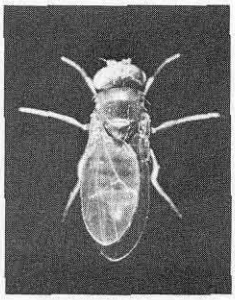


Where Behavior Begins

The genes, which so largely determine anatomical and biochemical characteristics, must surely interact with the environment to determine behavior. But how?

When the individual organism develops from a fertilized egg, the one-dimensional information arrayed in the linear sequence of the genes on the chromosomes controls the formation of a two-dimensional cell layer that folds to give rise to a precise three-dimensional arrangement of sense organs, central nervous system, and muscles. Those elements interact to produce the organism's behavior, a phenomenon whose description requires four dimensions at least. Surely the genes, which so largely determine anatomical and biochemical characteristics, must also interact with the environment to determine behavior. But how? In our group at Caltech, we have been applying tools of genetic analysis in an attempt to trace the emergence of multi-dimensional behavior from the one-dimensional gene.

Our objectives are to discern the genetic component of a behavior, to identify it with a particular gene, and then to determine the actual site at which the gene influences behavior and learn how it does so. In brief, we keep the environment constant, change the genes, and see what happens to behavior. Our choice of an experimental organism was constrained by the fact that the simpler an organism is, the less likely it is to exhibit interesting behavioral patterns that are relevant to man; the more complex it is, the more difficult it may be to analyze and the longer it takes. The fruit fly *Drosophila melanogaster* represents a compromise. In mass, in number of nerve cells, in amount of DNA, and in generation time it stands roughly halfway on a logarithmic scale between the colon bacillus *Escherichia coli* (which can be regarded as having a one-neuron nervous system) and man. Although the fly's nervous system is very different from the human system, both consist of neurons and synapses and utilize transmitter molecules, and the development of both is dictated by genes. A fly has highly developed senses of sight, hearing, taste, smell, gravity, and time. It cannot do everything we do, but it does some things we cannot do, such as fly and stand on the ceiling; its visual system can detect the movement of the minute hand on a clock.

One must not underestimate the little creature, which is not an evolutionary antecedent of man but is itself high up on the invertebrate branch of the phylogenetic tree. Its nervous system is a miracle of microminiaturization, and some of its independently evolved behavior patterns are not unlike our own. For analyzing the relation of specific genes to behavior, it is best to begin with a highly inbred, genetically uniform strain of flies and change the genes one at a time. This is done by inducing a mutation —an abrupt gene change that is transmitted to all subsequent generations.

A population of flies exposed to a mutagen (radiation or certain chemicals) yields some progeny with anatomical anomalies such as white eyes or forked bristles, and it also yields progeny with behavioral abnormalities. Workers in many laboratories (including ours) have compiled a long list of such mutants, each of which can be produced by the alteration of a single gene.

Let me use a defect in visual behavior to illustrate in some detail how we analyze behavior. The first problem is to quantitate behavior and to detect and isolate

BY SEYMOUR BENZER

Seymour is not the only Benzer with an interest in the fruit fly. His daughter Martha made this way-more-than-life-size model several years ago for a junior high school science project.

behavioral mutants. It is possible to handle large populations of flies, treating each individual much as a molecule of behavior and fractionating the group into normal and abnormal types. We begin—using the technique devised by Edward B. Lewis, Thomas Hunt Morgan Professor of Biology at Caltech—by feeding male flies sugar water to which has been added the mutagen ethyl methane sulfonate, an alkylating agent that induces mutations in the chromosomes of sperm cells. The progeny of mutagenized males are then fractionated by means of a kind of countercurrent distribution procedure, somewhat as one separates molecules into two liquid phases. Here the phases are light and darkness, and the population is "chromatographed" in two dimensions on the basis of multiple trials for movement toward or away from light. Normal flies—and most of the progeny in our experiment—are phototactic, moving toward light but not away from it. Some mutants, however, do not move quickly in either direction; they are *sluggish* mutants. There are *runners*, which move vigorously both toward and away from light.

A *negatively phototactic* mutant moves preferentially away from light. Finally, there are the *nonphototactic mutants*, which show a normal tendency to walk but no preference for light or dark. They behave in light as normal flies behave in the dark, which suggests that they are blind.

My colleague Yoshiki Hotta, who is now at the University of Tokyo, and I studied the electrical response of the *nonphototactic* flies' eyes and found that in one of the mutants the photoreceptor cells are normal in the young adults but that they degenerate with age. There are genetic conditions that produce this result in humans, and it may be that the fly's eye can provide a model system for studying certain kinds of blindness.

Now, if one knows that a certain behavior (*nonphototactic*, say) is produced by a single-gene mutation and that it seems to be explained by an anatomical fault (the degenerated receptors), one still cannot say with certainty what is the primary "focus" of that genetic alteration—that is, the site in the body at which the mutant gene exerts its primary effect. The site may be far from the affected organ. Certain cases of retinal degeneration in man, for example, are due not to any defect in the eye but to ineffective absorption of vitamin A from food in the intestine. In order to trace the path from gene to behavior one must find the true focus at which the gene acts in the developing organism. How? A good way to trouble-shoot in an electronic system—a stereophonic set with two identical channels, for example—is to interchange corresponding parts. That is in effect what we do with *Drosophila*. Rather than surgically transplanting organs from one fly to another, however, we use a genetic technique. We make mosaic flies, composite individuals in which some tissues are mutant and some have a normal genotype. Then we look to see just which part has to be mutant in order to account for the abnormal behavior.

One method of generating mosaics depends on a strain of flies in which there is an unstable ring-shaped X chromosome. Flies, like humans, have X and Y sex chromosomes; if a fertilized egg has two X chromosomes in its nucleus, it will normally develop into a female fly; an XY egg yields a male. In *Drosophila* it is the presence of two

X chromosomes that makes a fly female; if there is only one X, the fly will be male. The ring X chromosome has the property that it may get lost during nuclear division in the developing egg, so that some tissues retain only one X chromosome while others have both.

Such a mosaic fly is a system in which the effects of normal and of mutant genes can be distinguished in one animal. We arrange things so that both a behavioral gene and "marker" genes are combined on the same X chromosome. This is done through the random workings of the phenomenon of recombination, in which segments of two chromosomes (in this case the X) "cross over" and exchange places with each other during cell division in the formation of the egg. In this way we can, for example, produce a strain of flies that are *nonphototactic* and also have white eyes (instead of the normal red) and a yellow body color. Then we breed males of this strain with females of the ring X strain. Some of the resulting embryos will have one ring X chromosome and one mutation-loaded X chromosome. In a fraction of these embryos the ring X (carrying normal genes) will be lost at an early nuclear division. The XX body parts of the resulting adult fly will have one X chromosome with normal genes and one with mutations; because both the behavioral and the anatomical genes in question are recessive (their effect is masked by the presence of a single normal gene), the mutations will not be expressed in those parts. In the body parts having lost the ring X, however, the single X chromosome will be the one carrying the mutations. And because it is all alone, the mutations will be expressed. Examination of the fly identifies the parts that have normal color and those in which the mutant genes have been uncovered. We can select, from among the randomly divided gynandromorphs, individuals in which the dividing line falls in various ways: a normal head on a mutant body, a mutant head on a normal body, a mutant eye and a normal one, and so on. And then we can pose the question we originally had in mind: What parts must be mutant for the mutant behavior to be expressed?

When Hotta and I did that with certain visually defective mutants—for instance ones that produce no receptor potential—we found that the electroretinogram of the mutant eye was always completely abnormal, whereas the normal eye functioned properly. Even in gynandromorphs in which everything was normal except for one eye, that eye showed a defective electroretinogram. This makes it clear that the defects in those mutants are not of the vitamin A type I mentioned before; the defect must be autonomous within the eye itself.

In these mutants the primary focus of the *phototactic* defect is in the affected organ itself. More frequently, however, the focus is elsewhere. A good way to see how this situation is dealt with is to consider a *hyperkinetic*

A fly has highly developed senses of sight, hearing, taste, smell, gravity, and time

mutant that was studied by William D. Kaplan and Kazuo Ikeda at the City of Hope Medical Center. When such a fly is anesthetized with ether, it does not lie still but rather shakes all six of its legs vigorously. Flies that are mosaics for the gene shake some of their legs but not others, and the shaking usually correlates well with the leg's surface genotype as revealed by markers —but not always. The point is that the markers are on the outside of the fly. The genotype of the surface is not necessarily the same as that of the underlying tissues, which arise from different regions of the embryo. And one might well expect that leg function would be controlled by nervous elements somewhere inside the fly's body that could have a different genotype from the leg surface. The problem is to find a way of relating internal behavioral foci to external landmarks. Hotta and I developed a method of mapping this relation by extending to behavior the idea of a "fate map," which was originally conceived by A. H. Sturtevant, professor of biology at Caltech from 1928 until his death in 1970.

When Hotta and I undertook to map behavior in *Drosophila*, we began by preparing our own fate map of the adult external body parts based on the scores for 703 mosaic flies. Distances on the map are in "sturts," a unit that John Merriam, Hotta, and I have proposed in memory of Sturtevant. One sturt is equivalent to a probability of 1 percent that the two structures will be of different genotypes.

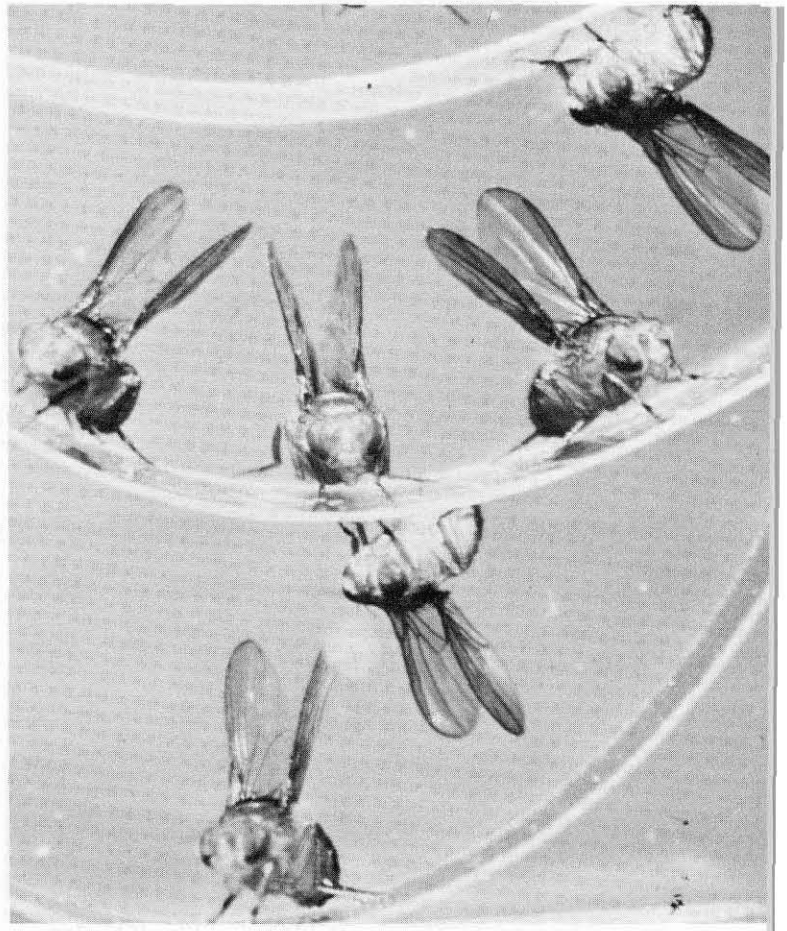
Now back to *hyperkinetic*. We produced 300 mosaic flies and scored each for a number of surface landmarks and for the coincidence of marker mutations at those landmarks with the shaking of each leg. We confirmed that the behavior of each leg (whether it shakes or not) is independent of the behavior of the other legs and that the shaking behavior and the external genotype of a leg are frequently the same—but not always. The independent behavior of the legs indicated that each had a separate focus. For each leg we calculated the distance from the shaking focus to the leg itself and to a number of other landmarks and thus determined a map location for each focus. They are near the corresponding legs but below them, in the region of the blastoderm identified by embryological studies as the origin of the ventral nervous system. This is consistent with electrophysiological evidence that neurons in the thoracic ganglion of the ventral nervous system behave abnormally in these mutants.

Another degree of complexity is represented by a mutant

we call *drop-dead*. These flies develop, walk, fly, and otherwise behave normally for a day or two after eclosion. Suddenly, however, an individual fly becomes less active, walks in an uncoordinated manner, falls on its back, and dies; the transition from apparently normal behavior to death takes only a few hours. The time of onset of the syndrome among a group of flies hatched together is quite variable; after the first two days the number of survivors in the group drops exponentially, with a half-life of about two days. It is as if some random event triggers a cataclysm. The gene has been identified as a recessive one on the X chromosome. Symptoms such as these could result from malfunction almost anywhere in the body of the fly, for example from a blockage of the gut, a general biochemical disturbance, or a nerve disorder. In order to localize the focus we did an analysis of 403 mosaics in which the XX parts were normal and the X body parts expressed the *drop-dead* gene and the surface-marker mutations, and we scored for *drop-dead* behavior and various landmarks.

Drop-dead behavior, unlike shaking behavior, which could be scored separately for each leg, is an all-or-none property of the entire fly. First we did a rough analysis to determine whether the behavior was most closely related to the head, thorax, or abdomen, considering only flies in which the surface of each of these structures was either completely mutant or completely normal. Among mosaics in which the entire head surface was normal almost all behaved normally, but 6 flies out of 97 died in the *drop-dead* manner; in the reciprocal class 8 flies of 80 with mutant head surfaces lived. In other words, the focus was shown to be near to, but distinct from, the blastoderm site of origin of the head surface. Similar analysis showed that the focus was substantially farther away from the thorax and farther still from the abdomen. Next we considered individuals with mosaic heads. In certain visual mutants the visual defect was always observed in the eye on the mutant side of the head; flies with half-normal heads had normal vision in one eye. For *drop-dead*, on the other hand, of mosaics in which half of the head surface was mutant only about 17 percent dropped dead. All the rest survived.

Now, a given internal part should occur in normal or mutant form with equal probability, as the external parts in these mosaics did. On that reasoning, if there were a single focus inside the head of the fly, half of the bilateral-mosaic flies should have dropped dead. We formed the hypothesis, therefore, that there must be two foci, one on each side, and that they must interact. Both of them must be mutant for the syndrome to appear. In other words, a mutant focus must be "submissive" to a normal one. In that case, if an individual exhibits *drop-dead* behavior, both foci must be mutant, and if a fly survives, one focus may be normal or both of them may be.



"Wings-up" flies are mutants that keep their wings straight up and cannot fly—behavior that might possibly be the result of flaws in wing structure, in musculature, or in nerve function. Hotta and Benzer's experiments have traced the defect to the muscle.

Mapping a bilateral pair of interacting foci calls for special analysis. By considering the various ways a mosaic dividing line could fall in relation to a pair of visible external landmarks (one on each side of the body) and a symmetrical pair of internal foci, one can set up equations based on the probability of each possible configuration. Using the observed data on how many mosaic flies showed the various combinations of mutant and normal external landmarks and mutant or normal behavior, it is possible to solve these equations for the map distance from each landmark to the corresponding focus and from one focus to the homologous focus on the other side of the embryo. The *drop-dead* foci turn out to be below the head-surface area of the blastoderm, in the area embryologists have assigned to the brain. Sure enough, when we examined the brain tissue of flies that had begun to exhibit the initial stages of *drop-dead* behavior, it showed striking signs of degeneration, whereas brain tissue fixed before the onset of symptoms appeared normal. As for mosaics whose head surfaces are half-normal, those that die show degeneration of the brain on

both sides; the survivors' brains show no degeneration on either side, a finding consistent with the bilateral-submissive-focus hypothesis. It appears that the normal side of the brain supplies some factor that prevents the deterioration of the side with mutant focus.

The mutants so far mapped provide examples involving the main components of behavior: sensory receptors, the nervous system, and the muscles. For some of the mutants microscopic examination has revealed an obvious lesion of some kind in tissue. Clearly the question is whether or not fate mapping is necessary; why do we not just look directly for abnormal tissue? One answer is that for many mutants we do not know where to begin to look, and it is helpful to narrow down the relevant region. Furthermore, in many cases no lesion may be visible, even in the electron microscope. More important, and worth reiterating, is the fact that the site of a lesion is not necessarily the primary focus. For example, an anomaly of muscle tissue may result from a defect in the function of

We have begun to study more elaborate behavior such as circadian rhythm, sexual courtship, and learning

nerves supplying the muscle. This possibility has been a lively issue in the study of diseases such as muscular dystrophy.

Another application of mosaics is in tagging cells with genetic labels to follow their development. The compound eye of *Drosophila* is a remarkable structure consisting of about 800 ommatidia—unit eyes containing eight receptor cells each. The arrangement of cells in an ommatidium is precise and repetitive; the eye is in effect a neurological crystal in which the unit cell contains eight neurons. Thomas E. Hanson, Donald F. Ready, and I have been interested in how this structure is formed. Are the eight photoreceptor cells derived from one cell that undergoes three divisions to produce eight, or do cells come together to form the group irrespective of their lineage? This can be tested by examining the eyes of flies, mosaic for the *white* gene, in which the mosaic dividing line passes through the eye. By sectioning the eye and examining ommatidia near the border between white and red areas microscopically, it is possible to score the tiny pigment granules that are present in normal photoreceptor cells but absent in *white* mutant cells. The result is clear: A single ommatidium can contain a mixture of receptor cells of both genotypes. This proves that the eight cells cannot be derived from a single ancestral cell but have become associated in their special group of eight irrespective of lineage. The same conclusion applies to the other cells in each ommatidium, such as the normally heavily pigmented cells that surround the receptors.

Not all cells have such convenient pigment markers. It would obviously be valuable to have a way of labeling all the internal tissues as being either mutant or normal, much as yellow color labels a landmark on the surface. This can now be done for many tissues by utilizing mutants that lack a specific enzyme. If a recessive enzyme-deficient mutant gene is recombined on the X chromosome along with the *yellow*, *white*, and behavioral genes, and mosaics are produced in the usual way, the male tissues of the mosaic will lack the enzyme. By making a frozen section of the fly and staining it for enzyme activity one can identify normal mutant cells.

In order to apply this method in the nervous system one needs to have an enzyme that is normally present there in a large enough concentration to show up in the staining procedure and a mutant that lacks the enzyme, and the lack should have a negligible effect on the behavior under study. Finally, the gene in question should be on the X chromosome. Douglas R. Kankel and Jeffrey C. Hall in our group have developed several such mutants. By scoring the internal tissues they have constructed a fate map of the internal organs of the kind made earlier for surface structures. We are now adapting the staining method for electron microscopy in order to work at the level of the individual cell.

Much of what has been done so far involves relatively simple aspects of behavior chosen to establish the general methodology of mutants and mosaic analysis. Can the methodology be applied to more elaborate and interesting behavior such as circadian rhythm, sexual courtship, and learning? Some beginnings have been made on all of these. By making flies that are mosaic for normal and mutant rhythms, Konopka has shown that the internal clock is most closely associated with the head. Looking at flies with mosaic heads, he found that some exhibited the normal rhythm and others the mutant rhythm but that a few flies exhibited a peculiar rhythm that appears to be a sum of the two, as if each side of the brain were producing its rhythm independently and the fly responded to both of them. By applying the available cell-staining techniques it may be possible to identify the cells that control the clock.

Sexual courtship is a higher form of behavior, since it consists of a series of fixed-action patterns, each step of which makes the next step more likely. The sex mosaics we have generated lend themselves beautifully to the analysis of sexual behavior. A mosaic fly can be put with normal females, and its ability to perform the typical male courtship steps can be observed. Hotta, Hall, and I found that the first steps (orientation toward the female and vibrating of the wings) map to the brain. This is of particular interest because the wings are vibrated by motor nerve impulses from the thoracic ganglion; even a female ganglion will produce the vibration "song" typical

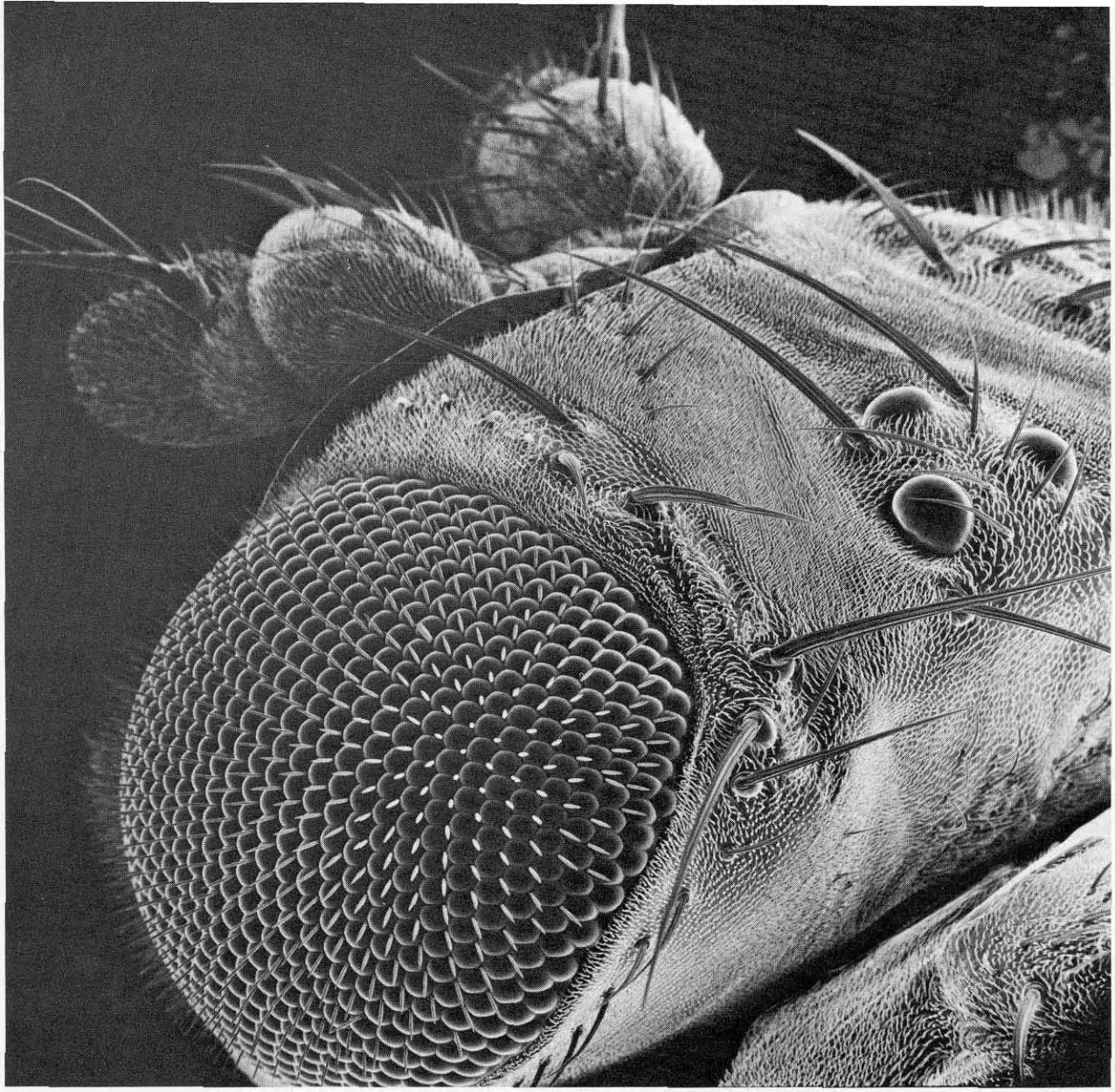


Photo by Robert Enns and Terri Martin

Drosophila melanogaster—as seen by the scanning electron microscope. This is a photograph of the fly's head, and the most prominent feature is the large compound eye—a remarkable structure consisting of 800 unit eyes, or ommatidia. Antenae are at the upper left, and the three globular objects on the right are simple eyes, or ocelli.

of the male if directed to do so by a male brain. It would appear that the thoracic ganglion in a female must "know" the male courtship song even though she does not normally emit it.

Sexual behavior in *Drosophila*, although complex, is a stereotyped series of instinctive actions that are performed correctly by a fly raised in isolation and without previous sexual experience. Other forms of behavior such as phototaxis also appear to be already programmed into the fly when it ecloses. Whether a fruit fly can learn has long been debated; various claims have been made and later shown to be incorrect. Recently William G. Quinn Jr. and William A. Harris in our laboratory have shown in carefully controlled experiments that the fly can learn to avoid specific odors or colors of light that are associated with a negative reinforcement such as electric shock. This opens the door to genetic analysis of learning behavior through mutations that block it.

In tackling the complex problems of behavior the gene provides, in effect, a microsurgical tool with which to produce very specific blocks in a behavioral pathway. With temperature-dependent mutations the blocks can be turned on and off at will. Individual cells of the nervous system can be labeled genetically, and their lineage can be followed during development. Genetic mosaics offer the equivalent of exquisitely fine grafting of normal and mutant parts, with the entire structure remaining intact. What we are doing in mosaic mapping is in effect "unrolling" the fantastically complex adult fly, in which sense organs, nerve cells, and muscles are completely interwoven, backward in development, back in time to the blastoderm, a stage at which the different structures have not yet come together. Filling the gaps between the one-dimensional gene, the two-dimensional blastoderm, the three-dimensional organism, and its multidimensional behavior is a challenge for the future. □