Farm labor. Leaf-cutter ants tend their fungus garden, a complex miniature ecosystem.

COMMUNITY ECOLOGY

All That Makes Fungus Gardens Grow

The discovery of a parasitic yeast draws attention to the ways that pathogens can stabilize ant agriculture and other symbiotic networks

Fifty million years ago, while the earliest primates were still scurrying from tree to tree, scrounging fruits and insects, attine ants were growing their own food. They were so adept at domesticating mushrooms that hundreds of species have descended from the original farmers, all of them cultivating fungi.

Humans could learn a lot from the ants' success. Over the past 10 years, researchers have come to realize that the fungus gardens thrive because of an intricate web of bacteria and fungi that includes both pests, such as a newly discovered black yeast, and partners, including bacteria that keep pathogens in check. By studying these relationships, biologists hope they'll uncover lessons about the evolution of such interactions, knowledge that will help humans better manage microbes in medicine and agriculture. "It's a system that works," says John Morrissey, a microbiologist at University College Cork in Ireland. "If you could develop a bacterial inoculant that was as successful in controlling a specific pathogen as the [beneficial bacteria] are for the ants, you'd be on to a real winner."

Complex network

Ant agriculture runs the gamut. For leaf-cutter ants, farming is big business. They're the most notorious of the more than 230 described species of fungus gardeners, forming colonies of millions of workers that can defoliate a tree or crop in mere hours. The ants use the harvest to fertilize hundreds of separate fungus gardens in an elaborate subterranean compound. Most attine gardens, however, are small-scale operations: Their inconspicuous colonies are tended by as few as a dozen workers that scavenge bits of detritus to feed a spongy handful of fungus.

But from the most primitive gardener to the dreaded leaf-cutter, all attines would starve if deprived of their fungal crops. When an ant queen leaves home to mate and found a new colony, she must take a little mouthful of the fungus with her to start a garden.

Although naturalists have known since 1874 that the attine ants are fungus gardeners, more than a century passed before Ph.D. student Cameron Currie began to chip away the microbial complexity underlying the ant-fungus symbiosis. While at the University of Toronto in Canada, he discovered that ant gardens often contained a second fungus, *Escovopsis*. When he grew it on culture plates with different food sources, Currie determined that *Escovopsis* is a pathogen with a sweet tooth for only the ants' cultivar. What's more, the pathogen's evolutionary tree had the same basic shape as those of the ants and their crop, indicating that all three had coevolved since the beginning of ant agriculture, Currie and colleagues reported in 2003 (*Science*, 17 January 2003, pp. 325, 386).

Although Currie isolated *Escovopsis* from up to 75% of the gardens of several attine species in Panama, this pathogen rarely seemed to do much damage. The reason, it turned out, was a fourth symbiont: Currie found that actinomycete bacteria, housed and nourished in pits on the ants' bodies, produce chemicals that keep *Escovopsis* in check.

The four-part garden symbiosis of ant, cultivar, pathogen, and bacteria interrupted a scientific tradition of studying symbionts two at a time—think corals and algae, for example, or soybeans and nitrogen-fixing bacteria. The discovery accelerated a transition toward thinking of interacting organisms in trios or networks, not pairs.

"When I got into this stuff, it was two symbionts," says Ted Schultz, an entomologist at the Smithsonian National Museum of Natural History in Washington, D.C., who has studied attine evolution for nearly 30 years. "I was stunned" when Currie identified two more.

Now, in a paper in this month's issue of *Ecology*, Currie and his colleagues introduce a fifth symbiont. "[It] just continues the trend of being repeatedly surprised by how complex this system is," Schultz says.

The first hint of the new player came when Currie, now at the University of Wisconsin (UW), Madison, cultured the actinomycete bacteria from an ant called *Apterostigma*. In addition to white bacterial spots, a black yeast often appeared on the same culture plates. Ainslie Little, now a postdoctoral fellow at UW Madison, took a closer look at the yeast. She treated worker ants in a vial coated with a selective antibiotic that would rub off on the ants and kill the yeast but not the other symbionts.

At first, it looked like the experiment was a bust: Getting rid of the yeast had no effect on the ants or their crop. But when Little spritzed half the ants' gardens with a solution of *Escovopsis* spores, the yeast suddenly revealed its true colors. Over 3 days, ants with black yeast infections lost twice as much of their crop to *Escovopsis* as the yeast-free ants. When Little grew the actinomycetes in petri dishes with the yeast, the yeast ate the bacteria, demonstrating that they rob ants of an important defense against *Escovopsis*.

DNA studies showed that the black yeasts are widespread on the attine ant family tree, thriving near the pits where the ants

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house the actinomycetes. Like the cultivar, *Escovopsis*, and actinomycetes, the yeast has been part of the attines' microbial balancing act since the ants first began to farm, Currie says.

"It's really exciting," Schultz says of the fifth symbiont. And Ulrich Mueller, an integrative biologist at the University of Texas, Austin, agrees: It's "interesting to what extent the presence of [another] symbiont can fundamentally change the interaction of two other symbionts."

No cheating allowed

Currie thinks that understanding three-, four-, and five-way interactions like the ones in the fungus gardens may ultimately revolutionize the way we think about the evolution of mutualism. Why two parties should cooperate—whether it's two species over evolutionary time or two people over the course of a day—is one of science's big mysteries (*Science*, 1 July 2005, p. 93). In a two-player partnership, cheaters should be able to get ahead by reaping benefits without paying their dues, destabilizing the agreement. But the ants have lived stably with two mutualists for millennia.

Moreover, the two antagonists rarely get too far out of line. Diseases and pests of humans and their crops, on the other hand, have evaded control measures in a matter of decades. So everyone, from crop scientists to basic evolutionary biologists, is itching to know the secrets to both cooperation and control in the ants' gardens.

Morrissey, for example, would like to incorporate beneficial microbes into human agriculture to reduce chemical input. The attine system "shows it is possible to set up a structured [microbial] community … over a very long term" for biological control, he says.

The "key question" for figuring out how to manage beneficial microbes, says R. Ford Denison, a crop ecologist at the University of Minnesota, St. Paul, is why the ants' actinomycetes never turn against their hosts. Antifungal compounds are expensive to make, and selfish bacteria that don't make the compounds should be able to reproduce faster and eventually overrun the helpers. But somehow the ant system is robust to cheaters.

This is where the multipart interactions come in, Currie says. He thinks there is a reason that each partnership in the garden has a parasite: *Escovopsis* intrudes on the ant-fungus mutualism, and the yeast disrupts the ant-actinomycete mutualism. These parasites may be the very thing that keeps the mutualists cooperating, Currie adds.

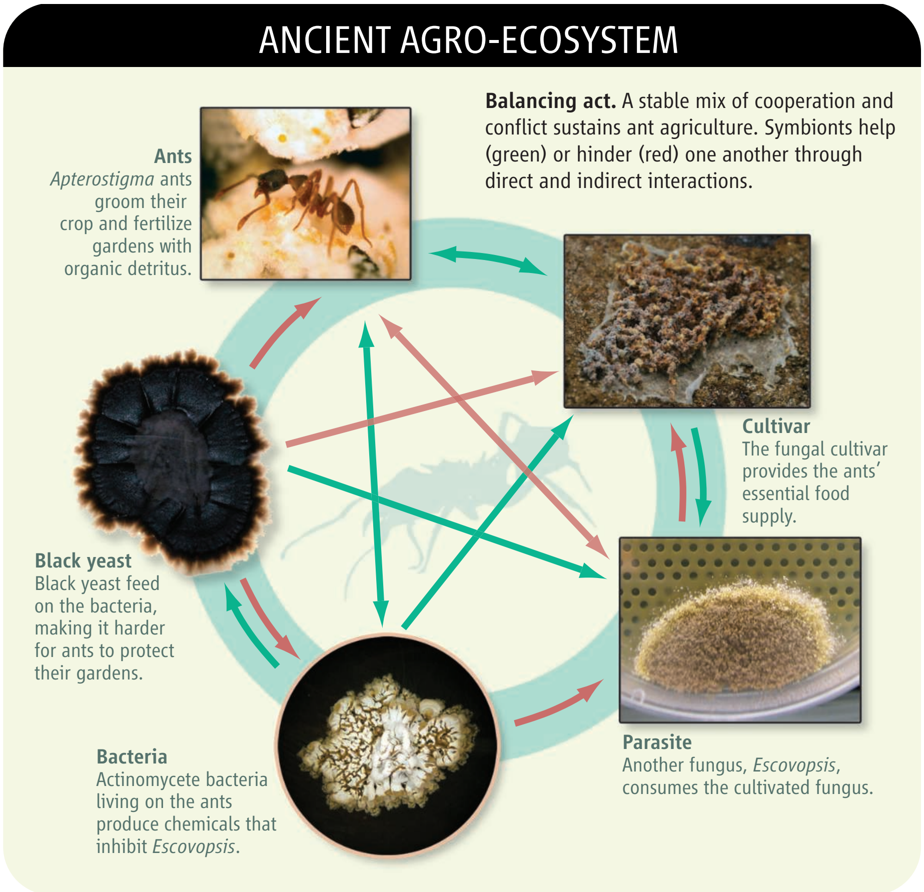
Currie and his colleagues tested this idea by forcing the fungus or the ants to cheat on each other. A selfish fungus would reproduce more and feed the ants less. To simulate this, Little removed specialized nutrient-laden structures from much of the cultivar. To shortchange the fungus, she reduced the proportion of fungus-tending workers in the colony.

With no *Escovopsis* around, the negative effects of cheating were minimal, suggesting that it could become common over time. But add *Escovopsis* and cheating was a disaster, Currie reported last summer at the Gordon Research Conference on Microbial Population Biology in Andover, New Hampshire. When either the ants or the fungus were cheating, *Escovopsis* took over more of the garden, killing the fungus and leaving the ants with little to eat. Parasites, Currie says, may play a crucial and underappreciated role in keeping cooperators honest by raising the costs of cheating.

But that doesn't explain what keeps the parasite itself from overrunning the fungus garden, the same way human pathogens have outpaced antibiotics. New results from Currie's lab, not yet published, indicate that *Escovopsis* does evolve resistance but never gets ahead because the actinomycetes themselves are so adept at evolving new antifungal compounds. And if they don't do it fast enough, the ants can acquire new actinomycete strains.

But other organisms, yet to be described, may also play a part in stabilizing fungus garden ecology. "There are additional biofilms associated with [the fungus], stuff that grows on the fungus, or in the substrate, wherever anything else can move in," says Mueller. When the ants transplant a garden, they do so by choosing a little piece of the existing garden, including any other microbes that are mixed in with it. "In a sense, they're selecting on an entire community that has desirable properties," Mueller says. The idea still needs to be tested. Meanwhile, only the daring would place bets on how many symbionts have yet to turn up. Currie expects a few more; Mueller predicts hundreds. Evolutionary biologist Jacobus Boomsma of the University of Copenhagen in Denmark says that regardless of the number of symbionts, the fungus gardeners are poised to answer critical questions about cooperation, conflict, and microbial ecology. "This system," he says, "will keep inspiring us for at least a decade more."

–ELSA YOUNGSTEADT



Balancing act. A stable mix of cooperation and conflict sustains ant agriculture. Symbionts help (green) or hinder (red) one another through direct and indirect interactions.

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