

A breakthrough innovation in animal evolution

Ulrich G. Mueller* and Christian Rabeling

Section of Integrative Biology, University of Texas, Austin, TX 78712

Among the many activities of animals, few seem more outlandish than the leaf-cutting and fungus-growing habits of attine ants of the New World tropics. In a typical rainforest, endless processions of workers transport leaf forage on cleared highways across the forest floor, each worker balancing a leaf fragment like an oversized green umbrella (Fig. 1). The ants do not eat the leaves, but they use them as garden compost to grow a nutritious fungus, the ants' main diet. So efficient is the conversion of leaves into fungal food that the ant-fungus symbiosis has been called "one of the breakthroughs in animal evolution" (1), on par with such major evolutionary innovations as the ungulate rumen or the powered flight of birds. Leaf-cutter ants consume more vegetation than any other comparable herbivore (2), making them major agricultural pests. Early Brazilian farmers were so frustrated in their battles against the *saúva* (leafcutter ants) that they concluded "Brazil must kill the *saúva* or the *saúva* will kill Brazil."

Leaf-cutter ants are the ecologically conspicuous representatives of a larger group of more than 200 fungus-growing (attine) ant species, most of which do not cut leaves and instead use leaf-litter debris for fungal cultivation (3, 4). The common feature of all fungus-growing ants is their astonishing proficiency in planting, manuring, weeding, and sheltering fungal gardens, but the specific fungicultural habits are remarkably diverse among ant species, suggesting a long and complex coevolutionary history between attine ants and their fungal crops. Now, Schultz and Brady (5) report in this issue of PNAS a comprehensive phylogenetic analysis of the attine ants, providing detailed insights into the evolutionary transitions leading from simple to complex fungiculture and, ultimately, to the breakthrough of leafcutter fungiculture.

Evolution of Fungus-Growing Ants. In the history of terrestrial evolution, ants represent one of the most successful insect families since arthropods conquered land. The recipe for success was a fine-tuned combination of social and ecological specialization, with some of the most diverse specializations evolving in the leaf litter stratum, the birthplace also of the fungus-gardening ants some 50 million years ago (5, 6). The original fun-

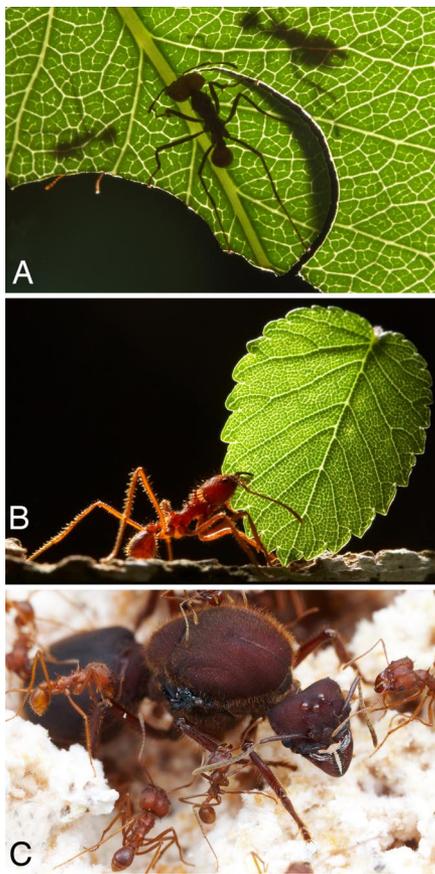


Fig. 1. Leaf-cutter ants. Workers cut leaves (A), transport the leaf fragments to their nest (B), and use the leaves as compost to grow a fungus for food (C). Leaf-cutter ant queens (C) are among the most fertile and long-lived queens of all social insects. Photographs by Alex Wild.

gus-growing ants were not leaf cutters, but debris collectors, using withered plant bits for cultivation of a relatively unspecialized mycelial fungus that retained close population-genetic ties to free-living fungal populations. Nest sizes were small, involving probably only dozens to hundreds of workers. The later evolutionary transition from debris collector to leaf cutter was accompanied by novel allocation of leaf-processing tasks to size-variable (polymorphic) worker castes and a dramatic increase in worker number produced by long-lived queens (Fig. 1C). Some extant leaf-cutter nests are estimated to live for 10–20 years, have 5–10 million workers, and maintain 500–1,000 football-sized fungus gardens in an underground metropolis occupying the volume of a bus.

Ant-Fungus Coevolution. Schultz and Brady's comprehensive reconstruction of

attine evolution (5) identifies several cycles of ant-fungus specialization followed by ant diversification. Three distinct specialized fungicultural systems arose out of the generalized ancestral attine fungiculture, and one of these specialists gave rise subsequently to the even more narrowly specialized leaf-cutter fungiculture. That is, more generalized systems gave birth to specialized systems, which, as the specialized descendants diversified, led to opportunities for further specialization. Such successive cycles of coevolutionary innovation followed by diversification were hypothesized more than 40 years ago (7), but empirical support for this model has accumulated only recently (e.g., refs. 8 and 9). Schultz and Brady provide a phylogenetic framework that will now allow testing of this model also for the attine ant-fungus symbiosis, hopefully stimulating investigations into biochemical or behavioral-physiological innovations that may drive speciation rates in the specialized fungicultural systems, such as the leaf-cutter ants.

One key evolutionary transition in attine fungiculture was the transition from the original open fungicultural system (allowing occasional genetic exchanges between domesticated and free-living fungal populations) to the more closed system of higher-attine fungiculture where domesticated cultivars exist disjoint from free-living relatives (4, 6). Schultz and Brady (5) date this transition to 30–40 million years after the origin of attine fungiculture. This delayed transition may seem surprising, but many comparable mutualisms also exist successfully as open systems, as if a pool of free-living microbes from which novel types can be recruited somehow enhances long-term evolutionary persistence. For example, nitrogen-fixing root bacteria or photosynthesizing algal symbionts are continuously acquired from the environment by, respectively, plants or corals. Long-term partnering and tight coevolution between host-microbe associates seems to be more an exception than the rule across life and, like-

Authors contributions: U.G.M. and C.R. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 5435.

*To whom correspondence should be addressed. E-mail: umueller@mail.utexas.edu.

© 2008 by The National Academy of Sciences of the USA

wise, also for attine ants and their fungal cultivars.

Breakthrough Transitions in Attine Evolution. The early diversification of the attine lineages dates to ≈ 45 –55 million years ago in the early Eocene (5), a period of rapid global warming, with maximum global temperatures and CO₂ levels never surpassed since then. South America was covered by humid forests with low seasonality, consistent with the traditional view that attine fungiculture originated in rainforest habitats (3, 10). These forests likely resembled current Amazonian forests, with leaf-litter accumulations that supported decomposing fungi and also provided humid nest opportunities for ants. Such conditions may have facilitated interactions between ants and leaf-litter fungi, leading eventually to the origin of attine fungiculture.

For the first 20 million years after the origin of attine fungiculture, Schultz and Brady (5) infer a period of evolutionary doldrums, with no apparent innovation in fungicultural habits. Innovations originating during this period were either lost because of extinction, or perhaps they remain to be discovered among the more primitive attine lineages, which are deplorably understudied. The next 20 million years between 25–5 MyPB (Miocene), however, emerge as the period of greatest fungicultural diversification in attine evolution, giving rise to yeast cultivation, a switch to a completely novel cultivar type in one attine lineage, and the emergence of leaf-cutter fungiculture. These fungicultural innovations

coincided with marked ecological transitions in South America, as grasslands expanded and forests receded with shifting precipitation patterns. The coincidence of grassland expansion with the origin of the leaf-cutting behavior is particularly intriguing because grass-cutting

Specific fungicultural habits are remarkably diverse among ant species.

specializations are common among leaf-cutting species and leaf-cutter diversity is greatest in grass-dominated habitats in South America (11). These patterns support the hypothesis that the first leaf cutters may have been grass-cutting specialists, with specializations on broadleaf plants evolving later (11).

The recent date of 5–15 MyBP for the origin of leaf cutters implicates a rapid radiation and geographic expansion of these ecologically diverse ants. For example, extant leaf cutters include species adapted to deserts or humid forests, species with subterranean or arboreal nesting habits, and species specialized on cutting grass or broad-leaved plants. Moreover, the most wide-ranging leaf-cutter species originated and expanded only within the last 1–2 million years (S. Solomon, M. Bacci, J. Martins, G. Gonçalves Vinha, and U. G. Mueller, unpublished data). This accelerated eco-

logical diversification and rapid geographic expansion of leaf-cutter ants underscores the belief that leaf-cutter fungiculture indeed represents one of the key innovations in animal evolution.

The Future of Attinology. The ubiquity of dynamic coevolutionary interactions predicts lineage-lineage reshuffling and novel microbe acquisition not only for ant-cultivar associations, but also for the associations between attine ants and their additional microbial symbionts, such as the actinomycete bacteria grown by many attine ants on their integument to procure antibiotic protection from these bacteria (12, 13). This expectation of dynamic ant-microbial coevolution is inconsistent with the narrow antibiotic specificities reported originally for the integumental bacteria (12), and further study into the diverse functions of the ant-associated bacteria may therefore reveal unsuspected complexities. It is likely that early conceptualizations of attine ant-microbe coevolution, derived from a study of limited samples (e.g., refs. 12, 14, and 15), will continue to be modified by more comprehensive analyses (13, 16–18). Taxonomic revision and phylogenetic analyses of attine ants will undoubtedly contribute to this enriched understanding of ant-microbe coevolution (5, 19, 20). Most importantly, microbial-ecological studies of the sister lineages of existing fungicultural specialists may reveal the existence of intermediate forms, similar perhaps to those stages that must have preceded the origin of the documented fungicultural innovations (5).

- Wilson EO (1986) in *Fire Ants and Leaf-Cutting Ants* eds Lofgren CS, Vander Meer RK (Westview, Boulder, CO), pp 1–9.
- Wirth R, Herz H, Ryel RJ, Beyschlag W, Hölldobler B (2003) Herbivory of leaf-cutting ants. *Ecological Studies*, Vol 164.
- Weber NA (1972) *Gardening Ants: The Attines* (Am Philos Society, Philadelphia).
- Mueller UG, Gerardo N, Schultz TR, Aanen D, Six D (2005) The evolution of agriculture in insects. *Ann Rev Ecol Syst* 36:563–569.
- Schultz TR, Brady SG (2008) Major evolutionary transitions in ant agriculture. *Proc Natl Acad Sci USA* 105:5435–5440.
- Mueller UG, Schultz TR, Currie CR, Adams RMM, Malloch D (2001) The origin of the attine ant-fungus mutualism. *Q Rev Biol* 76:169–197.
- Ehrlich PR, Raven PH (1964) Butterflies and plants: A study in coevolution. *Evolution* 18:586–608.
- Berenbaum MR, Favret C, Schuler MA (1996) On defining “key innovations” in an adaptive radiation: cytochrome P450s and Papilionidae. *Am Nat* 148:5139–5155.
- Wheat CW, Vogel H, Wittstock U, Braby MF, Underwood D, Mitchell-Olds T (2007) The genetic basis of a plant-insect coevolutionary key innovation. *Proc Natl Acad Sci USA* 104:20427–20431.
- Wheeler WM (1907) The fungus-growing ants of North America. *Bull Am Mus Nat Hist* 23:669–807.
- Fowler HG (1983) Latitudinal gradients and diversity of the leaf-cutting ants (*Atta* and *Acromyrmex*) (Hymenoptera: Formicidae). *Rev Biol Trop* 31:213–216.
- Currie CR, Scott JA, Summerbell RC, Malloch D (1999) Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* 398:701–704.
- Poulsen M, Erhardt DP, Molinaro DJ, Lin T-L, Currie CR (2007) Antagonistic bacterial interactions help shape host-symbiont dynamics within the fungus-growing ant-microbe mutualism. *PLoS One* 2:e960.
- Chapela IH, Rehner SA, Schultz TR, Mueller UG (1994) Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266:1691–1694.
- Currie CR, Wong B, Stuart AE, Schultz TR, Rehner SA, Mueller UG, Sung GH, Spatafora JW, Straus NA (2003) Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science* 299:386–388.
- Mueller UG, Rehner SA, Schultz TR (1998) The evolution of agriculture in ants. *Science* 281:2034–2038.
- Gerardo NM, Mueller UG, Currie CR (2006) Complex host-pathogen coevolution in the *Apterostigma* fungus-growing ant-microbe symbiosis. *BMC Evol Biol* 6:88–98.
- Mikheyev AS, Mueller UG, Abbot P (2006) Cryptic sex and many-to-one coevolution in the fungus-growing ant symbiosis. *Proc Natl Acad Sci USA* 103:10702–10706.
- Mayhé-Nunes AJ, Brandão CRF (2007) Revisionary studies on the attine ant genus *Trachymyrmex* Forel. Part 3: The Jamaicensis group (Hymenoptera: Formicidae). *Zootaxa* 1444:1–21.
- Rabeling C, Cover SP, Johnson RA, Mueller UG (2007) A review of the North American species of the fungus-gardening ant genus *Trachymyrmex* (Hymenoptera: Formicidae). *Zootaxa* 1664:1–53.