

Mosquitoes use several different kinds of odor sensors to track human prey

August 31 2010



Mosquito. Photo: MAP.

It now appears that the malaria mosquito relies on a battery of different types of odor sensors to mediate its most critical behaviors, including how to choose and locate their blood-meal hosts. In an article to be published next week in the online, open access journal *PLoS Biology*, researchers at Vanderbilt University have characterized two families of molecular odorant sensors in *Anopheles gambiae*, the mosquito responsible for the majority of human malaria transmission.

It now appears that the malaria mosquito needs more than one family of odor sensors to sniff out its human prey.

That is the implication of new research into the mosquito's sense of smell published in the Aug. 31 issue of the online, open-access journal

Public Library of Science Biology.

The experiments described in the paper provide striking new evidence that *Anopheles gambiae* - the species of mosquito that spreads malaria that infects some 250 million and kills 900,000 people annually - has a second set of olfactory sensors that are fundamentally different from the set of sensors that scientists have known about and have been studying for the last 10 years.

The discovery may help explain a puzzling question that has been plaguing scientists trying to develop new and more effective forms of mosquito lures and repellents:

"The ORs [odorant receptors] that were identified in the lab before don't respond to a lot of human odors," says Vanderbilt graduate student Chao Liu, who is the lead author on the paper. "Now that we have a new set of receptors, we may be able to fill in the picture."

There is a good chance that this new set of receptors may be specifically tuned to detect a number of the odorants given off by humans, adds co-author R. Jason Pitts, a senior research specialist and graduate student at Vanderbilt. "If this is the case then it is quite likely that it will play a critical role in attempts to develop improved lures and repellents to control the spread of malaria."

According to Pitts, they also have preliminary evidence that the mosquito's olfactory system may include additional families of sensors as well.

Vanderbilt Professor of Biological Sciences and Pharmacology Laurence Zwiebel, who was the principal investigator on the study, heads a major interdisciplinary research project to develop new ways to control by spread of malaria based on mosquito olfaction supported by the Grand

Challenges in Global Health Initiative funded by the Foundation for NIH through a grant from the Bill & Melinda Gates Foundation.

"It's not at all surprising that the mosquito's olfactory system is more sophisticated than we thought," says Zwiebel. "Olfaction is absolutely essential to the mosquito. If the female cannot find a host for a blood meal she cannot reproduce. As a result, mosquitoes have developed an uncanny ability to detect odors. This is true of all species of mosquitoes, not just Anopheles. So it is highly likely that the mosquitoes that spread West Nile, dengue fever, yellow fever and encephalitis also have similar sets of odor sensors."

About ten years ago, when the mosquito genome was first sequenced, scientists at Vanderbilt and Yale identified the genes and the structure of one set of Anopheles sensors, called odorant receptors (AgORs). At first, they thought that these receptors had the same basic design as the sensors found in the nose of humans and other mammals. But recent studies have found that the mosquito receptors, along with those of several other insects, have a distinctly different structure.

Researchers have identified about 75 different AgORs that respond to a variety of volatile compounds. These receptors are expressed on the surface of nerves located in tiny hollow spikes, called sensilla, located on the mosquito's antennae. When a target molecule wafts into the interior of one of these sensilla and comes into contact with the AgOR designed to detect it, the receptor causes the nerve to fire, signaling the compound's presence.

Earlier this year the Vanderbilt researchers and their colleagues at Yale succeeded in pairing more than 40 of the AgORs with the specific odorants that trigger them. In the process, the researchers discovered that these receptors are broadly tuned. That is, each receptor responds to a number of different compounds. They also overlap. More than one

AgOR responds to individual odorants.

In the new study, Liu and Pitts combined gene-silencing techniques with a new behavioral assay to confirm that DEET, the most commonly used commercial insect repellent, activates of a specific AgOR. Although the synthetic compound appears to effect mosquitoes in several different ways, there is no doubt that this olfactory effect explains much of its effectiveness as a repellent, the scientists say.

Despite all their efforts, however, mosquito researchers have been unable to find AgORs that react to a number of key human odorants, including ammonia, lactic acid and butylamine, all of which are given off by human sweat or breath. Scientists know that the mosquito can detect these compounds: When they "wire" the entire Anopheles antenna they can measure nerve activity when the antenna is exposed to these compounds. The researchers have traced the nerves that respond to several of these human odorants to a specific type of sensilla, called grooved pegs, which seem to lack AgORs.

As a result, last year when scientists at Rockefeller University announced they had discovered a second set of olfactory receptors in the fruit fly *Drosophila melanogaster*, an animal model for basic genetics, "it was like a light switched on," says Pitts. Because of the many parallels between the olfactory systems of the fruit fly and mosquito, the Vanderbilt researchers knew it was extremely likely that the mosquito had a second set of receptors as well. So they began searching for them.

The search was successful and the researchers identified genes that code for about 50 versions of the new type receptor. The new receptors appear to have a slightly different structure from that of AgORs: They are called "ionotropic receptors" (AgIRs) and they closely resemble the type of receptor found in the brain that responds to the common neurotransmitter, glutamate.

At this point, the researchers can only speculate about what effect this structural difference has on the way that the AgIRs function as odor detectors. However, they have managed to associate an AgIR with butylamine, a human odorant that AgORs do not appear to identify. Butylamine sensitivity is located in grooved peg sensilla, a type of sensory hair on the mosquito antennae. The correlation of AgIR to butylamine could indicate that AgIRs are responsible for grooved peg sensilla sensitivities to other human odors such as ammonia and lactic acid, an idea that the Zwiebel Lab has begun exploring.

The basic problem facing the mosquito searching for human prey - and the humans who are trying to figure out how it does it - is that none of the hundreds of odors given off by humans are necessarily unique. They are actually produced by the bacteria that live on human skin. But these bacteria live on other animals as well. So the current theory is that mosquitoes must identify a blend of different odorants that provide a unique signature for humans. Determining the way that the AgIRs work may be the key to identifying such a signature and that, in turn, could be the key to developing non-toxic, ecologically benign methods for combating malaria and other mosquito-borne illnesses.

More information: Liu C, Pitts RJ, Bohbot JD, Jones PL, Wang G, et al. (2010) Distinct Olfactory Signaling Mechanisms in the Malaria Vector Mosquito *Anopheles gambiae*. PLoS Biol 8(8): e1000467. [doi:10.1371/journal.pbio.1000467](https://doi.org/10.1371/journal.pbio.1000467)

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