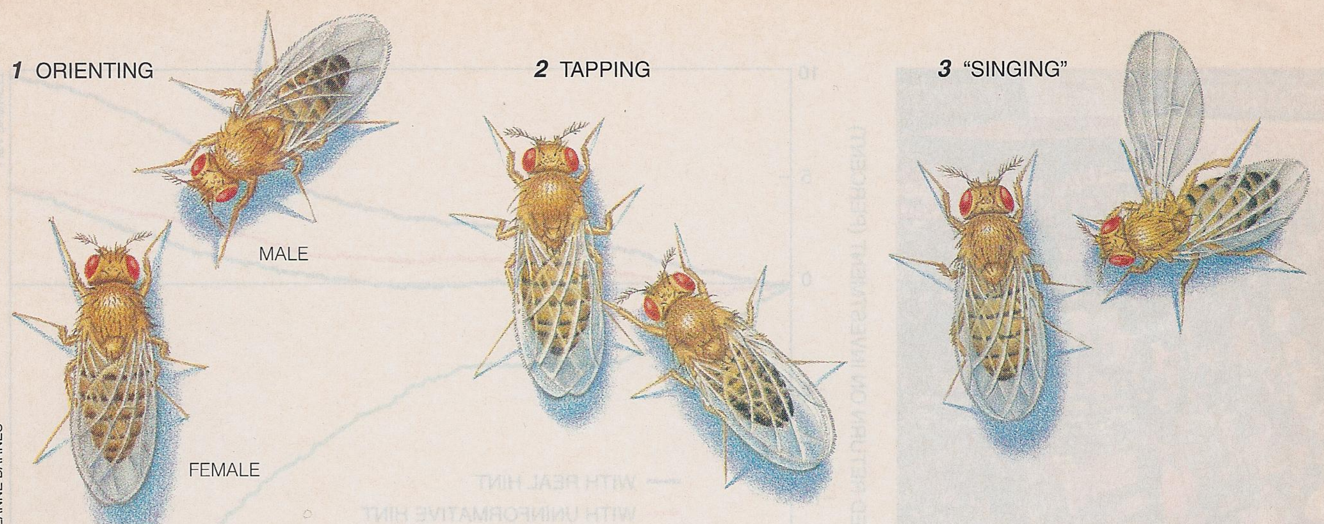


SUZANNE BARNES

Understanding the Genetic Construction of Behavior

Studies of courtship and mating in the fruit fly offer a window on the ways genes influence the execution of complex behaviors

by Ralph J. Greenspan

Within the first 15 years of this century, the newborn science of genetics had begun to give people their first glimpse of how heredity might work. Studies of such traits as flower color in plants and wing shape in fruit flies had confirmed Gregor Mendel's once obscure 1865 proposal that physical characteristics are passed from parents to progeny by discrete units of inherited material, or genes (the name these mysterious units were given in 1911). As commonly happens when a new discipline experiences its first flush of success, scientists and others soon began to apply the understandings of the budding field more broadly, and sometimes less carefully, to explain other phenomena—notably, the behavior of human beings. Often they claimed that complex behaviors were directed by single genes.

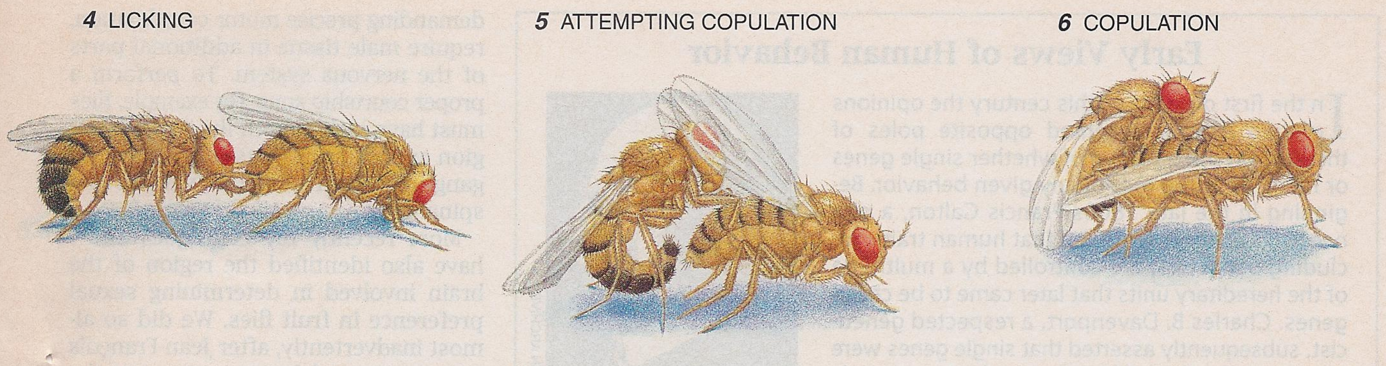
Yet even careful researchers have failed to link specific human behaviors convincingly to solitary genes or to small sets of genes. The reason could lie with methodology. When it comes to human behavior, there is virtually no way to disentangle unequivocally the influences of genes from those of culture and upbringing. On the other hand, if scientists could somehow manage to mask the effects of environment and focus solely on the genetic aspects of a behavior, they might still find the old assumptions flawed. Well-controlled investigations in simpler organisms suggest that a multitude of genes, some acting quite subtly, probably contribute to most behaviors.

Early Thinking on Humans

The question of whether human behavior is hereditary was initially asked more than a century ago. Francis Galton, a pioneer in the use of statistics, was among the first scientists to take up this issue. In the 1880s he analyzed various physical and behavioral traits in parents and their grown children. Using his newly invented "coefficient of correlation," he argued that behavioral traits are inherited. By comparing the distribution of traits in different generations, he concluded that each characteristic was the product of multiple donations from hereditary material.

A rather different view gained a following in the early 1900s on the heels of the rediscovery of Mendel's work, and it was embraced by such influential geneticists as Charles B. Davenport, a founder of Cold Spring Harbor Laboratory on Long Island. In the extreme, these researchers ascribed such ill-defined characteristics as musical ability, temperament or "feeble-mindedness" to individual genes. In 1921, for instance, Davenport asserted that "it appears probable, from extensive pedigrees that have been analyzed, that feeble-mindedness of the middle and higher grades is inherited as a simple recessive [trait], or approximately so." (In spite of their divergent views on the mechanisms of inheritance, both men regrettably drew similar, dangerous conclusions from their observations. Galton, who coined the term "eugenics," became a strong advocate of improving the human race by selective breeding between people having desirable traits. Davenport ardently supported that practice as well.)

Some of the first experiments designed to assess the impact of genes on behavior were carried out in the 1920s



MALE FRUIT FLY COURTS a female by executing a programmed sequence of steps. In the early stages he orients toward the female (*1*) and taps her abdomen with his foreleg (*2*). Next, he extends one wing and vibrates it to produce a "love song" (*3*). Then he licks the genitals of his partner (*4*), attempts to mount her (*5*) and, finally, mates with her (*6*). Analyses of this sequence suggest the genetic contributions to behavior are often surprisingly subtle.

on dogs. Those investigations examined, among other traits, pointing (indicating the location of prey) and vocalizing during hunting.

Dog breeds are as distinctive in their behavior as they are in appearance. The early studies crossbred dogs that differed in some behavioral characteristics and then mated their offspring to one another. If one or just a few genes controlled a chosen behavioral trait, investigators would expect to find that the animals of the final generation divided into discrete groups in which one group closely resembled the mother, a second closely resembled the father and perhaps one or a few groups behaved in an intermediate manner. If many genes were involved, workers would expect to find no discrete classes and a broad range of behavior in the offspring. The results were consistent with the last pattern, indicating that many genes underlay the appearance of each trait. Similar conclusions came from studies of maze running by laboratory rats.

Such analyses were informative but had major limitations. Breeding experiments cannot meaningfully address the genetic basis of behaviors that are relatively invariant in all members of a species. To delve into that problem and others, scientists needed ways to identify the specific genes involved in behaviors. Unfortunately, they would not have those techniques until many years had passed.

By the 1960s, however, many of the technical obstacles to the genetic dissection of behavior in animals had begun to fall. The structure of DNA had been deciphered in 1953. Studies of microorganisms had revealed that genes specify the makeup of proteins. When a gene is activated, it leads to the synthesis of the encoded protein. That protein, in turn, carries out some needed function in the body—such as helping to build and operate the nervous system (which itself ultimately shapes behavior). Such research had also clarified the steps by which genes give rise to proteins and had laid the foundation for development in the 1980s of many useful tools for isolating individual genes and determining the functions of their corresponding proteins.

Seymour Benzer of the California Institute of Technology was a leader in establishing that genes are linear segments of DNA. In the mid-1960s he also became one of the first investigators to go beyond linking genes to physical traits. Benzer began, in detailed studies of the fruit fly *Drosophila melanogaster,* to identify genes that affect behaviors. That effort is ongoing, particularly in the laboratory of Jeffrey C. Hall of Brandeis University, who was among the earliest researchers to work with Benzer in this new field. It also continues in my laboratory at New York University and elsewhere. I got involved in the mid-1970s, when I became Hall's first graduate student at Brandeis.

Spotlight on Fruit Flies

Among the behaviors receiving the most attention is the one the flies seem to do best: courting. This process consists of a series of actions, each of which is accompanied by the exchange of visual, auditory and chemosensory signals between males and females. The male is the more active of the dancers in this intricate ballet and has therefore been the focus of much of the research.

The ritual begins with a step called orientation. The male, who needs no instruction in this process, stands facing the female, about 0.2 millimeter away. Then he taps her on the abdomen with a foreleg and follows her if she moves away. Next, he displays one wing and flutters it to execute his form of a "love song." Depending on the female's level of interest at this point, he may go back and repeat his actions. But if all is going well, he unfurls his proboscis (a tubular appendage carrying the mouthparts at the tip) and licks the female's genitals. He may then mount her and, if she is receptive, copulate with her. Fruit flies will not mate unless the males have gone through this entire routine and the female has become receptive. Rape is uncommon in the fruit-fly world.

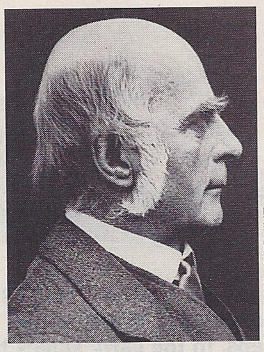
As a first step to finding the genes that might participate in courtship, Hall, initially working in Benzer's laboratory, set out to identify the parts of the central nervous system that control each element in the courting routine. He did so by producing extraordinary flies, called genetic mosaics, that carried mixtures of male and female cells.

The technique was based on an understanding of sexual development in fruit flies. In fly embryos, such development is controlled by the complement of X chromosomes within each cell. Cells that have one X chromosome give rise to male anatomical structures and behaviors in the fully formed fly; cells that have two X chromosomes lead to female anatomy and behavior. These differences arise because single-X (male) and double-X (female) cells activate separate, albeit overlapping, sets of so-called sex-determining genes. Hall knew that if a fly carried mainly female cells but harbored male cells in a particular site of the brain, any typically male

RALPH J. GREENSPAN, who earned his Ph.D. in biology from Brandeis University in 1979, is professor of biology and neural science at New York University and head of the W. M. Keck Laboratory of Molecular Neurobiology there. Outside the laboratory and N.Y.U.'s classrooms, Greenspan pursues an interest in making science accessible to the lay public. He teaches adult education courses at the New School for Social Research in New York City and helps to organize symposia for the Thrivers' Network of the Cancer Center at the University of California, San Diego, a group of cancer survivors and their families.

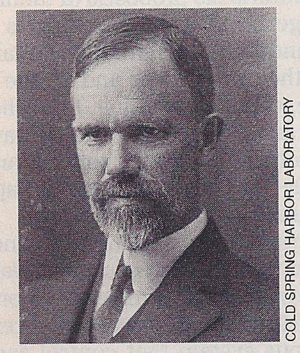
Early Views of Human Behavior

In the first decades of this century the opinions of two men represented opposite poles of thinking on the question of whether single genes or many lie at the root of any given behavior. Beginning in the late 1800s Francis Galton, a pioneering statistician, argued that human traits, including behaviors, are controlled by a multitude of the hereditary units that later came to be called genes. Charles B. Davenport, a respected geneticist, subsequently asserted that single genes were in control. Studies of fruit flies and other animals indicate Galton's view was probably the more accurate of the two.


BETTMANN ARCHIVE

Francis Galton

If the names of Galton and Davenport are familiar, it is because both are now notorious for their advocacy of eugenics, which in Galton's words involves checking "the birth-rate of the Unfit" and improving the human race by "furthering the productivity of the Fit." Galton introduced the term in the 1880s, and Davenport, who established a research center in human eugenics at Cold Spring Harbor Laboratory on Long Island, pushed the program forward. Davenport is sitting at the right on the bottom step in the photograph below, depicting a class of students he trained to carry out eugenics research.


COLD SPRING HARBOR LABORATORY

Charles B. Davenport


COLD SPRING HARBOR LABORATORY

Eugenics training class of 1914

courtship activities it displayed could be attributed to a male pattern of gene expression, or activation, in that site.

Once mosaics were produced, he monitored the animals' attempts at courtship. Then he froze the flies and painstakingly sliced the diminutive creatures (measuring just 1.5 millimeters long) into 20 thin sections, noting (with the help of a clever colorization technique) the distribution of male and female cells. The experiments were particularly nerve-racking in the 1970s because the method for creating mosaics had an inconvenient drawback: no two individuals ended up with exactly the same clusters of male and female cells. Each fly had to survive a battery of behavioral tests, and all 20 sections had to be analyzable. The uniqueness of each animal meant that the experimenter had no second chance.

After examining many of these mosaics, Hall concluded that initiation of courtship (orienting toward the female, tapping her abdomen, following, and extending a wing) required male cells in one side or the other of a relatively small region near the top and toward the back of the fly's brain. This region integrates signals from the fly's various sensory systems. In other words, male cells at that site somehow give rise to a trigger mechanism for courtship that is present in males but not in females. Later steps in courtship, especially those demanding precise motor coordination, require male tissue in additional parts of the nervous system. To perform a proper courtship song, for example, flies must have male cells in the "trigger" region as well as in parts of the thoracic ganglion, which is the fly's version of a spinal cord.

More recently my colleagues and I have also identified the region of the brain involved in determining sexual preference in fruit flies. We did so almost inadvertently, after Jean-François Ferveur in my laboratory (now at the University of Paris in Orsay) created entire strains of mosaic flies that were mainly male but that had female cells in selected areas of the brain. Before studying the courting behavior of these insects, we wanted to see whether full-fledged males would mistake our mosaics for females. The mosaics were not perceived to be female. To our surprise, however, a few strains displayed an odd behavior of their own: they courted males as vigorously as they courted females.

Examination of the brains in these unusual insects, undertaken in conjunction with Klemens F. Störtkuhl and Reinhard F. Stocker of the University of Fribourg, revealed that sexual discernment was altered when either of two parts of the central nervous system was female: the antennal lobe or the mushroom body of the brain. Both these regions, the second of which lies close to the trigger site for courtship, participate in processing olfactory signals. If either or both of these centers for analyzing odors were female, the fly lost the ability to distinguish males from females and became equally interested in both.

Genetic Influences on Courtship

The discovery that so many different regions of the central nervous system are involved in male courtship suggests that a variety of genes also participate in the process. Indeed, more than a dozen have been discovered, mainly by Hall and his colleagues. For instance, the *fruitless* gene influences sexual preference. Mutation in this gene affects male flies in much the same way as having female cells in the antennal lobe or mushroom body does: it causes males to court other males as avidly as they court females. The gene is also needed in the late stages of courtship; males carrying a mutant gene never attempt to copulate with females.

Hence, the picture beginning to emerge is more consistent with Galton's view than with Davenport's. Oddly enough, no one has yet identified any gene involved in courtship that is dedicated solely to that behavior. Growing evidence suggests an explanation neither Galton nor Davenport would have predicted. It may be that most genes underlying courtship (and other behaviors) serve more than one function in the body. Identical genes may also be used for somewhat different purposes in males and females.

Consider, for example, one of the three genes known to participate in the male's courtship song. It is called *period* and has been studied most extensively by Hall and Charalambos P. Kyriacou of the University of Leicester.

Hall and Kyriacou decided to examine *period* when they discovered, in 1980, that the male song has a distinct rhythm to it. They already knew, from research done by Benzer's graduate student Ronald J. Konopka, that the gene affects the fly's circadian rhythms—the timed cycles, such as for waking and sleeping, that are characteristic of all living things. This property led them to wonder whether *period* might also affect the rhythm of the courtship song.

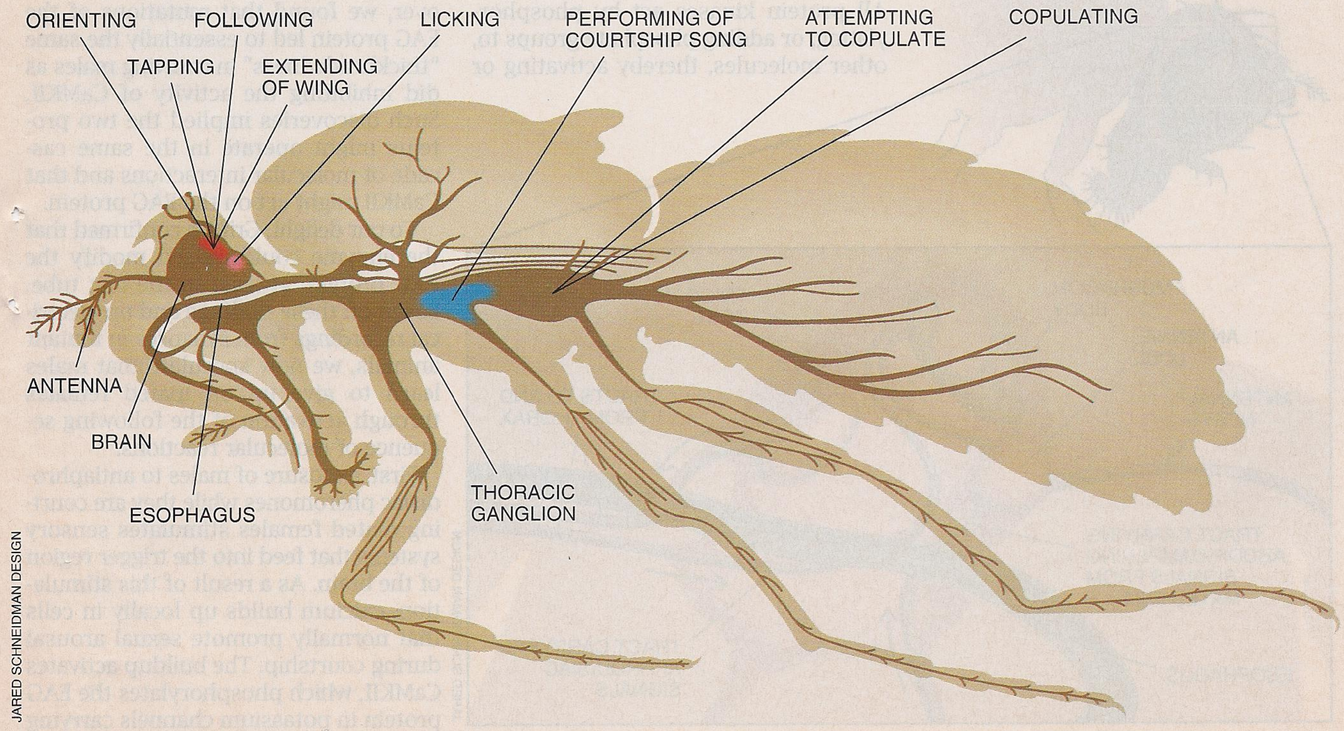
The song performed by fluttering the wing is not very musical to our ears, but it does have a detectable pattern. As the insect raises and lowers its wing once, the up-and-down motion produces a characteristic sound, or pulse, that can be picked up with a recording device. For approximately 27 seconds, the male gradually increases the interval between each successive pulse. Then, over another 27 or 28 seconds, he gradually decreases the interval, so that a plot of the intervals over time yields a smooth sinusoidal curve.

Hall and Kyriacou found that males carrying a normal *period* gene produce a normal song that makes females more receptive to their advances. In contrast, males carrying an inactive gene generate a song that lacks the usual smooth rhythm and is apparently less effective at stimulating females: when computer-generated simulations of normal and rhythmless songs were played for lone females who were then paired with a male, the females exposed to the aberrant song proved less receptive to the male's advances. Similarly, less drastic mutations in the gene allow rhythmicity to be retained but stretch or shrink the sine curve; in the process, they reduce the song's power over a female.

The subtlety of *period's* effects on the overall courtship routine, and on the song itself, adds credence to the notion that courtship—and other complex behavior—is regulated by multiple genes acting together. And the fact that the *period* gene participates in setting other clocks, and is also expressed in many parts of the central nervous system, supports the idea that any given gene may affect more than one behavior.

In a fascinating turn of events, Hall, Kyriacou and Michael Rosbash, also at Brandeis, have recently pinpointed the exact part of the gene that controls song rhythm. A small region in the middle is devoted to the song, and the balance of the gene controls other rhythms. That division of labor was deduced in part from the fact that a different species of fruit fly, *D. simulans,* has the same 24-hour cycle of activity and rest as is found in *D. melanogaster* but performs a song that differs in the intervals between pulses. The *period* gene in both species is similar, except for small differences in the middle region. What is more, genetically engineered flies that carry a hybrid *period* gene made by replacing the middle region of the *D. melanogaster* gene with the corresponding segment of *D. simulans* will "sing" just like *D. simulans.*

Although sexual preference and courtship behavior are certainly programmed in fruit flies, males and females have the ability to modulate their activity in response to one another's reactions. In other words, they can learn. Just as the ability to carry out courtship is directed by genes, so, too, is the ability to learn during the experience. Studies of this phenomenon lend further



SITES IN THE CENTRAL NERVOUS SYSTEM (*brown*) that control the steps of courtship in male fruit flies have been mapped by studying flies that consist of a mixture of genetically male and female cells. To perform the initial steps of the mating routine (orienting, tapping and wing extension) and to follow peripatetic females, flies must have male cells in a small trigger zone (*red*) at the back of the brain. They also need male cells nearby (*pink*) to perform licking, in part of the thoracic ganglion (*blue*) to produce their song and in many different sections of the thoracic ganglion to copulate.

support to the likelihood that behavior is regulated by a myriad of interacting genes, each of which handles diverse responsibilities in the body.

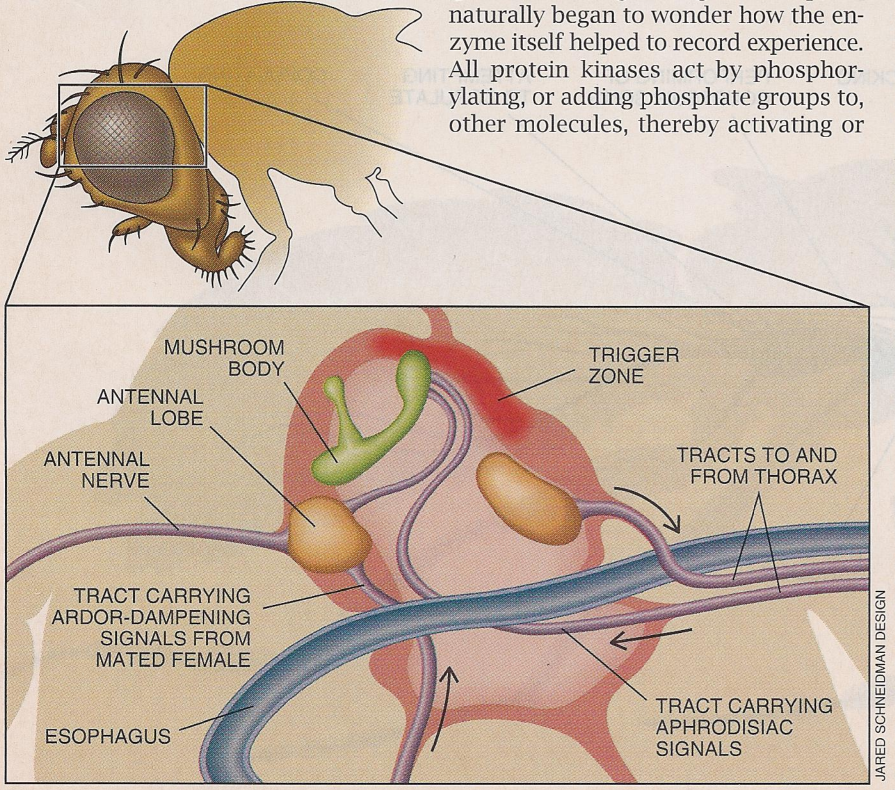
Learning from Experience

One thing a male can learn during courtship is not to waste time on a female who has already mated and who, consequently, will not be receptive. As Hall and Richard W. Siegel of the University of California at Los Angeles found, male flies will court virgin females tirelessly but will lose interest in mated females after about 30 minutes or an hour—when they finally become impressed by the inhibitory pheromone, or scent, emitted by mothers-to-be. Once males give up the chase, they become uninterested in all females, virgin or not, for a few hours. If there is discernible evolutionary logic to this behavior, it may be that the presence of a mated female in a group of females is a sign that most or all of them have already mated; hence, a male's efforts would be better spent elsewhere.

My explorations of the genetic underpinnings of this response began a few years ago and were undertaken with Leslie C. Griffith, who is now at Brandeis. We knew from the work of other investigators that an enzyme called calcium/calmodulin-dependent protein kinase II (CaMKII) can help record the effects of experience in neurons, in the process inducing molecular changes that are likely to be essential to learning. We therefore decided to see whether male fruit flies needed this protein—and thus the corresponding gene—in order to respond appropriately to mated females.

As a first step, Griffith engineered a strain of flies whose CaMKII protein could be quieted simply by increasing the body temperature of the flies. Sure enough, when the enzyme's activity was reduced even mildly, males of this strain behaved oddly. They were as avid as normal flies in their courtship of virgin females, and they lost interest in mated females after the usual hour or so, but they seemed to forget their rejection almost immediately. If they were placed with females soon after being with a mated one, they began their pursuit anew. When CaMKII was inhibited even more, the males did not learn at all: they pursued mated females unabated for hours. (Even in the world of fruit flies, it seems, some men never learn.)

Once we knew that the *CaMKII* gene, through its enzyme product, did participate in learning during courtship, we naturally began to wonder how the enzyme itself helped to record experience. All protein kinases act by phosphorylating, or adding phosphate groups to, other molecules, thereby activating or inactivating the targets. But what was the kinase's target in neurons, and what happened after that target was phosphorylated? Such questions ultimately led us to demonstrate that a second gene expressed in neurons—*eag*—is instrumental in such learning as well.



SEAT OF ATTRACTION to females resides in two sites in the male fruit fly's brain (*shown schematically*). One is the antennal lobe (*gold, left*); the other is the mushroom body (*green*), which lies close to the trigger zone responsible for initiating courtship. The importance of these regions was discovered when males engineered to contain female cells in either site began courting males as well as females.

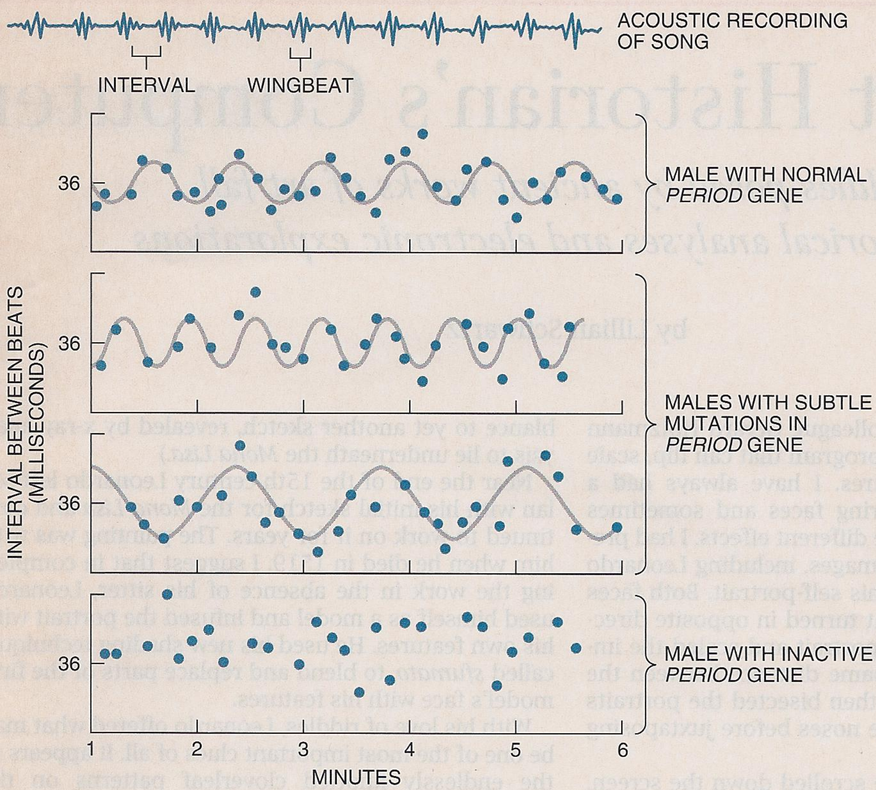
Another Learning Gene

The protein product of this gene is a component of certain membrane-spanning channels that regulate the flow of potassium ions out of neurons. Opening these channels helps to control excitability and the release of neurotransmitters, which carry messages from cell to cell. (The name derives from the fact, discovered in the 1960s, that when flies bearing mutated *eag* genes are anesthetized, their legs shake: in a sign of the times, the discoverers called the gene *ether-a-go-go.*)

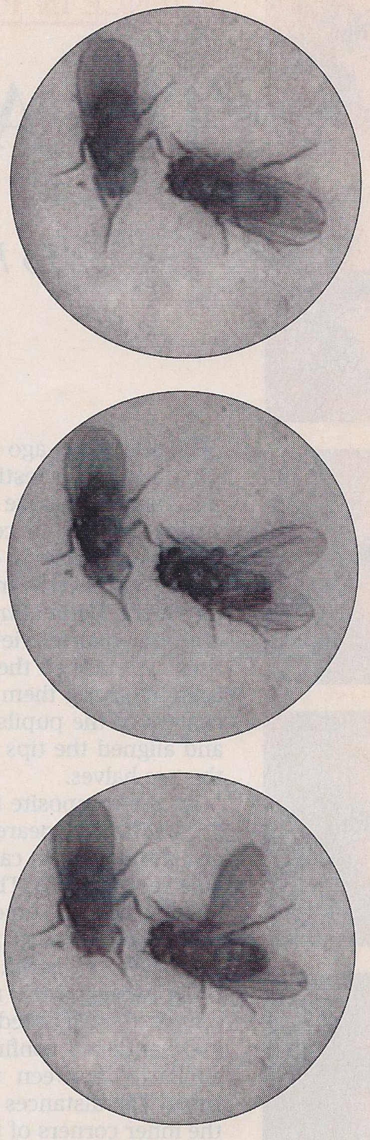
On the basis of a number of clues from our own research, from that of our collaborators Jing Wang and Chun-Fang Wu of the University of Iowa and from others, Griffith and I began to think the CaMKII enzyme might participate in learning by modifying the EAG protein in potassium channels. For instance, Eric R. Kandel and his colleagues at Columbia University had shown in the marine mollusk *Aplysia* that one kind of potassium channel is modified by a kinase during a simple form of learning. Moreover, we found that mutations of the EAG protein led to essentially the same "thickheadedness" in courting males as did inhibiting the activity of CaMKII. Such discoveries implied the two proteins might operate in the same cascade of molecular interactions and that CaMKII might act on the EAG protein.

To our delight, Griffith confirmed that the enzyme could indeed modify the EAG protein, at least in the test tube. Based on these findings and on electrical recordings from synapses in mutant animals, we now speculate that males learn to give up on mated females through activation of the following sequence of molecular reactions.

First, exposure of males to antiaphrodisiac pheromones while they are courting mated females stimulates sensory systems that feed into the trigger region of the brain. As a result of this stimulation, calcium builds up locally in cells that normally promote sexual arousal during courtship. The buildup activates CaMKII, which phosphorylates the EAG protein in potassium channels carrying that protein. Such modification causes the channels to open, allowing potassium ions to flow out of the neurons, thereby quieting the cells and reducing their ability to release neurotransmit-



JARED SCHNEIDMAN DESIGN; source: CHARALAMBOS P. KYRIACOU AND JEFFREY C. HALL



NANCI KANE, SUSAN BROUGHTON AND KAREN RAPHAEL *New York University*

MALE FLY EXTENDS A WING (*photographic sequence at right*) before vibrating it to produce his song. In the normal song the interval between each beat (*highlighted in an acoustic recording at top*) increases gradually over about 27 seconds and then decreases equally gradually; a plot of the intervals resembles a sine curve (*top curve*). This rhythmicity has been shown to be controlled by a gene called *period.* Flies carrying a healthy *period* gene display the usual song. But those with mildly defective genes produce abnormal rhythms (*middle curves*), and those harboring an inactive gene completely lose their ability to "carry a tune" (*bottom*).

ters. As the cells become silent, the males lose interest in mating. Conversely, flies carrying defects in the genes for either protein presumably retain misplaced interest in mated females because the potassium channels remain closed in the critical cells, allowing the neurons to become hyperactive.

The *CaMKII* and *eag* genes turn out to be just two of several known to affect learning and memory in fruit flies. Some of the others also participate in courtship—a finding that meshes rather well with the view that behaviors arise from the interactions of vast networks of genes, most of which take part in many different aspects of an organism's biology.

Lessons for Humans?

Do the lessons from genetic studies of fruit-fly behavior bear any relevance to human beings? I think they do—within limits. There is every reason to believe that the genetic influences on behavior will be at least as complicated in people as they are in fruit flies. Hence, the notion of many, multipurpose genes making small contributions is likely to apply. And many of the gene products that function in the brains of flies will probably turn out to be important in the human brain. Human counterparts have already been discovered for a number of genes originally identified in the fly, such as *eag.* These findings should provide insights into the molecular interactions that enable the central nervous system to produce behavior.

New technologies hold promise for detecting the contributions of individual genes to human attributes. The techniques are already being applied to a variety of complex traits, including musical ability—though more carefully than in Davenport's day. Such work, and extrapolations from animal research, can probably help pinpoint some of the genes that contribute to specific human behaviors. But any research claiming to explain human activity in purely genetic terms must be viewed with caution. Society's well-founded unwillingness to rear human subjects in perfectly controlled environments makes it virtually impossible to prove the validity of such claims.

FURTHER READING

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