Mosquito sperm have 'sense of smell'

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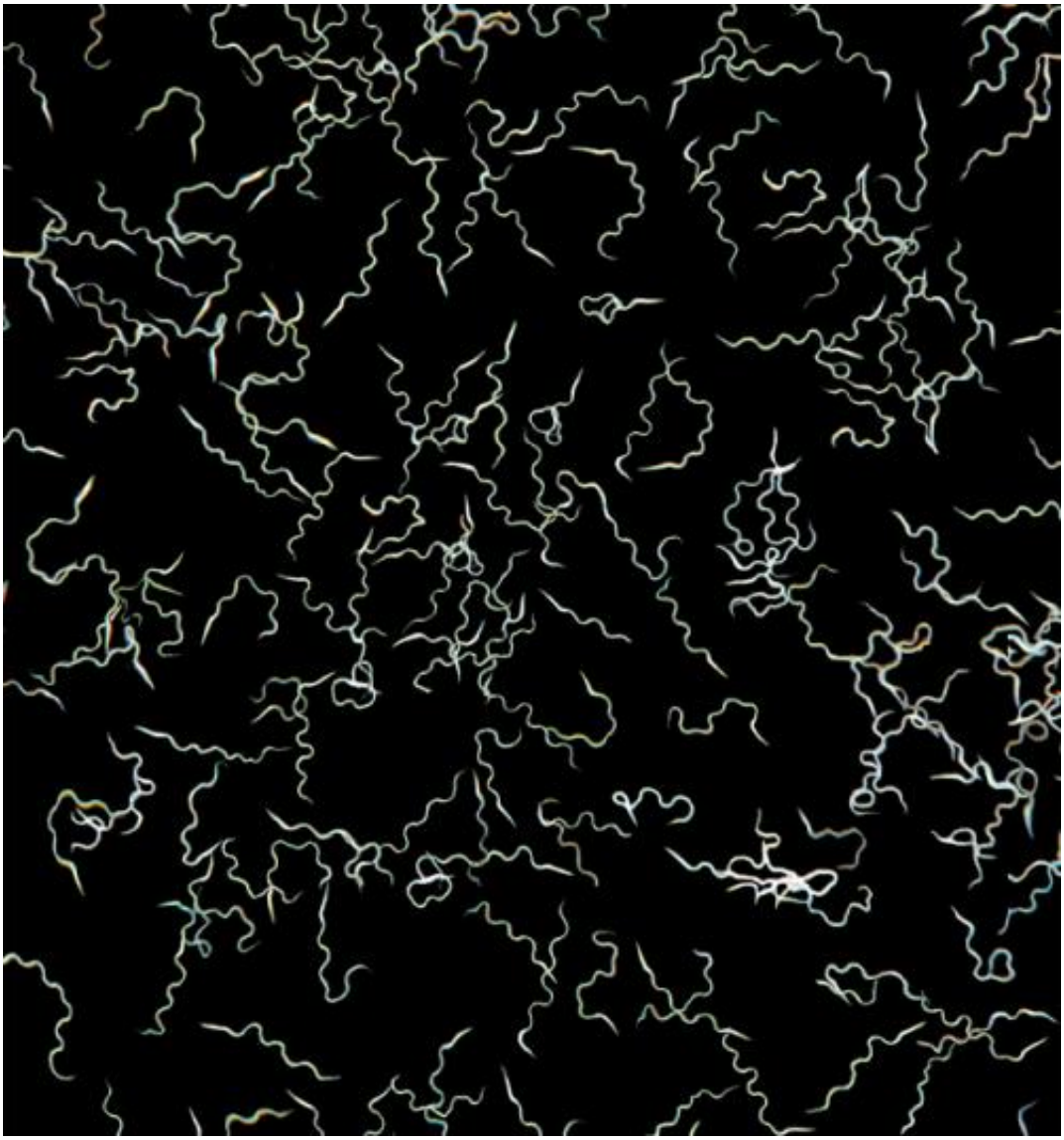

This is a photomicrograph of the sperm of the mosquito *Aedes aegypti* magnified 50 times. Mosquito sperm is substantially larger than human sperm. Credit: Jason Pitts, Vanderbilt University

Vanderbilt biologists have discovered that mosquito sperm have a "sense of smell" and that some of same chemicals that the mosquito can smell cause the sperm to swim harder.

This unexpected discovery is reported in an article published in the online Early Edition of the *Proceedings of the National Academy of Sciences* for the week of Feb. 3 by a team of Vanderbilt University biologists.

The scientists report that they have detected a suite of specialized chemical sensors called odorant receptors (ORs) in mosquito sperm. These are the same as the sensors that play a central role in the mosquito's olfactory system, which is found on the insect's antennae.The researchers found that the odorant receptors in the sperm are expressed along their tails where they drive the rapid increase in the movement (beating) of the sperm tails.

"This discovery is really 'out of the box' for us," said L.J. Zwiebel, the Cornelius Vanderbilt Chair in Biological Sciences who directed the study. "It is the first time that insect ORs have been found to function in non-sensory cells or tissue. We think this could be an entirely new paradigm for how insect reproduction is regulated. If it is, it could provide a powerful new approach for controlling populations of insects of medical and/or economic importance."

In nature, evolution often reuses successful structures after they have arisen. In this case, it is likely that the ORs evolved first in the reproductive system and then were used to form the basis of the mosquito's complex adult olfactory system. If this is the case, then it is also likely that the use of insect odorant receptors to modulate sperm behavior is a fundamental aspect of insect biology.

Female mosquitoes, which live for about a month, only mate once. They

store the male's sperm in special organs called spermathecae. After mating, females require a blood meal to get the basic compounds they need to produce eggs. That is why they bite humans and other animals and, in so doing, act as vectors for globally important diseases such as malaria and Dengue fever. Once the eggs have developed, they are fertilized by the sperm stored within the female's reproductive tract.

"The sperm may need a chemical signal to become ready for fertilization," said Research Assistant Professor Jason Pitts. "There are reports that within one day after insemination, the sperm begin swimming around in the spermathecae. There must be one or more signals that activate this movement and our findings suggest that odorant receptors may be the sensor that receives these signals."

Pitts, graduate student Chao Liu, and postdoctoral fellow Xiaofan Zhou are co-first authors of the paper.

The origin of the discovery was an observation the researchers made several years ago as part of their research into the olfaction system of the malaria mosquito Anopheles gambiae. They found unusually high expression of a group of odorant receptors in the bodies of male mosquitoes. This caught the researchers' attention because they were receptors that females use so they didn't expect them to be enhanced in males. It took them several years to follow up on the observation. When they did, they tracked these receptors to the males' testes and ultimately to the sperm themselves.

The finding sparked their interest further because of controversial research that has reported finding olfactory receptors in human sperm. "Evidence for the presence of these receptors in human sperm is very solid. What is controversial is whether they play any role in human reproduction," Pitts said.

Because of ongoing research that the Vanderbilt researchers have been conducting aimed at discovering new and more effective mosquito repellents, they had developed the tools they needed to determine if the odorant receptors in the mosquito sperm were functional. In particular, they had identified specific chemical compounds that specifically activate insect odorant receptors as well as others that prevent them from activating.

Liu and Zhou used these compounds to design a novel video-based bioassay. They found when the mosquito sperm were exposed to odorant receptor activators as well as chemical cues like fenchone, a natural organic compound found in fennel, the sperm tails started beating much more frequently. However, the sperm did not respond to the same compounds when they were simultaneously exposed to an agent that blocks the odorant receptors. The researchers also showed the ability to activate sperm beating was absent in a mutant strain of *Aedes aegypti* mosquitoes that are genetically altered to lack functional odorant receptors.

"This provides compelling evidence that the odorant receptors are involved in the reproduction process," said Zwiebel. Their tests also found that cyclic-AMP, which has been shown to cause sperm beating in mammals, also increased the rate of beating in mosquito sperm. But its effect was not prevented by the OR blockers, indicating that it was linked to a different set of receptors in the sperm.

"We know there is a lot more going on. We have just scratched the surface," said Zwiebel.

The researchers have also tested three additional insect species – the Asian Tiger mosquito *Aedes albopictus*, the fruit fly *Drosophila melanogaster*, and the jewel wasp *Nasonia vitripennis* – and found that their sperm also contain odorant receptors. According to the researchers,

this suggests that ORs have a general function in reproduction across most, if not all, species of insects.

Undergraduate researcher Juan Malpartida has begun surveying other insect species to find out whether the reproductive role of odorant receptors is indeed universal or if it is limited to specific insect groups.

At the same time, the researchers will be exploring the possibility that their discovery can provide improved ways to control insect populations. If they can find a compound that renders males sterile, for example, it could be used for the sterile insect method of biological control. The method involves releasing overwhelming numbers of sterile males of an insect pest into the wild. The sterile males compete with the wild males to mate with females to reduce the size of the next generation. It has been successfully used to eradicate the screwworm fly from areas of North America and to control the Medfly and Mexican fruit fly. However, it is difficult to do correctly and it can be very expensive.

More information: Odorant receptor-mediated sperm activation in disease vector mosquitoes, *PNAS*, www.pnas.org/cgi/doi/10.1073/pnas.1322923111

Provided by Vanderbilt University

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