Bisexual Bugs

Added DNA changes fruit fly behavior, stirs up controversy

By JOHN TRAVIS

Andy Warhol once said that everyone would enjoy 15 minutes of fame, but even he could not have predicted the notoriety surrounding some fruit flies now frolicking in a laboratory on the National Institutes of Health campus in Bethesda, Md. These *Drosophila melanogaster* bask in Warholian celebrity for a reason that seems at first glance more like talk-show fodder than a topic of serious scientific discussion.

In this group of six-legged critters, says Ward F. Odenwald of NIH's National Institute of Neurological Disorders and Stroke, "the males are bisexual."

That observation, combined with the fact that Odenwald and his colleague Shang-Ding Zhang produced the abnormal sexual behavior by manipulating the genes of the flies, garnered widespread media attention last month. To Odenwald's dismay, TIME magazine trumpeted the story under the headline "Search for a Gay Gene" the day before his research appeared in the June 6 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

"What does this say about the origins of homosexuality?" the magazine's provocative subhead continued.

Probably nothing, say *Drosophila* researchers upset about what they assert is a misguided attempt to relate the research to the controversial issue of what determines human sexual orientation.

"There's so many speculations built upon speculations. I think people have to be careful about what inferences they draw from this work," says Ralph Greenspan of New York University, who studies the regions of the brain involved in the courtship behavior of *Drosophila*. "The relevance to humans is not something one can assume. I'm very cautious about making human conclusions from fruit flies. The fly brain has virtually no anatomical homology to the human."

The story of NIH's bisexual bugs began not as a search for a gay gene, but as an inquiry into how the fruit fly's central nervous system develops. Because it breeds quickly, the insect is a popular organism for biologists to study.

Odenwald and Zhang planned to examine the role of a gene called *pollux*. This gene, researchers believe, directs cells to produce a protein critical to the maintenance of axon fascicles, the long, winding cables that nerve cells use to transmit messages.

The NIH duo wondered what would happen if they disturbed the gene's normal schedule of activity, making it express its protein at inappropriate times. "We thought by misexpressing *pollux* we could learn something about its function," says Odenwald.

To achieve that, he and Zhang created an artificial genetic sequence that couples *pollux* to other strips of DNA. A crucial part of this construct is a span of DNA that turns on after exposure to high temperatures.

By linking this so-called heat shock promoter to *pollux* and inserting the combination into the genome of *Drosophila*, the NIH researchers would be able to turn on *pollux* by exposing the flies to heat. With this technique, they hoped to activate *pollux* at different times during the fruit flies' development. Over the last decade, many researchers have taken a similar approach to studying other genes, notes Odenwald.

The insects' mating mischief started after he and Zhang took their genetically engineered flies, housed in large bottles, and warmed the insects to 37°C for an hour. Afterwards, says Odenwald, "we began to see very long courtship chains traversing throughout our bottles." Some chains even turned back on themselves, forming long-lasting circles or intertwined chains of fruit flies.

Oldenwald and Zhang

Largely shunning females, male fruit flies form courtship chains (left) and circles when they misexpress a gene called white. *If the gene is not expressed at all, the insect's eyes are white instead of red.*

That in itself was unusual. In normal mating, says *Drosophila* researcher Laurie Tompkins of Temple University in Philadelphia, a courting male fruit fly pursues a female, rubbing his genitals upon her from behind, licking her, and singing a "love song" by vibrating his wings.

Another male might tag along behind, in case the female rejects the first suitor by kicking his face, flicking her wings disdainfully, or running away. These triples are relatively common, she says, but larger courtship groups are rare and never last long.

When Odenwald and Zhang took a closer look at their frenzied insects, a bigger surprise emerged. The lead flies in most of the chains were male. In fact, almost all the courting behavior they observed involved only males. Females avoided the male-dominated courtship chains, tending instead to cluster at the tops and bottoms of the bottles.

For almost a year, the two researchers tried to puzzle out the cause of the unusual sexual behavior. "We were dead certain it had to be *pollux*," says Odenwald, because no one had reported similar behavior in other transgenic flies. But when they completed another experiment, in which they added to *Drosophila* a genetic construct that obstructs rather than promotes the manufacture of *pollux*'s protein, they still documented male-male courtship.

Eventually, Odenwald and Zhang realized that *pollux* was a red herring. Like many other researchers, they had included in their construct a shortened version of a gene called *white*. This marker gene provides an easy visual proof that a construct has successfully integrated itself into a fly's genome, because the gene codes for a protein that helps make the insect's eyes red instead of white.

With few alternatives left, the NIH scientists theorized that misexpression of *white*, not *pollux*, had generated the unexpected sexual behavior. They ultimately confirmed the hypothesis when they added only *white* to *Drosophila*, which produced male-male courtship, and then fed them a chemical to disrupt the gene's expression, which eliminated the behavior.

Despite this proof, the identification of *white* as the culprit shocked Odenwald and Zhang. The gene is one of the best-known in *Drosophila* research. In 1910, Thomas Morgan, who pioneered the study of genetics in the fruit fly, described a natural mutant strain that he called white because the insects lacked pigmentation and had colorless eyes. Geneticists eventually discovered that the strain stems from a flaw in a gene, which they also called *white*.

From studying the white fruit fly strain, researchers concluded that the gene aids pigment production in a few types of cells, such as eye cells. In their experiments, Odenwald and Zhang apparently "turned on the gene all over the fruit fly," says Jeffrey Hall, who studies the genetics of *Drosophila* sexual behavior at Brandeis University in Waltham, Mass.

One clear message from the research, says Odenwald, is that "everyone should be very leery of the second gene they have in their construct."

That bit of advice, however, is not likely to be as controversial as the issues of how misexpression of *white* generates male-male courtship and what that might mean for other organisms. Hall, for instance, praises the observations of Odenwald and Zhang but dismisses any suggestion that *white*'s normal function sways sexual behavior in fruit flies, let alone higher organisms.

"It's completely silly. Nobody between now and doomsday will think *white* is going to have anything to do with behavior in mammals. The chance of this is 1 over the number of neutrons in the universe," he says.

First, as Hall points out and Odenwald and Zhang take pains to stress, the male fruit flies display bisexuality, not homosexuality. They seem perfectly willing to court females when given the chance. Second, no one has studied whether the males sing their love songs to other males, a courtship ritual integral to the flies' normal sexual behavior. Moreover, the misexpression of *white* does not trigger unusual sexual behavior among female flies; in other words, they do not become lesbians or bisexuals.

Tompkins chimes in with the observation that *white* misexpression may not selectively affect sexual behavior. "As far as we know, a lot of their behaviors are screwed up," she says.

Still, Odenwald and Zhang can be held somewhat responsible for the media buzz connecting their research to human homosexuality. In their report, they outline a path of "complete speculation," as Odenwald freely calls it, that links *white* to possible chemical changes in the mammalian brain that might influence sexual behavior.

The two note that the protein produced by *white* appears to join with other proteins to distribute the amino acid tryptophan and other molecules into cells or into compartments inside cells. While tryptophan is needed to generate red pigment, Odenwald and Zhang point out that it can play another role as a precursor of serotonin, a chemical essential to the transmission of messages in the mammalian brain.

As a result, they wonder out loud, could the misexpression of *white* lower the amount of serotonin in the brain cells of the fruit fly? That would be interesting, they observe, because a few pharmacological studies in the early 1970s found that "depletion of tryptophan in rats and rabbits lowers serotonin levels and triggers male homosexual mounting behavior." Studies around the same time also showed that a drop in serotonin caused homosexual behavior in cats, they add.

To finally bring the issue around to humans, Odenwald and Zhang cite work by James Croop of the Dana Farber Cancer Institute in Boston, who has found a gene in people that bears some similarity to the fruit fly's *white* gene.

The *white*-homosexuality hypothesis rests more on questions than on facts, however. No one knows, says Croop, what his human gene does or whether it's active in the brain. The same holds true in fruit flies as well, says Hall. No one has studied whether the insects' brain cells express *white*.

Odenwald and Zhang's speculation has another hole: They did not attempt to discern whether *white* influences serotonin concentration in fruit flies. Even if it does, *Drosophila* researchers have no data supporting the idea that serotonin functions similarly in the very dissimilar brains of humans and insects. In fact, they know little about serotonin's role in fruit flies.

"We know much more about what serotonin does in the human brain," says Hall, who studies a gene called *fruitless*. (When mutated, *fruitless* causes male fruit flies to court only other males. Researchers have shown that *fruitless* functions in the insect brain.)

In any case, speculation about serotonin remains moot for the moment, because no one knows how the altered sexual behavior comes about. Some researchers suggest that genetic manipulation disturbs the production of chemicals, called pheromones, that males produce to signal other males that they are not female.

Tompkins doubts that possibility, however, since the genetically manipulated male flies also pursue unaltered males, which presumably exude the repelling pheromones. She wonders, instead, if *white* misexpression changes the way the male insect brain interprets the male pheromones. In essence, the male fly may not get the hint to shove off. One point against that hypothesis is that unaltered male fruit flies, after some hesitation, join in the sexual chains and other male-male courtship behavior when placed with their genetically manipulated kin.

Though *Drosophila* researchers shy away from linking *white* to sexual behavior in humans, they are eager to get their hands on the insects created by Odenwald and Zhang. Tompkins hopes to investigate the role of pheromones in the bugs. Jay Hirsh at the University of Virginia in Charlottesville plans to examine their serotonin.

Hall cannot wait to compare them with his *fruitless* insects. He also wants to place them in a specially designed instrument that will let him listen in on the male-male courtship. "Do they sing at all, and what is the nature of the singing?" he asks.

Only further study will determine whether Odenwald and Zhang's bisexual bugs are important or much ado about nothing. "It could be an interesting biological process they've stumbled upon, but we just don't know," says Greenspan. □