Researchers create technique for opening insects' exoskeletons to study living cells

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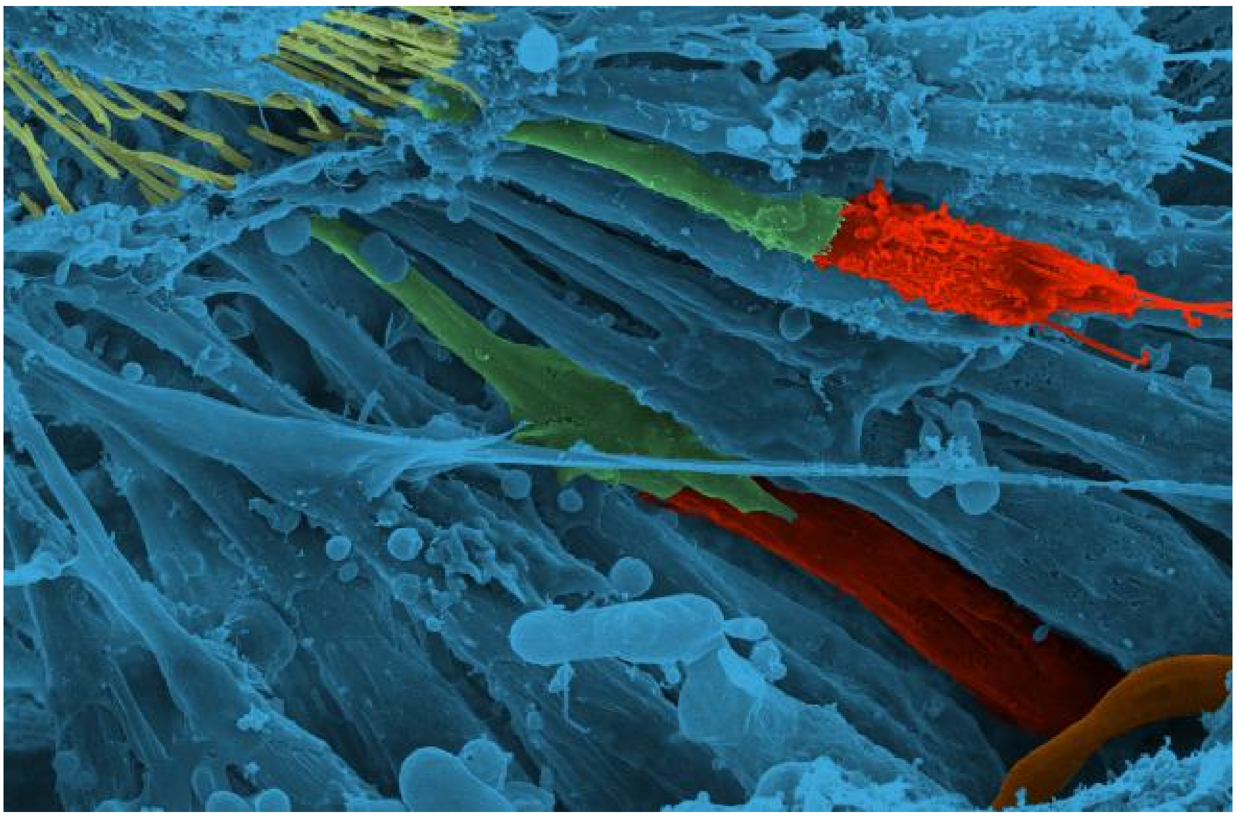

University of Iowa researchers have developed a technique to open insect exoskeletons to study live cells, such as the sensory cells in a fruit fly's antenna, which carry nerve signals to the brain and are shown in this image using scanning electron microscopy. Photo courtesy of Elena Sivan-Loukianova, Alan Kay, and Daniel Eberl.

Scientists believe that hearing in fruit flies and in humans is remarkably similar at the cellular level, which is why they'd like to explore the fruit fly's ear to learn more about how humans hear.

But they can't easily get at the fly ear.

Research led by the University of Iowa has a found a way to access the fly's ear—as well organs in other insects—even though it is protected by a hardened outer coating known as an exoskeleton. The researchers describe a technique that encloses the insect in a hard resin, similar to the way some ancient insects were preserved in amber. Unlike those permanently entombed animals, however, the new technique allows researchers to open the exoskeleton and study living organs and cells.

Besides using the technique to study fruit fly hearing, the researchers say it can be used also to study eyesight, brain function, and other biological processes with live cells from the test animals.

"We have developed a simple procedure for opening up the exoskeleton of arthropods, which exposes the live tissue and neuronal components," the authors write in the paper, published in the open-access journal *Frontiers in Physiology*. "We believe that the procedure will be of great value in helping to reveal the secrets of sensory organs and other cells trapped within the confines of very small cuticular compartments."

An insect's exoskeleton is like a combination of bones and skin, providing a double-plated body of armor that protects the organs inside from predators, disease, and other threats. Because these organs also are often located in tiny, hard-to-reach spots, scientists have struggled to expose them in a way that allows them to be studied in a live state.

The fruit fly ear is a good example. It is tucked away in a section of the fly's antenna that's only 80 microns wide, less than the width of a human hair. The compartment is sheathed in a part of the exoskeleton as well, exacerbating the challenge of plumbing its contents.

"The essential problem is that the sensory cells are tucked inside the very small antennae, which prevents scientists from accessing the sensory cells," says Alan Kay, UI biology professor and the paper's corresponding author.

Kay turned to dentistry for a solution. His father was a dentist, and Kay remembers being fascinated as a child by all the novel gizmos and materials that his dad used. He wondered whether he could enclose the fly's head in a kind of glue, where it would be fixed in space and yet remain alive so he could study the cells and organs.

"There's nothing like having the detailed internal 3-D topology that's just right there."

Christopher Barawacz and Steven Armstrong, from the UI's College of Dentistry, provided resins for Kay to try. Jon Scholte and Allan Guymon, in the UI Department of Chemical and Biochemical Engineering, determined the resins' chemical properties.

The final product was a resin with a honey-like consistency engineered so that a droplet would envelop the fly's head and hold it in place without bonding to it. Kay then hardened the resin with light so precise cuts could be made to isolate an area without destroying the delicate cells nestled inside. The researchers also added a nutrient solution to keep the tissue and cells alive during the cutting.

From there, the cut section could be wedged into wax and observed and probed from any angle, like a suspended living specimen.

"There's nothing like having the detailed internal 3-D topology that's just

right there," says Daniel Eberl, a UI biologist who studies fruit fly hearing and is a co-author on the paper.

The researchers have demonstrated that the technique works on ants, mosquitoes, water fleas (Daphnia), and mites. Eberl and Kay plan to take advantage of the technique to learn, for example, why cells within the fly's ear respond differently to outside stimuli.

"Some of the sensory nerve cells respond to vibration, while others respond to simple deflection, as would happen in a breeze," Eberl says.

These basic hearing processes, whether in flies or in humans, remain mostly a mystery.

"The remarkable thing is a fly ear and a human ear look vastly different, but there's a lot of similarity in how these organs work," Eberl says.

Davide Raccuglia and Michael Nitabach from Yale University are contributing authors. The UI's Office of the Vice President for Research and Economic Development funded the work.

Provided by University of Iowa

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