New findings in insect fungiculture

Have ants developed non-food, agricultural products?

Jérémie Lauth,1 Mario X. Ruiz-González2,3 and Jérôme Orivel4,*

1Université des Antilles et de la Guyane; UMR Ecologie des Forêts de Guyane; Campus Agronomique; 2CNRS; EDB (Laboratoire Evolution et Diversité Biologique); Université Toulouse III; Toulouse, France; 3Abiotic Stress Department; Integrative and Systems Biology Group; Instituto de Biología Molecular y Celular de Plantas; Valencia, Spain; 4CNRS; UMR Ecologie des Forêts de Guyane; Campus Agronomique, France

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.

Addendum to: Ruiz-González MX, Malé PJG, Leroy C, Dejean A, Gryta H, Jargeat P, et al. Specific, non-nutritional association between an ascomycete fungus and Allomerus plant-ants. Biol Lett 2011; 7:475–9; PMID: 21084334; DOI: 10.1098/rsbl.2010.0920.
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. 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. 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. 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. 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. 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.

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. 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. 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. Thus, the ant galleries seem to provide both a favorable substrate and environmental conditions for the development of these specific Chaetothyriales fungi. 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 clausal foundation. Moreover, it is absent from plants that have not been associated with ants and does not survive long after the colonies die. 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.

Acknowledgments

We are grateful to the Laboratoire Environnement de Petit Saut and the Nouragues Research Station for furnishing logistical help, to Celine Leroy for her help in the field, and to Andrea Yockey-Dejean for editing the manuscript. Financial support was provided...
by a research program of the French Agence Nationale de la Recherche (research agreement n°ANR-06-JCJC-0109-01), by the ESF-EUROCORES/TECT/BIOCONTRACT program (06-TECT-FP-007), by a fellowship from the Fondation pour la Recherche sur la Biodiversité (research agreement n°AAP-IN-2009-050) and by a Nouragues research grant from the CNRS.

References
1. Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR. The evolution of agriculture in insects. Annu Rev Ecol Evol Syst 2005; 36:563-95; DOI:10.1146/annurev.ecolsys.36.102003.152626.
2. Farrell BD, Sequeira AS, O’Meara BC, Normark BB, Chung JH, Jordal BH. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution 2001; 55:2011-27; PMID:11761062.
3. Aanen DK, Eggleton P, Rouland-Lefèvre C, Guldborg-Froslev T, Rosendahl S, Boomsma JJ. The evolution of fungus-growing termites and their mutualistic fungal symbionts. Proc Natl Acad Sci USA 2002; 99:14887-92; PMID:12386341; DOI:10.1073/pnas.222313099.
4. Rohfritsch O. Plants, gall midges and fungi: a three-component system. Entomol Exp Appl 2008; 129:116.
5. Holldobler B, Wilson EO. The ants. Berlin: Springer 1990.
6. Schultz TR, Brady SG. Major evolutionary transitions in ant agriculture. Proc Natl Acad Sci USA 2008; 105:5435-40; PMID:18362345; DOI:10.1073/pnas.0711024105.
7. Mueller UG, Schultz TR, Currie CR, Adams RMM, Malloch D. The origin of the attine ant-fungus mutualism. Q Rev Biol 2001; 76:169-97; PMID:11409051; DOI:10.1086/393867.
8. Ruiz-González MX, Malé PJG, Leroy C, Dejean A, Gryta H, Jargeat P, et al. Specific, non-nutritional association between an ascomycete fungus and Allomerus plant-ants. Biol Lett 2011; 7:475-9; PMID:21084334; DOI:10.1098/rsbl.2010.0920.
9. Dejean A, Solano PJ, Ayroles J, Corbara B, Orivel J. Insect behaviour: Arboreal ants build traps to capture prey. Nature 2005; 434:973; PMID:15846335; DOI:10.1038/434973a.
10. Leroy C, Sejalon-Delmas N, Jaunceau A, Ruiz-González MX, Gryta H, Jargeat P, et al. Trophic mediation by a fungus in an ant-plant mutualism. J Ecol 2011; 99:583-90.
11. Kahlenporh M. Actinobacteria as mutualists: general healthcare for insects? Trends Microbiol 2009; 17:529-35; PMID:19853457; DOI:10.1016/j.tim.2009.09.006.
12. Sejalon-Delmas N, Leroy C, Ruiz-González MX, Orivel J, Yu DW, Hutchings MI. Fungus-growing Allomerus ants are associated with antibiotic-producing actinobacteria. Antonie van Leeuwenhoek 2011; In press; PMID:21748399; DOI:10.1007/s10482-011-9621-y.
13. Mayer VE, Voglmayr H. Mycelial carton galleries of Azteca brevis (Formicidae) as a multi-species network. Proc Biol Sci 2009; In press; PMID:19596257; DOI:10.1098/rspb.2009.0768.
14. Bailey IW. Some relations between ants and fungi. Ecology 1920; 1:174-89; DOI:10.2307/1929134.
15. Voglmayr H, Mayer V, Maschwitz U, Moog J, Djetel-Lordon C, Blatrix R. The diversity of ant-associated black yeasts: insights into a newly discovered world of symbiotic interactions. Fungal Biol 2010; 115:1077-91; DOI:10.1016/j.funbio.2010.11.006.