The Co-Evolution of a Beetle and a Plant

DNA evidence shows survival of ancient association

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The Bursera leaf squirts a poison under high pressure when the vein is cut.

The flea beetle genus Blepharida includes more than 70 species, about half from Africa and the other half from the tropical Americas, especially Mexico.

With each evolutionary change, the insect has adapted behaviorally to circumvent the flow of poison, figuring out how to disarm the plant's squirting mechanism.

For the past 112 million years an insect and a plant have faced off in a deadly dance where neither quite finishes off the other. If the flea beetle bites a leaf, the leaf vein squirts a glue-like resin under high pressure to poison and immobilize its attacker. The beetles have learned to sever leaf and stem veins to disarm the squirting mechanism so they can continue to feed. In response, the plant has developed different poisons and toxin delivery systems over time.

Back and forth, the beetle genus *Blepharida* and the host plant genus *Bursera* have coevolved, each never quite destroying the other, weathering separations of continents and the extinctions of fellow species.

For all its drama, this battle and its exquisite history may have gone unnoticed if Judith Becerra hadn't gotten a terrible case of chigger bites in the Tehuacán Valley of Mexico in 1991. She was taking students from the National Autonomous University of Mexico on a field trip to explore arid environments. Her swollen, itching ankles forced her to fall behind and she began to notice a distinctive fragrance coming from a nearby shrub. As she snapped a leaf, it squirted a resinous, sticky substance that smelled like pine, beginning a research adventure that has continued for 12 years.

The results of her study, published in the October 2003 issue of the *Proceedings of the*

National Academy of Sciences, show the resilience of this co-evolutionary system that is based on defense and counterdefense strategies.

"At first I was just having fun," says Becerra, who is now an assistant professor in the University of Arizona Department of Entomology. "I wondered why the plant did this, so I went looking for something that might be eating it. I found these beetles." She conducted field and laboratory experiments, beginning with the beetles and plants she found that day, and later expanded her studies to include other beetle species from the Blepharida genus, and other Bursera species. She traveled to the south of Mexico, Sonora, southern Baja, Yucatan and South Africa to collect specimens for laboratory studies. In all, Becerra analyzed 40 different plant-beetle associations from around the world to get a better picture of their interaction over time.

She used fossils and biogeographic information to calibrate insect and plant molecular clocks and to date this ancient interaction. Becerra and her research team analyzed DNA base pairs to compare the timelines for change in the insect and the plant, developing time-scaled reconstructions to date the origin of the interaction and also of some of the defense innovations in the plants, with their corresponding beetle counterdefenses. The evolutionary changes

coincided, and the interaction began before the African and South American continents separated.

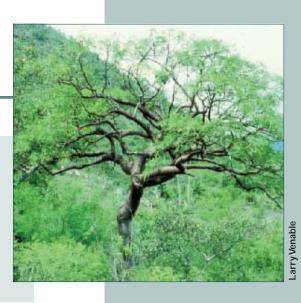
As the Blepharida and Bursera species diversified, the plant developed the highpressure squirting mechanism and poisonous compounds to repel or disarm the beetle's attack strategies. Becerra and her team have snapped leaves and measured toxin squirts up to two meters long from the central vein. The secondary veins can also squirt. In some cases the leaf canals don't pump the resin under high pressure, but rather drench and cover the leaf to protect it. Some of the plants that don't spray produce chemicals instead that taste bad or poison the beetle. Africa and South America were at one time part of a supercontinent known as Gondwana. When they separated about 100 million years ago the plant-herbivore association survived with similar adaptations as the species evolved.

"Our questions are actually more fundamental than studying squirts," Becerra says. "It wouldn't matter if the plants squirt or not. It's more about what the impacts of insects have been on the evolution of plants and the effect of plant defenses in the evolution of host shifts by insects." In response to the insects' feeding, some of the plants have completely changed their chemistry. Most *Bursera* produce turpenes (turpentine is a relative), but others in the last five to ten million years have developed alkenes (ethylene is an example), according to Becerra.

It is still unclear how *Bursera* developed the high pressure system. Becerra and her group have calculated that some of the leaves contain 15 to 25 percent resin by weight, compared to the more typical 1 to 5 percent toxin content in most poisonous plants. By examining the leaf canals they may find an elastic mechanism where the plants accumulate a lot of resin, Becerra suggests.

With each evolutionary change, the insect has adapted behaviorally to circumvent the flow of poison, figuring out how to disarm the plant's squirting mechanism. Yet it's still a one-to-one combat between each individual flea beetle and *Bursera* plant every time they meet, and there's just enough failure on both sides to keep the association going. The insects have indeed adapted to stopping the flow of resin by cutting the leaf

The 100 species of flowering Bursera are found in roughly the same geographic range as the Blepharida beetle. Although some are small plants, most are medium-sized trees that have colorful trunks—red, green, yellow, blue—with bark that exfoliates in sheets. They have a distinctive canopy.



canal with tiny bites. They may take an hour to do this, and then polish off the leaf itself in about 15 minutes. Although they are able to eat, this time investment costs them in other ways as the beetles and larvae are left exposed on the leaf for predators to find. In another scenario the small young larvae start mining the leaves, trying to eat between the veins instead of cutting them, but sometimes they goof.

"Every once in a while you see them drowning in the resin, a source of mortality for the young stages," Becerra says. "Insects breathe through their skin. Their mandibles get stuck and they can't breathe or move."

The effect on humans is less severe, but still notable, since the toxin can be absorbed through the lungs and skin. Becerra and her students usually have pounding headaches and nausea after working with the plant's odiferous resins in the laboratory, but like the chigger bites that once slowed Becerra down, the results have been worth it. This research currently offers the oldest known example of synchronous, reciprocal adaptation between an insect predator and plant host.

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Blepharida larva feeding on Bursera leaf must avoid severing veins that release the turpenoid poison.

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