Entomologists at Cornell and Utah Receive $710,263 Grant from the NIH

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by Peter Seem

GENEVA, NY: After 500 million years of evolution, what could the brains of a tobacco budworm moth and a mouse have in common? Very likely, it is something that humans share as well, which is why the National Institutes of Health (NIH) have recently given a $710,000 grant to Charles E. Linn, working in Wendell Roelofs' lab in the department of entomology at the New York State Agricultural Experiment Station, and Neil Vickers, assistant professor in the biology department at the University of Utah. The three-year grant, "Discrimination of Complex Mixtures in Olfactory Signaling," involves microsurgery, behavioral testing and neurophysiological analysis of moths.

Suggested caption: Kathy Poole perfotmed the transplant of antennal lobes between larvae of the Tabacco Budworm and the Corn Earworm, *Heliothis virescens* and *Helicoverpa zea*, respectively.

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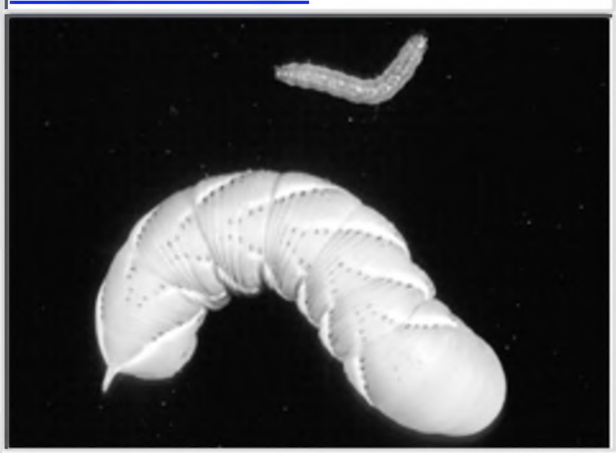

The scientists study the neurobiology and behavior of sex pheromone communication systems among insects. Pheromones are chemical signals produced by insects to attract mates in blends that are unique to that species. A major research problem is to understand how sensory information for individual chemicals is integrated in the brain and recognized as a specific pheromone blend. A key structural feature in the antennal lobe of insect brains, and in the olfactory bulb in mouse brains, are the olfactory glomeruli. The glomeruli route specific sensory inputs to different areas of the brain. In the antennal lobe of the male insect there is a specific Macroglomerular Complex-a set of glomeruli involved in processing the pheromone signal. Each glomerulus in the complex receives sensory input of one specific pheromone component.

Suggested caption: The research tean successfully adapted the transplant procedure, first performed on Sphinx Moth larvae (bottom), to the much smaller Corn Borer (top).

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Suggested caption: Adult moths used in antennal imaginal disk transplant experiments. TOP: the tobacco budworm, *Heliothis virescens*. BOTTOM: the corn earworm, *Helicoverpa zea*.

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To find out how the routing occurs Linn and Vickers are transplanting antennal imaginal disks between different moth species. The imaginal disks develop into the antennae of the donor species in the adult moth. At the Experiment Station in Geneva, Kathy Poole, a technician in Roelof's group, performs an interspecies transplant-the first of its kind-of the imaginal disks from larvae of two moth species, the tobacco budworm, *Heliothis virescens*, and the corn earworm, *Helicoverpa zea*. Several weeks after the transplant, adult moths emerge. One day after they emerge, the moths are tested in a flight tunnel for response to each of the two species' pheromones. After testing they are shipped overnight to the University of Utah, where Vickers completes the neurophysiological component of the research the next day.

The project began as inter-race transplants in the European Corn Borer at Geneva, without Vickers' collaboration. Linn received a two-year seed grant from the National Science Foundation to allow development of the transplant procedure. After successfully developing the technique Linn and Roelofs realized that future funding would require expertise in neurophysiology. It was at a pheromone conference in Europe that the collaboration with Dr. Vickers, who specializes in the study of the neural underpinnings of insect behavior and olfaction, was established. At this point, they also switched from the European Corn Borer to the Tobacco Budworm and Corn Earworm because of the depth of background information available for the two new moth species.

A new proposal was submitted to the National Institute of Deafness and Communicative Disorders (NIDCD), which has a chemical senses sub-program, and was eventually granted a Shannon Award-a two-year, $50,000 per year grant given to projects that the director feels are close to obtaining full funding. The award keeps the project alive until a new proposal can be submitted for full funding, as happened in the case of Linn and Vickers. In 2001, the proposal received the three-year funding.

The NIH picked up on the project because of its underlying significance to their goal of understanding the sense of smell. It addresses questions relating to neuronal targeting, how the neurons' development and connections are directed. The olfactory glomeruli are particularly important because they play a very similar role in both invertebrates and vertebrates. "If you look at a flow diagram of a mouse brain and a flow diagram of a moth brain the steps involved in the sensory detection-1st and 2nd and 3rd levels of processing-are all completely analogous, so [NIH] is interested in glomerular organization in the same way we are," said Linn. Additionally, the moth brain is simpler and, as such, is a more attractive candidate for this type of study.

TRANSPLANT PROCEDURE PERFECTED

The transplant procedure was first developed and performed by Anne Schneiderman as part of her Ph.D. research at the Harvard Medical School in the early '80s. She transplanted the imaginal disks from male Sphinx Moths, *Manduca sexta*, to females of the species. Poole accepted the challenge of adapting the transplant procedure to the Corn Borer, and challenge it was, mainly because the size of the insects. The larvae of the Sphinx Moth can be as large as six cm. The Corn Borer is approximately 1.5 cm, and the imaginal disks being transplanted are no larger than the point of a pencil. Schneiderman's description of the procedure notes that when making one of the incisions, which runs along the mandible, care must be taken not to damage the mouth parts as they are essential for the development into adult. That became even more difficult for Poole because European Corn Borner larvae are a fraction of the size of the Sphinx Moths. The surgical sealant Schneiderman used to close the incisions had to be heated, which killed the European Corn Borer, presenting yet another hurdle. "In the beginning it took a long, long time just to get larvae to survive and come through as successful pupae," said Poole.

After solving several problems, the procedure is now at a point where better than 70 percent of the transplants emerge as adults. "Without Kathy's persistence this project would never have left the ground. Her dedication to the project is a key element in its success," Linn noted.

TESTING RESPONSES IN THE FLIGHT TUNNEL

Linn and Karrie Cotropia test adults that develop successfully, with at least partial antennae, in a flight tunnel. An airflow is established in the tunnel and a moth is released down wind of either the recipient's or the donor's pheromone blend. The blends have a common major component and different minor components. If the moth orients itself toward the pheromone source and flies upwind, it is a positive response to that blend.

"The minor component of one is an antagonist for the other species; the minor component of the second species the first species can't even recognize, so they'd never cross attract. In the flight tunnel we've never seen normal males of one species go toward the pheromone of the other. Our transplants go to both." Although the number of moths doing so is small, Linn cautiously notes they are too numerous to be explained by chance. "One of the really exciting things that is coming out of this is that we're now seeing behavioral responses that were not predicted, which means that rearrangements have occurred in the neural pathways in the antenna and the brain."

Vickers shared Linn's enthusiasm for the research. "It's a nice project that's going to turn up some interesting results." In Vickers' lab, moths are tested in two ways. Individual neurons are monitored with a micro-electrode to test their response to particular odors. When a neuron is identified as responding to an odor of interest, it is injected with a fluorescent die and mapped using a process called confocal microscopy. Thus it is possible to tell where a particular neuron arborizes-to what region of the brain the neuron sends it signal. As part of the dissection, it is also possible to tell, visually, which of the two species' glomerular array has developed in the transplant.

"Now that both labs have stable funding we are looking forward a very productive period of research," noted Linn. "Using a novel experimental paradigm that involves behavior, neuroanatomy, and neurophysiology to address key problems that are relevant to a wide range of animal groups is very exciting and bound to produce a number of discoveries. Additionally, the more we learn about discrimination of pheromone blends, the better we can utilize pheromones in pest management strategies. And that is an equally important part of our research program at the Experiment Station."

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