PHYS ORG

Walk in the park yields biological treasure

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Noah Whiteman examines leaves for tunnels made by the leaf-mining fly, Scaptomyza. (Photo by Beatriz Verdugo/UANews)

A newly identified relationship between a fly and a weedy mustard-type plant promises to answer many long-standing questions surrounding the evolutionary arms race between plant-eating insects and their host plants.

Scientists trying to get a grip on the arms race between plant-eating insects and the defenses put up by their hosts just got a boost from new research by a University of Arizona entomologist published in the early view edition of *Molecular Ecology.*

Noah Whiteman, an assistant professor in the UA's department of ecology and evolutionary biology, has found a miniature ecosystem consisting of a plant and a tiny fly that spends its entire life cycle on the plant.

What makes this system special is the fact that both its key players – the plant and the insect – are what scientists call genetically tractable model organisms: holy grails of any serious science that aim to unravel biological mechanisms down to the level of genes and proteins and signaling molecules.

Decades of research and knowledge rest upon two of the most famous and widely used workhorses in genetics research: Arabidopsis thaliana, an unassuming, weedy plant in the mustard family, and Drosophila melanogaster, familiar to many as the tiny, red-eyed fruit flies hovering around the produce aisle.

However, until now, scientists wanting to study interactions between plant-eating insects and the plants they befell were out of luck: Fruit flies, as the name implies, feed on rotting fruit and couldn't care less about Arabidopsis plants, and vice versa.

A plant-insect model system: Scaptomyza flava flies mating on Arabidopsis leaf. (Photo: Noah Whiteman)

Enter Scaptomyza flava, a fly so closely related to Drosophila melanogaster it shares most of its genes, and with a strong appetite for

Arabidopsis. Female Scaptomyza flies prick a hole into the plant tissue and lay their eggs inside. Once the larvae hatch, they spend their childhood as leaf miners: tunneling their way through the leaf, munching on the nutritious plant tissue.

A fruitful walk in the field

Although the science underlying plant-insect interactions is no walk in the park, Whiteman's discovery started out as just that.

While a postdoctoral research fellow at Harvard, Whiteman became frustrated by the lack of a model system to study plant-insect interactions on a genetic and molecular level.

After an extensive literature search, he went out into the field and started looking for mustard plants that had flies living in and on them.

"One of the first places I started to look was Fresh Pond, which provides a lot of the water supply for Cambridge. There was a vacant lot so I pulled up my car and looked for yellow flowers. And sure enough, there was this plant called Barbarea vulgaris growing there, which is introduced from Europe and closely related to watercress."

"I looked for mines in the leaves. I brought some leaves and larvae to the lab, wrapped them in paper towels, put them in cages and let them go."

Eventually, flies started coming out. Some of them had red eyes.

"I keyed them out and they turned out to belong to the genus Scaptomyza, an outdated classification because we now know they belong to the Drosophila genus. ‘This is exciting,' I thought. I put them all in a cage with Arabidopsis plants. They started attacking the plants; not only the larvae, but the adult females, too. They make holes in the leaves with their ovipositors and drink the juices that come out."

Then, one day, while walking his dog in one of Boston's oldest city parks, Whiteman again came across the plants with the telltale tunnels in their leaves.

"I found the same plant there and the same flies in it. There were actually two Scaptomyza species coming out of this plant. One is a true herbivore and was making the mines, and the other was living in the mines of the miner. I sent the flies to the Smithsonian USDA Insect Identification Lab for confirmation but they sent them back and said, ‘There is nobody here who could identify this.'"

"There seems to be this idea that there is this big convention where people decide what becomes a model organism, when in fact it's just individuals who decide what can be collected and what will work. The problem with these flies is you can't freeze the eggs, you can't store them. You need to keep things alive."

Sharing a hotel room with flies

When Whiteman traveled across the country to join the faculty at the UA, the flies traveled with him.

"I kept them in the back of my car, in cages. I was bringing them into hotel rooms."

Next, Whiteman's research group had to show that the flies and Arabidopsis plants could be used as a model system.

"There is a lot of research going on trying to figure out what biochemical pathways plants use to cope with insect attacks," said Whiteman. "Now we can tackle these questions in much more detail."

As one might expect, over the course of evolution, host plants have developed numerous ways to ward off parasites, such as chemicals that are toxic to the insects or throw a wrench into their development in some way.

Whiteman and his coworkers found that Arabidopsis plants ramp up their production of proteins that mess with the insects' digestive tract.

"The idea is that the plant is making it difficult for the insect to digest it," Whiteman explained. "It's very complicated, we don't really know what's going on at a molecular level."

To address this question, the researchers compared how well larvae fared on defenseless, mutant plants unable to make the indigestive proteins compared to their kin raised on wild-type plants.

"From those analyses, we do know that it has an effect on the larvae; they don't do as well," Whiteman said. "The plants put up their defense in response to the insects being present. If you're a fly, living on something that is trying to kill you is different from living on, say, a rotting apple," Whiteman said.

"We also know that once a plant turns on one defensive pathway, others shut down. They inhibit each other," he added. "To the ecologist this is confusing, but of course, you can't be good at everything at the same time."

"Until now, we weren't able to tease these mechanisms apart, to answer questions like, why is this insect feeding less? We didn't have any tools to study these interactions in a controlled fashion."

"You could put any plant-eating insect on Arabidopsis to study this interaction from the plant side," he said. "In order to make our new model system compelling from the insect side, we had to use some tools from Drosophila genetics."

Hunting down defense genes

His team tested the Scaptomyza flies for the activity of genes known from Drosophila to be important in dealing with toxic plant compounds, and how they responded to the presence or absence of specific host plant defense molecules.

The researchers found that when they reared larvae in knockout plants unable to produce defense molecules, the flies dialed back their expression of detoxification genes.

"It makes sense for them to not turn on those genes unless the plant's defenses are up," Whiteman said.

Conversely, putting larvae on plants with intact defenses turned on their detoxification genes.

To put the new model system on a broad basis, Whiteman and his colleagues determined the genetic code of all the active genes in larvae reared on plants with different abilities to fend off insects.

"Now we have a couple of billion of base pairs of data, and our goal is to identify genes that are selectively induced or repressed in larvae depending on what kind of plant they are reared on," he said. "We found 400-500 significantly induced ones."

As often in ground-breaking research, the initial discovery stirred up a myriad of questions.

"Now we have to figure out, are those genes functionally important?"

Whiteman said. "We can show that there is resistance in Arabidopsis and we can identify which pathways are involved in that resistance. The next question is: How is the leaf miner responding to the presence or absence of these toxic molecules? Does it care? Clearly, it does. There is a cost for detoxifying, but what is it?"

Ecological questions are waiting to be answered as well.

"We know that the leaf mining habit has evolved probably 25 times in insects," Whiteman said, "mostly in beetles, butterflies, moths, some wasps and saw flies. It's not present in the other insect orders, but why? How has selection shaped the ability of these insects to colonize an organism with a potent defense response? We want to develop the Scaptomyza lineage as a system for answering questions like these. This is our lab rat system."

Provided by University of Arizona