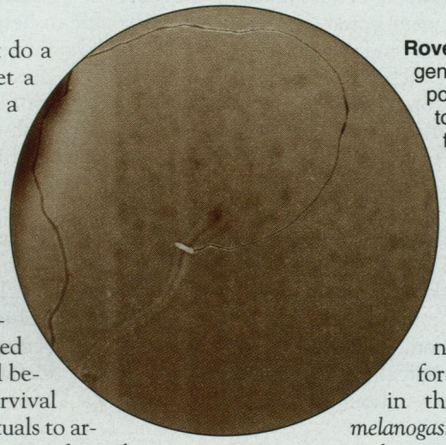
ANIMAL MODELS

From Fruit Flies, Rats, Mice: Evidence of Genetic Influence



Rovers and sitters. A single gene in fruit flies predisposes feeding larvae either to roam around on their food *(left)* or to stay put *(below)*.

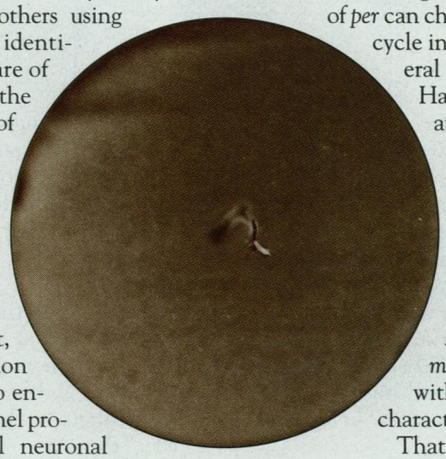
Here's a puzzle: What do a rat tapping a bar to get a shot of alcohol and a male fruit fly serenading its mate have in common? Not much, you say? In fact, both organisms are displaying behaviors governed largely by their genes. Behavioral researchers have realized for decades that animal behaviors ranging from survival instincts and mating rituals to artificially induced patterns such as alcohol consumption are at least partly under genetic control, and they have surmised, therefore, that some human behavior may also have a genetic component. Driven by that knowledge, they have searched for animal systems that could illuminate the question of just how genes influence behavior.

Gaining insight into the genetic control of human behavior isn't the only motivation for developing these animal models. Researchers interested in evolution would like to understand how genetic change leads to behavioral change—and potentially to the creation of a new species. Neuroscientists would like to use genetic alteration of behavior to understand the nervous system, the "middleman" between genes and behavior. But for many researchers, the most compelling motivation for exploring animal systems is the hope that they can help us to understand the roots of human behavior.

The road to that goal, however, is long and treacherous. In the first place, there is an ongoing debate over how one defines a "behavioral gene." A mutation that renders an animal blind, for example, will clearly change the animal's behavior—but many would argue that that doesn't make it a behavioral gene. In addition, even in the simplest animal, most behaviors will be governed by many genes in concert, not by a single gene. And until recently, geneticists had no good way to trace the multiple genes involved. But in recent years the pace of the field has quickened dramatically, thanks to determined experimental work and to the development of some remarkable methods for identifying the contributions of groups of genes to behavior.

Behind the rapidity of recent advances, however, lies decades of slow but steady progress, much of it based on the strategic choice of simple animal systems. In the 1960s, for example, Seymour Benzer of the California Institute of Technology began searching for behavioral mutations in the fruit fly *Drosophila melanogaster*. Benzer and his group began inducing mutations in fruit flies, selecting those impaired in behaviors such as attraction to light. Benzer reasoned that his approach would yield insights into how genes govern behavior and also provide a window on the nervous system.

Over the course of nearly 30 years, Benzer's group (and others using these methods) has identified many genes that are of great importance to the normal functioning of the nervous system. One example is the gene *Shaker*, mutations in which cause flies to shake violently under anaesthesia. Such shaking is a sign of a nerve-cell defect, and the normal version of *Shaker* turned out to encode a potassium-channel protein vital for normal neuronal function.



PHOTOS BY G. TEMPLE AND M. SOKOLOWSKI

But genes like *Shaker* raise the much-debated issue of how to prove that a particular gene is, in fact, involved in determining behavior. At the core of the question, says Cory Bargmann of the University of California, San Francisco, is the fact that "genes don't generate behavior—the nervous system generates behavior." Therefore, a mutation such as *Shaker*, which changes nervous system function, will obviously affect behavior. But is it actually a behavioral gene—the kind of gene natural selection would work on to effect subtle changes in a species' behavior?

For Benzer and many of his colleagues, the answer isn't so crucial, since they're largely interested in the nervous system. But ethologists, whose primary interest is behavior, have laid down narrower standards—standards genes like *Shaker* don't pass, because mutations in these genes don't alter a specific behavior; they just make the fly sick. To satisfy the more stringent criteria, says Brandeis University behavioral geneticist Jeffrey Hall, a gene must be shown to affect a defined behavior in a specific way. "The [normal form of the] behavior in question must be something the normal animal does in an active, real sense, part of the ethology of the animal," says Hall. "Not shaking is not a [behavior]," he says; "it is just well-being."

One gene identified in Benzer's lab that does fit Hall's criterion is *period* (*per*), which controls the fly's 24-hour circadian rhythm. The *per* gene has been cloned and sequenced, but it is not yet known exactly how it exerts its effects. Even so, says Bambos Kyriacou, who studies *per* at the University of Leicester in England, "*per* is probably the best current example of a behavioral gene." Flies without a functional *per* gene are healthy—but different from their normal cousins. "If you completely remove the gene, the flies are fine," says Kyriacou; "they're just not rhythmic."

The lack of rhythm extends to many areas of life, including a key species-specific behavior: courtship. Males with *per* mutations sing courtship songs with an altered rhythm. Even more striking, laboratory manipulation of *per* can change the courtship song cycle in a predictable way. Several years ago, the groups of Hall and Michael Rosbash at Brandeis, along with Kyriacou, reported that they could replace part of the *per* gene from *Drosophila melanogaster* with a fragment of the gene from a related *Drosophila* species, *D. simulans*, and make *melanogaster* males sing with the shorter rhythm characteristic of *simulans*.

That dramatic result shows *per* is just the kind of gene ethologists are looking for: a gene whose variations produce subtle behavioral differences that can be acted on by natural selection, perhaps ultimately resulting in a new species. Indeed, Kyriacou has found that fly populations in northern and southern Europe show systematic differences in a small region of *per*. Kyriacou says he has evidence that these variations cause behavioral alterations that are under selection pressure in the contrasting climates of northern and southern Europe. The PER protein is part of an internal clock, says Kyriacou, and the changes he sees may buffer the clock against temperature differences that would throw off its timing.

In the *per* story, scientific success came from finding a gene and then discovering its context in nature. Other researchers are re-

A New Tool for Examining Multigenic Traits

The reason behavioral genetics is so difficult is that behavioral traits are generally governed by more than one gene. And tracking down the location of many genes linked to a single trait is impossible using standard genetic methods. But recently several techniques have been developed that make it possible to follow these forked genetic trails. One of the most promising is quantitative trait locus (QTL) analysis, proposed 5 years ago in a theoretical paper by Eric Lander and David Botstein, then both at the Massachusetts Institute of Technology. QTL analysis can be done on any species of animal or plant for which there are inbred strains. It's not limited to exploring the genetic roots of behavior, but it is now being exploited for that purpose in mice and rats by groups tracking the genes behind traits ranging from alcohol addiction to learning ability.

The accompanying figure shows how a researcher with two inbred strains of mice, A (red) bred for aggressiveness, and D (blue) bred for docility, could use QTL analysis to find genes contributing to aggression. Each strain has been inbred by mating brother to sister for many generations until all members of the group are genetically identical (as identical twins are), and also have two uniform sets of chromosomes.

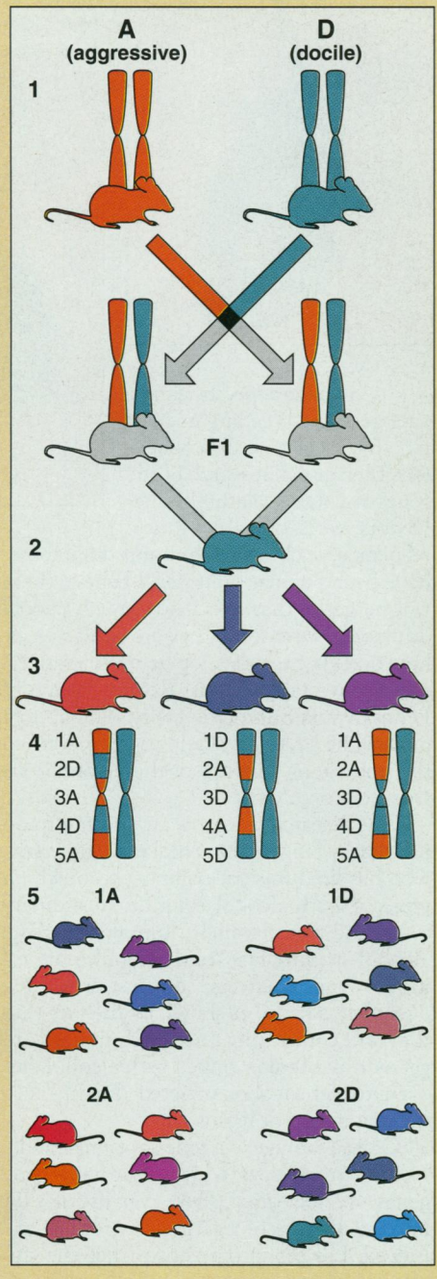


ILLUSTRATION: D. DEFRANCESCO

1. The researcher breeds A mice to D mice, producing a first generation (F1) of hybrid offspring in which every mouse has one chromosome set from each parent. In the F1 generation, chromosomes exchange material in the cells that produce eggs and sperm. Segments of the mother's and father's DNA are recombined on individual chromosomes.

2. The F1 generation is now bred back to D mice, producing offspring with one recombinant set of chromosomes and one set that is pure D. Each offspring will carry, on its recombinant chromosome, a unique mix of genes from both original strains. This allows the researcher to examine the effects of those genes. The fact that the second set of chromosomes is pure D means it is unvarying from mouse to mouse—and therefore won't complicate the analysis.

3. Each second-generation mouse is ranked by its level of aggressiveness (shades of purple). Because more than one gene determines aggressiveness, these second-generation mice will show a range of aggression depending on the genetic mix in their recombinant chromosome set.

4. To find the QTLs, sites in the genome that contain genes that contribute to aggression, the researcher searches each mouse's DNA for genetic markers, landmarks scattered throughout the genome that are known to differ between A and D strains. For each marker, the researcher determines whether the mouse has inherited A-type or D-type DNA at that location.

5. For each marker, the researcher sorts the mice into those that have A-type DNA at that spot and those that have D-type DNA, and checks the aggressiveness scores for the mice in the two groups. If the A-type group is significantly more aggressive than the D-type group (as in the case of marker 2), that marker represents a QTL that may contain a gene contributing to aggressiveness. But the quest doesn't end here. Each QTL contains many genes, and the researcher must use other methods to sort through those genes for the one in each QTL responsible for its contribution to aggression.

There is a statistical "price to pay" for making multiple comparisons, says Lander. If, for example, you use a statistical confidence level of 95%, which is reasonable for an analysis at a single genetic site, but instead analyze 10 sites, the possibility rises from 5% to an unacceptable 50% that one of the sites may show a correlation with aggressiveness merely by chance. That likelihood continues to rise as you add genetic sites. The solution, according to Lander and Botstein's theoretical analysis, is to increase the number of animals until the confidence level reaches 99.9%, a level that provides ample certainty no matter how many genetic markers are tested.

–M.B.

versing that approach by looking for behaviors that seem to take several forms in nature, then searching for the genes that underlie them. Marla Sokolowski of York University in Toronto, for example, observed two forms of feeding behavior in the larvae of wild fruit flies. The larvae she dubbed "rovers" moved around continually on their food; others, called "sitters," sat in one spot to savor their meal. To Sokolowski, the fact that the behavior showed two distinct forms suggested it might be governed largely by variation in a single gene.

To test that hypothesis, Sokolowski bred pure strains of rovers and sitters and found she was correct. Most of the difference between the strains could be explained by variation in a gene she named *foraging* (*for*). Like *per*, the *for* gene is subject to natural selection, even in the lab. Crowded conditions favor rovers, apparently because they range farther in search of food; sparsely populated situations favor sitters, which conserve energy by staying put.

In her hunt for the gene controlling this behavior, Sokolowski was fortunate, because *for* appears to map to a gene for a known enzyme, cyclic GMP-dependent protein kinase, which is important in intracellular signaling pathways. Alternative RNA splicing allows several versions of the enzyme to be made from the same gene; sitters seem to have an alteration that affects just one form of the enzyme and has a specific influence on foraging without harming the fly's overall well-being. As with *per*, that makes for an ideal gene to be acted on by natural selection, and Sokolowski is eager to test her hypothesis that crowded conditions naturally select for the rover form of the gene in the wild.

But despite the satisfying progress she has made in tying genes to behavior, Sokolowski says she doesn't support the view that genes are solely responsible for behavior—in animals or in humans. "Just because an animal is a rover genetically, it may not always behave as a rover," she says. If, for example, a rover larva is deprived of food before being given a chance to feed, it will probably behave like a sitter (perhaps due to internal signals that tell it to conserve energy). "We have to get away from the idea of genetic determinism—that the genes determine our behavior" independent of the environment, says Sokolowski. "Behavior," she adds, "is strongly influenced by the environment."

Mice and rats: More like us

The fruit fly has the virtue of simplicity as a model for behavioral genetics, but it also has the drawbacks attendant on being far removed from us on the evolutionary scale. Researchers who crave a more specific understanding of the genes underlying human behavior are therefore turning to rats and mice, which, as mammals, are much closer evolutionarily to *Homo sapiens*. Those working on mice have gotten a big boost from the development of a technique for making "knockout mice," in which specific genes can be inactivated to study their effects. That method has already shed light on several genes involved in behavior.

One example comes from René Hen of INSERM in Strasbourg, France, who reported at last November's meeting of the Society for Neuroscience that he and his colleagues have knocked out the gene for one type of receptor for serotonin, a neurotransmitter. The resulting mice display aggressive behavior toward other mice (*Science*, 19 November 1993, p. 1211). Humans have an equivalent type of serotonin receptor, and Hen's results have focused suspicions on that receptor as the possible root of some abnormally aggressive behavior in humans.

Hen and his colleagues were fortunate in having a candidate gene to work with. But not all are so fortunate, and some researchers who don't have a specific candidate gene are breeding mice or rats that display certain behaviors, then going in search of the genes involved. Naturally, behaviors with obvious clinical relevance, such as alcohol and drug abuse, have been popular subjects. "I believe there will be genes that affect how much people drink or how sensitive they are to alcohol or how quickly they develop acute tolerance," says behavioral geneticist Robert Plomin of Pennsylvania State University. The animal models, he says, may "help us get closer" to the underlying genetics of these conditions (see story on p. 1696).

PHOTOS BY RENE HEN *ET AL.*, CNRS-LGME, INSERM

Aggression gene? Mice missing a type of receptor for the neurotransmitter serotonin attack other mice more aggressively than their normal peers. Photos show hyperaggressive mice fighting.

One researcher who is attempting to do just that is Ting Kai Li of Indiana University, who has bred four rat strains, two of which prefer a 10% alcohol solution to ordinary water and two of which shun alcohol. The alcohol-swilling rats "find alcohol reinforcing for its drug effects," not just as a thirst quencher, says Li, as shown by the fact that they will continually press a bar not only to get a drink of water spiked with alcohol, but even to get alcohol injected directly into their stomachs or brains.

One advantage of a genetic animal model such as this one, says Li, is that before any genes are identified, "you can use it...for looking at ways of treating the condition." Indeed, Li's group has shown that the tippling rats have intrinsically low levels of serotonin and dopamine (another neurotransmitter) in the "reward center" of their brains. Drugs such as Prozac, which enhance serotonin's effects, diminish the rats' craving for alcohol, suggesting the serotonin deficiency is related to their alcoholic tendencies.

Prozac doesn't block alcohol craving in all imbibing rats, however. A strain bred in Finland doesn't show the serotonin defect—and doesn't respond to Prozac, either. That, says David Overstreet, who studies alcohol-drinking rats at the University of North Carolina, suggests there may be a variability in the human condition as well. Some human alcoholics may have a serotonin deficiency and respond to Prozac, he argues; others may not.

These contrasting rat strains show the influence genes can have on a complex behavior. Until recently, however, there was little hope of actually locating the multiple genes that govern the craving for alcohol. Now, thanks to new genetic techniques for analyzing complex traits, several groups are starting to track down those genes (see article by Crabbe on p. 1715); other behavioral geneticists are exploiting the new techniques to find genes for behaviors aside from imbibing.

One of those researchers is Jeanne Wehner of the Institute for Behavioral Genetics in Boulder, Colorado, who is searching for genes involved in spatial learning in mice. Wehner has been studying two common lab strains of mice that differ dramatically in their ability to learn and remember the location of a submerged platform in a tank of water. Mice in one strain, C57, are good learners; those in a second strain, DBA, are not.

Acting on a hunch, Wehner checked the two strains of mice for the level of activity of protein kinase C (PKC), a key cellular signaling enzyme. She looked in the hippocampus, one of the brain's learning centers, and found that the DBA "slow learners" had much lower levels of PKC than the "gifted" C57s. But that didn't mean PKC had anything to do with learning; it might have been a coincidence that the enzyme varied between the two strains.

To find out whether the enzyme really does influence the ability of the mice to learn, Wehner turned to one of the popular new genetic tools: recombinant inbred (RI) mice, so called because they are inbred strains formed by recombining or mixing the genes from two commonly used laboratory mouse strains. Each RI strain has a unique mix of genes from the two parent mouse strains from which it was formed. Produced at Jackson Laboratory in Bar Harbor, Maine, RI mice have been used for decades for analyzing genetic traits caused by single genes; only recently have researchers begun to use them to analyze multigenic traits.

Wehner chose a collection of RI strains that have random assortments of genes from DBA and C57 mice. In a blinded study, her group tested 11 RI strains, one investigator scoring the mice for learning

ability, a second for PKC levels. "When we broke the code, we found a very significant correlation between activity of PKC in the hippocampus...and performance," Wehner says. And that finding suggests the PKC gene is one of the many genes that influence spatial learning.

RI mice are convenient to use because they can be ordered from Jackson Lab, sparing the researcher the laborious job of making and analyzing genetic crosses. And since all the mice in any RI strain are genetically identical, researchers from different teams can easily compare their work to see if they are on the trail of the same genes. The drawback of using RI mice, however, is that there are only a few dozen RI strains derived from any two parent strains such as C57 and DBA. And that severely limits the statistical power of any analysis that uses the mice to link genes or regions of DNA to behavioral traits.

So before drawing any firm conclusions, Wehner plans to take her analysis further using another new technique, quantitative trait locus (QTL) analysis, proposed 5 years ago by Eric Lander and David Botstein, then both of the Massachusetts Institute of Technology. QTL makes it possible for researchers to identify multiple regions of DNA that contain genes that contribute to a single trait in rats, mice, or any other species for which inbred laboratory strains are available (see box on page 1691).

Wehner's QTL analysis will involve crossing DBA and C57 mice and genetically characterizing hundreds of second-generation mice with random assortments of DBA and C57 genes, then testing them for learning ability and enzyme levels. That's a daunting amount of work, says Wehner, but she thinks that in this field it's better to be safe than sorry. "Learning is very difficult to study because of both genetic and environmental influences, and I don't want to have to say, 'Oh, those were putative loci, [but] none of them panned out'"—as has happened repeatedly with candidate behavioral genes both in animals and in humans.

The caution Wehner is showing seems to come with the territory in studying genes and behavior. Though many behavioral genes have been identified in animals, the indirect relationship between genes and behavior, along with the influence of the environment, makes hunting for behavioral genes a risky business. And once researchers manage to find a gene or group of genes that shape behavior, they may still be a long way from knowing precisely how those genes exert their influence. Given the difficulties, it is safe to say that persistence is a quality (perhaps even a genetically determined one) that will be required of behavioral geneticists for many years to come.

–Marcia Barinaga

MANIC DEPRESSION

Highs and Lows on the Research Roller Coaster

A decade ago, many researchers were confident they would soon have proof that a mutation in a single gene can have a dramatic effect on complex human behaviors. They had found many mutations that can influence at least some forms of behavior—those that cause profound retardation, for example—but they wanted to find a gene more directly involved in causing subtler effects. Specifically, they thought they would be able to find a gene for manic depression. Encouraged by the triumphs of internal medicine, several groups set out in the 1980s in high hopes of tracking down the putative manic-depression gene, using techniques that had proven spectacularly successful in locating marker DNA for Huntington's disease and Duchenne muscular dystrophy in 1983 and markers for retinoblastoma and cystic fibrosis 2 years later.

Among the behavioral disorders, manic depression was at the top of the list of new targets because its symptoms are clear-cut. The roller-coaster ups and downs of manic depression, researchers believed, would make it relatively easy for clinicians to identify patients with an inherited illness. Through "linkage analysis"—looking for common genetic markers among patients from families with a high incidence of the disorder—they expected to find a gene. Not everyone in the field agreed; some thought a disorder as complex as manic depression must be produced by multiple genetic traits interacting with one another and the environment. But the medical model had been so successful that by the mid-1980s the hunt was on in earnest.

Since then, researchers looking for a manic depression gene have been riding an emotional roller coaster of their own. The high came early: Between 1987 and 1988, psychiatric geneticists published several reports linking manic depression to specific regions of the human genome. By 1989, however, that exhilarating "up" gave way to a vertiginous decline. Authors began revising their work and challenging others' findings. One by one, the statistical results linking manic depression with a particular region of the human genome began melting away, leaving a residue of failure.

Today, there are signs that optimism is building again, as gene hunters acquire better maps of the chromosomes and faster methods of testing DNA. The field also has received a vote of confidence from the Charles A. Dana Foundation, which last year awarded $2.5 million to a new consortium searching for a manic-depression gene. This infusion of cash and enthusiasm hasn't yet overcome all the disappointments of the past 10 years, however. Psychiatric researchers still hope to find a gene for manic depression, but they're less confident than they were in the 1980s. Indeed, like their colleagues looking for genes for alcoholism (see story on p. 1696), they've become somewhat gun-shy, although there's little doubt that the disease has a genetic component.

Elliot Gershon of the National Institute of Mental Health (NIMH) acknowledges that he and his peers have become hesitant to publish. When Gershon spoke with *Science*, he was awaiting publication of his new finding in the *Proceedings of the National Academy of Sciences*—a report with Wade Berrettini of Thomas Jefferson University in Philadelphia linking manic depression to a region of human chromosome 18. Gershon says they "had a lot of fear and trepidation" about sending the paper out. Caution and

PHILADELPHIA MUSEUM OF ART: THE WILLIAM L. HELFAND COLLECTION

Demons of depression. A 19th-century engraving shows depression (or melancholia, as it was then known) in the form of blue devils.