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New findings in insect fungiculture

Have ants developed non-food, agricultural products?

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The interaction between Allomerus plant-ants and an ascomycete fungus growing on and strengthening their galleries is not opportunistic. We previously demonstrated that this association is highly specific as only one fungal species represented by a few haplotypes was found associated with the ants. We also discovered that the ants' behavior revealed a major investment in manipulating and enhancing the growth of their associated fungus. We have growing evidence that this specificity is consistent with selection by the ants. Here, we discuss this selection within the framework of insect agriculture, as we believe these ants fulfill all of the prerequisites to be considered as farmers. Allomerus ants promote their symbiont's growth, protect it from potential pathogens and select specific cultivars. Taken together, we think that the interaction between Allomerus ants and their cultivar might represent the first case of insect fungiculture used as a means of obtaining building material.

Key words: agriculture, ant-plant-fungus interaction, selection, *Allomerus decemarticulatus*, Trimmatostroma sp

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Agriculture refers to highly specialized mutualisms in which two partners (i.e., a farmer and a cultivar) exchange specific services. To be considered a "farmer," an organism must fulfill at least three major criteria: (1) the habitual planting of a cultivar on a favorable substrate and improving its growing conditions, (2) protection from potential parasites and competitors, and most importantly, (3) the selection of specific cultivars.[1] In return, the cultivar is used by the farmer as a source of nutrients or as a trade good. These mutualisms have evolved only a few times: once for humans and at least 10 separate times for insects from four orders (i.e., ants, termites, ambrosia beetles and gall midges).[1-4] Although humans cultivate a large array of crops, using them in diversified ways (e.g., alimentation, pharmacology, construction, ornamentation), known cases of agriculture by insects are restricted to the cultivation of fungi for food. Among them, the fungiculture developed in the higher Attine ("leaf-cutter" ants) has been the best studied.[5] In this system, the interaction is obligatory for each partner and the vertical mode of fungal propagation is thought to have played a decisive role in the coevolutionary history of both partners, even leading to the emergence of new fungal structures (e.g., gongylidia).[6,7] The ecological success of this fungiculture facilitated the great diversification of fungus-growing ants. The production of goods, however, remains known only for humans.

We recently described a non-food based mutualism between the plant-ant *Allomerus decemarticulatus* Mayr (Myrmicinae) and the ascomycete fungus Trimmatostroma sp (Chaetothyriales).[8] In this interaction, the ants build galleried structures pierced with holes along their host plant's stems that they then use to ambush for prey.[9] The galleries are made with the trichomes and vegetal dough of their host plant and are further reinforced by the presence of the associated fungus.[9] We demonstrated that this interaction is highly specific because only a few fungal

haplotypes were found associated with the ants, with one major haplotype representing 75% of all of the fungi sampled among geographically-distant populations in French Guiana.[8] Moreover, the ants' behavior reveals a major investment in manipulating and enhancing the growth of their associated fungus. We have growing evidence to support the idea that specific molecular and behavioral traits are both indicators of a true fungiculture in Allomerus. This ant-fungus interaction fulfills most, if not all, of the required conditions to be considered the first non-human case of agriculture aimed at the production of a cultivar for structural purposes.

Choosing the Right Substrate and Improving Growing Conditions

Ants select favorable substrates for the development of this fungus. The workers prepare pellets of chewed vegetable matter extracted from the inner wall of domatia (i.e., plant structures in which the ants nest) and paste them around sheaves of trichomes in the foundations of the galleries. The fungus spreads from these areas. Once the mycelia prosper on the galleries around the trichomes, the ants paste prey debris and more vegetable matter on the galleries from which the mycelium absorbs nutrients.[10] In this case, the ants apply the debris as if spreading manure to fertilize the substrate for the fungus. Direct observations of the ants' behavior demonstrate they are actively manipulating the fungal mycelia in at least two ways. First, the fungal growth is constrained to the galleries and the mycelium does not overrun the galleries, except for during a short period if the ants are experimentally removed from the plant.[9] Second, when the walls of the domatia are artificially damaged, the ants transport packets of pure mycelia from inside the domatia that they paste along the cuts (JL, personal observation). In less than 24 h, the wound is completely filled with pieces of mycelium.

Protection of the Cultivar from Potential Parasites

When ants are present, only the associated fungus grows on the galleries. An examination of the infrabuccal pellets of the workers highlights the presence of many fungal spores, suggesting the existence of a weeding and/or grooming behavior by these ants. Indeed, when the ants are removed from the plant, many alien fungi, most probably from the latent spores that are present, rapidly grow and overrun the galleries.[8,9] The ants can control the contaminants in either of two ways: first, by physically removing the fungal spores and mycelia; and, second, through potentially antibiotic secretions and/or through other symbionts secreting antifungal compounds.[11] The latter hypothesis still needs to be demonstrated, although recent studies have shown that the antifungal producing actinobacteria present on *A. decemarticulatus* cuticles inhibit the growth of some fungi whose spores were isolated from the galleries.[12]

Selection of Specific Cultivars

Our study, based on the genetic population structure of more than 114 Allomerus colonies with their fungal cultivars, highlighted the occurrence of a single fungal species from the order Chaetothyriales in the galleries of the two Allomerus species investigated.[8] In total, 16 fungal haplotypes were isolated, of which one was over-represented across the area sampled. Six and seven haplotypes were strictly associated with *A. decemarticulatus* and *A. octoarticulatus* colonies, respectively. The other three haplotypes were found associated with both ant species. Although more exhaustive sampling is needed to understand the selection of the cultivars at the population level and the mode by which the association is transmitted, the interaction with a single fungal species points to the strong selection of the cultivars. Comparatively, the molecular investigation of the mycelia growing in similar carton galleries built by the ant *Azteca brevis* revealed the presence of several species, suggesting that there is a weaker level of selection than in Allomerus.[13] It should be noted, however, that the species found in the carton tunnels of *Az. brevis* were also phylogenetically placed within the order Chaetothyriales, close to the Trimmatostroma species associated with Allomerus. These fungi are saprophitic or grow on insect secretions.[14] Thus, the ant galleries seem to provide both a favorable substrate and environmental conditions for the development of these specific Chaetothyriales fungi.[15] This ecological filter may partly account for the observed specificity between the ants and the Chaetothyriales, but does not explain why Allomerus ants are specifically associated with a few haplotypes from a single fungal species. Direct selection by the ants, as is the case for the Attine, would result in such specificity. Indeed, the fungus associated with Allomerus is always present at the very first stage of colony development when the queen starts claustral foundation. Moreover, it is absent from plants that have not been associated with ants and does not survive long after the colonies die.[8,9] All in all, this argues in favor of fungal selection by codispersal and the vertical transmission of the cultivar across ant generations.

The association between Allomerus ants and the fungus that they manipulate in their galleries seems to result from an agricultural process as the ants fulfill all of the prerequisites to be considered farmers. They develop favorable substrates and promote their symbiont's growth, protect it from potential pathogens and actively manipulate it for use as construction material. Most importantly, the specificity between the ants and the symbiont is consistent with two different levels of selection by the ants. First, the specific substrate created by the ants induces a loose and indirect selection through an ecological filtering process. Second, the potential codispersal might further add to the niche specificity through the direct selection of the fungal cultivars. Current studies are focusing on the demonstration of a vertical mode of transmission to fully demonstrate this first case of insect fungiculture as a means of obtaining building material.

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