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Mutant Fly Hints at Evolution of Carbon Dioxide Sensing

For humans, carbon dioxide (CO₂)

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) is an odorless, tasteless gas that our sensory organs cannot detect. In the insect world, however, CO₂

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- S. Lawrence Zipursky

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is an important mode of communication, and it means different things to different bugs. Mosquitoes sense the CO₂

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that seeps from the skin of animals, homing in on it to find the source of their blood meals. Fruit flies, on the other hand, secrete it themselves as part of an odor that warns other flies to flee in the face of danger.

Now, Howard Hughes Medical Institute (HHMI) researchers have discovered a mutant fruit fly whose misguided nerve cells are providing scientists with a fascinating look at the evolution of systems that detect this ubiquitous gas.

Insects detect CO₂ using specialized neurons located in their olfactory system. Fruit flies normally have the CO₂-sensing neurons only in their antennae, while blood suckers like mosquitoes and tsetse flies house them in

the other half of their “noses”--a structure near their mouthparts called the maxillary palp.

While examining flies under a microscope for an unrelated study, the researchers, led by S. Lawrence Zipursky, an HHMI investigator at the University of California, Los Angeles, noticed something amiss--one of their flies had what looked like CO₂-sensing neurons in its antennae and maxillary palp. In the February 29, 2008, issue of the journal *Science*, Zipursky, his postdoctoral fellows Pelin Cayirlioglu, Ilona Grunwald Kadow, and Xiaoli Zhan, and their colleagues report on the identification of a genetic mutation that causes this curious developmental mixup and its implications for the evolution of CO₂-sensing in insects.

The CO₂-sensing neurons in the antennae of the mutant flies were normal, said Zipursky. As expected, they expressed only CO₂ receptors and made connections with the area of the fly's brain that is known to be associated with CO₂ sensing. However, he said, the strange neurons in the maxillary palp “weren't actually kosher.” According to Zipursky, “They also expressed some receptors that *are* typically expressed in the maxillary palps,” that smell other things like food. In addition, he said, they made connections to the brain both at the same place as the CO₂-sensing neurons, and at a location usually associated with the other olfactory neurons generally found in the maxillary palps. “So they weren't CO₂ neurons, and they weren't typical maxillary palp neurons. They were something in between,” he said.

The hybrid neurons were the result of a genetic mutation, which the team tracked to a short stretch of DNA that encoded not a protein, but a molecule known as a micro RNA. Micro RNAs bind to and silence the RNA copy of another gene so it cannot be made into a functional protein. Zipursky's team found that a specific microRNA, *miR-279*, normally interferes with production of a protein called nerfin-1, which itself regulates the transcription of genes in neurons. When *miR-279* malfunctioned, nerfin-1 became activated in the maxillary palp cells, and the fly developed CO₂ receptors in an area it wasn't supposed to have them. According to Zipursky, presumably these receptors developed only in this one errant region because of similarities in the genetic programs that regulate the development of maxillary palp olfactory neurons and CO₂ neurons in the antennae.

The team was intrigued by the way this simple genetic mutation turned a very specialized neuron with only one type of receptor into a more generalized neuron with two types of receptors. And that made the researchers wonder if they were witnessing evolution in reverse. “It makes a very interesting story,” Zipursky said. “You could imagine that there was an evolutionary intermediate,” during the evolution of insects' CO₂ sensory apparatus, he

said. That cell might have looked just like the one they found: one that expressed both a CO₂ receptor and another type of maxillary palp receptor, and then connected to two different regions of the fly's brain, said Zipursky.

Over time, he said, this neuron would have evolved to become more specialized. Depending on whether it was in the antennae or in the maxillary palp, it would have lost either its CO₂ or olfactory receptors, and it would have restricted its connections to one area of the brain. These changes could well have helped determine whether the modern fly's ancestor was attracted to the scent of CO₂ or repelled by it.

According to Zipursky, the microRNA that was turned off in their mutant may have evolved as part of this process. However, he stressed, other steps must have happened, as well. For instance, he said, other genes must specify that CO₂ receptors form in the fruit fly's antennae—their normal location.

Zipursky said that one of their experiments confirmed that these hybrid neurons do send signals to the brain when exposed to CO₂. But, he said, no one has yet looked at whether the odd way these neurons connect to the brain is enough to change a fly's behavior—perhaps attracting them to CO₂ like a mosquito—or whether other changes are required. “It's a great question,” he said.

According to Zipursky, his group didn't set out to tease apart the evolution of the CO₂ sensory system; their hybrid fly was a serendipitous find that Cayirlioglu, Grunwald Kadow, and Zhan made while looking for mutants that might help them understand how the olfactory system is wired in flies. When they realized that their mutant regulated the type of neurons that formed in the maxillary palp rather than specifying wiring patterns of olfactory receptor neurons Zipursky said he discouraged his postdocs from investigating further. “I was skeptical about what we'd learn from it. I said, ‘We're interested in wiring...and this is clearly not a wiring mutant,’” he said. “It's not something I thought I was particularly interested in.

“But they were much more imaginative than me,” he said. “They came to me and said, ‘It really is an interesting issue from an evolutionary perspective’... and they pushed very hard to pursue it,” he chuckled. “I'm glad they did; it came to be a beautiful paper and a beautiful understanding.”