Science Observer

How a Fungus Boosts a Beetle's Invasion

Microbial evolution helps explain why a mild-mannered American beetle has become a tree killer in Asia

When China opened its borders to international commerce in the late 1970s, it got more imports than it really wanted. Among the extras was the red turpentine beetle *(Dendroctonus valens)*, a wood-boring species native to North America. In its home range, *D. valens* is an unremarkable forest dweller that mainly colonizes dead and dying trees. But in China, it has wiped out more than seven million vigorous pines in the past dozen years, and it looks poised to spread through much of Eurasia.

What caused this unfortunate personality change has been an open question for years. And that question applies not only to *D. valens* but also to its many relatives that have increasingly colonized—and ravaged—new habitats around the globe. *D. valens* belongs to the beetle subfamily Scolytinae, a group of several thousand species known as bark and ambrosia beetles. These insects are very good at stowing away in internationally traded timber products, and their troublemaking is not restricted to China. More than 50 introduced scolytine species are also established in the United States, and the subfamily accounts for more than half of the exotic insects intercepted at U.S. ports—a figure disproportionate to the group's abundance on the planet.

When they travel, the beetles don't go alone. *D. valens*, like many of its relatives, harbors a menagerie of bacteria, fungi and mites on the hairs and pores of its rice-grain-sized body. When the insect drills through the bark of a tree to lay eggs, it escorts these microscopic hitchhikers into the plant's sensitive vascular tissues. There, beetle larvae and fungi develop side by side—the fungus breaking down the innermost layer of bark (the phloem) and the insects consuming both fungus and phloem.

What you don't see in this photograph is just as important as what you do. The wood-boring red turpentine beetle *(Dendroctonus valens)* carries fungal spores, invisible to the naked eye, that help it colonize the trees where its larvae develop. Evolution of one of its associated fungi may have been instrumental to the beetle's emergence as a destructive, invasive species in China. (Photograph courtesy of Runzhi Zhang.)

All that boring and digesting is bad for trees. But most healthy plants fend off infestations or simply aren't attacked. So it seemed to be a fluke when the now-notorious Dutch elm disease, an Asian fungus conveyed by European bark beetles, began to devastate otherwise-healthy trees in North America and Europe in the mid-1900s. Alas, that outbreak was the harbinger of a growing wave of rogue beetle-fungus pairs being introduced and reshuffled around the globe. An insect-fungus couple can "suddenly become this crazy killer" when it's relocated, says Jiri Hulcr, an entomologist who studies symbioses at North Carolina State University. "It's a mystery why that happens," he says. "We don't exactly know what changes."

For *D. valens*, however, scientists are hot on the trail of what it is that's changed. A team of biologists, including Min Lu, Michael Wingfield, Nancy Gillette and Jiang-Hua Sun, have discovered that, in China, the beetle's most common fungal partner has evolved several new strains that make trees sicker and, in a vicious cycle, force them to attract still more beetles.

The fungus in question is *Leptographium procerum*, a species that also lives in parts of North America with *D. valens* and other bark beetles. In their latest study, published in the November issue of *Ecology*, Sun and his team worked with 96 *L. procerum* samples from both China and North America. They showed that the Chinese strains included just a subset of the genetic diversity found in the United States, confirming that the fungus, like the beetle, originated in the latter country. But in China, that limited genetic material had been rearranged into at least 24 novel combinations. Most of those new genotypes—including the most common *L. procerum* strains in China—were more damaging than their American counterparts.

The researchers infected hundreds of two-year-old Chinese pines *(Pinus tabuliformis)* with the various Chinese and American strains by inserting a bit of fungal culture into each stem. After three weeks, the Chinese strains had spread farther along the tree trunks than had the American strains, suggesting that they had a greater potential to injure the trees. But the real kicker was how the fungus changed the seedlings' aroma.

Throughout their range in North America and China, *D. valens* beetles home in on pine trees by following the fragrance of 3-carene, a compound that adds a sweet, camphorous scent to pine sap. Sun's team wanted to know whether the fungal strains had different effects on tree odor. They extracted 3-carene from the phloem of some of their infected seedlings; others they kept alive and sealed inside plastic bags to collect the compound directly from the air around each tree. Both methods showed that trees infected with the novel Chinese genotype produced, on average, about 20 times more 3-carene than did those infected with North American genotypes. A behavioral test confirmed that the beetles were more strongly attracted to the higher concentrations.

If the same phenomena play out in adult trees, the first few beetles may launch a positive feedback loop in which the Chinese fungus strains cause the tree to release more 3-carene, which attracts even more beetles to a now-weakened plant. Ultimately, the pine succumbs to the damage that the beetles and fungi together inflict on its vascular system. That feedback loop wouldn't happen with the North American fungus genotypes, which don't prompt the tree to produce as much 3-carene.

But what if those new Chinese strains made it back to America? "It's a two-way street," warns Sun. If *D. valens* returns to the United States with its new fungal partner, he thinks "it will become a pine-killer" here, too. He and his collaborators are already preparing to test that hypothesis—in quarantine—with American ponderosa pines.

Meanwhile, other introduced beetle-fungus couples are killing trees around the globe, and it's not clear whether any principles of the *D. valens* case may generalize to the other species. Rarely do biologists study the beetles until they become pests, so their native habits and transitions into pesthood are poorly documented. "Every one of them requires a lot of effort to figure out," says Hulcr. That makes it very hard to predict which species will launch the next epidemic, and how they'll do it.—*Elsa Youngsteadt*

Relative Risk, One Result at a Time

Evidence mounts for endocrine effects of a compound used in many antibacterial bar soaps

When research suggests that a single chemical may cause harm, public concern rises, as it has for the plastic additive bisphenol A (BPA) in recent years. But many more of the 83,000 or so humanmade chemicals used in the United States receive little attention. The possible effects of chemicals in combination get still less scrutiny, even though the potential that some chemicals will interact is high, given their numbers.

This may be due in part to the staggering amount of work required to discern those effects. It would be a very difficult task to keep up with research on all of these substances, much less evaluate their relative risk as new results appear. The U.S. Environmental Protection Agency (EPA) has put considerable effort into this under the Toxic Substances Control Act, but the Act has not been updated since its passage in 1976 and excludes many substances from the agency's purview.

Substances that have the potential to disrupt development in an organism are of special concern. The results of exposure to such chemicals can range from birth defects to developmental irregularities that don't appear until later in life. Determining whether a substance is an endocrine disruptor, how strongly it acts and at what concentrations, not to mention deciphering hormone pathways themselves, takes a great deal of time and resources. Studies in the lab can't be directly extrapolated to real-life situations, but they can offer clues about new routes to explore, along with help in evaluating the risk posed by various chemicals.

Heather Patisaul, a biologist at North Carolina State University, studies the effects of BPA and other compounds suspected to disrupt hormonal processes, using female rats as models. "The biggest unknown," she says, "is if human harm is indeed resulting from exposure to these chemicals at low doses. If it is, it requires a major paradigm shift in how we approach toxicology, because the current strategies are ill equipped to deal with endocrine disruptors."

A new study adds several more pieces to the puzzle. In a September 2011 study in the *Proceedings of the National Academy of Sciences of the U.S.A.*, Eunah Chung, Maria C. Genco, Laura Megrelis and Joan V. Ruderman chose a less known, but widely used, substance to investigate: triclocarban (3,4,4′-trichlorocarbanilide, or TCC).

TCC has been used as an antimicrobial in consumer products since the 1950s. A 2001 study found that it was present in 84 percent of antimicrobial bar soaps sold in the United States. It's often mentioned in the same breath with triclosan: Both are halogenated carbons used in soaps and other products, but their chemical identities are unique. The EPA reports that between 1 and 10 million pounds of TCC were used in the United States in 2002. People who shower with soap containing TCC absorb it through their skin. It is metabolized quickly by humans but persists in surface waters and in sewage sludge that is spread on agricultural fields.

Ruderman and her coauthors looked at the gene aromatase-B (AroB) in the brains of developing zebrafish embryos. AroB is regulated by estrogen, among other compounds, and is expressed in subregions of the brain including the hypothalamus and preoptic areas. To