Ancient fungal farmers of the insect world

Not only humans practise agriculture. As **Garret Suen** and **Cameron R. Currie** describe, ants have amazing systems of growing fungal crops in their 'gardens' too.

◀ A worker of the leaf-cutting ant *Acromyrmex octospinosus* tends to her fungus garden. These ants grow bacteria on their body and use the antibiotics the bacteria produce to protect their gardens against infection from invading pathogens. *Heidi Horn*

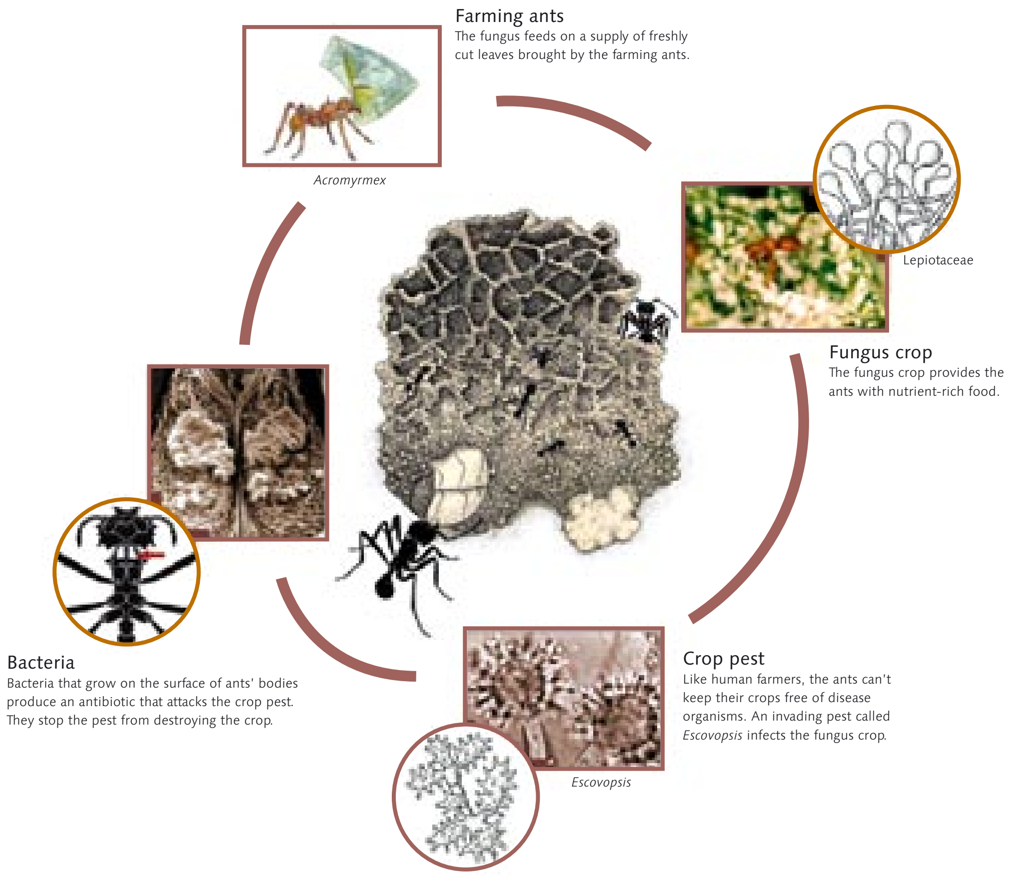
Take a stroll through a rain forest in South America and you might find yourself walking in a river, not of water, but of leaves. Leaf-cutter ants swarm in the underbrush, carrying their precious cargo back to their nest with an apparent single-minded determination. This conspicuous behaviour has made these ants one of the most dominant herbivores in the Neotropics, and one of the most successful social insects in nature. A closer look at the ants reveals that they are ancient farmers, having developed the secret of agriculture over 50 million years ago. Using their freshly-cut leaves, they incorporate them into gardens where they grow a specialized fungus that they consume for food. This relationship between ant and fungus has been described as a breakthrough in animal behaviour, and parallels the practice of sustainable agriculture in humans, arguably the most important development in human civilization that, in our opinion, resulted in the dominance of humans on planet Earth.

Leaf-cutting ants are the most highly-derived group of ants that practice fungus growing. A total of four other fungus-growing ant agricultural systems have been described, spanning over 200 different species of ants, each based on the type of fungus grown and the material incorporated into their gardens. The vast majority of fungus-growing ants do not cut leaves, but instead collect fruit, leaf litter and decomposing organic material, such as caterpillar dung, to grow their fungus.

Fungus-growers also have diverse colony sizes, with some species containing only a few hundred workers, while many leaf-cutting ant species can contain upwards of 5 million. All species, however, follow the same life cycle. Organic material is brought into the colony by foragers and is then processed to form a garden matrix where the fungus grows. New material is continuously incorporated into the gardens in order to propagate the fungus, and old material is removed by the ants and placed in special refuse dumps away from the colony. In many groups of fungus-growing ants, the fungus produces specialized packets of nutrients called gongylidia that the ants eat and feed to their developing brood. At the start of the rainy season, the colony produces male and female winged reproductives called aletes, which mate in a spectacular display of flying ants. The newly-mated queens then go on to found new colonies. Young queens transport a small piece of the fungus garden in a special organ known as an infrabuccal pocket when they leave the nest for their mating flights, and thus ensure that they can successfully start a fungus garden in the new colony.

Garden microbiology

Until about a decade and a half ago, research on fungus-growing ants focused primarily on the ants and their foraging behaviour. It wasn't until the early 1990s that this focus shifted to the fungus gardens and their associated microbial communities. Since the ant gardens are maintained in soil chambers, they are routinely exposed to a number of potential pathogens



◀ Fig. 1. The fungus-growing ant system. The ants grow a fungus crop for food in gardens, which often get attacked by invading crop pests. The ants deal with these attacks by growing bacteria on the surface of their bodies that produce antibiotics capable of stopping the pest. *Cara Gibson & Angie Fox*

▼ Fig. 2. A fungus garden from a 1-year-old colony of *Acromyrmex echinatior*. Note that many of the garden workers are covered with the antibiotic-producing bacteria. *David R. Nash*

▶ Fig. 3. Rivers of leaves. Foragers of the leaf-cutter ant *Atta cephalotes* bring freshly-cut leaves back to their nest. *Alexander Wild*

that could infect and overtake a garden. In fact, many of the ant colonies do become overgrown by fungal pathogens, often resulting in the death of the colony. Intensive sampling of the fungal communities within the gardens revealed that a specialized microfungal pathogen selectively attacks the gardens of the fungus-growing ants. These fungi, which belong to the genus *Escovopsis*, directly attack and kill the crop fungus, and can overrun the garden in a similar fashion to the way weeds and pests can ruin human gardens.

A curious observation that researchers noted was that some workers had a white wax-like substance across their bodies. It was thought that this substance was a wax produced by the ants themselves, with an unknown function. However, when viewed under a microscope it was discovered that this covering was not a wax, but a bacterium! Isolation of these bacteria revealed that they belong to the genus *Pseudonocardia*, which are part of the actinobacteria, a group of prokaryotes that produces over 80 % of the antibiotics used by humans. Further work on this ant-associated actinobacteria has shown that it produces antifungal compounds that inhibit the specialized microfungal pathogen that attacks the garden. As a result, it is now known that these ants employ these bacteria as a source of pesticides to control the invading pathogenic fungi. Interestingly, the spread of the actinobacteria on

worker ant bodies is correlated to the incidence of infection. At the onset of invasion by *Escovopsis*, the actinobacteria will cover the workers' bodies, presumably to increase the production of the pesticide. This discovery was the first demonstrated example of an animal, other than humans, that employ bacteria to produce antibiotics in order to deal with pathogens.

As a second line of defence, the ants have also adopted the practice of weeding. Anyone who has ever weeded a garden can readily identify with this onerous task! When *Escovopsis* is detected by garden workers, there is an immediate flurry of activity as ants begin to comb through the garden matrix. Upon finding the pathogenic fungus, they weed them out and discard them into their refuse dumps away from the garden. By weeding and applying pesticides, the ants have developed a system to keep their gardens pest-free, an impressive feat given that they grow their fungal crop in monoculture, an ability which has evaded human agriculturalists.

Mutualism happens

The interaction between the ants and their fungus crop, and the ants and the bacteria is known as a mutualistic relationship. In general a mutualism is established when both members of the interaction derive a benefit from the association. In the ant–fungus mutualism, the ants obtain nutrients from the fungus, and use this to feed the entire colony. This mutualism is so tight, that the loss of fungus by the ants results in the death of the entire colony. In return, the fungus receives a continuous supply of growing material, protection from the environment, and the removal of disease-causing agents and competitors through the ants' weeding behaviour and pesticide application.

So what do the bacteria get out of producing pesticides for the ants? For starters, they get food. Many species of fungus-growing ants have evolved special crypts on their bodies where the bacteria live and grow. It is thought that the ants provide nutrients to the bacteria through glands connected to these crypts. Furthermore, the bacteria gain a protected environment in which to grow, away from the intense competition they would face if they lived in other environments such as the soil. Since the ants are invested in these bacteria as a producer of pesticides, they are carried by young queens that found new nests, and thus gain access to new resources that ensure their continued existence and survival.

A chemical arms race

Research in our laboratory has revealed a number of interesting properties between the bacteria and the pathogenic fungus. The bacteria appear to be specially suited to inhibiting the pathogenic fungi that infect the ants' fungus garden. Even though these parasitic fungi belong to a single genus, they are differentiated into various species and strains that are each associated with particular groups of ants. We have found that the actinobacteria associated with any given species of fungus-growing ant is effective at inhibiting some strains of pathogenic fungi, but not all; they tend to be most effective against the pathogenic fungus that specifically infects the gardens of the ants they are associated with. Interestingly, the tight association between ant, bacteria and pathogen will sometimes result in the pathogen winning. This interplay has been described as a chemical 'arms race' between the bacteria and fungus, with one side beating the other as new compounds are evolved. At the moment, we are beginning to understand the chemical warfare at the genetic level, and it is likely that these types of interactions are more prevalent in nature than previously thought.

So how exactly does an ant go about forming partnerships with a fungus and a bacterium? No one really knows. With new advances in molecular and genetic technologies, such as whole-genome sequencing, we will hopefully begin to understand how these associations were established, and gain further insight into how these interactions resulted in the remarkable fungus-growing ability of the ants.

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Further reading

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