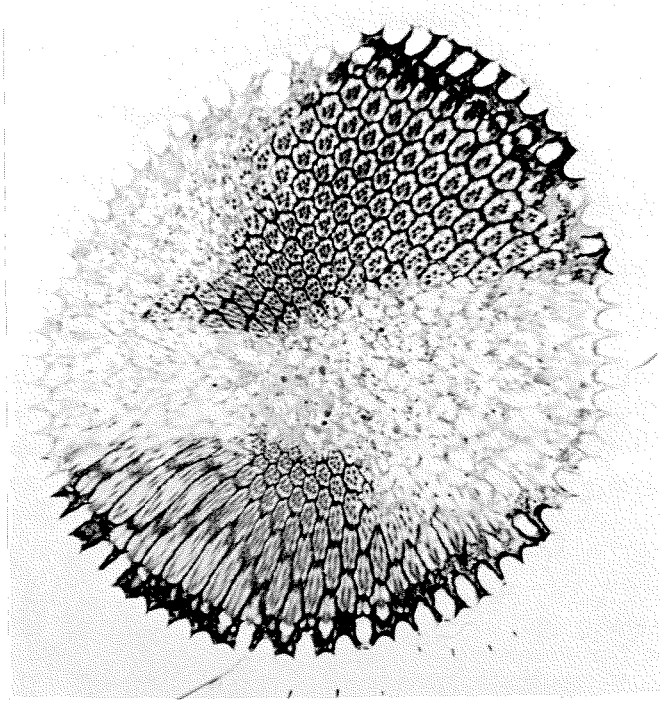
From the Gene to Behavior

by Seymour Benzer



Mosaicism—the mingling of patches of tissue of unlike genetic constitution—is one of the most promising fields for genetic research. In this section of a Drosophila *compound eye, the normal areas show precise arrays of photoreceptor elements; mutant areas show extensive degeneration. This degeneration can be used as a cell marker to trace the lineage of the photoreceptor cells.*

Splitting the gene and running its map into the ground was exciting while it lasted, but molecular genetics, pursued to ever lower levels of organization, inevitably does away with itself: The gap between genetics and biochemistry disappears. Recently, a number of molecular biologists have turned their sights in the opposite direction, i.e., up to higher integrative levels, to explore the relatively distant horizons of development, the nervous system, and behavior.

When the individual develops from an egg, the one-dimensional information contained in the linear sequence of genes on the chromosomes is somehow translated into a two-dimensional blastula, which later folds and produces a precise three-dimensional array of sense organs, central nervous system, and muscles. Finally, the ensemble interacts to produce behavior, a phenomenon which requires four dimensions, at the least, to describe. The genes contain the information for the circuit diagram, but little is known about the relationship between this primary information and the end result. How the tags of specificity are parceled out among the neurons so that they form the proper network, or even what kinds of molecules carry the specificity are, at present, complete mysteries. The problem of tracing the emergence of multi-dimensional behavior from the genes is a challenge that may not become obsolete so soon.

It is well established that the genes speak strongly in determining anatomical and biochemical features. It should not be surprising if, to a large degree, the genes also determine behavioral temperament, although, of course, environmental influences can also play a large role. All behavior is inevitably the resultant of both components. To discern the genetic contribution clearly, the thing to do is to keep the environment constant and change the genes. This is not easy to do with human beings; they are notoriously uncooperative and unwieldy

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Seymour Benzer, professor of biology, is distinguished for his work on genes in viruses and bacteria—studies for which he received the 1971 Lasker Award in Basic Medical Research. "From the Gene to Behavior" is adapted from his talk on the occasion of receiving that award and describes the work in which he is currently engaged.

experimental subjects, particularly if one must wait generations for the results. For this reason, the molecular biologists who have turned to studying behavior have cast around for more favorable model organisms. There immediately arises the problem that the simpler an organism is, the less likely it is to exhibit behavioral patterns that are relevant to man, while the more complex it is, the more difficult it may be to analyze.

Because of its short generation time and small size, plus the fact that it could be raised on simple laboratory food, the fruit fly, *Drosophila*, was chosen by the school of genetics that flourished half a century ago. The lessons learned from this model organism about the linkage of genes into linear arrays on the chromosomes, the production of mutations by x-rays and chemicals, recombination of genes by crossing over, sex determination by X and Y chromosomes, and the role of genes in development carry over almost directly to human genetics, although there are, of course, variations in detail.

While the fly's nervous system differs vastly from ours, it does work via neurons, synapses, and transmitter molecules, and its development is dictated by genes. A fly has highly developed senses of sight, hearing, taste, smell, gravity, and time. While it does not do everything that man does, it can do a few things that we cannot, such as flying or standing on the ceiling. One must not underestimate the little creature. Perhaps you have seen the remarkable film, *Hellstrom Chronicle*, of which the theme was that the insects were here well before man's arrival and already have seen the dinosaurs come and go. It should be recalled that the fly is not an evolutionary antecedent of man, but is high up on the invertebrate branch of the phylogenetic tree. Some of its independently evolved behavioral patterns are not unlike our own.

For example, sexual courtship in *Drosophila* begins with an encounter between individuals of opposite sex. The male, spying a female, orients toward her, faces her head from one side, holds out and vibrates one wing, produces a species-specific song. After this overture, the male usually runs to the other side and repeats the performance with the other wing, always using the wing closer to the female's head. There follows a series of steps that are only too embarrassingly anthropomorphic. In both fly and man, sexual courtship is a chain of action patterns, each dependent on the previous one for activation of the nervous system to be responsive to the next step.

The role of the genes becomes evident in fly mutants. There exists a class of what may be called *savoir-faire* mutants, where the males are unsuccessful in courtship, due to inadequate performance of one or another of the steps. In a mutant known as *fruity*, discovered by K. Gill, the males pursue each other. A pathetic case is the mutant *stuck*, described by C. Beckman, in which the male is unable to withdraw his penis after copulation. Obviously, most of these mutants would not stand a chance in the competitive natural environment. In the laboratory, however, they can be maintained and studied. Even genes having the most drastic effects can, of course, be maintained in heterozygotes, provided they are recessive.

The richness of the behavioral repertoire of *Drosophila* and its genetic basis is illustrated by some of the known kinds of behavioral mutants, listed in the table below. All the types listed can be produced by altering single genes. Some mutants are congenitally

Some Behavioral Mutants of Drosophila

Locomotor	**Sexual**
sluggish	savoir-faire
Hyperkinetic	fruity
flightless	stuck
uncoordinated	
nonclimbing	**Visual**
	nonphototactic
Response to stress	negative phototactic
easily shocked	nonoptomotor
Shaker	negative optomotor
freaked-out	
paralyzed	**Nerve and muscle abnormality**
parched	photoreceptor degeneration
Circadian rhythm	lamina degeneration
arrhythmic	wings-up
short-period	drop-dead
long-period	

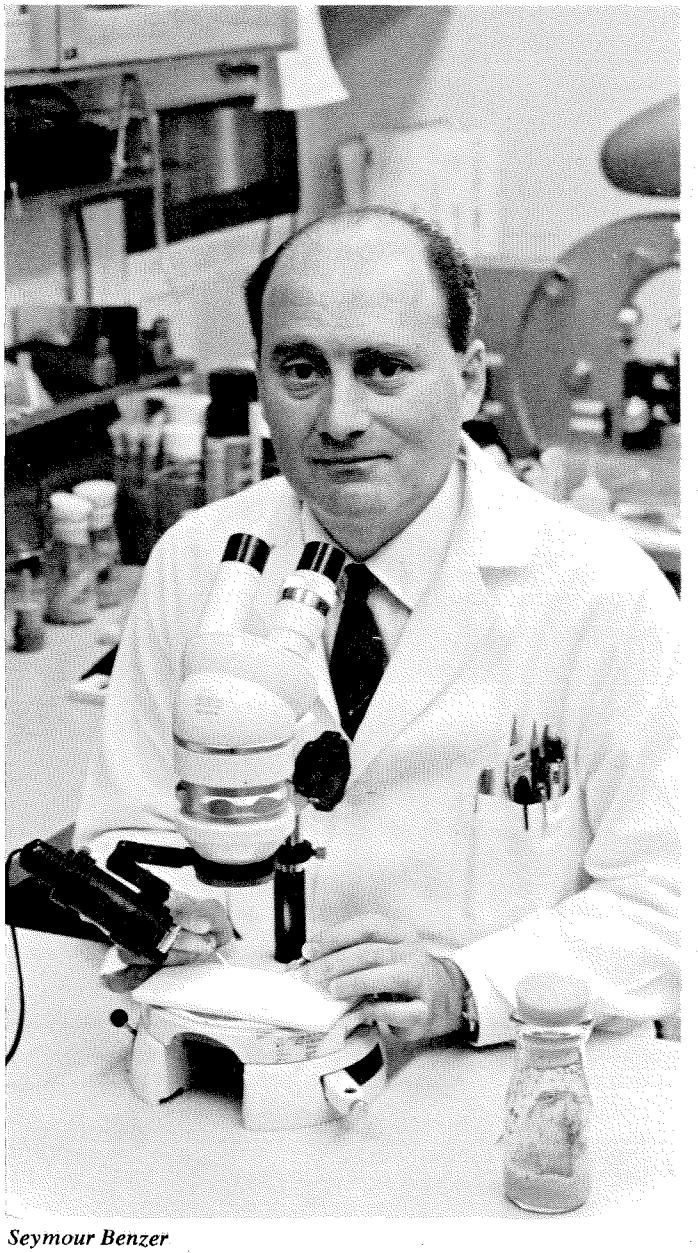
sluggish; others, as W. Kaplan has shown, are *hyperkinetic*. There are mutants that do not fly, though they have perfectly well developed wings. Some mutants are uncoordinated; they stagger over themselves and each other. Others do not climb up a vertical surface, in contrast to normal flies.

Individuals that appear quite normal in ordinary circumstances may harbor hereditary idiosyncracies that show up only under stress. For example, the mutant, *easily shocked*, when subjected to a mechanical jolt, displays a syndrome not unlike an epileptic seizure: The fly takes a few faltering steps, falls on its back, flails its legs and wings wildly, and coils its abdomen under. A male exudes a droplet of fluid; a female is likely to extrude an egg. The fly then goes into a coma, lasting some minutes, after which it revives and walks around as if nothing had happened. This routine can be repeated many times. The mechanism is unknown. We do know that there exist several different genes on the X chromosome alone, which, if mutated, can produce this syndrome.

Some abnormalities become manifest only under anesthesia. In working with *Drosophila*, one often etherizes the flies for examination under the microscope. While normal flies lie quietly for five or ten minutes, mutants known as *shakers* vigorously vibrate all their legs. Another type is one which we call *freaked out*, because, under the influence of ether, it performs grotesque, random gyrations. It is not inconceivable that mutants such as these could shed light on the mechanism of anesthesia and the genetic factors involved in individual idiosyncracies.

Gene changes in flies also produce marked differences in response to extremes of temperature and humidity. A spectacular mutant found by D. Suzuki is *paralyzed (temperature-sensitive)*. It collapses above a critical temperature, normal flies being unaffected. Several such mutants are now known, each with its own critical temperature. Another kind of mutant, *parched*, dies within a few minutes after being placed in a low-humidity atmosphere, whereas normal flies survive much longer.

An important feature of behavior in a wide range of organisms is the endogenous 24-hour rhythm controlling



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activity, which has become personally evident to everyone in this day of jet displacement to new time zones. The fruit fly, too, shows a natural circadian (around one day) rhythm, and here it is possible to clearly demonstrate the role of the genes. The name *Drosophila*, by the way, means "lover of dew." Adults normally eclose from the pupal stage around dawn, when all is moist and cool. The young fly must expand its folded wings and harden its cuticle, and it is important to time emergence carefully, to minimize the risk of desiccation or easy visibility to predators until it is able to fly. The fly has, in fact, evolved a highly ingrained biological clock, which has been studied extensively by C. Pittendrigh and others. In a cycle of 12 hours of light and 12 hours of darkness, adults emerge mostly during a few hours around dawn; those that miss this interval tend to wait until dawn on the following or successive days. The rhythm persists even in constant darkness (provided the pupae have previously been exposed to light); once primed, the internal clock continues to run on its natural cycle. The activity of an adult fly, once emerged, is similarly controlled by an internal clock. Maintained in constant darkness, its locomotion can be monitored by a photocell, using infrared light, which the fly cannot see. At a certain time, the fly begins to walk around for about 12 hours, then becomes very quiet, as if asleep on its feet. Next day, at the same time, within an hour or so, activity begins anew.

The genetic control of this clock is clearly shown by the fact that one can obtain mutants with abnormal rhythms, or even no rhythm, as my student Ronald Konopka has done. *Arrhythmic* mutant flies eclose at arbitrary times of day. After emergence, they are typical insomniacs; in constant darkness, their locomotor activity is spread randomly over time. A *short-period* mutant has an excellent rhythm, but runs on a natural cycle of 19 hours rather than 24. A *long-period* mutant runs on a 28-hour cycle. In a normal world, these mutants would appear always to wake up too early or too late. One need not look far to find human analogs of these types. It is possible that genetics may be a strong component of this personality trait.

Suppose that one wishes to analyze the visual system genetically. To obtain blind mutants, say, one can select for the loss of the fly's normal response of running toward light when agitated. The progeny of mutagenized males are readily fractionated by means of a counter-current distribution technique, analogous to partition chromatography for separating molecules between two liquid phases, except that the two phases in this case are light and darkness. The population can be "chromatographed" in two dimensions, based on multiple trials for movement toward light, then away from light. Normal flies consistently move toward light but not away from it. However, one also obtains mutants that respond differently. Certain mutants run neither toward light nor from it. These *sluggish* types in some cases show obvious anatomical defects, but in others they appear outwardly normal. Some mutants are *runners*, and move very vigorously, whether to or from light (or even in the dark). One also obtains the reverse of normal, i.e., negatively phototactic. Finally, there is even the kind that acts simply *non-phototactic*, showing a normal tendency to walk, but irrespective of whether it is to or from light. These flies behave in light just as do normal flies in the dark, suggesting that they are blind.

Such *non-phototactic* mutants have been studied by W. Pak and M. Heisenberg as well as by our group at Caltech. In certain mutants, the photoreceptor cell responds, but fails to trigger off excitation of the next step in the visual pathway. In other cases, the genetic lesion affects the response of the primary photoreceptor cells so that no signal at all is observed. In another type, the signal is small and greatly delayed. Histology of the latter reveals that the photoreceptor elements, present in the young adult, degenerate with age, not unlike genetic conditions known in man. Thus, the fly's eye may provide a model system for various kinds of blindness. Although many different mechanisms could result in such disorders, mutant material provides perturbations which can be used to analyze normal function.

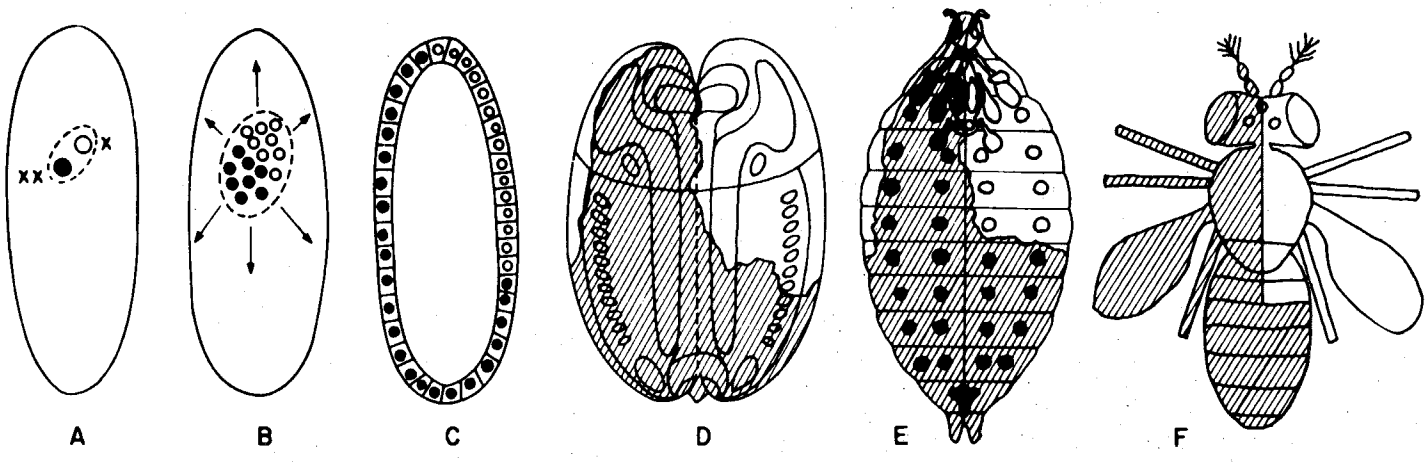
A basic difficulty in pathology, whether in fly or man, is to identify the primary defective focus that causes an observed condition. This focus may lie in an altogether

The fly has evolved a highly ingrained biological clock that governs its daily activity rhythm; the rhythm is genetically determined.

different region of the body from the affected organ. Certain cases of retinal degeneration in man, for instance, are due not at all to a defect in the eye, but are caused by insufficient absorption of dietary vitamin A by the gut. This question recalls the familiar conflict in medical history between humoralism and solidism. A neat way to make the test would be to excise the defective organ and transplant it in place of the corresponding one in a normal individual. If it is a matter of a circulating humor, the transplanted organ should function normally. If the solid organ is at fault, it should still be defective in a normal host.

In *Drosophila*, one can, in effect, do precisely such experiments by using genetic techniques; mosaic individuals can be produced that are composed of parts having different genotypes. One way of doing this is to utilize a fly strain which has a special ring X chromosome that tends to get lost during an early nuclear division of the developing egg, as shown in the drawing below. If the experiment starts with a female egg which has this ring for one of its two X chromosomes, this produces one daughter nucleus which still has both X chromosomes and another that contains only one X chromosome. In *Drosophila*, the latter (XO) type nucleus produces male tissues. The nuclei, after about a dozen divisions, migrate to the surface of the egg, forming a blastoderm. The groups of nuclei stay roughly intact, so that the XX (female) group tends to populate one area of the surface, while the XO (male) group covers the remainder. Since, in *Drosophila*, the orientation of the first nuclear division spindle is arbitrary with respect to the axes of the egg, the dividing line between XX and XO tissues can cut the embryo in any way, in some cases longitudinally down the middle, in others transversely, or at an angle. When, after larval growth and metamorphosis, the adult fly emerges, it is a gynandromorph, i.e., consists of female and male parts.

To adapt this system to the problem at hand, given a recessive behavioral gene, that gene is recombined on the

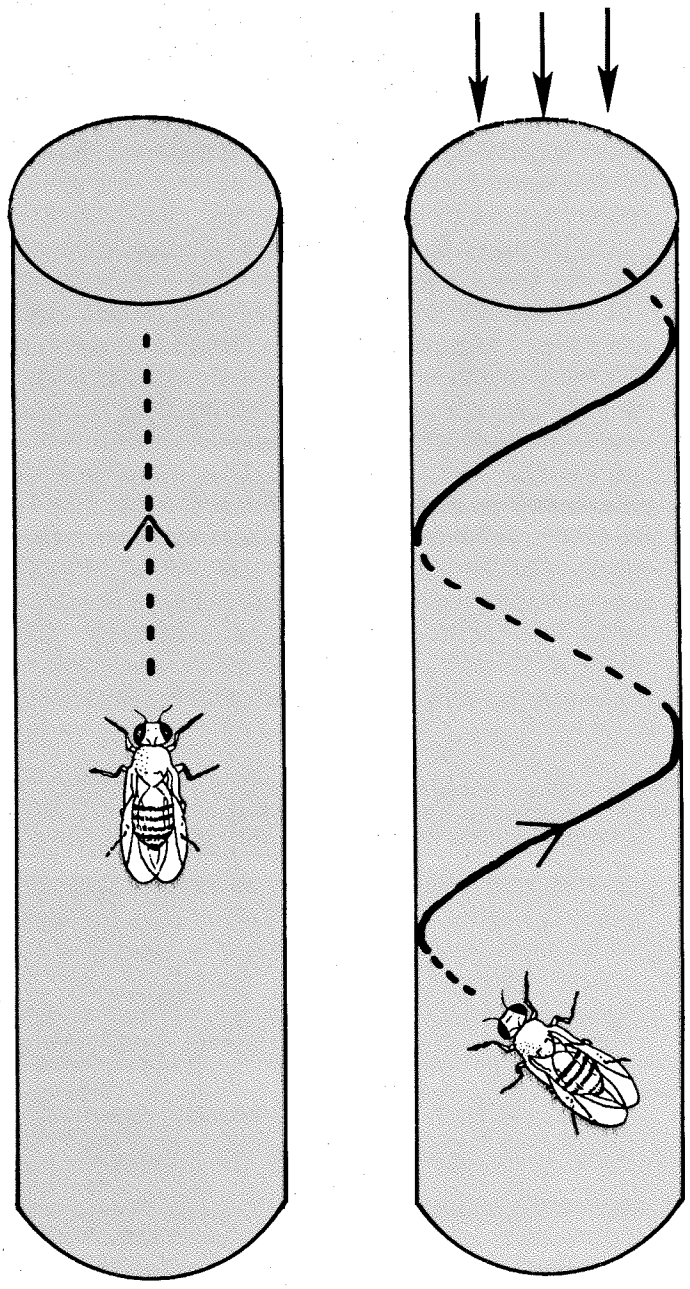


A mosaic fly may be formed by the loss of one X chromosome during the first nuclear division (A). The nuclei then migrate to the surface of the cell (B), and form a composite blastula (C). The fate map of the embryo (D) presages the map of larval structures destined to form the adult body parts (E), and the mosaic fly after metamorphosis (F).

(non-ring) X chromosome with other recessive marker genes that affect phenotypes visible in the adult, such as white eyes, yellow body color, and forked bristles. In the XX body parts, these marker genes will be dominated by the corresponding normal genes on the second X chromosome, but in the XO parts, the mutations will be expressed. Examination of the surface of the fly identifies the parts that are normal and those in which the mutant genes have been uncovered. One can then select, from among the random gynandromorphs produced, ones in which the dividing line falls in desired ways. Thus, individuals can be obtained having a normal head on a mutant body, or vice versa, or even flies having one mutant eye and one normal one.

A normal fly, when placed in a vertical tube in the dark, climbs more or less straight up, utilizing gravity as a cue. If there is a light source on top, the fly still climbs straight up, since the phototactic orientation response, which the fly achieves by moving in such a direction that the light intensities on the two eyes are kept equal, indicates a direction consistent with the gravity cue. Yoshiki Hotta and I have investigated flies that are mosaic for various nonphototactic genes. If one puts a 50-50 mosaic (one eye good, the other eye bad) into the tube in the dark, it climbs straight, as a normal fly does, since its gravity sense is unimpaired. But if a light at the top is turned on, the fly now traces a helical path, always turning the mutant eye toward the light in a futile attempt to balance input signals (right). The electroretinogram likewise is defective, showing that the action of the mutant gene is upon the eye itself, and not via lack of some circulating substance.

The gynandromorph technique also can be used to good effect in analyzing behavioral phenomena. For instance, where is the origin of the circadian rhythm in the fly? Some preliminary work has been done by Konopka with gynandromorphs in which part of the body has one rhythm gene combination, while the rest of the body has a different one. The results indicate that the clock is closely associated with the head; a fly with a mutant head runs on a mutant rhythm, even if all the rest of the body is normal. An especially interesting case arises when half



The climbing path of a mosaic fly with one blind eye—in darkness—is straight (left), but with a light shining from above the same fly will trace a helical path (right), turning the mutant eye toward the light in a futile attempt to balance input signals.

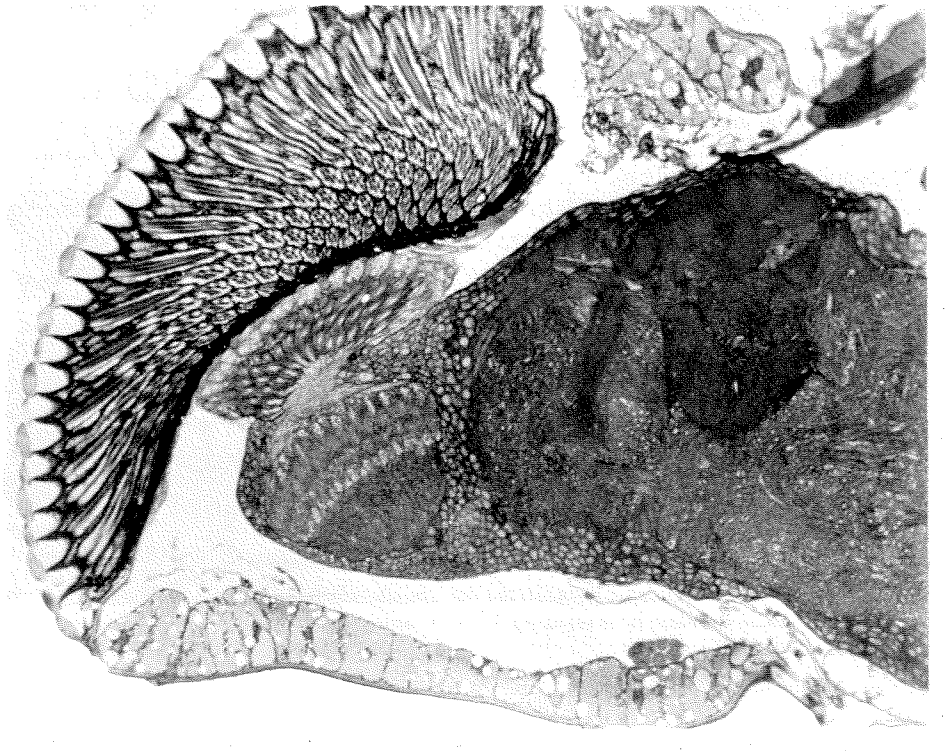
Experience thus far with the fly as a model system for unraveling the path from the gene to behavior is encouraging. In any case, it is fun.

the head is normal, the other half mutant. In such "split-brain" flies, the rhythms observed seem to be neither one nor the other but more complex. Just as Roger Sperry has done for human split-brain subjects, it may prove possible to learn how the two "hemispheres" of the fly brain interact to produce normal behavior.

The pursuit of the primary focus of a behavioral phenotype may lead to unexpected results. One mutant, which we call *wings-up*, raises its wings shortly after emergence to a position perpendicular to the body axis, and keeps them permanently in that position. Is this a defect in the wing itself, its articulation, the wing-controlling muscles, or a "psychological" quirk of the nervous system? Hotta and I studied mosaic flies and found that the character is more closely associated with the thorax of the fly than with the head or abdomen. However, it does not reside in the wings or, indeed, anywhere on the thorax cuticle, for in some mosaics the entire thorax surface may be normal, yet the wings are held up, and vice versa. In the fly, the raising and lowering of the wings during normal flight occurs indirectly, by alternating action of vertical and longitudinal muscles that change the shape of the thorax. In the *wings-up* mutant, all these indirect flight muscles are defective, while other muscles are quite normal. Electron microscopy shows an almost complete lack of myofibrils in the affected muscles. In flies heterozygous for this gene, myofibrils are present, but in contrast to the very precise striations in normal flight muscle, the Z-bands are highly irregular, as if there were a deficit in the amount of Z-band substance produced. If this is the case, it calls to mind the syndrome in man of nemaline myopathy, in which the converse seems to apply, the genetic defect causing an excess of Z-band molecules.

Another recently discovered mutant that Hotta and I have investigated is one we call *drop-dead*. For the first day or two after emergence, the adults show normal behavior, such as walking, flying, geotaxis, phototaxis, and mating. At some unpredictable time, each individual becomes less active, walks in an uncoordinated manner, falls on its back with limbs struggling, and dies. While the transition from apparently normal behavior to death occurs within only several hours, the time of onset of the

This horizontal section of the head of a normal Drosophila *shows the eye at the extreme left, the optic ganglia at the left of center, and the brain.*

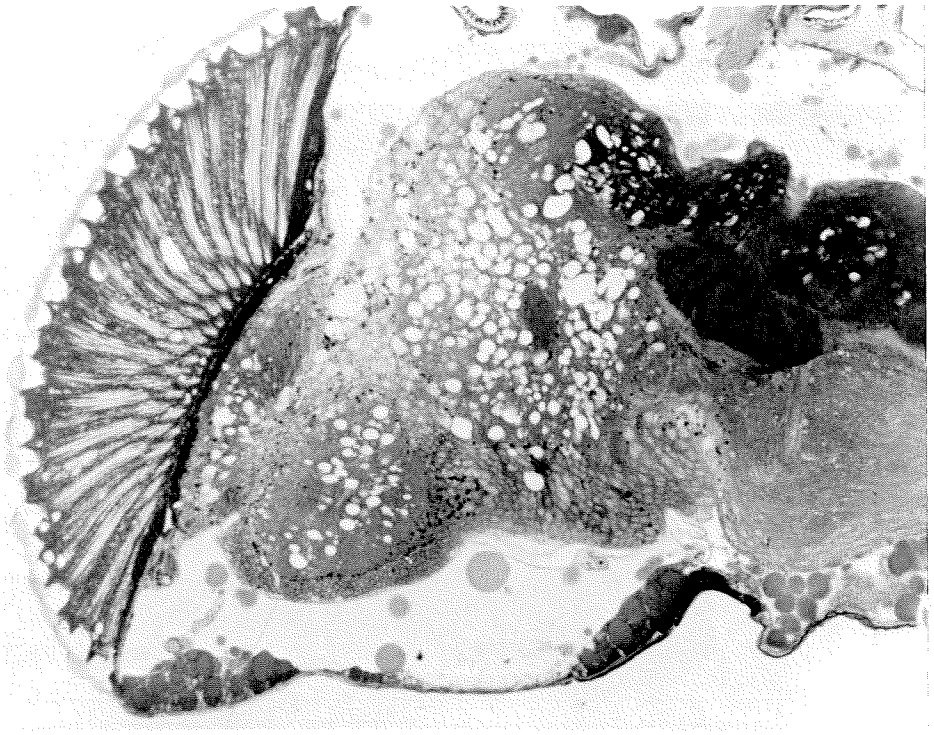


syndrome is highly variable. It is as if some random event triggers off a cataclysm. The death rate depends surprisingly little on temperature.

Mosaic analysis shows that whether a fly drops dead or not is most closely correlated with the genotype of the head, rather than the thorax or abdomen. However, there do occur occasional mosaics where the entire head surface is normal, yet the fly drops dead, and vice versa. This suggests looking inside the head to the brain. Histology of *drop-dead* mutant flies, fixed before staggering has set in, shows fairly normal appearance of the brain. However, whenever a fly that is already demonstrating staggering symptoms is examined, the brain is found to be shot full of holes. The holes tend to be concentrated around certain regions of the brain, as shown below.

This syndrome recalls the many kinds of hereditary brain degeneration in man. For instance, the gene for Huntington's disease leads to degeneration which appears to start in a specific brain region and is followed by more general deterioration, production of involuntary movements, incapacitation, and death. Although the gene is sooner or later expressed in all individuals carrying it, the age of onset of symptoms is highly variable. In fact, the distribution of incidence versus age for *drop-dead* is roughly similar to that for Huntington's disease, one day in the life of a *drop-dead* fly being roughly equivalent to a decade for an affected human. One must not, of course, push analogies such as these too far. The gene for Huntington's disease is dominant and autosomal, while *drop-dead* is recessive and sex-linked, and, needless to say, a fly is not a man.

In summary, gene changes can alter behavior in many different ways, and by very diverse mechanisms, by affecting the development and function of sense organs, the central nervous system, or motor output. Mutations provide an incisive tool for producing perturbations by which the normal system may be dissected and analyzed. Genetic tricks such as production of mosaic individuals are powerful in pinpointing the relevant components. Experience thus far with the fly as a model system for unraveling the path from the gene to behavior is encouraging. In any case, it is fun.



In horizontal section, the brain of a drop-dead *mutant at the stage of pronounced staggering is shot full of holes. The holes extend into the optic ganglia.*