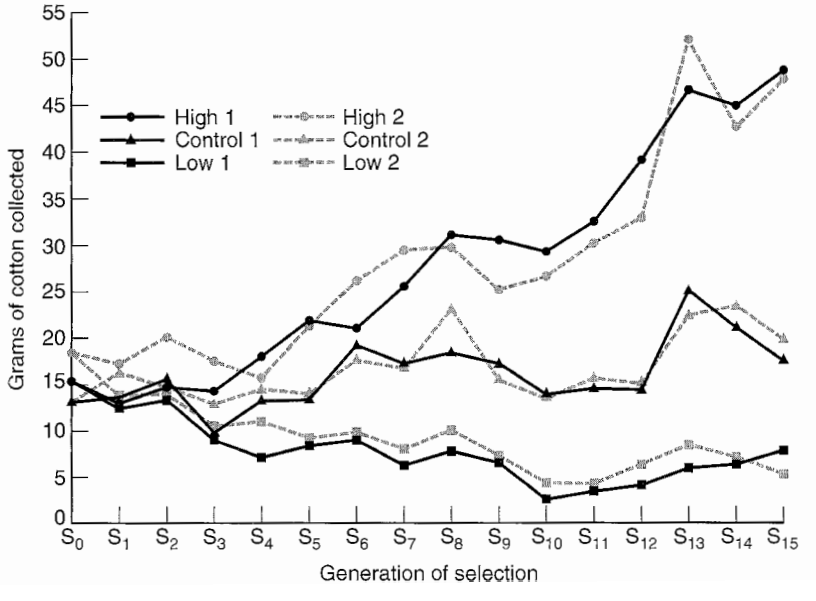


FIGURE 3.7 Artificial selection for large or small nest size in house mice. The nests of mice in the original population consisted of between 13 and 18 grams of cotton. Individuals who built the largest nests were bred with others who built large nests to create a high nest-building line. Individuals who built the smallest nests were bred with others who built small nests to create a low nest-building line. A control line was developed by randomly mating individuals of each generation. After 15 generations, the nests of the high line were an average of 8 times larger than those of the low line—40 grams and 5 grams, respectively. The nests of mice in the control line were roughly the same size as those built by mice in the initial population (15 grams). (Data from Lynch 1980.)

tions to produce the same adaptive behavior (Bult and Lynch 1996).

In some cases, selection affects behavior by affecting genes that code for proteins that affect the structure or function of the nervous system. For example, it is possible to selectively breed mice to be very active and to explore an open test arena or to be less active. Such selection results in specific differences in brain structure between the high and low activity lines: Specific regions of the hippocampus are more developed in the mice that are selected for high activity (Hausheer-Zarmakupi et al. 1996).

HYBRIDIZATION

Hybridization is a third way to demonstrate a genetic influence on behavior. The usual procedure is to mate two individuals that display a particular type of behavior in distinct but different ways. Once interbreeding has occurred, the behavior in the hybrid offspring is then observed. Depending on the number of genes influencing the behavior, the hybrids may perform the action in the same manner as one of the parents or as a combination of the parental types.

Rover and Sitter Fruit Fly Larvae

Most of us would not find foraging fruit fly larvae of great interest, unless it was taking place in a fruit bowl on the kitchen table. But, it is fascinating to many behavioral geneticists. The interest began when Marla Sokolowski noticed two forms of feeding behavior in natural populations of larval fruit flies (*D. melanogaster*) in the vicinity of Toronto, Canada. Whereas the so-called "rover" larvae move around continually on their food, "sitter" larvae travel only short distances. In fact, when these larvae were brought into the laboratory and allowed to feed on yeast paste in a petri dish, the distances traveled by rovers were nearly four times longer than those of sitters. Sokolowski immediately suspected that a behavior with two distinct forms would be genetically controlled.

To investigate the genetic basis of these foraging strategies, Sokolowski performed a series of cross-breeding experiments using adult rovers and adult sitters. The results are consistent with the idea that the trait is controlled by a single gene and that *rover* is dominant to *sitter*, as shown in Figure 3.8. Sokolowski began by crossing adult rovers with other rovers and adult sitters with other sitters, creating parental strains of sitters and rovers. The responses of male larvae resulting from these crosses are shown in Figure 3.8*a*. Notice that the frequency distribution of path lengths of rovers is easily distinguished from that of rovers. Next, Sokolowski crossed adults of sitter larvae with adults of rover larvae. The path lengths of the F_1 offspring of these crosses are shown in Figure 3.8*b*. Nearly all the larvae were rovers. When the F_1 adults were crossed with one another, the resulting F_2 larvae consisted of both rovers and sitters in a ratio of three rovers to one sitter (Figure 3.8*c*). Thus, most of the variation in foraging strategies could be explained by variation in a single gene, dubbed *foraging* (*for*) (de Belle and Sokolowski 1987).

Several aspects of the *for* gene make it attractive to study. First, behavioral variants of the gene occur in natural populations; It isn't necessary to use mutants created in a laboratory. Second, the gene is subject to natural selection, and the form of the trait favored depends on environmental conditions. Rovers, who will search farther for food, are favored in crowded conditions. On the other hand, sitters are favored when the population density is low because they conserve energy by limiting movement (Barinaga 1994). Sitters don't move slowly because they are energy-deficient or sick. When they aren't feeding, there are no significant differences in locomotion between sitters and rovers (Sokolowski and Hansell 1992). Also, the developmental time and growth rates of sitters and rovers are essentially identical (Graf and Sokolowski 1989). Third, the expression of the trait illustrates that genes and environment interact to produce the observed behavior. For example, if a rover is deprived of food before it has an opportunity to forage, the chances are good that it will behave as a sitter and conserve energy (Barinaga 1994). Fourth, we are beginning to see glimpses of the mechanisms by which *for* alters the feeding strategy. *For* is located on chromosome 2 (de Belle, Hilliker, and Sokolowski 1989), and its location seems to match that of a gene for an enzyme (cyclic GMP*-dependent kinase) that is known to be important in signaling pathways within the cell (reviewed in Barinaga 1994). Moreover, it is speculated that the change in this enzyme affects behavior by altering some aspect of information transfer between sensory input and motor output that influences the way in which the larvae perceive and respond to the quality of food in the area where they are foraging (Pereira et al. 1995).

LOCATING THE EFFECTS OF GENES THAT INFLUENCE BEHAVIOR

The breeding experiments we have discussed so far were the primary tools used by classical behavior geneticists, but they are actually not very powerful tools. Inbreeding, selection, and hybridization may indicate that the behavior in question has a genetic basis and perhaps that it is controlled by few or many

*Guanosine monophosphate

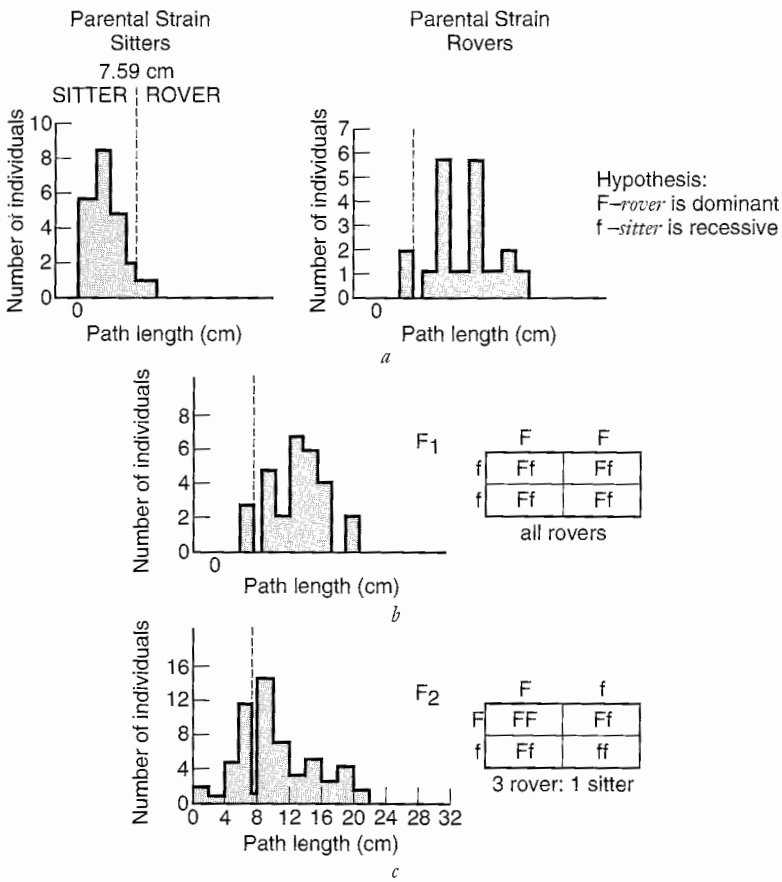


FIGURE 3.8 **The results of mating experiments on fruit flies showing "rover" or "sitter" foraging strategies as larvae. Rover larvae forage over significantly longer distances than do sitter larvae. (*a*) The parental strains were created by crossing rovers with rovers or sitters with sitters. (*b*) The frequency distributions show foraging path distances of male (F_1) larvae of crosses between adults of the parental rover strain and adults of the parental sitter strain. Almost all the resulting larvae were rovers. (*c*) The frequency distributions show foraging path distances of male (F_2) larvae resulting from crosses between F_1 flies. The results are consistent with the hypothesis that the trait is controlled by a single gene, called *foraging (for)*, and that the *rover* allele is dominant to the *sitter* allele. Punnett squares of the crosses are shown on the right. (Data modified from de Belle and Sokolowski, 1987.)**

genes, but it tells us nothing about where or how the genes are exerting their effects. More modern techniques such as inducing single-gene mutations, mutation and mosaic analysis, and the use of recombinant DNA have filled in some of the gaps in our understanding of the relationship between genes and behavior.

SINGLE-GENE MUTATIONS

One way to find the links between behavior and genes is to induce a mutation in a single gene, screen the population for individuals who behave abnormally, and then search for the anatomical or physiological differences between the mutant and the normal organisms.

A mutation, a change in a gene's instructions for producing a protein, can be induced by agents that change the DNA bases. When organisms are exposed to mutagenic agents, some of them become behaviorally aberrant and can be separated from the population of normal individuals on this basis. Appropriate genetic crosses can then determine whether the behavioral change is caused by an alteration in a single gene. Even a small change may result in a difference in a specific aspect of an anatomical structure or a physiological process that mediates a behavior. Identifying the anatomical or physiological differences between mutant and normal individuals brings us closer to understanding how genes can influence behavior.

Studies on learning in the fruit fly, *D. melanogaster*, have helped fill in a few of the missing links between genes and behavior. You have probably never met a fruit fly with remarkable intelligence, but some mutant strains are so poor at olfactory learning that they have earned the epithet *dunce*. Before considering the deficiency caused by the *dunce* mutation, olfactory learning in normal fruit flies and the way of demonstrating it should be described. Normal fruit flies can associate an odor with an unpleasant event, such as an electric shock, and learn to avoid that odor if it is encountered again. This should not be surprising because odors are important in the daily life of fruit flies for locating both food and appropriate mates. William Quinn and his colleagues (1974) demonstrated this avoidance conditioning by shocking a group of flies for 15 seconds in the presence of one odor but not in the presence of a second odor. Then they presented the odors, one at a time, to the flies without shocking them to see how many would avoid each odor. Most normal flies avoid only the odor connected with the electric shock, an association that lasts for three to six hours (Dudai et al. 1976).

Two mutants, *dunce*^{1} and *dunce*^{2}, were isolated by

exposing a population of *D. melanogaster* to a chemical known to cause mutations and then screening the flies on the basis of their learning ability. The *dunce* mutations are alleles, or alternate forms, of a single gene on the X chromosome. In contrast to normal flies, *dunces* fail to learn to avoid odors associated with shock when they are taught with Quinn's experimental design (Dudai et al. 1976). Even larval *dunces* are deficient in olfactory learning (Aceves-Pina and Quinn 1979).

Why don't *dunce* fruit flies learn as well as normal flies? The first guess, that the sensory system was defective so that the *dunce* flies could not detect either the odors or the shock, was incorrect. Experiments revealed that the mutants are able to detect both (Dudai et al. 1976). In spite of this, mutant flies are unable to remember the association between the shock and an odor.

Apparently the *dunce* mutants have a problem with the early stages of memory formation. If they are shocked in the presence of an odor without subsequent exposure to a second odor, they do associate the odor with the aversive stimulus and avoid it if tested immediately after training, but the association fades quickly. The association between the shock and an odor is short-lived, and the experience with a control odor during the interval between the training and the test of learning seems to eliminate associations that may have formed. For these two reasons, it is concluded that an early stage of memory formation is defective in the *dunce* mutants (Dudai 1979).

What does the *dunce* gene do? It codes for a form of an enzyme called cyclic AMP (adenosine monophosphate) phosphodiesterase. This enzyme is important because it breaks down cyclic AMP (cAMP), which is a mediator of many processes in different types of cells. Thus, we see that the *dunce* mutation causes a reduced level of cAMP phosphodiesterase and, therefore, an increased level of cAMP. In addition, the mutation impairs an early stage of memory formation in olfactory learning. This should lead you to suspect that the enzyme or cAMP might play a role in this type of memory formation, an idea that has been tested by inhibiting cAMP phosphodiesterase in normal flies and testing the olfactory learning abilities. When treated in this way, normal flies learn no better than *dunces* (Byers, Davis, and Kiger 1981). Thus, the behavior of *dunce* flies suggests that cAMP has a role in learning and memory.

Another single-gene mutation in fruit flies, *rutabaga*, also causes poor learning and memory. This mutation has filled in some of the details of the connection between cAMP and memory formation. The level of cAMP within a cell actually depends on two enzymes. As we've seen, cAMP phosphodiesterase breaks down cAMP, lowering its concentration. In contrast, another enzyme, called adenylyl cyclase, raises

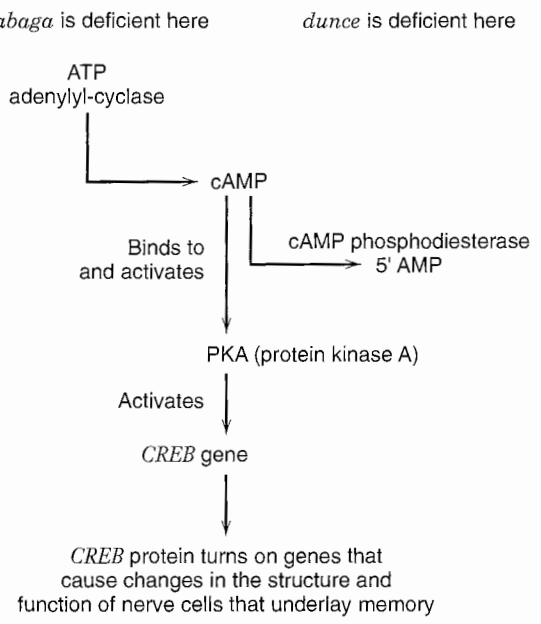


FIGURE 3.9 A summary of the molecular events that accompany memory formation in fruit flies. Single-gene mutations that result in poor olfactory learning were helpful in uncovering many of the details of memory formation. The *dunce* mutant has a defective form of cAMP phosphodiesterase, an enzyme that breaks down cAMP. *Rutabaga* has a defective form of adenylyl cyclase, an enzyme that forms cAMP from ATP. Thus both mutations affect the levels of cAMP within nerve cells. The cAMP binds to another enzyme, PKA (protein kinase A), and activates it. Active PKA then turns on the *CREB* gene, whose protein regulates the activity of other genes so that new connections can be made among nerve cells. These connections are responsible, in part, for long-term memory.

cAMP levels by causing the formation of cAMP from ATP (adenosine triphosphate) (Figure 3.9). *Rutabaga* mutants have a defective form of adenylyl cyclase (Levin et al. 1992). As a result, *rutabaga*'s adenylyl cyclase is not activated by the stimulus involved in learning in the way it would be in a normal fly. Therefore, the learning stimulus doesn't cause cAMP levels to rise. Thus *dunce* and *rutabaga* have opposite effects on the level of cAMP within a cell.

As is often the case in science, the more we learn, the more questions we have. In this case, the *dunce* and *rutabaga* mutants prompt us to wonder how cAMP is involved in memory formation. A pathway involving cAMP that is thought to be important for memory formation is summarized in Figure 3.9. Notice that cAMP binds to another enzyme, protein kinase A (PKA), and activates it. All protein kinases work by adding a phosphate group to another molecule, activating or inactivating the other molecule. In this case, PKA activates the *CREB* gene, which codes for the protein CREB

(*c*AMP *re*sponse *b*inding protein) that, in turn, activates other genes. These other genes then control the growth of connections between brain cells, changes in the nervous system that are responsible for memory (Davis et al. 1995).

It is interesting to note that cAMP and the *CREB* gene play a key part in memory formation in other organisms. Marc Klein and Eric Kandel (1978) have shown that in the sea hare *Aplysia californica*, changes in the levels of cyclic AMP in certain nerve cells play a role in sensitization of the gill-withdrawal reflex. Sensitization, an increase in neuronal responsiveness to a stimulus because of previous exposure to another intense stimulus, might be considered a simple form of memory. Also, preventing CREB proteins from activating other genes blocked long-term memory formation in *Aplysia* (Dash, Hochner, and Kandel 1990). In mice, the story is similar—long-term memory formation depends on normal *CREB* gene activity (Bourtchuladze et al. 1994).

MUTATION AND MOSAIC ANALYSIS

In the study of *dunce* fruit flies, luck was on the side of the investigators. When a gene for the mutant behavior was mapped, it turned out to be in the same chromosome region as a gene whose ultimate product was known. This helped to pinpoint cyclic AMP phosphodiesterase as a link between the mutant behavior and the gene. However, we are not always that fortunate, so other techniques must be used to determine how or where particular genes are acting.

Why is it so difficult to determine where a gene is acting? First, examining a population of mutants to look for the defective structure or physiological problem is like looking for the proverbial needle in a haystack. Where do you begin? It would be helpful to know which part of the haystack to search first. Furthermore, knowing where to look for a problem is crucial when the effect of the mutation cannot be seen directly, as would be the case if the gene's activity resulted in abnormal neural connections; an inability to absorb an important precursor; or an inability to produce a neurotransmitter, enzyme, or hormone. Knowing where to look for a defect is also helpful when there are several structures that logic would tell us could be affected by a mutation and cause a behavioral anomaly. For example, there is a mutant fruit fly called *wings up* that cannot fly because its wings are always held straight up. Think for a moment about where the fly's problem might be. Obviously the wings are the immediate problem, but what causes the wings to be held in an abnormal position? Is the defect in the wing structure itself, in the wing muscles, in the nerves that control the wing muscles, or in the messages sent from the brain? Not only is it difficult to determine which of several possible sites is the source of the problem, it is also hard to find the site of gene action when it is not in the structure that is outwardly affected. In the *wings up* fruit fly, for example, the wings are up because the indirect flight muscles that attach to them are either abnormal or missing (Hotta and Benzer, 1972). Another example, one in which a gene has its primary effect on a structure distant from the site of the problem, is seen in some humans. An inability of the cells of the gut to absorb enough vitamin A may cause the retina of the eye to degenerate, resulting in a sensory deficiency with obvious consequences for behavior. In this case, the gene's action is in the small intestines even though the defect is seen in the eye. In both of these examples, it would be easy to misidentify the site of gene action as the structure with the obvious defect.

It is possible to locate the primary site of gene action by using mosaic analysis, which is a comparative study of mosaics, individuals composed of some sections of normal wild-type tissue and some pieces of mutant tissue. Although mosaic analysis does not always reveal what the gene is doing—that is, the nature of the problem—it does locate where the gene is working. The procedure is to compare the distribution of normal and mutant tissue in all the mosaics displaying the mutant form of a behavior to identify the region of mutant tissue common to them all. Those regions are then implicated as the sites of action of that gene.

You have probably used the same reasoning used in mosaic analysis in solving other problems. Suppose, for example, you sit down to read in your favorite seat but, much to your frustration, the lamp next to the chair will not go on. How do you locate the source of the problem? First, you might suspect that there was no power in the outlet you were using. So you plug the lamp into an outlet that you know is functional. The lamp still does not work. Second, you check the bulb and find that it was not screwed in tightly, but screwing in the bulb does not solve the problem. In this analogy, the electrical connection between the bulb and the socket is considered defective, or "mutant." However, correcting that problem or "mutation" did not make the light go on, so that defect is not the primary source of the problem. Third, you replace the bulb with a new one. No luck. Finally, you try replacing the plug. The light goes on! Even if you could not see anything wrong with the plug, you know it was the cause of the problem because when it was replaced by a good plug, the light worked. What you did was to determine which of several components of the system was the source of the problem by replacing possible defective parts with good ones until you found the one that caused the problem.

When you want to determine which part of an animal showing mutant behavior is the source of the problem, you look at the distribution of normal and mutant tissue in mosaics and determine which structures must

be mutant for the aberrant behavior to be seen. For example, suppose you compare all the mosaic fruit flies that show the mutant behavior and find that in some, but not all, the brain is made up of mutant tissue. In some mosaics, "nature" replaces a mutant part with a normal part. Since the animal still behaves abnormally, even when the brain is made up of normal tissue, clearly the brain cannot be the source of the problem. However, if the thoracic ganglion is made up of mutant tissue in every individual showing the aberrant form of the action, and if the presence of normal tissue in the thoracic ganglion is associated with the normal form of the action, then the conclusion is that the thoracic ganglion is the structure altered by the gene. When the thoracic ganglion is defective, the behavior is abnormal. Conversely, when nature puts in a normal, rather than mutant, thoracic ganglion, the animal behaves normally. This is the equivalent to replacing the plug in the lamp analogy. Furthermore, as in the analogy, the site of the problem can be determined even if the specific anatomical or physiological defect in that structure cannot be detected without further testing. Mosaic analysis may not identify all the structures necessary for the expression of a particular behavior, but it does point out those that are always altered in individuals showing the mutant, rather than the wild-type, behavior. This technique has been useful in locating the sites of gene action in both fruit flies and mice.

Drosophila

When tracing the path between a gene and behavior by mosaic analysis, the first step must be to obtain mosaic animals. A common way to acquire a mosaic fruit fly (*Drosophila*) is by producing a gynandromorph, a fly that has some sections of male tissue and some pieces of female tissue. A *Drosophila* cell is female if it contains two X chromosomes. Normal male cells contain one X and one Y chromosome, but cells containing only a single X chromosome are also male. A gynandromorph results from an XX (female) zygote when one of the X chromosomes is lost from one of the two nuclei created by the first cell division. The chances that the X chromosome will be lost are increased if it has an abnormal ring shape. When an X chromosome is lost, one of the resulting nuclei and all its descendants have two X chromosomes and express female characteristics, but the other daughter nucleus and all its descendants have only one X chromosome and express male characteristics. The fly that develops from this embryo is, therefore, half male and half female.

To produce a gynandromorph for behavioral analysis, a female who contains a ring X chromosome and who is also homozygous for a dominant allele producing the normal wild-type behavior is mated with a male who has a recessive mutation for that behavioral trait on his X chromosome. For example, a female with normal wings and flight might be mated with a male bearing the *wings up* mutation. Remember, male fruit flies have only one X chromosome. As a result, a male with a recessive mutation can be identified, because he displays the mutant form of the behavior. In this example, he would be identified by the position of his wings. It is necessary to have the female bear a dominant allele and the male a recessive allele so that female (normal) tissue can be distinguished from male (mutant) tissue in the mosaic. Notice in Figure 3.10 that when this male's X-containing sperm joins with an ovum containing a ring X, a female zygote results. If the ring X chromosome is lost during the first cell division, half the cells—those containing only the X chromosome that came from the father's gamete—are male. The remaining cells, those containing an X chromosome from the father in addition to the ring X chromosome from the mother, are female. The mutation can be expressed only in male cells because they lack an X chromosome with the dominant wild-type allele. None of the female cells of the gynandromorph expresses the mutation. In other words, the gynandromorph is a special type of mosaic, a fly with patches of both male (mutant) and female (normal) tissue.

Although all gynandromorphs are produced in the same way, by the loss of an X chromosome during one

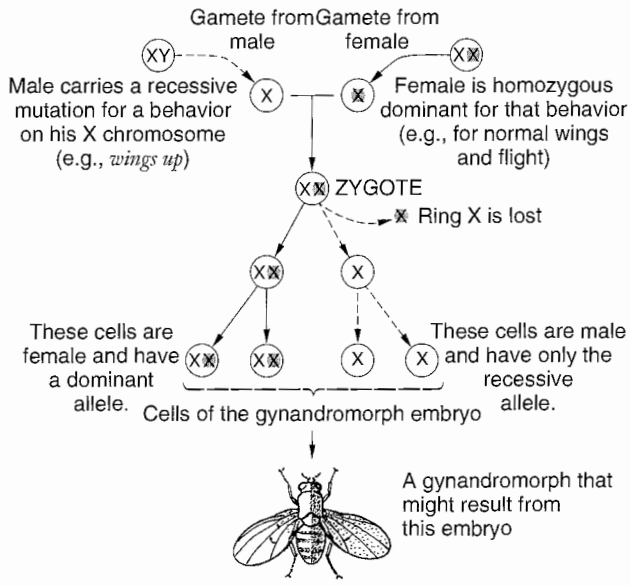


FIGURE 3.10 The development of a fruit fly gynandromorph because of the loss of a ring X chromosome during the first cell division of a female zygote. When the ring X chromosome is lost, one of the daughter nuclei then has only one X chromosome and is, therefore, male. Male cells are shaded. The other daughter nucleus has two X chromosomes and is female. The unshaded cells are female. These cells develop into a gynandromorph, an individual composed of half male cells and half female cells.

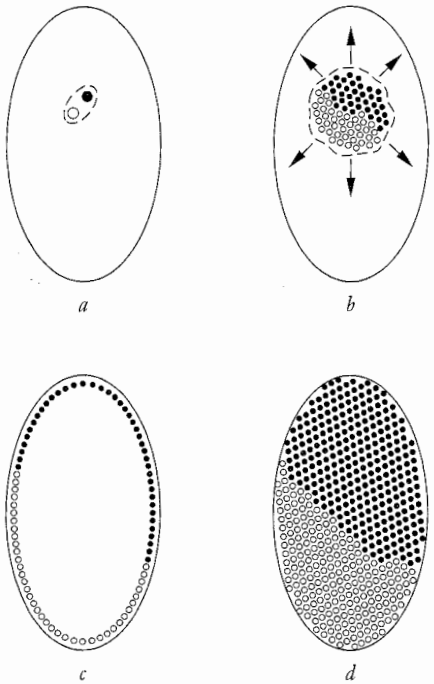


FIGURE 3.11 Early embryonic development of a fruit fly gynandromorph. (*a*) The products of the first nuclear division of the zygote. The male nucleus is indicated as a black circle, and the female nucleus is indicated as a white circle. (*b*) A view of the surface of the early embryo. The nuclei continue to divide and migrate to the surface of the egg. During the divisions and the migration, nuclei tend to stay near their neighbors. As a result, all the female nuclei are on one half of the embryo and all the male nuclei are on the other half. (*c*) A cross section of a slightly older embryo. The nuclei are enclosed in cells at this point. The embryo is still divided so that one half has male cells and the other half has female cells. (*d*) A later stage of development. An irregular imaginary line divides the embryo into a male half and a female half.

of the divisions of the embryo, they are not all identical. The male (mutant) and female (normal) tissue may be divided in a variety of ways because of the way insects develop. In Figure 3.11 you can see that daughter nuclei from each mitotic division tend to remain near one another. After several divisions, the nuclei migrate to the surface, forming a single layer of cells around the yolk. In a gynandromorph, approximately half the cells of this layer are female and the remainder are male. Because the nuclei stay near their neighbors even as they migrate, all the male cells are on one side of the embryo and the female cells on the other. However, the orientation of the dividing line between male and female cells varies because the first cell division can be in any direction on the surface of the egg. As a result, there are many ways in which the embryo may be divided into male (mutant) and female (wild-type) parts.

Furthermore, as in all fruit fly embryos, the destiny of each cell in this single layer is determined by its position. As a result, the orientation at the first cell division determines which structures will consist of male cells and which of female cells. A fate map indicating the structures that will eventually be formed is shown in Figure 3.12. If we mentally draw a line to separate the fate map in half, the orientation of this line will determine which structures will be composed of male cells and which of female cells. In a real embryo, the orientation of the initial cell division varies, so there are innumerable ways in which the adult fly may be divided into male and female chunks (Figure 3.13).

The sex of the cells making up each structure is important because it will determine whether the mutation under investigation can be expressed in those cells. If the cells of the anatomical structure in which the gene is normally expressed are female, the mutation will be hidden by the wild-type allele on the second X chromosome and the tissue will be normal. For example, the *wings up* mutation mentioned earlier will not be expressed in female cells, so when the wing muscles consist of female cells the gynandromorph has normal use of its wings. However, if the anatomical structure affected by the mutant gene falls in a male region of the adult, the mutation can be expressed and the tissue will be mutant. In the *wings up* example, the mutant allele can be expressed in the flight muscles of gynandromorphs that hold their wings up because those muscle cells are male. The next step is to select the gynandromorphs that show the abnormal behavior and compare the distribution of male and female tissue in each animal. If the insect shows the mutant behavior, each anatomical structure altered by the gene must be made up of male cells. The structure that is male in every gynandromorph displaying the mutant behavior is considered to be the site of action for the gene in question.

To make this comparison, there must be a way to identify male or mutant cells. These cells can be distin-

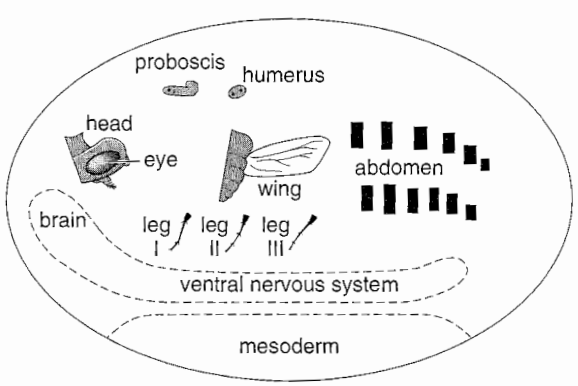


FIGURE 3.12 A diagrammatic representation of a fate map of a fruit fly embryo, indicating the structures that will eventually be formed from cells in various locations in the embryo.

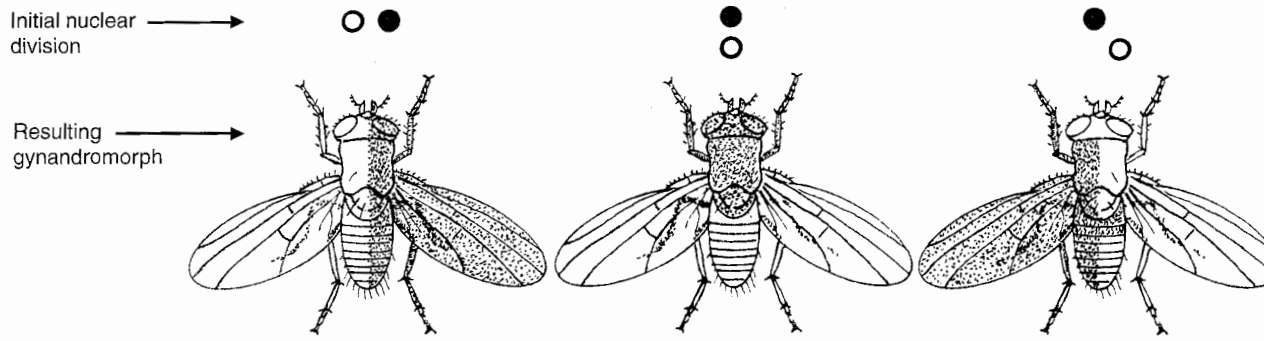
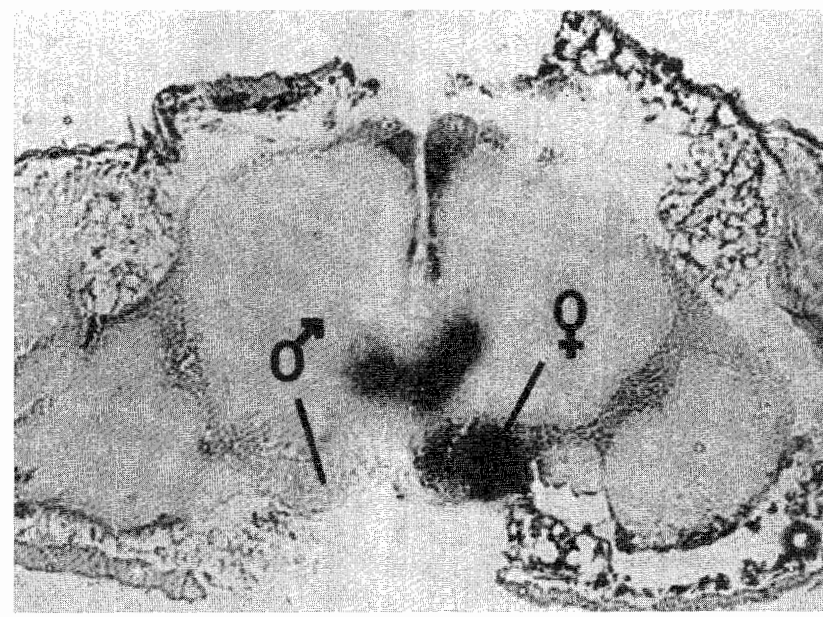


FIGURE 3.13 Diagrams of the gynandromorphs that might develop if the first division of the zygote nucleus were oriented as shown above the individual.

guished if the male who fathered the gynandromorphs had another recessive allele on his X chromosome, one that produces a trait we can see in each male cell. Genetic variants of enzymes are particularly useful tissue markers. Although these variants catalyze the same reaction within the cell, they have different physical and chemical properties. Because each variant has different biochemical properties, it is sometimes possible to find a stain that reacts with only one form of the enzyme. Then the cells producing that genetic variant can be distinguished from cells producing other forms. Variants of enzymes such as acid phosphatase (Kankel and Hall 1976) and succinate dehydrogenase (Lawrence 1981) are examples of tissue markers useful for labeling internal tissues. When histological markers such as these are used, the fly is sectioned and stained to highlight the form of the enzyme present in each cell. If the female cells have one form of the enzyme and the male cells another, the sex of the cells (and, therefore, the presence of the mutant tissue) can be determined at a glance. An example of a section of the brain of a gynandromorph that showed courtship behavior typical of males is shown in Figure 3.14. The stained cells are female, and the unstained cells are male.

Before looking at examples of the use of mosaic analysis, it may be useful to summarize the steps in the procedure. A female whose ring-shaped X chromosome bears a dominant allele that produces a normal behavior is mated with a homozygous recessive male who behaves abnormally. If the ring X chromosome is lost during one of the first cell divisions of a female embryo, a gynandromorph is produced. Because the mutation can be expressed only in male cells, the gynandromorph is also a mosaic of mutant and normal tissue. The mutant cells are also labeled with a tissue marker so that they can be distinguished from normal ones. Then all

FIGURE 3.14 A section through the brain of a gynandromorph. Variants of an enzyme were used as tissue markers. The female cells were set up genetically to produce a form of the enzyme that leads to a staining reaction. The male cells are unstained because they carry a mutant form of the enzyme gene.



the mosaic flies showing the mutant behavior are compared. The region that is male in every gynandromorph that behaves abnormally is where the gene is acting.

Mosaic analysis has been used to locate the primary site of action of genes that influence a variety of behaviors. This information is often useful in directing future investigations to determine the exact nature of the anatomical or physiological cause for the mutation's effect on behavior. For example, mosaic analysis has been used to pinpoint particular regions of the nervous system that are key to specific components of courtship and mating in the fruit fly.

Courtship and mating are activities that fruit flies do well, even though the choreography is fairly complex. The dance begins with orientation, during which the male faces the female and taps her on the abdomen with his foreleg. If she wanders away, he follows her. Next, he begins to "sing" a courtship song by fluttering a single outstretched wing. If the female does not show interest, he will repeat these actions. When the female seems receptive, he extends his proboscis (a tubular structure, bearing the mouthparts) and licks the female's genitalia. Next, he will try to copulate with her. If the attempt fails, he will wait a few moments before starting the ritual from the beginning (Hall 1994). It sounds simple, but remember, the minuscule nervous system of a fruit fly is orchestrating the whole thing.

Mosaic analysis has been helpful in identifying brain regions important in aspects of courtship. Male behavior could be expressed only in brain regions that consisted of male cells. Jeffrey Hall examined many mosaic flies and identified brain regions important in the beginning of courtship (orienting, tapping, following, and extending a wing). These stages of courtship required male cells, on one side of the brain or the other, in a small region near the top rear of the fly's brain. This brain region is important in integrating signals from the various sensory systems (Hall 1977, 1979). Tapping through wing extension will occur only if the dorsal posterior brain consists of male tissue. The fly will attempt to copulate only if its thoracic nervous system is male. And even if a fly has female genitalia, if its brain has the appropriate male regions, it will court any fly whose abdomen is genetically female. Furthermore, the antennae of a fly can be male or female, but the brain must be male if courtship is to occur. We can conclude, then, that the antennae of both males and females detect olfactory courtship cues, but genetically male brain cells process these cues differently from genetically female brain cells (Siegel et al. 1984).

More recently, mosaic fruit flies have been produced in another manner. By inserting a transposon (a segment of DNA that causes change in nearby genes) into specific regions of the nervous system of male flies, it is possible to convert those regions into female tissue. When certain regions of the nervous system that are involved in processing olfactory information were feminized, otherwise male fruit flies would court males or females with equal vigor. Thus these regions of the nervous system (portions of the antennal lobes or of the mushroom bodies) are important in discriminating males from females, probably on the basis of odors (Ferveur et al. 1995).

Mice

Although mosaic mice are used in the same way as mosaic fruit flies to determine where genes act to control behavior, they are generated through different procedures. A mosaic mouse, like a mosaic fruit fly, is made up of two genetically different types of cells, cells that were originally from two different embryos. A mouse begins its life as a single cell, called a zygote, but that cell soon begins to divide rapidly and forms a cluster of cells called a morula. A morula can be removed from the mother's oviduct and separated into individual cells by treatment with the appropriate enzyme. To produce a mosaic mouse, a morula is removed from each of two females with different genetic makeups. There must be two differences in the genotypes of the donor females. First, they must differ in the alleles for the behavior under investigation. The genetic information in one of the morulae would cause a normal behavior pattern, and the genes in the other would cause an aberrant action. Second, the morulae would have to differ in some trait that could be used as a marker to identify which morula gave rise to each cell in the resulting mouse. The morula removed from each female is dissociated, and the cells from both are mixed together. While this mixture is incubated for several hours, the cells reassociate to form a new morula composed of cells from both of the original morulae. Amazingly, this new embryo continues its development. When it reaches a slightly more advanced stage of development, the blastocyst stage, the embryo is implanted in the uterus of another female, where it will continue to develop into a genetically mixed-up mouse.

Mosaic mice differ from mosaic fruit flies in two ways. The first difference is in the percentage of normal and mutant cells in the mosaic. About half of the cells in a mosaic fruit fly are normal and the remainder are mutant. No such predictions can be made about the proportion of cells in a mosaic mouse that are derived from a particular morula. Although different morulae sometimes make equal contributions to a mosaic mouse, it is also possible that the resulting mouse is not a mosaic at all. Its cells may be all one genotype. Any mixture is possible. One reason for the difficulty in predicting the percentage of cells derived from each morula is that some of the cells of the mosaic morula will develop into extraembryonic structures and others

will give rise to the fetus. As a result, there is an even greater potential for variety in mosaic mice than there was in mosaic flies. A second difference in mosaics of these two species is in the way cells of different genetic composition are divided. Remember that mosaic fruit flies were made up of chunks of normal or mutant tissue. Usually a whole structure such as a limb or wing is composed of one genotype. In contrast, the cells from different morulae are more thoroughly intermingled in mosaic mice (Stent 1981).

Mosaic analysis provides a way to locate the primary site of action of some of the genes that result in neurological defects in the cerebellum of mice. Nerve cells interact with one another more than cells of most other systems do, so it is particularly difficult to determine which cells in the nervous system are being affected by a particular gene. When a nerve cell degenerates or behaves in an abnormal manner, the problem could originate within that cell or within another cell that then fails to interact with it properly. Mosaic mice have been used to distinguish between these possibilities.

One recessive mutation that results in neurological difficulty for mice is called *pcd* because it causes *P*urkinje *c*ell *d*egeneration. Purkinje cells are large neurons that provide the only output from the cerebellum, the part of the brain that coordinates bodily movement. Animals that are homozygous for the *pcd* alleles seem normal at birth because they have Purkinje cells. However, these neurons gradually die, and as they degenerate the mice become uncoordinated. Richard Mullen (1977b) wondered whether the *pcd* mutation affects the Purkinje cells directly or whether the Purkinje cells are responding to an abnormality in some other cell. To answer this question, Mullen studied mosaic mice in which some cells were homozygous normal and other cells were homozygous for the *pcd* mutation. An enzyme tissue marker was used so that the normal cells could be distinguished from the *pcd* cells. Mullen reasoned that if the *pcd* mutation had its primary effect on the Purkinje cells, any Purkinje cells bearing the mutation would degenerate and all those with the normal dominant allele would survive. However, if the *pcd* mutation directly affected some other cell type, making it incapable of interacting with Purkinje cells in the proper manner, then the population of surviving Purkinje cells would have mixed genotypes. When the brains of mosaic *pcd*/normal mice were sectioned and stained, Mullen found that all the surviving Purkinje cells were normal, but those with the *pcd* allele had degenerated. Therefore, the Purkinje cells were directly affected by the *pcd* gene. We now know that *pcd* is located on chromosome 13, which will help us determine the biochemical mechanism by which the gene causes the problem (Campbell and Hess 1996). In any event, with the *pcd* gene, it seems that justice prevails. The fate of the Purkinje cells depends on their own genotype.

However, the *reeler* mutation shows that life, even for a neuron, is not always fair; the fate of the Purkinje cells of *reeler* mice is determined by the genotype of other neurons. By the time *reeler* mice are two weeks old, they can be identified because they walk as if they were drunk, similar to the way normal mice move while they are recovering from anesthesia (Sidman, Green, and Appel 1965). A *reeler* mouse has trouble walking because during its development, the Purkinje cells fail to migrate to the proper positions in the cerebellum. In contrast to the direct effect of the *pcd* mutation, mosaic analysis suggests that the *reeler* allele affects the Purkinje cells indirectly. Some of the Purkinje cells in the *reeler*/normal mosaic mice do migrate properly, but others do not. Of those that move to the correct locations, some have the *reeler* allele and others have the normal allele. In addition, many of the Purkinje cells that were in the proper position had the *reeler* genotype (Mullen 1977a). The best explanation for this is that the *reeler* mutation has its effect on some other type of cell.

Indeed, it is true—the neurons in brains of reeler mice are scrambled because of a defect in another type of cerebellar cell (the Cajal-Retzius cells). These cells normally produce an extracellular protein called Reelin, which acts as a signpost that guides Purkinje cells to their proper locations within the brain. Cajal-Retzius cells with the *reeler* mutation do not produce this protein, and therefore the Purkinje cells never migrate to their proper locations (D'Arcangelo et al. 1995). Furthermore, there is another protein involved in the migration of the Purkinje cells. This protein is the product of a gene called *mouse disabled-1* (*mdab1*) that was identified in *scrambler* mice, which behave like *reeler* mice and have similarly scrambled brains. It is thought that the mDab protein may act in migrating Purkinje cells to relay the message from Reelin (Howell et al. 1997; Sheldon et al. 1997).

GENETIC ENGINEERING

The relationships between genes and behavior are now being considered by using techniques of genetic engineering. Of primary importance is the production of recombinant DNA, that is, DNA from two different organisms. Specifically, it is possible to insert certain genes into organisms to determine their effect on behavior. In addition, recombinant research has revealed some of the ways in which genes control behavior.

The *Drosophila per* Gene

In nature, *Drosophila* eclosion, the emergence of adult flies from their pupal cases, occurs around dawn each day. Any pupae that fail to develop enough to eclose during the dawn hours must wait until the following morning. Eclosion is described as rhythmic because

flies in a population tend to emerge in a repeating pattern—many emerging at dawn and then very few until the following dawn. It is interesting that the pupae do not require environmental time cues to remain rhythmic. When pupae are maintained in a laboratory without normal time cues such as light or temperature cycles, they still emerge at approximately 24-hour intervals. Since the ability to measure the passage of time is not dependent on these obvious environmental cues, we attribute it to a biological clock (see Chapter 9). The biological clock is still running in adult fruit flies; their locomotor activity is cyclic, with a 24-hour period. The *per* gene affects the biological clock, governing both eclosion and activity.

The genetic roots of the biological clock of *Drosophila* have been studied by Ronald Konopka and Seymour Benzer (1971). They induced mutations and screened the population for individuals whose biological clocks had been altered. As you can see in Figure 3.15, one mutation created flies whose biological clock runs fast, resulting in eclosion and activity rhythms with shorter period lengths. This mutation, per^s, results in rhythms with a 19-hour period. Another mutation, per^l, has the opposite effect—a rhythm with a long, 28-hour period. The third mutation, per^0 creates arrhythmic flies; they emerge from their pupal cases and are active at random times throughout the day. These three mutations seem to be in the same functional gene, the *per* gene, which is located on the X chromosome. To determine where this gene has its primary effect, mosaic flies were created. The head region in flies with aberrant rhythms was always composed of mutant cells, indicating that the *per* gene has its primary effect in the brain.

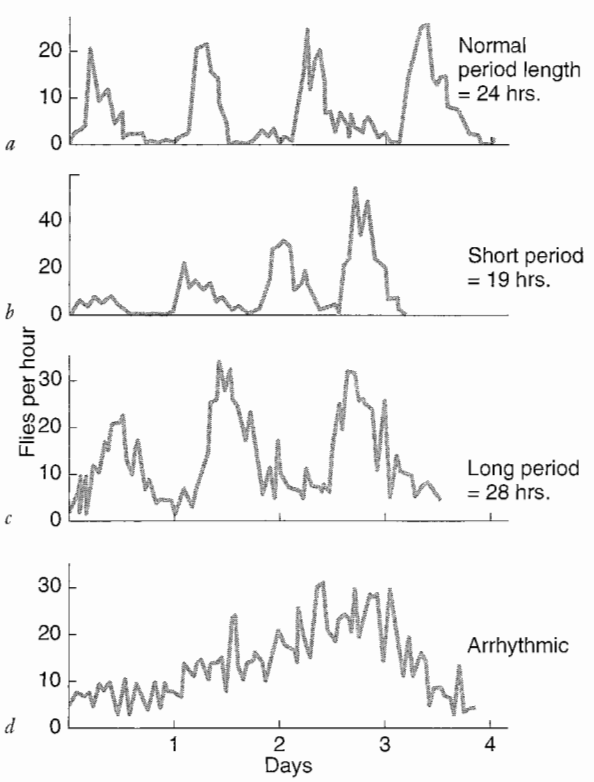


FIGURE 3.15 The eclosion rhythms of normal and three mutant strains of fruit flies studied in constant conditions. The top curve (*a*) shows the number of flies emerging each hour in a population of normal fruit flies. Peaks of eclosion occur every 24 hours. The lower curves depict the number of flies that emerge each hour in a population of (*b*) short-period (19-hour) flies, (*c*) long-period (28-hour) flies, and (*d*) arrhythmic flies. (Data from Konopka and Benzer 1971.)

Now let us see how recombinant DNA techniques have been used to study behavior. Recombinant DNA has made it possible to insert the per^+ allele into a per^0 fly. A normal per^+ fly has 24-hour rhythms, but a per^0 fly is arrhythmic. In these experiments, a fragment of DNA containing the *per* gene along with the dominant allele for rosy eye color was inserted into a plasmid (a small circular piece of DNA). These recombinant plasmids were then injected into the pole cell region of early embryos of per^0 flies that also had the recessive alleles for eye color. Some of the cells in the pole region develop into the cells that go on to form gametes when the embryo becomes an adult. The flies that developed from embryos treated in this way were mated with arrhythmic (per^0) flies that were homozygous recessive for the rosy eye color gene. In the resulting offspring, rosy-eyed individuals could have gotten their eye color only from the DNA in the recombinant plasmid since both of their parents were homozygous recessive for that eye color gene. Therefore, the presence of rosy eyes indicates not only that the plasmid is present in those individuals but also that the inserted genes could be expressed. The eye color serves as a marker that can be used to identify flies that have incorporated the plasmid. But our interest is really in the per^+ gene. Flies that incorporated the plasmid were not only rosy-eyed but also rhythmic. Their eclosion and activity rhythms resembled those of per^+ flies (Bargiello, Jackson, and Young 1984). Using recombinant DNA is, as you can see, a rather precise way to demonstrate that a particular gene is necessary for the expression of a complex behavior pattern, in this case biological timing.

Is the *per* gene needed for the daily functioning of the clock or does it influence the clock during development, perhaps by creating a brain-damaged fly? This question has also been answered by using recombinant DNA techniques. Recall from the discussion at the beginning of this chapter that genes are not always active; they can be turned on or off. One way in which this may occur is through the activity of another gene, which is called a promoter gene. Through recombinant techniques, a segment of DNA was created that contained the per^+ gene, as well as a promoter gene to regulate its activity. When induced to do so by a temperature shock of 27°C, the promoter turned on the per^+

gene. This segment of DNA was injected into a *per*^{0} embryo, where, as in the previous example, it was incorporated into almost all the adult cells. When these adult flies were kept at 18°C, they were arrhythmic. However, when the temperature was raised to 27°C, the promoter turned on the *per*$^{+}$ gene, so the flies became rhythmic. The animals quickly became arrhythmic again when the temperature was lowered. This indicates that the product of the *per* gene is not very stable. Rhythmicity can be turned on or off by changing the temperature. The conclusion is that the product of the *per* gene must be present for the clock to function (Ewer, Rosbash, and Hall 1988).

Another question that has been addressed by recombinant DNA techniques concerns the number of clocks present in a multicellular organism. As previously mentioned, mosaic analysis revealed that the *per* gene affects a certain cluster of cells in the brain. Certainly, the brain is the site of one clock. Are there more? Research using recombinant techniques suggests that there are many clocks. A clock might be located wherever the *per* gene is active. Since there is no stain for the *per* gene product, the *per* gene was fused with a gene that would produce a product that could be stained, the β-galactosidase gene taken from the bacterium *E. coli.* The fused genes were inserted into a *per*^{0} embryo and became incorporated into adult cells. The activity of fused genes is linked, so the product of the bacterial gene, galactose, could be stained green to reveal the tissues that were expressing the *per* gene. In this way, it was shown that the *per* gene becomes active shortly before eclosion. In the adult, the *per* gene is expressed in many tissues, including the antennae, eyes, optic lobe, central brain, thoracic ganglia, Malpighian tubules, and ovarian follicle cells (Liu et al. 1988). Another study used antibodies specific for the *per* gene product to determine where the gene is expressed. Similar results were obtained (Siwicki et al. 1988). Because the *per* gene is expressed in so many tissues, it seems likely that there are multiple independent clocks. (The role of the *per* gene in biological timing is discussed further in Chapter 9.)

Inactivating and Adding Genes—Knockout and Knock-in Mice

It is now possible to explore the mechanisms that underlie behavior by deleting or adding extra copies of specific genes. Mice in which a specific gene has been inactivated, or knocked out, are aptly named "knockout mice." Knockout mice completely lack the product of the disabled gene. Thus, by selectively disrupting the function of a single gene, this technique should allow one to determine the gene's function.

The production of a knockout mouse is tricky. The gene of interest must first be identified, isolated, and disabled by mutating it. The gene is then delivered to a target cell from an early mouse embryo (Figure 3.16). These target cells, called embryonic stem cells, will take up foreign DNA, such as the inactivated mutant gene. The mutant gene finds its complement on the stem cell chromosome and switches places with it. In this way, the mutant stem cell incorporates a copy of the inactivated gene. Furthermore, embryonic stem cells are still capable of differentiating into any tissue type. Thus, they can be injected into another, otherwise normal early embryo and continue developing as part of it. The resulting embryo is then implanted into a surrogate mother.

The newborn mice will have some cells that contain the inactivated gene and some cells that contain the functional gene. This type of animal, one that contains a mixture of cells with different types of genes, is called a chimera. If the mutant stem cells differentiate into cells that will become eggs or sperm, some of the gametes will contain the mutant inactivated gene. When the chimeric mice are bred with wild-type mice, some of the offspring will be heterozygous for the gene of interest. The heterozygous mice are bred with one another, and according to the laws of genetics one-fourth of the offspring will be homozygous for the inactivated gene; these are the knockout mice.

The use of knockout mice in behavioral research has some drawbacks. It is not uncommon for all of the knockouts to die as early embryos or soon after birth because the product of the inactivated gene is essential. Even when inactivating a gene isn't lethal, it can cause gross anatomical and physiological abnormalities, making it difficult to determine the direct cause of the resulting changes in behavior (R. J. Nelson 1997). Genes can interact in complex ways, making it difficult to pinpoint the effects of one of them. Thus, another problem is that the mutant organism might have physiological or developmental processes that have been altered and compensate for the missing gene. And, knocking out the same gene in different species may have different effects on behavior (Crawley 1996).

For technical reasons, up to now most knockout mice have been created by using embryonic stem cells from one strain of mice (the 129/SV strain), but the chimeric mice were mated to mice of a different strain (the C57BL/6 strain). During the genetic recombination that occurs as gametes are formed (discussed in Chapter 4), the genes from chromosomes of two different strains may be mixed. The genes that contain alleles from the 129/SV strain and those that contain alleles from the C57BL/6 strain may differ among littermates. Put simply, the genetic backgrounds of the knockout mice littermates will differ from one another and from the wild-type control mice. Thus, observed differences in behavior between the knockout mice and mice with a functional gene may actually be caused by differences in other genes (Gerlai 1996). As newer techniques are developed that use the same strain of mice for stem cells

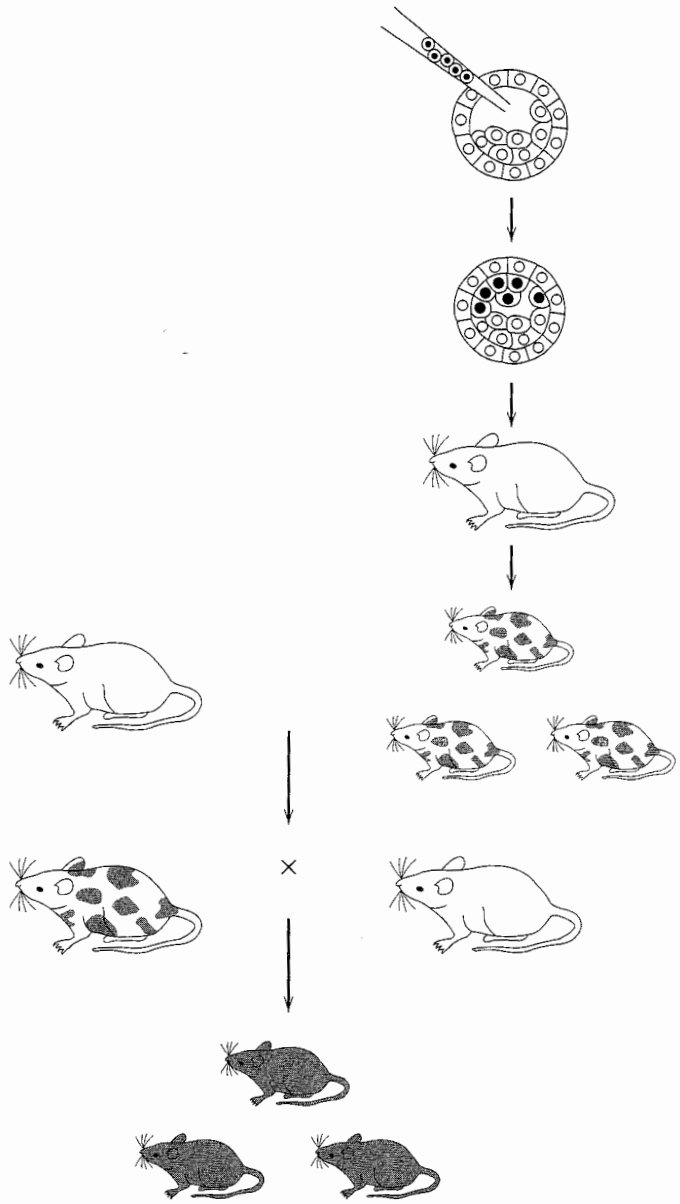


FIGURE 3.16 The creation of a knockout mouse—a mouse in which a specific gene has been inactivated.

and as donors, differences in the genetic background of mice will be less of a problem (R. J. Nelson 1997)

Currently, there are knockout mice for many genes that influence behavior (e. g. reviewed in Nelson and Young 1998). We will consider how knockout mice have been used to explore the role of hormones in regulating behavior in Chapter 7. Later in Chapter 3, we will see how knockout mice have been useful in understanding the way that genes influence nurturing behavior in mice. So, here we will look at the way knockout mice have been used to shed light on the mechanisms of learning and on the role of the hippocampus in mammalian learning and memory.

Although we are still far from completely understanding how we, or even mice, learn and remember, recent studies using knockout mice and knock-in mice to which extra copies of genes have been added have made significant advances. Many studies have demonstrated that a brain region called the hippocampus is important in the formation of long-lasting memories. According to one hypothesis a molecular mechanism by which memories are acquired and stored involves a protein that spans the membrane of nerve cells and serves as a receptor for glutamate, a chemical signal used by certain nerve cells to communicate with one another. Like most receptors for such neural chemical signals, this one, called NMDA (N-methyl-D-aspartate), opens molecular gates to tiny channels through the nerve cell

membrane, allowing ions to pass through. Specifically, when the neurotransmitter glutamate binds to NMDA, calcium channels are opened. However, NMDA differs from most other receptors in an important way. It requires *two* signals before it opens the calcium channel. One signal is glutamate, which is released by a neighboring cell. The other signal is electrical, generated within the cell. When the two stimuli occur at about the same time, the calcium channels are opened and calcium ions flow into the cell. As a result, it is easier to stimulate the cell the next time the stimuli occur. Because it requires two signals, NMDA is a molecular mechanism for associating stimuli, which is the basis for a memory.

The hypothesis that the NMDA receptor is a keystone for learning and memory was tested using genetically engineered mice. Susumu Tonegawa created knockout mice in which the gene that codes for NMDA was inactivated in the nerve cells of the hippocampus. These knockout mice had difficulty passing a standardized test of spatial learning called the hidden-platform water test. In this test, the mice are placed in a pool of an opaque liquid. They must swim until they locate a platform on which they can rest, which is hidden beneath the liquid's surface. Not only were the knockout mice slower than normal mice to learn the location of the platform in the initial trials, they also had difficulty remembering the location of the platform in subsequent trials (Silva et al. 1992; Tonegawa 1994, 1995).

More recently, Joseph Tsien and his colleagues (Tang et al. 1999) provided additional support for the hypothesis by creating mice that overexpress the *NR2B* gene that codes for the NMDA receptor. The ability of these mice to learn and remember was then tested in a variety of ways and their performance compared to their normal, wild-type littermates. In the hidden-platform test of spatial memory described earlier, mice with the enhanced *NR2B* genes escaped to the hidden platform more quickly than normal mice (Figure 3.17). After three training sessions, the mice were placed in the pool without a platform. The genetically-enhanced mice spent more time searching in the quadrant of the pool where the platform was previously located than did their normal littermates. In similar tests after six training sessions, the difference in performance between genetically-enhanced and normal mice was smaller. The enhanced mice were just learning more quickly than normal mice were.

The ability of the mice to remember a familiar object was also tested. The mice were placed in an area and allowed to explore two objects for five minutes, becoming familiar with them. Several days later, the mice were returned to the test area, but one of the objects had been replaced by a new one. Normal mice spend about as much time exploring each object. Apparently, normal mice forget their previous experience with one of the objects. In contrast, the mice with the enhanced *NR2B* genes spend most of their time exploring the novel object. They don't waste time exploring the familiar object.

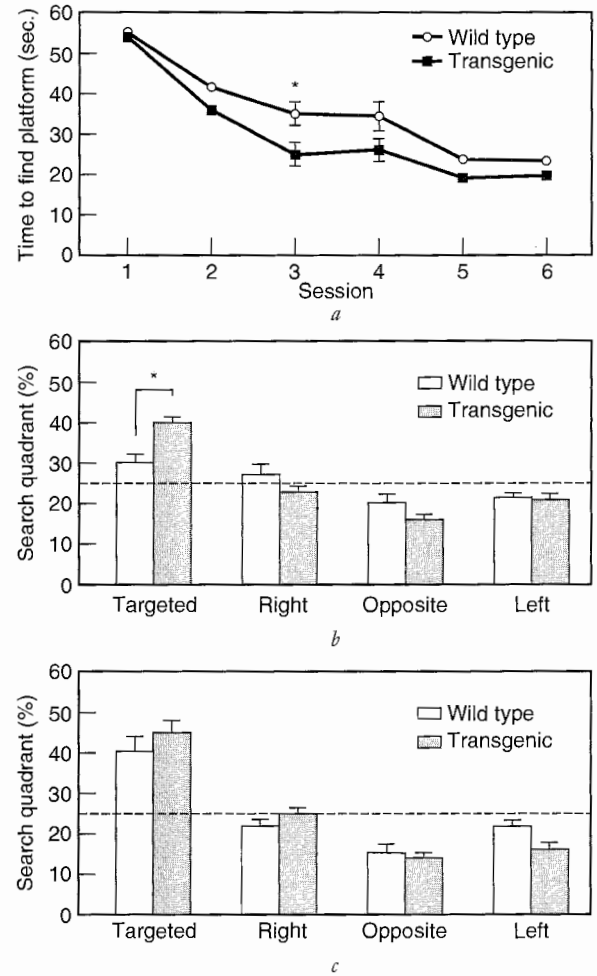


FIGURE 3.17 Transgenic mice that overexpress the gene for the NMDA receptor on nerve cells in the hippocampus of the brain perform better than their normal, wild-type littermates in the hidden-platform water test. The mice are released into a pool of an opaque liquid and must swim until they find a platform hidden under the surface. (*a*) The genetically-enhanced mice are able to escape to the hidden platform more quickly than normal mice. However, after six training sessions, the normal mice perform almost as well as the enhanced mice. The mice were then tested in a pool without a platform. (*b*) After three training sessions with a hidden platform, the genetically-enhanced mice spent more time swimming in the quadrant of the pool where the platform had been hidden in previous tests than did normal mice. (*c*) The differences in performance between enhanced and normal mice were smaller after six training sessions. (Data from Tang et al. 1999.)

The emotional memory of the genetically-enhanced mice was also compared with that of normal

mice. The mice were placed in a chamber where they received electrical shocks to their feet. After a few trials, the mice associated the chamber with shock and showed signs of fear when placed in it. When they were returned to the chamber after one hour, one day, or ten days, the genetically-enhanced mice showed more pronounced fear responses than did normal mice. The mice with enhanced *NR2B* genes also remembered an association between an audible tone and an electric shock better than normal mice. We see, then, how the use of knockout and knock-in mice have helped us understand one of the mechanisms underlying learning and memory.

MECHANISMS OF CONTROL

Keeping what we have learned about the relationship between genes and behavior in mind, we can now reconsider the mechanisms through which genes orchestrate specific behaviors.

APLYSIA EGG LAYING

The reproductive behavior of the sea hare *Aplysia* involves a number of fixed action patterns, such as the coordinated actions involved in the laying of eggs (Figure 3.18). When the snail is about to produce an egg string, it stops normal activities such as moving and eating. There are also several physiological changes at this time: The heart and respiratory rates increase, and the muscles of the reproductive ducts contract as the animal expels a string of eggs. The record-holding sea hare laid a string 17,520 cm long at a rate of 41,000 eggs per minute! When the egg string first appears, the snail grasps it in a fold of its upper lip and, waving its head in a stereotyped pattern, helps to pull the string out, winding it into an irregular mass. During this process, the egg string is coated with sticky mucus. Finally, the tangled egg string is attached to a rock or other firm support with a characteristic wave of the head (Kandel 1976).

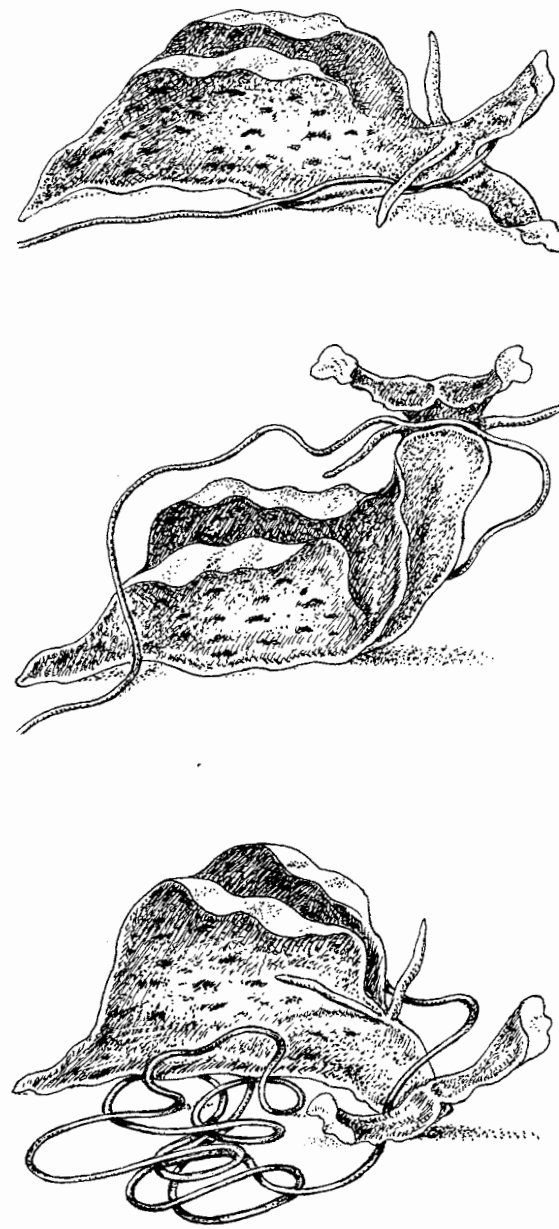


FIGURE 3.18 **The egg-laying behavior of the sea hare *Aplysia*. (*a*) The snail grasps the end of the egg string in a fold of its upper lip. (*b*) Waving its head, it helps pull the egg string out. (*c*) The tangled mass of egg string is attached to a solid surface. (Scheller and Axel 1984.)**

The neuroendocrine link between the genes and egg-laying behavior is known. Two small proteins, peptide A and peptide B, are produced by the atrial gland in the reproductive system. When these peptides are injected into an animal, they stimulate two clusters of neurons on top of the abdominal ganglion, called bag cells, to release other peptides (Figure 3.19). One of the bag cell products is egg-laying hormone (ELH), which acts as an excitatory neurotransmitter and increases the firing rate of neurons in the abdominal ganglion. In addition, ELH acts as a hormone that causes the smooth muscle of the reproductive ducts to contract and expel the egg string. It may also cause the changes in heart and respiratory rates. Simultaneously, the bag cells release alpha bag cell factor, an inhibitory transmitter that decreases the rate of specific neurons (L2, L3, L4, and L6), and beta bag cell factor, an excitatory transmitter that increases the firing rate of certain other neurons (L1 and R1). The stimulation or inhibition of particular neurons presumably coordinates the egg-laying behavior pattern (Scheller and Axel 1984).

How do genes fit into this scenario? The *ELH* gene is translated into a large protein that is broken down into the shorter functional proteins, ELH, alpha bag

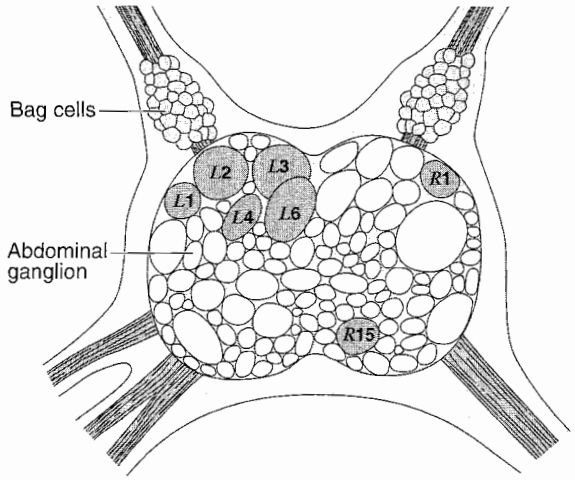


FIGURE 3.19 The abdominal ganglion and the bag cells of *Aplysia*. The *ELH* (egg-laying hormone) gene is expressed in the bag cells. Bag cells produce several substances, including ELH, alpha bag cell factor, and beta bag cell factor, that coordinate the actions involved in egg laying. Some of the large neurons in the abdominal ganglion that are affected by bag cell products are labeled. (Redrawn from Scheller and Axel 1984.)

factor, and beta bag factor. Thus a single gene is activated, and because its products act as neurotransmitters and hormones, that gene can orchestrate a complex behavioral sequence. Furthermore, if the gene is activated, all the components of the behavior pattern appear, as is typical of a fixed action pattern.

It was the sequence of bases on the *ELH* gene that revealed the mechanism of this gene's control of egg-laying behavior, but sequencing the *ELH* gene involved many steps. First, the *ELH* gene had to be identified and cloned. To accomplish this an *Aplysia* DNA library was created. A DNA library is made up of clones of different recombinant plasmids, containing fragments of all of the DNA of an organism. Second, the plasmids that bear the *ELH* gene had to be identified. This was done by making cDNA from mRNA in the bag cells and from mRNA in nonneural cells and then labeling each with a radioactive marker so that it could be identified. The cDNA from both sources was hybridized with the *Aplysia* DNA in each clone of recombinant plasmids. The key to hybridization is the specificity of base pairing. When a DNA molecule is heated, the molecule unzips down the middle by breaking the bonds that hold the two strands together. This separates the base pairs and leaves two single strands of DNA. As the DNA cools, each strand usually finds another strand with the complementary sequence of bases, and these match up to re-form double-stranded DNA. Because the bag cells produce ELH, they will have many mRNA molecules coding for ELH. Therefore, the cDNA from bag cells will have the gene for ELH. Nonneural cells do not produce ELH, and consequently the cDNA made from their mRNAs will lack the *ELH* gene. As a result, the recombinant plasmids that hybridized with cDNA from bag cells, but not with cDNA from other cells, were identified as those containing the *ELH* gene. Third, the exact piece of DNA comprising the *ELH* gene was identified by hybridizing the clones known to contain the gene with mRNA from the bag cells. The predominant mRNA in the bag cells would code for ELH. The *ELH* gene could be spotted on an electron micrograph as a region of hybridization. Once the gene was isolated, it was sequenced.

The sequence of bases in the *ELH* gene revealed that it codes for a large polyprotein precursor that is chopped into several functional proteins. The sequence of bases on the *ELH* gene codes for 271 amino acids, but ELH itself has only 36 amino acids. The sequence of bases that would be translated into ELH could be seen in the precursor, and it was bordered by signals that indicated that ELH would be clipped out of the larger protein (Figure 3.20). What came as a surprise was that the alpha bag factor and beta bag factor were also flanked by cleavage signals. In fact, the gene codes for 11 different proteins, each bordered by cleavage signals, and 4 of these are known to be released by the bag cells. In other words, a single gene codes for a large protein that is broken down into at least three smaller active proteins with different roles in producing a behavior pattern. The role of the genes in generating this fixed action pattern is to produce peptides that act locally as neurotransmitters or distantly as hormones. These peptides stimulate or inhibit specific structures to coordinate a stereotyped behavior pattern (Scheller and Axel 1984).

NURTURING IN MICE

Genes are also involved in behaviors less stereotyped than egg laying in *Aplysia*. For example, consider the role of the *fosB* gene in nurturing in mice. After giving birth, a female mouse normally nurtures her young, which involves creating a nest, cleaning the pups, bringing the pups back to the nest if they become scattered, and huddling over the young to keep them warm and to nurse them. These activities are crucial to the survival of the pups.

Jennifer Brown and her colleagues (1996) demonstrated the importance of the *fosB* gene in nurturing by creating mice with a knockout mutation in that gene. Brown noticed that the pups of mutant mice with an inactive *fosB* died when they were one or two days old. Furthermore, the pups of mutant mice were scattered throughout the cage and neglected (Figure 3.21). The mutant mothers investigated pups but seemed unable to process the cues that bring about nurturing in normal mothers.

Clearly, *fosB* is important in nurturing behavior, but

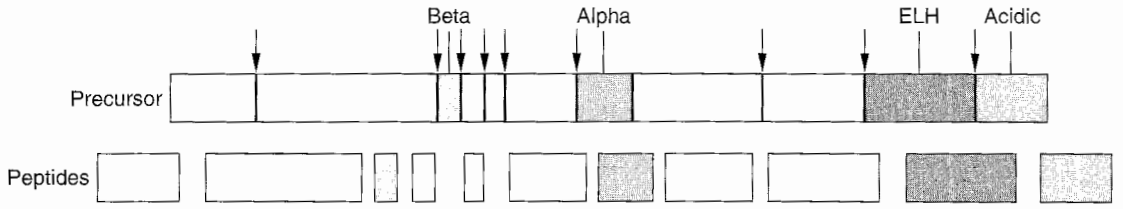


FIGURE 3.20 The products of the *ELH* gene. This gene codes for a large polyprotein precursor that is later cleaved into several smaller functional peptides. (Redrawn from Scheller and Axel 1984.)

what is the mechanism of its action? The development of normal nurturing has two components, both involving the same brain regions. One component depends on sensory input from touching and hearing newborn pups, and especially from smelling them. We know that in mice who nurture normally, *fosB* becomes active following experience with pups. The other component of nurturing depends on hormones. The hormone levels of *fosB* mutant mice are normal. Therefore, it is thought that the mutant *fosB* interferes with the first component of the nurturing response, the one built through experience with the pups.

We are certain of only a few of the details in the link between nurturing and *fosB. FosB* is an immediate-early gene. You may recall from our previous discussion of *zenk* and song learning that immediate-early genes produce proteins that activate other genes, which are then directly involved in changing behavior. The preoptic area of the hypothalamus (POA) is a brain region critical to the nurturing response, and it is here that input from several senses is integrated. *FosB* is activated in the POA neurons following experience with pups. In turn, the protein coded for by *fosB* activates a program of gene expression that leads to structural changes in the neuron circuits responsible for nurturing. In mutant mice, however, *fosB* cannot be activated. Therefore, normal nurturing does not develop.

It is possible that one of the genes activated by *fosB* codes for oxytocin receptors. If so, a chain of events that strengthens the neural circuit involved in nurturing might be this: Sensory input from experience with pups raises oxytocin levels; oxytocin activates *fosB; fosB* then turns on other genes and leads to the production of more oxytocin receptors; the increase in oxytocin receptors would then make the neurons more sensitive to the hormone, strengthening the neural pathway for nurturing (Brown et al. 1996).

RETROSPECTIVE

We have seen that each gene codes for a specific protein. Because proteins play so many functional and structural roles in the body, genes can influence behavior in a myriad of ways. The gene may alter a structural protein, as we saw in *Shaker* fruit flies, which have

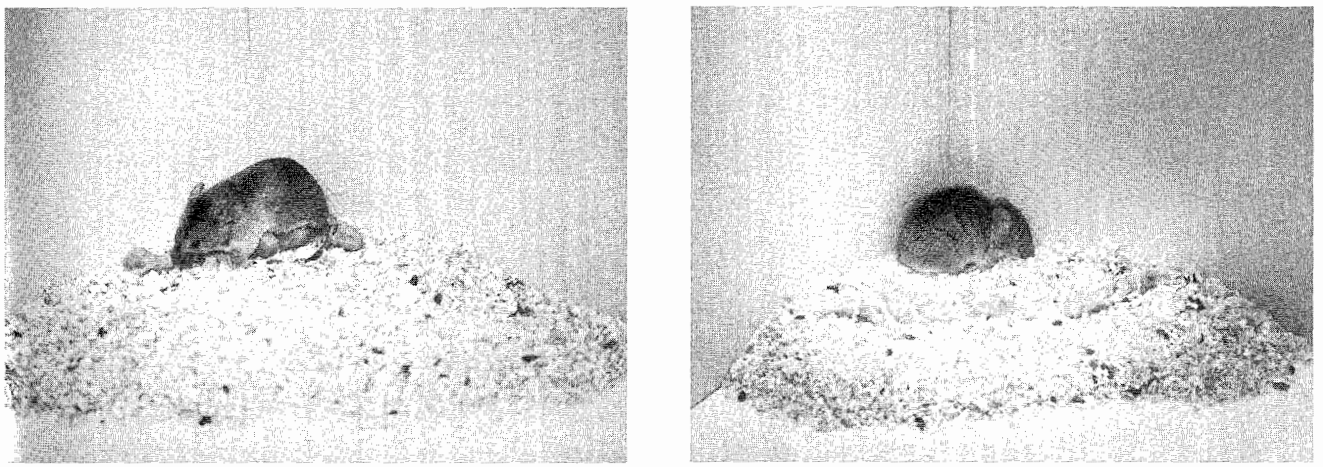


FIGURE 3.21 Mice with an inactive *fosB* gene fail to nurture pups appropriately. Normal mice nurture their pups after birth—they clean, retrieve, and huddle over pups (*left*). Mice with an inactive *fosB* gene do not show appropriate nurturing behavior and their pups are scattered (*right*). The pups of mutant mothers usually die when they are one or two days old (Modified from Brown et al, 1996).

altered channels through nerve cell membranes, and *reeler* mice, which have altered extracellular proteins that fail to guide nerve cells to their correct positions. Alternatively, the gene may code for an enzyme, as we saw in *dunce* and *rutabaga* fruit flies. The altered enzymes influenced levels of cAMP and, therefore, learning. Furthermore, the immediate-early genes, *zenk* and *fosB*, illustrated that a gene may even code for a protein that regulates the activity of other genes. We see, then, that we can look for behavioral effects of genes virtually anywhere proteins are found (which is everywhere).

In some cases, a single gene influences several different behaviors, a phenomenon called pleiotropy. For instance, the *dunce* mutation in fruit flies not only alters olfactory learning but also impairs courtship. A female fruit fly who has already mated produces a pheromone (a chemical that sends a message to other members of the species) that inhibits courtship by normal males. This makes sense in the evolutionary scheme of things. Why should a male waste time and energy in courting an already inseminated female when she can block most attempts at copulation? Nonetheless, *dunce* and *rutabaga* males never learn to cease courting a mated female, in spite of her persistent signals (Hall, 1994). Furthermore, the *per* gene, which alters the period length of a fruit fly's circadian rhythms, also changes the rhythm of the male's courtship song (Kyriacou and Hall 1980).

Complex behaviors result from the combined activities of many genes. Consider, for instance, the genes that have effects on aggressive behavior in mice. The list of genes with demonstrated effects include the genes for (1) monoamine oxidase on the X chromosome, (2) a serotonin receptor on chromosome 9, (3) the estrogen receptor on chromosome 10, (4) α-calcium calmodulin kinase II on chromosome 18, (5) the androgen on the X chromosome, and (6) a t-complex gene on chromosome 17 (reviewed in Maxson 1996).

Finally, different gene combinations can result in the same expression of a particular behavior. We saw this in the results of the selection experiments that led to the strains of mice that built large nests and small nests (Bult and Lynch 1996). As a result, populations that are adapted to specific environmental conditions will still retain genetic variability. This genetic variability will be important if environmental conditions change, as we will see in Chapter 4.

SUMMARY

Genes control behavior by directing the synthesis of proteins. The instructions for making a protein, which is a long chain of amino acids, are in the order of bases in the DNA. Many of those proteins influence anatomy or physiology—the foundation for behavior. Some genes cause permanent changes in behavior. For example, the *Shaker* gene in fruit flies changes a protein in the ion channels through nerve cell membranes. The change affects nerve activity and causes flies to shake under anesthesia.

If an animal has "a gene for some behavior," it means that the gene makes a difference in the performance of a behavior. However, even if an individual has a gene for a behavior, the action may not be observed. One reason is that not all genes are expressed all the time. They are turned on and off by several regulatory mechanisms. An important class of regulatory substances is the steroid hormones. In addition, nerve activity in response to stimuli can affect gene activity. For example, hearing the species song turns on the *zenk* gene in zebra finches. The resulting protein then regulates the activity of other genes, which are important in the growth of nerve cells that is essential for the formation of long-term memories of the species song.

The goals of behavior genetics are to demonstrate that there is a genetic basis to a behavior and then to determine the mechanisms by which the gene affects behavior. The classical methods of experimentally demonstrating the genetic basis of behavior include inbreeding, artificial selection, and hybridization. Inbreeding is the mating of close relatives. When mating is arranged in this way, the siblings of successive generations become increasingly similar genetically until 98% of their genes are identical. The genetic similarity of individuals in inbred strains is the key to their usefulness in behavioral genetics. When inbred strains that have been raised in similar environments are compared, any difference in behavior must be due to differences in their genes. Therefore strain comparisons are a means of demonstrating that genes can make a difference in the form of a behavior. For example, two inbred strains of paradise fish larvae respond differently to a predator model. This difference in behavior is due to genetic differences in the inbred strains.

Artificial selection is a different breeding regimen. Here individuals showing a desired behavior are bred with one another. If the frequency of the trait in the population increases when the appropriate breeders are mated, then the behavior must have a genetic basis. Artificial selection is the way breeds of dogs are created. Artificial selection for nesting behavior in house mice has demonstrated a genetic basis to nest building. These experiments have also revealed that natural selection can select for different combinations of alleles in different populations to produce the same observed adaptive behavior. It is important to keep in mind that natural selection alters behavior by acting on the structure or function of the nervous system.

Hybridization is another breeding system used to demonstrate that a given behavior has a genetic basis. Individuals that display the behavior in distinct but different ways are mated with one another, and the behavior of the hybrid offspring is observed. One such series of experiments demonstrated that foraging strategies of fruit fly larvae have a genetic basis. These strategies, called rover and sitter, occur in natural populations. Furthermore, the strategy favored by natural selection varies with environmental conditions.

Although these breeding programs demonstrate that genes influence the expression of many behaviors, they do not show where or how the genes act. Techniques that have been useful in pointing out what genes do to alter behavior include inducing mutations, mosaic analysis, and producing recombinant DNA.

A mutation is a change in the DNA. Mutations often result in the production of different proteins. Some mutations cause the individuals to show abnormal behavior patterns. When these mutants are separated from the general population, they can be studied to learn how anatomy or physiology has been altered to modify the behavior. The poor memory of the *dunce Drosophila* mutant is thought to be related to its lack of an enzyme, cyclic AMP phosphodiesterase. The learning problems of the *rutabaga* mutant are caused by a defective form of another enzyme, adenylyl cyclase. These mutants have helped us discover some of the links between genes, cAMP, and memory.

Mosaic analysis is another way to pinpoint the primary site of a gene's action. A mosaic is an individual made up of some mutant and some normal cells. When many mosaic animals are compared, the structure that is always composed of mutant tissue in the individuals that show the mutant form of a behavior is thought to be the primary site of action for that gene. The production of mosaics has been useful in studying certain behaviors in both fruit flies and mice. Mosaic analysis of mutant male fruit flies who had difficulties in courtship identified some specific regions of the nervous system involved in each component of courtship behavior. The primary site of action of genes that cause neurological disorders in the cerebellum of mice has also been studied in this way. The *pcd* mutation affects cerebellar Purkinje cells directly, but the *reeler* mutation seems to have its primary effect on cells that direct the migration of Purkinje cells during development of the brain.

Producing and cloning recombinant DNA is another way in which the relationship between genes and behavior has been clarified. For example, the *per* gene was shown to be an essential factor in the expression of circadian rhythmicity in fruit flies. When the per^{+} allele was isolated and inserted into arrhythmic flies, it restored the rhythms in eclosion and activity.

The mechanisms through which genes influence behavior are varied. One example is provided by egg laying in *Aplysia*. The gene coding for egg-laying hormone (ELH) in *Aplysia* has also been cloned and identified. When it was sequenced, some interesting facts about genetic control of the fixed action pattern of egg laying were learned. The *ELH* gene codes for a polyprotein precursor that could be broken down into 11 separate proteins. Four of these are known to be released by bag cell neurons, cells that initiate egg laying. The roles of three of them, ELH, alpha bag cell factor, and beta bag cell factor, are known. They all act as neurotransmitters that either excite or inhibit specific cells in the abdominal ganglion. In addition, ELH serves as a hormone that causes the ducts of the reproductive system to contract and expel the eggs.

The *fosB* gene in mice affects nurturing. It is thought that *fosB* in the preoptic area of the hypothalamus is normally activated by experience with newborn pups. The protein coded for by *fosB* then activates other genes that are responsible for strengthening the neural circuits necessary for nurturing behavior. If *fosB* is mutant, proper nurturing does not develop.