What Makes Fruit Flies Roam?

After many years' work, a Canadian geneticist pins down a gene linked to differences in foraging behavior in fruit fly larvae and adults. It codes for a known cell-signaling protein

In 1976, Marla Sokolowski, then an undergraduate at the University of Toronto, began noticing that some of the fruit flies she was studying seemed a bit lazy. Some feeding fruit fly larvae would sup a little yeast paste, then wiggle in ever wider circles in search of more food, covering about 5 centimeters in a 10-minute period. But other, seemingly lazy, larvae did little roaming. Through breeding experiments, Sokolowski determined that these "sitter" variants, as she called them, and their more energetic "rover" cousins had different versions of a gene that she called *foraging* (*for*). Now, after 2 decades of searching, Sokolowski and her colleagues at York University in Toronto have finally uncovered the *for* gene.

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> **—James Truman**

On page 834, they report that they have tracked down its location and found that it is actually a gene called *dg2*, which codes for a protein that helps to relay chemical messages inside cells. Mutations leading to lower-than-normal levels of this protein, one of the enzymes known as cyclic GMP–dependent protein kinases (PKGs), cause the sitter behavior.

And these mutations aren't just a laboratory curiosity. Shortly after Sokolowski induced the sitter mutation into her lab fly larvae, she found that adult flies, including those in the wild, showed just as pronounced differences in their food-foraging behaviors. That she has now been able to use observed differences in a real-life behavior to track down the molecular underpinnings of that activity "is a first," comments Bambos Kyriacou, a behavioral geneticist at Leicester University in the United Kingdom.

The discovery that this "foraging" gene codes for a generic protein also points to a larger lesson. Researchers should not expect to find unique genes for behaviors, says neurogeneticist Ralph Greenspan, now at the Neurosciences Institute in San Diego. After all, a different set of generic messenger proteins, the cyclic AMP–dependent protein kinases, has turned out to play a role in learning and memory in both fruit flies and mammals. Behaviors, too, are likely to be shaped by genes that code for proteins with broad activities, affecting multiple traits.

"The organism [would be] crazy to use a gene for just one behavior," agrees Jeff Hall, a behavioral geneticist at Brandeis University in Waltham, Massachusetts. It's much more cost effective, so to speak, to use the same gene in different cells, at different times, and in combination with other genes to generate an organism's behavioral repertoire.

Because PKG proteins are widely distributed across species, similar genes are likely to be important in shaping behavior in other organisms, perhaps even in people. "This is not something that's going to just apply to insects," predicts James Truman, a neurobiologist at the University of Washington, Seattle.

Sokolowski had been slowly homing in on the *for* gene since 1981. She decided it was worth pursuing, she recalls, after finding that the behavior was linked to one gene and that 70% of the fruit flies she collected from local Toronto orchards were rovers, while the rest qualified as sitters. The persistence of both behaviors in natural populations suggested that each confers some benefit, and although Sokolowski did not know at the time what those benefits might be, she did know that she had a rare opportunity to explore the role of this gene in a behavior critical to the fly's survival.

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Sitter or rover? A change in a single gene determines whether a fruit fly larva, such as this one, will wander far in search of food or be a "sitter."

As a first step toward finding *for*, Sokolowski did a series of breeding experiments that tracked the gene to the left arm of fruit fly chromosome 2. Then, to further narrow down *for*'s location, Sokolowski and her colleagues developed a way to introduce an easy-to-trace mutation into the fly's chromosomes, one that was lethal once the fly larvae became pupae. By watching how often the lethal mutation was inherited together with sitter behavior, the group pinned down its quarry in a particular 150-kilobase section of the chromosome, in or near the location where other researchers had found the *dg2* gene. But Sokolowski still needed to prove that *for* and *dg2* are the same.

Help came in 1995. At the time, Kim Kaiser of the University of Glasgow in Scotland had been making mutant fruit flies by allowing a movable piece of DNA called a transposable element to insert randomly into their genomes. In one case, the transposable element had sneaked into the region that Sokolowski was studying. Kaiser offered to send the mutant strain to Sokolowski, who found that they acted like sitters. By using restriction enzymes to dissect that part of the chromosome, she was able to see that the insertion was right in the middle of the *dg2* gene, suggesting that *for* and *dg2* are one and the same. Further evidence for that came when Sokolowski's team removed the extra DNA from the gene. The fruit flies "reverted back to rovers," she says. That showed that "[Sokolowski] had the right gene," Kyriacou notes.

But Sokolowski herself was not convinced until her team inserted four extra copies of *dg2* into the nuclei of eggs from a sitter fruit fly. The larvae that developed from these eggs began moving around as if they were rovers, the group reports, suggesting that the level of gene expression is what influences the behavior.

Confirmation came from experiments in which the group teamed up with Greenspan and his colleagues at New York University to analyze the PKG activity in rover and sitter fruit flies and in the larvae with the extra genes. The activity levels were higher in the rovers than sitters, and the transgenic animals had levels similar to that of the rovers. "There was a nice correlation between behavior and the amount of PKG [activity]," says Sokolowski.

The activity levels didn't differ all that much, however. The sitters still retained about 75% of the PKG activity found in the rover fruit flies. "The enzymatic abnormalities are incredibly mild," Brandeis's Hall notes. But Greenspan and others say that's what you would expect for an important enzyme like PKG—flies with a serious deficit of the protein are unlikely to survive.

The researchers do not yet know exactly how the varying levels of PKG activity change foraging behavior, but other work suggests that the PKG signaling pathway affects the excitability of nerve cells. Higher levels of the enzyme could thus cause nerve cells involved in sensing food or in controlling foraging movements to fire more readily, increasing foraging behavior.

Meanwhile, other recent work by Sokolowski and her colleagues has pointed to why the sitter variant exists in wild populations. In her early experiments she had shown that sitters aren't really lazy. When there is no food, sitter larvae and adults wander just as far as rovers do to look for sustenance. The different behaviors come into play only after the flies have eaten, and results reported by the Sokolowski team in the 8 July issue of the *Proceedings of the National Academy of Sciences* help explain why.

Starting with wild populations of fruit flies, Sokolowski and her colleagues put mixtures of rovers and sitters into jars that contained either 50 or 800 to 1200 individuals. The researchers removed extra flies to keep those numbers steady for 73 generations. In that time, the ratio of rovers to sitters increased in the crowded jars and decreased in the uncrowded ones. Those changes make sense, says Sokolowski. Moving takes a lot of energy, so larvae that do not have fierce competition for food do better to move less, while those in crowded conditions gain an edge if they go out searching for food ahead of their jarmates. "It seems empirically that [the sitter behavior] has an adaptive significance," says Hall.

However, Sokolowski says that many questions remain to be answered. She still has to pin down the specific cells that control foraging behavior. In addition, the *dg2* gene produces three different versions of the enzyme, and it's not yet known whether just one or all three forms of the kinase are involved. Most likely, other, as yet unidentified genes will prove important to foraging behavior as well. But even with these unknowns, Sokolowski's colleagues salute her persistence. "She had the tenacity and whiz to stick with it," says Hall. "She's to be congratulated."

–**Elizabeth Pennisi**

PLANETARY SCIENCE

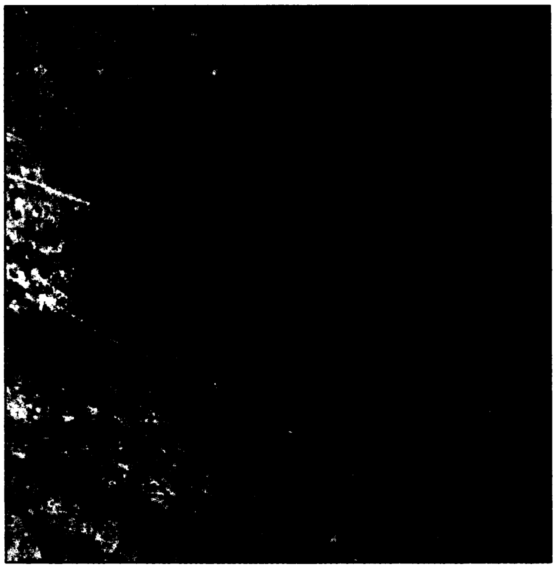
Once, Maybe Still, an Ocean on Europa

BOSTON—Does the mightiest ocean in the solar system lie beneath the ice-sheathed surface of Jupiter's moon Europa? Planetary scientists are piling up the evidence, and even the skeptics are now finding it hard to resist. Images of the moon's tortured surface returned by the Galileo spacecraft had persuaded some, but not all, researchers that an ocean tens of kilometers deep lies beneath the ice (*Science*, 18 April, p. 355). But the evidence was indirect, and skeptics argued that a solid layer of ice slightly warmed by Europa's internal heat could mimic the effects of a deep ocean by slowly churning, reshaping the surface without ever melting.

At last week's annual meeting here of the Division for Planetary Sciences of the American Astronomical Society, however, researchers presented new analyses of Europa's surface scars that had even the skeptics agreeing that, either now or in the recent past, an ocean stirred just beneath the icy surface. "It's likely that at the time that the surface we see formed, there was an ocean," says Robert Pappalardo of Brown University, a Galileo team member and self-described skeptic. That may not have been so long ago, he says, judging from the fresh appearance of the surface.

A deep pool of seawater on another world is sure to nurture speculations about the possibility of life there. But Pappalardo stresses that Europa's deep waters could have frozen up since they left their traces on the surface: "I'm still a skeptic [about a present-day ocean]; we have to prove it by some direct technique." That may be more than Galileo can deliver, even when it shifts its orbit about Jupiter late this year to begin an extended mission devoted to Europa.

Making even the skeptics edge toward tentative belief in an ocean are geologic features such as fractures pulled open in the ice, stress cracks formed from the weight of ridges, and disrupted crustal blocks. The sizes and distributions of these features imply that when the features formed, the brittle outer crust of ice was less than 6 kilometers thick—and perhaps as thin as a few hundred meters, says Pappalardo. Below this thin, brittle crust was presumably a layer of warmer, and therefore ductile, churning ice, he says, which implies that temperatures were rising so sharply from the surface into the interior that liquid water would have been found not much farther down.



JPL/NASA

Telltale blemish. The debris-ringed 140-kilometer impact scar Tyre (in false color) is almost perfectly flat, suggesting it formed on a thin skin of ice floating on water.

Supporting the idea that the ductile ice beneath the surface gave way in turn to liquid water is a different set of features: 7- to 15-kilometer-wide pits, domes, and spots. James Head of Brown, Pappalardo, and their Galileo teammates argue that heat-driven convection in the ductile ice beneath the surface is responsible for these blemishes. Plumes of warm, rising ice hit the underside of the cold, brittle surface layer, they say, pushing the surface upward to form a dome. Plumes that carried enough heat could have caused some of the surface ice to sublimate away or melt, forming pits and spots.

If all these features do share a common origin, their spacing—every 5 to 20 kilometers, on average—says something about the thickness of the ductile ice layer. Convection tends to space its rising or falling plumes about as far apart as the depth of the convecting layer, the researchers note. The 20 kilometers of ice inferred from the spacing would leave plenty of room for liquid water, because the subtle gravity variations Galileo detected during its Europa flybys imply that the water or ice layer sheathing the moon's rock core is some 150 kilometers thick.

Places where large impacts have battered Europa's icy shell also point to a deep ocean beneath. At the meeting, Jeffrey Moore of NASA's Ames Research Center in Mountain View, California, and his colleagues argued that some of the so-called maculae of Europa—huge dark splotches 60 to 140 kilometers across—are debris-encircled craters produced by asteroid or comet impacts. Unlike the high-rimmed, bowl-shaped impact craters that form in solid rock, maculae are flat—the 45-kilometer crater Callanish, for example, is roughened by just 100-meter variations in the height of the surface. The lack of relief implies that Europa's icy surface was weak, and therefore