

By integrating studies in genomics, neuroscience, and evolution, researchers are beginning to reveal some of the mysteries of animal behavior

A Genomic View of Animal Behavior

Why a dog—or a human for that matter—cuddles up with one individual but growls at another is one of life's great mysteries, one of the myriad quirks of behavior that has fascinated and frustrated scientists for centuries. Here's another: are we hard-wired to tend our young or culturally indoctrinated to have family values?

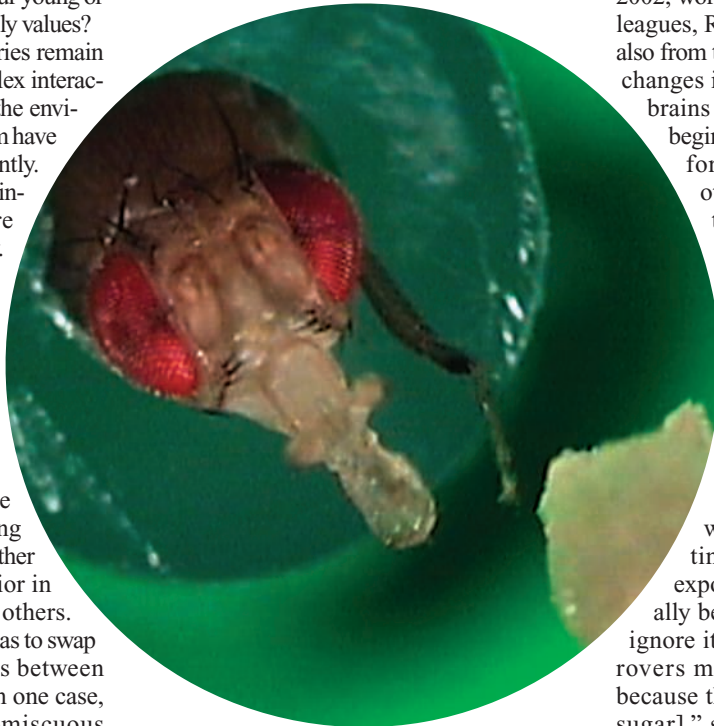
It's no surprise that such mysteries remain unsolved. They are rooted in complex interactions between multiple genes and the environment, and the tools to tackle them have largely been unavailable until recently. But behavioral researchers are beginning to apply techniques that are transforming other areas of biology. They are using microarrays—which can track hundreds or thousands of genes at once—to learn, for example, why some honey bees are hive workers and others are foragers, and what makes some male fish wimps and others machos.

They are also comparing the sequenced genomes of the growing menagerie of animals, probing whether genes known to influence behavior in one species play similar roles in others. Investigators have even gone so far as to swap gene-regulating DNA sequences between species with different lifestyles; in one case, they transformed normally promiscuous rodents into faithful partners.

While these comparative approaches are de rigueur for evolutionary biologists, they are something new for many neuroscientists and others who typically study behavior in a single model organism, says Gene Robinson, an entomologist at the University of Illinois, Urbana-Champaign, who is trying to encourage more crosstalk between disciplines. "There is this clear gulf between people who are using modern genetic techniques to study very specific questions and the people who are studying natural diversity," adds Steve Phelps from the University of Florida, Gainesville. But as more behavioral scientists take up the tools of genomics and comparative biology, the payoff may be a deeper understanding of the molecular basis of behavior in animals—even people—and how behaviors originally evolved. The field "is very ripe for a productive synthesis," says Phelps.

Foraging for genes

As gene sequencers turn their attention to deciphering the genomes of dozens of evolutionarily diverse species, a deluge of genome data is beginning to transform some aspects



Sweet "tooth." A gene that prompts roving in fruit flies also makes them more eager to sip sugar.

of behavioral science. Instead of just probing the minutiae of how a gene works in one organism, scientists are increasingly investigating how a particular gene operates in multiple species.

Take the story of a wanderlust gene studied by Marla Sokolowski of the University of Toronto, Ontario, Canada. Almost 25 years ago, Sokolowski and her colleagues discovered that a then unidentified gene, which they dubbed *forager* (*for*), controlled how much a fruit fly wandered. One variant of the gene makes a fly a more active forager—a "rover"—while another variant causes a fly to be less active, a "sitter." In 1997, her team finally cloned this gene, which codes for a protein called cGMP-dependent protein kinase (PKG), an important cell-signaling molecule.

(*Science*, 8 August 1997, pp. 763, 834) The rover variant turned out to generate higher quantities of the signaling protein.

This gene has recently proved key to feeding behavior in other invertebrates as well. In 2002, working with Sokolowski and her colleagues, Robinson and Yehuda Ben-Shahar, also from the University of Illinois, found that changes in the activity of *for* in honey bee brains prompted hive-bound workers to begin to change roles and start actively foraging for food. That same year, other researchers demonstrated that this gene influenced how likely nematodes were to explore their environment.

In the May-June 2004 issue of *Learning and Memory*, Sokolowski and her colleagues demonstrated that the *PKG* gene affects another behavior—how readily fruit flies respond to sugar. Rover flies are quick to extend their proboscis when exposed to sugar and continue to be stimulated by repeated exposure to sugar, while sitters gradually become used to the sweet stuff and ignore it, they reported. "It suggests that rovers may keep on searching for food because they don't [become indifferent to sugar]," says Sokolowski. This constant movement may be an evolutionary advantage for rovers in places where fruits and other foods are scattered.

Given the apparent importance of *for* in the behavior of fruit flies and other species, Sokolowski and Mark Fitzpatrick from the University of Toronto, have now looked across the animal kingdom for the gene and others related to it. They searched public gene databases, and earlier this year, in the February *Journal of Integrative and Comparative Biology*, they reported finding 32 *PKG* genes from 19 species, including green algae, hydra, pufferfish, and humans. The strong sequence conservation of the genes between many species hints that they may play a role in food-related behavior in many organisms. "By studying [*for*] in additional species, we will find out how it modulates foraging behavior in different evolutionary scenarios," says Sokolowski.

The buzz about microarrays

Comparative genomics is helping researchers pinpoint specific genes involved in some behaviors, but scientists are also using microarrays to cast a broader net. For example, Robinson, behavioral geneticist Charles Whitfield, and their colleagues at the University of Illinois are using these gene expression monitors to study honey bee behavior. They first used microarrays to look at the differences, beyond the *PKG* gene, that distinguish bees that tended the hives from bees that left the hive for pollen (*Science*, 10 October 2003, p. 296). Of the 5500 genes examined, they found 2200 whose brain activity varied between the two types of bees.

Now they have begun to tease out the role of the hive environment in stimulating “nurse” or “forager” genetic regimes—finding genes that help regulate the *PKG* gene’s activity. They raised newly emerged bees with no exposure to other bees, then used microarrays to test how certain chemicals known to change bee behavior alter the isolated insects’ genetic activity. Last year, Christina Grozinger, now at North Carolina State University in Raleigh, showed that a hormone produced by the queen bee shifted gene expression toward the nurse profile, possibly by suppressing the *for* gene. Ben-Shahar conducted a similar experiment using a hormone that promotes foraging behavior. About half of the genes in the isolated bees shifted in a forager-like direction—and those typically active in hive worker bees turned off.

“We had no genes going in the wrong direction,” says Whitfield. Now he and his colleagues are looking at gene expression patterns in bees that either build combs or remove dead bees from a hive. The effort may provide a handle on which genes might promote these construction and undertaker behaviors.

Neurobiologist Hans Hofmann of Har-



Social status. It takes many genes to transform hive workers into foragers.

vard University uses microarray technology to probe the behavior of fish. He’s investigating the genetic basis for the presence of studs and social outcasts among male cichlids. Some macho males sport bright colors, bully their peers, and court females. Others, the wimps, have small gonads and spend most of their time feeding or swimming in schools with other wimps. In certain circumstances, however, wimps become studs and vice versa, switches that seem to be driven by changing environments.

In the traditional approach, Hofmann would have tried to track individual genes involved in these transformations. Instead, he turned to microarrays and, in less than a year, has identified 100 genes that likely shape the male’s social status. Some are genes that Hofmann had expected to be involved, but others, such as a number for ion channels, were surprises. He and his colleagues are now looking more closely at cichlid brains for differences in expression patterns between genes identified in the array studies. “Some of these genes that we decided to follow up, we would not have looked at without this approach,” Hofmann notes.

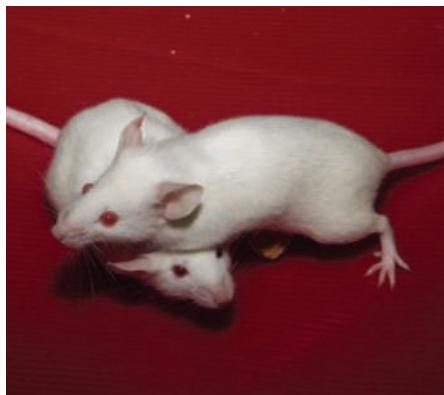
For both Robinson and Hofmann, microarrays have changed the way they investigate genes and behavior. In the pre-genomics era, both chased after candidate genes—those they had reason to suspect were important. But that tunnel vision “doesn’t give you a perspective of how many other [genes] are involved,” Whitfield explains.

Pathways to behavior

The genetic bounty provided by microarrays poses its own challenges, however. The devices can turn up many genes involved in even a simple behavior, and the molecules those genes encode need to be tied together into a logical pathway. Piecing together that jigsaw puzzle is no easy task.

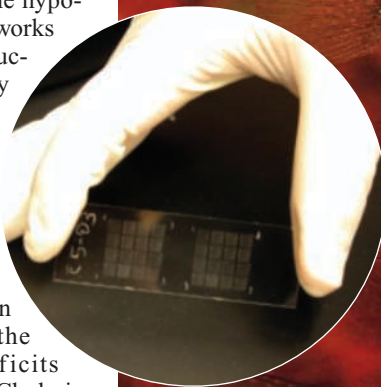
Elena Choleris from the University of Guelph has taken on that challenge and has worked out the relatively simple pathway underlying one behavioral response in a rodent. She, Martin Kavaliers at the University of Western Ontario, London, Canada, and Don Pfaff from Rockefeller University in New York have shown the genetic interactions necessary for one mouse to recognize another and to react in a friendly or unfriendly manner.

Researchers have known for several years that at least four proteins are involved in this process of social recognition: two estrogen receptors, located in different parts of the brain, and a neuropeptide, oxytocin, and its receptor. Choleris looked at the interplay of these molecules by breeding mutant mice lacking each component. In different groups of mice, she and her team disabled one of the genes encoding the receptors or oxytocin. No matter the genetic defect, the outcome was the same: The mutant mice couldn’t tell a familiar mouse from a stranger and were no longer worried about newcomers.



Close contact. Overly friendly mutant mice helped clarify the genetic pathway involved in reactions to strangers.

From additional experiments, Choleris has deduced some of the protein connections in what she calls a micronetwork, or micronet: One of the estrogen receptors controls oxytocin production in the hypothalamus, while the other receptor works in the amygdala to control the production of oxytocin's receptor. If any component of this micronet is interrupted, the whole pathway breaks down. The micronet exemplifies "how multiple genes act in parallel in an orchestrated manner between different systems and different brain areas," says Choleris. In the wild, a breakdown of this particular micronet and the resulting social recognition deficits could have powerful implications. Choleris and colleagues have recently found that her mutant mice have a diminished ability to sense and stay away from nearby mice carrying parasites, for example.



Beyond the gene

Microarrays are powerful tools for spotting genes that underlie different behaviors, but the way those genes are regulated may be just as important as the proteins they produce. Take the case of the prairie vole and the meadow vole.

The prairie vole (*Microtus ochrogaster*) is faithful to its mate; meadow voles (*Microtus pennsylvanicus*) are not. Yet the DNA sequence for vasopressin, the neuropeptide governing this trait, is the same in both species, as is the sequence of the gene for the hormone's receptor protein. There are, however, significant species differences in the number of brain receptors for vasopressin: Prairie voles have a lot more.



Mother's touch. Standoffish mother rats cause chemical changes in DNA bases that make pups timid adults.

In 1999, Larry Young, a neuroscientist at Emory University in Atlanta, Georgia, noticed that a regulatory region, a DNA sequence that sits at the beginning of the receptor gene, was longer in the monogamous species. When he put the prairie

All-out gene search. Microarrays (inset) are helping to uncover the genes that make some male cichlids more macho (lower fish) than others.

vole's vasopressin receptor gene and its regulatory region into mouse embryos, the resulting adult rodents were more faithful than is typical for that particular mouse species. The same has now proved true for meadow voles, he and his colleagues reported in the 17 June *Nature*. When he put the full prairie vole gene, including the regulatory region, for this receptor into meadow voles, males abandoned their promiscuous ways and began acting like faithful prairie voles.

Michael Meaney from McGill University in Montreal, Quebec, has found that a different regulatory region, called a promoter, is pivotal in another social relationship, the one between parents and their offspring. In the early 1990s, he and others had demonstrated that when a mother rat fails to lick and groom her newborn pups, those pups grow up timid and abnormally sensitive to stress.

The key seems to be methylation, a process in which DNA sequences are chemically modified by the addition of methyl groups to cytosine bases. This often suppresses the activity of a gene. Meaney's team discovered that in mice, a mother's behavior alters the typical methylation of the promoter for the gene for the glucocorticoid receptor in her offspring. In the brain, this receptor protein helps set off the cascade of gene expression that underlies the stress response.

Before birth, there's no methylation of this gene promoter. But in mice neglected by their mothers, the promoter is methy-

lated shortly after birth, Meaney and his colleagues reported in the 27 June online *Nature Neuroscience*. This increased methylation causes less of the receptor to be produced, creating anxious animals. And because DNA methylation tends to last the life of the animal, it could explain why the pups' personalities don't change as they mature, Meaney notes.

While most behavioral genetics researchers have concentrated on non-human species, some are now slowly venturing into the murky waters of human behavior. Meaney's team, for example, is following 200 mothers and their children, looking at the interplay between maternal care and activity in key genes in the offspring. "The extent to which researchers are finding similar patterns" between animals and people is quite promising, notes Stephen Suomi, a psychologist at the National Institute of Child Health and Human Development, Laboratory of Comparative Ethology, Bethesda, Maryland.

These patterns are prompting new research alliances. Genes can represent a common ground, increasing "the links between individuals interested in [neural] mechanisms and the people who are interested in behavior," explains Andrew Bass, a neuroethologist at Cornell University in Ithaca, New York. With this common ground will come a greater understanding of the brain as it relates to behavior, says Pfaff. And that, he adds, "is exciting to the nth degree."

—ELIZABETH PENNISI

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