Coevolved Crypts and Exocrine Glands Support Mutualistic Bacteria in Fungus-Growing Ants

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Attine ants engage in a quadripartite symbiosis with fungi they cultivate for food, specialized garden parasites, and parasite-inhibiting bacteria. Molecular phylogenetic evidence supports an ancient host-pathogen association between the ant-cultivar mutualism and the garden parasite. Here we show that ants rear the antibiotic-producing bacteria in elaborate cuticular crypts, supported by unique exocrine glands, and that these structures have been highly modified across the ants' evolutionary history. This specialized structural evolution, together with the absence of these bacteria and modifications in other ant genera that do not grow fungus, indicate that the bacteria have an ancient and coevolved association with the ants, their fungal cultivar, and the garden parasite.

Attine ants are a New World tribe having obligate associations with fungi that they cultivate for food. The ants' fungal gardens are host to specialized and virulent parasitic microfungi in the genus *Escovopsis* (Ascomycota, Hypocreales) (*1*–*3*). Infected colonies experience a significant reduction in garden growth rate and production of new workers, and under some conditions *Escovopsis* can completely overgrow the fungus garden (*1*, *2*). The symbiotic association between attine ants, their fungal cultivars, and the specialized garden parasite *Escovopsis* originated about 50 to 65 million years ago and has subsequently been shaped by millions of years of coevolution (*4*–*6*). The ant-cultivar-*Escovopsis* host-pathogen coevolution has resulted in ancient evolutionary congruence: Specific groups of attine ants are specialized on specific groups of cultivated fungi, and these fungi are host to specific groups of *Escovopsis* parasites (*4*). In addition, even at the finer phylogenetic level, there is parasite specialization on fungal cultivar genotypes (*7*).

To help defend their cultivar from the garden parasite, attine ants have a mutualistic association with filamentous bacteria that produce antibiotics with potent antagonistic properties against *Escovopsis* (*3*, *8*, *9*). The filamentous bacteria are in the genus *Pseudonocardia* (*10*); belonging in the order Actinomycetales, a group well known for its ability to produce antibiotics (*11*). *Pseudonocardia* bacteria are associated with all attine-ant species examined, and occur on specific locations on the cuticle of a given ant species. The bacterium is carried by gynes (female reproductive ants) on their mating flights and is thereby transmitted from parent to offspring colonies (*8*). Individual ant nests are associated with a single strain of *Pseudonocardia*, but genetically distinct strains

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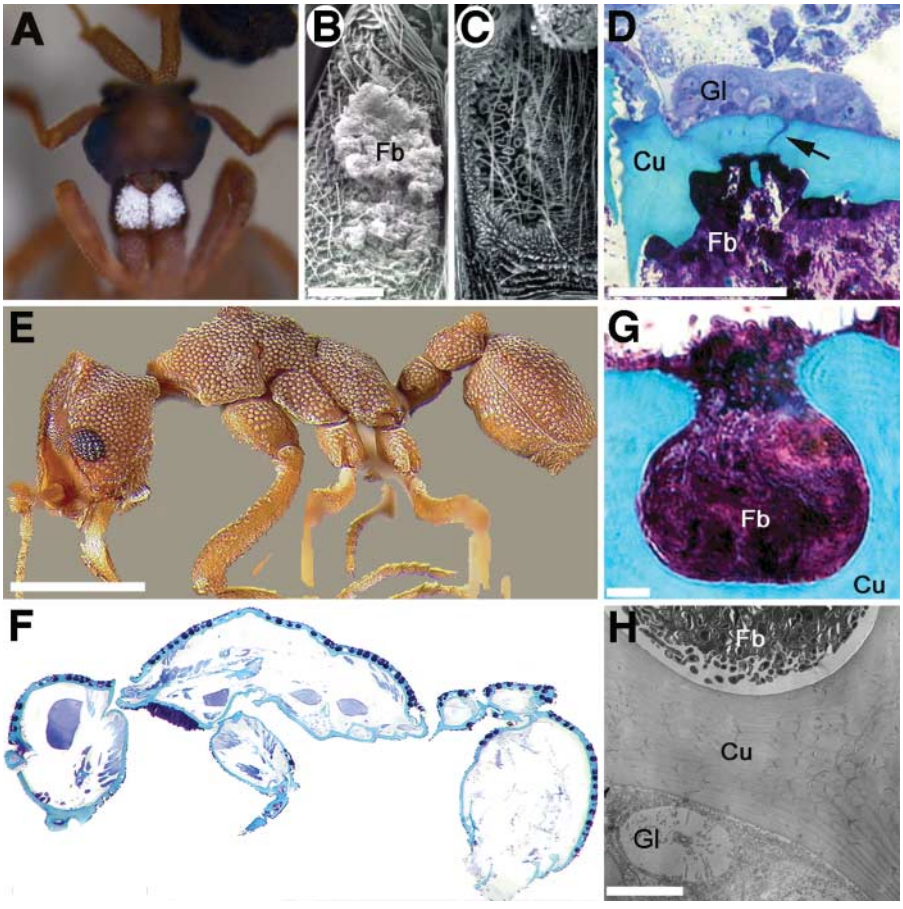

Fig. 1. (**A**) Photograph of *Cyphomyrmex costatus* showing the bacteria on the propleural plates. SEM of the plates in *C. muelleri*: The left plate is covered with bacteria (**B**), whereas they have been removed from the right plate, revealing the underlying fovea (**C**). (**D**) Light micrograph of a semithin cross section through the propleural plate of *C. longiscapus* showing the gland (Gl) and duct cells (black arrow) associated with the fovea and the bacterium (Fb) on the plate (Cu for cuticle). (**E**) Photograph of *C. longiscapus*, illustrating foveae openings covering most of the cuticle. (**F**) Sagittal semithin section through a *C. longiscapus* worker, illustrating foveae outlining nearly the entire body of the ant. (**G**) Light micrograph of a single fovea within the cuticle (Cu) illustrating the abundance of mutualistic bacteria (Fb) within the crypt. (**H**) TEM of the lower section of a fovea showing a single glandular cell (Gl) and bacteria (Fb) within the crypt. Scale bars: 50 μm (A to C), 5 μm (E and G), and 0.5 mm (F). [Photograph in (A) by A. Little]

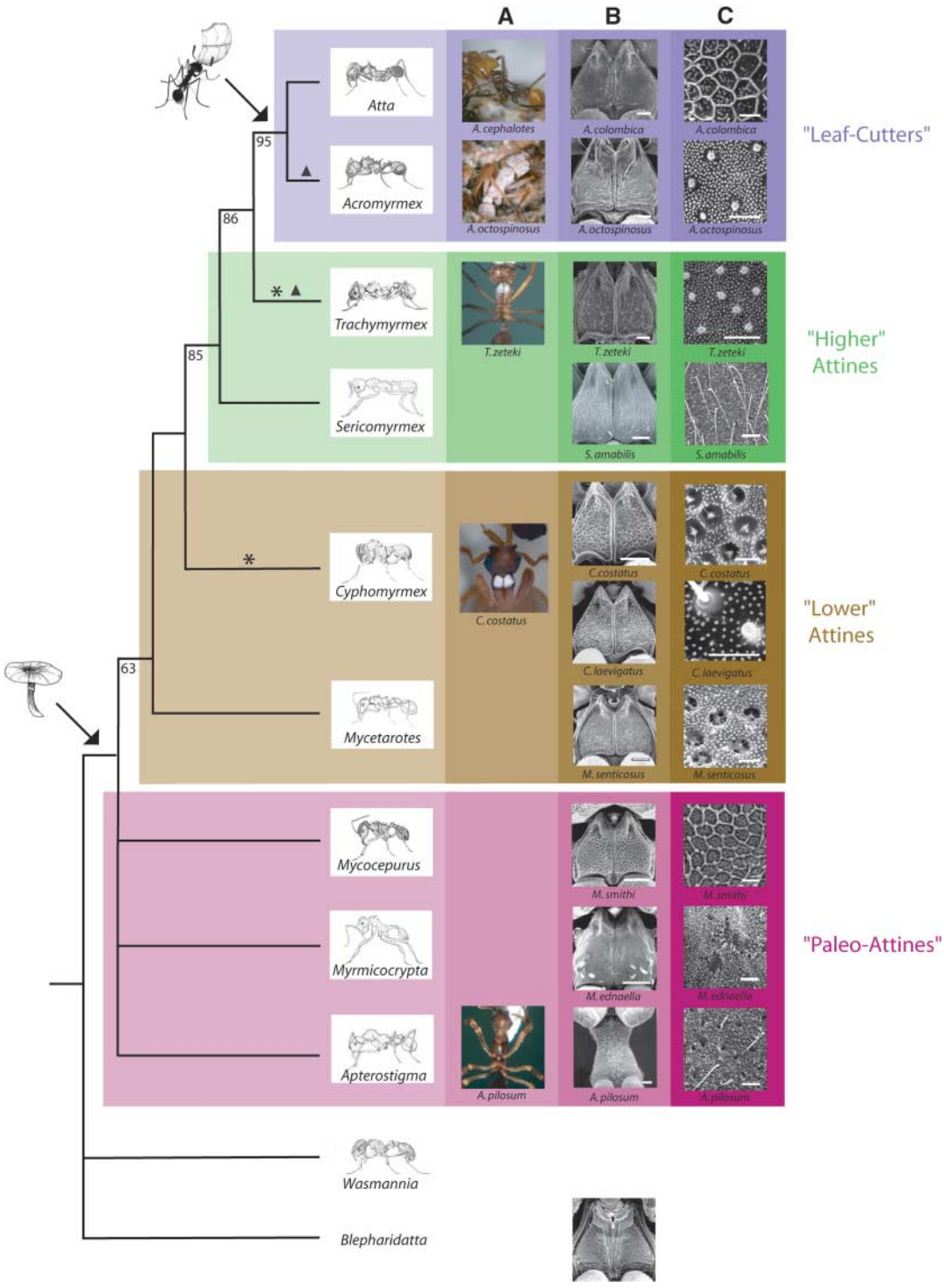

Fig. 2. Genus level phylogeny of fungus-growing ants [adapted from (*15*, *16*)] illustrating the location and modifications of the exoskeleton for maintaining the mutualistic bacteria. The origin of fungus growing by attine ants and the leaf-cutters is represented by the Lepiotaceous mushroom and the worker carrying a leaf fragment, respectively. Major groups of attine ants are depicted by colored boxes, illustrating the phylogenetically basal genera in the "paleo-attines" (red), the "lower" attine genera (brown), the "higher" attines (green), and the leaf-cutters (blue). (Column A) Photographs illustrate the location of the bacterium under the forelegs in the paleo-attines, on the propleural plates in the "lower" and "higher" attines, the presence all over the integument in the genus *Acromyrmex*, and absence on the cuticle in *Atta*. (Column B) SEM micrographs of the location of the bacterium, under the forelegs in *Apterostigma* and on the propleural plates in other groups. (Column C) SEM micrograph close-ups for the structures presented in (B), showing the specific structural modifications for different groups of fungus-growing ants. Presence of foveae (star) and tubercles (triangle) all over the body in some species within a genus are indicated by the corresponding symbol above the branch on the phylogeny. Scale bars: 0.5 mm (B), 10 μm (C). [The line drawings of *Wasmannia auropunctata*, *Cyphomyrmex rimosus*, *Trachymyrmex septentrionalis*, *Acromyrmex versicolor*, and *Atta texana* were made by Smith (*20*); those of *Myrmicocrypta ednaella* and *Sericomyrmex amabilis* were made by Weber (*21*); and those of *Apterostigma pilosum*, *Mycocepurus smithi*, and *Mycetarotes* sp. were made by A. Little. Photographs of *Acromyrmex octospinosus* and *Cyphomyrmex costatus* in (A) were taken by A. Little.]

and/or species of bacteria can occur within populations of the same species and between species of ants (*12*). The diversity and mode of transmission predict congruence between the ant and bacteria phylogenies; however, the complete evolutionary history of ant-associated *Pseudonocardia* still remains to be determined. Here we examine the presence and evolution of specific cuticular structures on attine ants to house and maintain the parasite-inhibiting bacteria (*8*).

To investigate this, we first examined ant species in the genus *Cyphomyrmex* because of the conspicuous white "bloom" of bacterium present on the propleural plates (Fig. 1A). Scanning electron microscopy (SEM) of *Cyphomyrmex longiscapus* and *C. muelleri* workers with the filamentous bacterium removed revealed the presence of a previously unnoticed large crescent-shaped cavity (fovea) on each propleural plate (*13*) (Fig. 1, B and C; fig. S1A). The foveae are porous and occupy a significant proportion of the surface area of the propleural plates; the filamentous bacteria grow directly within these crypts (Fig. 1C).

Our investigations further revealed, in the semithin sections of the propleural plates in *C.*

longiscapus, the presence of a previously unknown exocrine gland located on the inner surface of the cuticle, just below the foveae. The gland consists of bicellular units, each formed by a gland cell and duct cell (*14*). The duct cells cross the cuticle and open within the foveae where the bacteria are cultured (Fig. 1D; fig. S1, A to C).

In addition to foveae occurring on the propleural plates, *C. longiscapus*, *C. muelleri*, and *C. costatus* ants also have bacteria-filled foveae covering most of the surface of worker exoskeletons, including the head, thorax, abdomen, and legs (Fig. 1, E to G). These crypts have small openings to the external surface of the ant, with minute microtrichia (hair-like cuticular projections) that appear to shield the opening of the crypt (fig. S1D). At the bottom of each fovea is a porous tubercle (integumental protrusion) (fig. S1E), connected via a duct cell to the corresponding gland cell directly beneath the crypt (Fig. 1H).

The locality of bacteria on the cuticle varies across fungus-growing ant species (Fig. 2, column A). Examination of specialized structures for bacterial maintenance across the phylogenetic diversity of attine ants revealed several broad evolutionary patterns (Fig. 2). Ant genera closely related to attine ants, *Wasmannia* and *Blepharidatta* (*15*, *16*), do not have filamentous bacteria, fovea, or tubercles (Fig. 2). In the most phylogenetically basal attine ants (paleoattines), such as the genus *Apterostigma*, the filamentous bacterium occurs on the mesopleura (under the forelegs), where it grows directly on the cuticle over the pores of duct cells connected to the corresponding gland cells (Fig. 2, fig. S1F). In most species of "lower" attine ants, mutualistic bacteria occur on the propleural plates (e.g., *Cyphomyrmex costatus*, in Fig. 2), in which the bacterium grows on tubercles within foveae. Similarly, the bacteria are also concentrated on the propleural plates in the "higher" attine genus *Trachymyrmex* and the leaf-cutter genus *Acromyrmex*, although in these two genera the bacteria grow on gland cell–associated tubercles directly on the exoskeleton rather than in foveae (Fig. 2).

Several species of plants and animals engaged in mutualistic associations with microbes have evolved structures to house their symbionts. For example, root nodules in legumes house *Rhizobium*, squid light organs are filled with bioluminescent bacteria, aphids have modified bacteriocytes that form organlike structures to rear *Buchnera*, and some beetles and woodwasps have specialized structures (known as mycangia) to house mutualistic fungi (*17*–*19*). Our findings indicate that the exoskeleton of attine ants is modified to house mutualistic bacteria, apparently supporting their growth through glandular secretions. In addition, our phylogenetic examination of the structures across the fungus-growing ant tribe revealed that, like the cultivar and garden parasite, the mutualistic *Pseudonocardia* bacteria was apparently present at the earliest stages of fungus cultivation by ants. This is supported by the presence of the bacteria and bacteria-associated glands and duct cells in the most phylogenetically basal genera (e.g., *Apterostigma)*, in contrast to their absence in closely related ants that do not cultivate fungus gardens (*Blepharidatta* and *Wasmannia)*.

The apparently early evolutionary origin of the bacteria within the fungus-growing ant symbiosis, in combination with bioassay results confirming that filamentous bacteria isolated from across the phylogenetic diversity of attine ants are effective at inhibiting their corresponding garden parasites (*8*, *10*, *13*), indicate that the bacteria have provided an efficient defense against *Escovopsis* for millions of years. This raises the question of how the antibiotics have remained effective without rampant evolution of resistance in the parasite over the long evolutionary history of this symbiosis.

References and Notes

1. C. R. Currie, U. G. Mueller, D. Malloch, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7998 (1999).
2. C. R. Currie, *Oecologia* **128**, 99 (2001).
3. C. R. Currie, *Annu. Rev. Microbiol.* **55**, 357 (2001).
4. C. R. Currie *et al.*, *Science* **299**, 386 (2003).
5. I. H. Chapela, S. A. Rehner, T. R. Schultz, U. G. Mueller, *Science* **266**, 1691 (1994).
6. U. G. Mueller, T. R. Schultz, C. R. Currie, R. M. M. Adams, D. Malloch, *Q. Rev. Biol.* **76**, 169 (2001).
7. N. M. Gerardo, U. G. Mueller, S. L. Price, C. R. Currie, *Proc. R. Soc. London Ser. B.* **271**, 1791 (2004).
8. C. R. Currie, J. A. Scott, R. C. Summerbell, D. Malloch, *Nature* **398**, 701 (1999).
9. C. R. Currie, A. N. M. Bot, J. J. Boomsma, *Oikos* **101**, 91 (2003).
10. M. J. Cafaro, C. R. Currie, *Can. J. Microbiol.* **51**, 441 (2005).
11. M. Goodfellow, T. Cross, *The Biology of Actinomycetes* (Academic Press, London, 1984).
12. M. Poulsen, M. Cafaro, J. J. Boomsma, C. R. Currie, *Mol. Ecol.* **14**, 3597 (2005).
13. Materials and methods are available as supporting material on *Science* Online.
14. J. Billen, E. D. Morgan, in *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*, R. K. Vander Meer, M. D. Breed, M. L. Winston, K. E. Espelie, Eds. (Westview Press, Boulder, CO, 1998).
15. T. R. Schultz, R. Meier, *Syst. Entomol.* **20**, 337 (1995).
16. J. K. Wetterer, T. R. Schultz, R. Meier, *Mol. Phyl. Evol.* **9**, 42 (1998).
17. A. E. Douglas, *Symbiotic interactions* (Oxford Univ. Press, Oxford, 1994).
18. L. Margulis, R. Fester, *Symbiosis as a Source of Evolutionary Innovation* (MIT Press, Cambridge, MA, 1991).
19. S. Paracer, V. Ahmadjian, *Symbiosis: An Introduction to Biological Associations* (Oxford Univ. Press, Oxford, 2nd ed., 2000).
20. M. R. Smith, *Am. Midl. Nat.* **37**, 521 (1947).
21. N. A. Weber, *Gardening Ants: The Attines* (American Philosophical Society, Philadelphia, 1972).

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A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells

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Macrophages and dendritic cells (DCs) are crucial for immune and inflammatory responses and belong to a network of cells that has been termed the mononuclear phagocyte system (MPS). However, the origin and lineage of these cells remain poorly understood. Here, we describe the isolation and clonal analysis of a mouse bone marrow progenitor that is specific for monocytes, several macrophage subsets, and resident spleen DCs in vivo. It was also possible to recapitulate this differentiation in vitro by using treatment with the cytokines macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. Thus, macrophages and DCs appear to renew from a common progenitor, providing a cellular and molecular basis for the concept of the MPS.

Macrophages (MΦs) and dendritic cells (DCs) are involved in the scavenging of dying cells, pathogens, and molecules through phagocytosis and endocytosis and the use of pattern recognition receptors (*1*). As a result, both cell types make a vital contribution to immunity and inflammatory responses to pathogenic microorganisms (*2*).