# How drones find queens: Odorant receptor for queen pheromone identified

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The mating ritual of the honey bee is a mysterious affair, occurring at dizzying heights in zones identifiable only to a queen and the horde of drones that court her. Now a research team led by the University of Illinois has identified an odorant receptor that allows male drones to find a queen in flight. The receptor, on the male antennae, can detect an available queen up to 60 meters away.

This is the first time an odorant receptor has been linked to a specific pheromone in honey bees. The findings appear in the *Proceedings of the National Academy of Sciences*.

The "queen substance," or "queen retinue pheromone," was first identified decades ago, but scientists have only recently begun to understand its structure and role in the hive. The pheromone is a primary source of the queen's authority. It is made up of eight components, one of which, 9-oxo-2-decenoic acid (9-ODA), attracts the drones during mating flights. It also draws workers to the queen and retards their reproductive growth.

Principal investigator Hugh Robertson, a professor of entomology, said the research team pursued the receptor for the queen retinue pheromone because it was the "lowest hanging fruit" of the known honey bee odorant receptors. Robertson was among the research group that last year published the entire honey bee genome, a feat that allowed his lab to identify 170 odorant receptors in honey bees.

Robertson and his colleagues knew that male drones probably had little use for most of these receptors. The drones don't forage and so do not need to detect the subtle scents of flowers. Their social role within the hive is virtually non-existent. They have only one task: to find and mate with a queen. Once they have accomplished this, they die.

Using a functional genomics approach, entomology postdoctoral researcher Kevin Wanner was able to determine which odorant receptors were more dominant in males than females. He found four receptors that were expressed in much higher quantities in males than females.

"These proteins are expressed in the membranes of the olfactory neurons way up in the tips of these little sensilla in the antennae of these males," Robertson said. "A neuron goes all the way from there to the brain. Now the brain gets a signal that says, 'I've smelled this chemical.' If the chemical is 9-ODA, for the drone that means one thing and one thing only: 'There's a queen somewhere! Go get her!' "

Determining which of the four primary receptors in males was actually responding to 9-ODA was a formidable challenge.

"That's where we were very, very lucky," Robertson said.

By chance, at a conference on the science of olfaction, Wanner met Charles Luetje, a neuroscientist at the University of Miami who had expertise with precisely this type of problem. Luetje had perfected a technique for expressing mammalian odor-sensing receptors on the outer membranes of frog oocytes (eggs) and testing them to see which compounds activated them. When he heard of Wanner's work in honey bees, Luetje offered to use this technique to test the four primary odor receptors of honey bee drones.

After refining and testing the technique in insects, Luetje's graduate student Andrew Nichols exposed each of the drone odorant receptors to 9-ODA. Only one of the four receptors responded. When it bound 9-ODA, the protein receptor's conformation changed, setting off a measurable shift in the membrane potential.

None of the four primary male odorant receptors responded to the other components of the queen pheromone. Only the 9-ODA elicited a response in one of the four, said Robertson, a discovery he called, "thrilling."

"We grabbed the lowest hanging fruit and we got it," Robertson said. "Of course, ultimately, we've got another 169 receptors to go."

Scientists have spent decades exploring the mysteries of insect smell, but the newest tools make such research much more promising, Robertson said.

"Like so many biologists, we are wonderfully caught up in the genomic revolution," he said. "We can sequence genomes. We can use functional genomics to narrow it down. We've got these assays, such as the frog oocyte, and other assays. And the genomic revolution has opened up this black box of the molecular biology of insect smell. Finally now we can peer inside."

Source: University of Illinois at Urbana-Champaign

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