repressed, as expected, because Notch activation inhibits photoreceptor differentiation (*23*). Similarly, in the eyes of adult flies in which clones of cells ectopically expressing SER or DL have been induced, scars of tissue lacking ommatidia occur. Reversals of ommatidial chirality occur along the equatorial sides of these Notch signaling–induced scars (Fig. 4B). Although these observations show that Notch activation influences ommatidial chirality, the absence of D-V midline expression of SER or DL behind the morphogenetic furrow, together with the inhibition of photoreceptor development associated with Notch activation behind the furrow, indicate that this influence must be indirect. The equatorial bias in the influence of ectopic Notch activation implies that the equator is the normal source of a Notch-dependent, chirality-determining signal (*11*).

D-V signaling in the eye shares many similarities with D-V signaling in the wing (*2*). In both cases an initial asymmetry is set up by Wingless expression. They then go through a distinct intermediate step, as in the wing Wingless represses the expression of a positive regulator of *fng*, Apterous (*3*, *24*), whereas in the eye Wingless promotes the expression of *mrr* (*13*), which encodes a negative regulator of *fng* (*25*). They then share a FNG-SER-DL-Notch signaling cassette to effect signaling between dorsal and ventral cells and establish Notch activation along the D-V midline. Local activation of Notch leads to production of diffusible, long-range signals that direct growth and patterning, which in the wing include Wingless, but in the eye remain unknown. At least one downstream target of D-V midline signaling is also conserved, as *fj* is also expressed in the wing (*14*, *15*), and its expression there is indirectly influenced by Notch (*26*).

fng-related genes appear to play analogous roles in D-V signaling during the development of vertebrate limbs (*27*). This conservation, together with conservation of other molecules involved in D-V patterning (*2*, *28*), and the similar deployment of Hedgehog- and Decapentaplegic-related proteins to pattern the anterior-posterior axes of *Drosophila* and vertebrate limbs, led to the suggestion that a common ancestor used these signaling cassettes to control patterning and growth in some appendage or tissue (*28*). Notably, Decapentaplegic and Hedgehog signaling are also required for the growth and patterning of the developing eye along its anterior-posterior axis (*29*). Thus, three disparate structures, *Drosophila* wings, *Drosophila* eyes, and vertebrate limbs, use the same signaling pathways along both anterior-posterior and D-V axes to effect patterning and growth. Although a role for D-V signaling in vertebrate eye development has not yet been described, genes that specify eye fate in *Drosophila* and vertebrates are homologous (*30*). The similarity between the molecular mechanisms that control eye and appendage development may reflect their descent from a common ancestral organ. Alternatively, the entire patterning system may have been co-opted by one of these organs from the other. In either case, the similarity seems beyond coincidence, and our results imply an evolutionarily relationship between eyes and appendages.

References and Notes

1. W. J. Brook, F. J. Diaz-Benjumea, S. M. Cohen, *Annu. Rev. Cell Dev. Biol.* **12**, 161 (1996).
2. K. D. Irvine and T. F. Vogt, *Curr. Opin. Cell Biol.* **9**, 867 (1997).
3. K. D. Irvine and E. Wieschaus, *Cell* **79**, 595 (1994).
4. R. J. Fleming, Y. Gu, N. A. Hukriede, *Development* **124**, 2973 (1997).
5. V. M. Panin, V. Papayannopoulos, R. Wilson, K. D. Irvine, *Nature* **387**, 908 (1997).
6. J. A. Campos-Ortega, M. Waitz, *Wilhelm Roux's Arch. Dev. Biol.* **184**, 155 (1978).
7. D. F. Ready, T. E. Hanson, S. Benzer, *Dev. Biol.* **53**, 217 (1976).
8. A. Tomlinson, *Development* **104**, 183 (1988).
9. A. P. Jarman, *Trends Genet.* **12**, 121 (1996).
10. L. Zheng, J. Zhang, R. W. Carthew, *Development* **121**, 3045 (1995).
11. M. Wehrli and A. Tomlinson, *ibid.* **125**, 1421 (1998).
12. H. McNeill, C.-H. Yang, M. Brodsky, J. Ungos, M. A. Simon, *Genes Dev.* **11**, 1073 (1997).
13. U. Heberlein, E. R. Borod, F. A. Chanut, *Development* **125**, 567 (1998).
14. M. H. Brodsky, H. Steller, *Dev. Biol.* **173**, 428 (1996).
15. J. L. Villano and F. N. Katz, *Development* **121**, 2767 (1995).
16. G. Struhl, K. Fitzgerald, I. Greenwald, *Cell* **74**, 331 (1993).
17. K. Ito, W. Awano, K. Suzuki, Y. Hiromi, D. Yamamoto, *Development* **124**, 761 (1997).
18. T. R. Parody and M. A. T. Muskavitch, *Genetics* **135**, 527 (1993); D. L. Shellenbarger and J. D. Mohler, *Dev. Biol.* **62**, 432 (1978).
19. S. A. Speicher, U. Thomas, U. Hinz, E. Knust, *Development* **120**, 535 (1994).
20. X. Sun and S. Artavanis-Tsakonas, *ibid.* **122**, 2465 (1996). Although animals in which the ventral eye is distorted and reduced are recovered when *fng* mutant clones are induced, this appears to reflect a requirement for *fng* activity outside of the eye primordia. *fng* mutant clones within the eye do not lead to significant reductions in size.
21. Evidence for Notch activation along the D-V midline has been observed in eye discs of UAS-GFP larvae expressing a modified version of the Notch^{+}-Gal4-VP16 (N^{+}-GV3) protein described in G. Struhl and A. Adachi, *Cell* **93**, 649 (1998) (G. Struhl and A. Adachi, unpublished observations).
22. S.-I. Higashijima *et al.*, *Genes Dev.* **6**, 50 (1992).
23. M. E. Fortini, I. Rebay, L. A. Caron, S. Artavanis-Tsakonas, *Nature* **365**, 555 (1993).
24. J. A. Williams, S. W. Paddock, S. B. Carroll, *Development* **117**, 571 (1993).
25. H. McNeill, R. Chasen, V. Papayannopoulos, K. D. Irvine, M. Simon, unpublished observations.
26. C. Rauskolb, V. Papayannopoulos, K. D. Irvine, unpublished observations.
27. E. Laufer *et al.*, *Nature* **386**, 366 (1997); C. Rodriguez-Esteban *et al.*, *ibid.*, p. 360.
28. N. Shubin, C. Tabin, S. Carroll, *ibid.* **388**, 639 (1997).
29. J. E. Treisman and U. Heberlein, *Curr. Top. Dev. Biol.* **39**, 119 (1998).
30. R. Quiring, U. Walldorf, U. Kloter, W. J. Gehring, *Science* **265**, 785 (1994).
31. *fng* expression was detected by in situ hybridization. Immunostaining was performed as described (*5*), except that SER was detected with a rat antisera (diluted 1:1000) (produced by T. Correia). *mrr* expression was detected with an enhancer trap (*12*).
32. Ectopic *fng* expression was generated by use of the Flip-out system to produce clones of cells expressing *Gal4* in animals that also carried *UAS-GFP* and *UAS-fng* transgenes (*17*). Clones of cells mutant for *fng* were produced by mitotic recombination between a *fng*^{13} FRT80B chromosome (*3*) and FRT80-3. *fj* was detected by an enhancer trap (*14*), Myc was detected with a rabbit antiserum (A14, Santa Cruz) diluted 1:100.

33. We thank E. Wieschaus for assistance during the initial phases of this project; H. McNeill and G. Struhl for communication of unpublished observations; Y. Hiromi, D. Doherty, E. Knust, U. Walldorf, H. McNeill, H. Stellar, T. Laverty, G. Struhl, and the Bloomington stock center for *Drosophila* stocks; E. Knust, M. Muskavitch, S. Artavanis-Tsakonas, K. Saigo, and T. Correia for antibodies; and R. Padgett for comments. C.R. is a Foundation for Advanced Cancer Studies Fellow of the Life Sciences Research Foundation. This work was supported by NIH grant GM-R01-54594 to K.D.I.

20 May 1998; accepted 24 August 1998

The Evolution of Agriculture in Ants

Ulrich G. Mueller,* Stephen A. Rehner, Ted R. Schultz*

Cultivation of fungi for food by fungus-growing ants (Attini: Formicidae) originated about 50 million years ago. The subsequent evolutionary history of this agricultural symbiosis was inferred from phylogenetic and population-genetic patterns of 553 cultivars isolated from gardens of "primitive" fungus-growing ants. These patterns indicate that fungus-growing ants succeeded at domesticating multiple cultivars, that the ants are capable of switching to novel cultivars, that single ant species farm a diversity of cultivars, and that cultivars are shared occasionally between distantly related ant species, probably by lateral transfer between ant colonies.

Fungus farming by ants of the tribe Attini originated in the early Tertiary (*1*, *2*) and thus predates human agriculture by about 50 million years (*3*). During its extensive evolutionary history, this symbiosis between "attine" ant farmers and their fungal cultivars has acquired an astonishing complexity, involving secretion of antibiotic "herbicides" to control weed molds and elaborate manuring regimes that maximize fungal harvests (*4*, *5*). Of the over 200 known

extant attine species, all are obligate fungus farmers. Cultivars are propagated vegetatively (as asexual clones) within nests, and between parent and offspring nests. In the few studied cases, the foundress queen carries in her mouth a pellet of fungus from the natal nest that she uses to start her own garden (*2*). This mode of propagation suggested the long-standing hypothesis that attine fungi are ancient clones that have strictly coevolved with their hosts (*2*).

Most attine cultivars are propagated as a mycelium (multicellular phase), but those cultivated by a group in the ant genus *Cyphomyrmex* are maintained as masses of yeast (unicellular phase) (*2*). The great majority of these fungi, including the yeasts, are members of the tribe Leucocoprini (Basidiomycotina: Agaricales: Lepiotaceae) (*6*, *7*), a large, poorly known group of predominantly tropical mushrooms (*8*). The cultivation of a nonlepiotaceous fungus by some ants in the genus *Apterostigma* is the only exception to this ancestral association with lepiotaceous fungi and indicates a historically unique switch to a cultivar outside the Lepiotaceae (*7*).

Domestication of novel cultivars and switching between cultivars may be ancient themes in attine agriculture, which presumably began with an ancestral ant that facultatively associated with fungi (*1*, *2*, *5*). Indeed, repeated and possibly recent domestication events were suggested by phylogenetic patterns (*7*). First, some attine cultivars appeared to be more closely related to free-living Leucocoprini than to other cultivars; and second, ant and cultivar phylogenies were topologically incongruent. Both patterns are inconsistent with a scenario of a single domestication event followed by strict clonal propagation. Support for these conclusions was weak, however, and other processes can generate topological incongruence, including cultivar transfers between ant lineages.

To distinguish between these diverse scenarios of ant-fungus evolution, we surveyed Neotropical free-living and ant-cultivated fungi and sequenced representative lineages for phylogenetic analysis. The survey focused on the seven genera of "lower" [phylogenetically basal (*9*)] attine ants, those most likely to have retained the least modified forms of the ancestral farming behavior (*10*). We surveyed sympatric Panamanian communities of lower attine cultivars (n = 337) and free-living Leucocoprini collected as fruiting bodies (mushrooms) (n = 309 collections) (*11*), and an additional 216 cultivars along an axis intersecting the United States (n = 96), Costa Rica (n = 13), Trinidad (n = 65), Guyana (n = 39), and Brazil (n = 3) (*12*). Collections were screened for restriction fragment length polymorphisms (RFLPs) (*13*), and representative RFLP types (25 free-living and 57 ant-cultivated fungi) were sequenced for two nuclear ribosomal DNA (rDNA) gene regions [internal transcribed spacer (ITS) and large subunit (25*S*) (*14*)]. Phylogenetic analyses using parsimony and maximum likelihood criteria produced results identical in the features emphasized below (Fig. 1) (*15*).

DNA sequences of one field-collected, free-living leucoprinoid mushroom matched exactly (PA-234: 100% sequence identity) (Fig. 1B) and a second matched nearly exactly (PA-302: differing in one base pair) (Fig. 1A) the sequences of two separate, ant-cultivated fungi. This establishes the existence of free-living counterparts of two attine cultivars. During our year-long survey, four individuals of one counterpart (PA-302) were collected within a 2-week period, and the other counterpart (PA-234) was collected only once. This rarity suggests that free-living analogs may also exist for other ant cultivars, but were simply not encountered. The discovery of two free-living counterparts, each matching a cultivated fungus in a fast-evolving gene (ITS), suggests that these cultivars were domesticated recently. The alternative explanation, that the free-living counterparts represent "escapes" from a monolithic, clonal lineage that originated with a single domestication event 50 million years ago, is inconsistent with observed levels of allele sequence divergence (see below) and theory predicting genetic degradation of unexpressed fruiting (mushroom) structures under long-term asexuality (*16*). These objections to sexual forms arising from ancient clones do not, however, preclude repeated instances of domestication and escape that occur over time spans too short to permit degradation of underlying fruiting genes.

The majority of lower-attine cultivars fall into two monophyletic groups, but two unique cultivars (*Mycocepurus smithi* S60 and *Myrmicocrypta infuscata* G11) (Fig. 1) fall outside these clades. This pattern suggests four independent domestications and contradicts the hypothesis of a single domestication followed by long-term clonal propagation (*2*). Statistical tests in which cultivars were constrained to form less than four independent lineages resulted in highly significant support for a minimum of three independent domestications (*17*). Perhaps more compelling, the statistical tests conservatively excluded the two free-living counterparts of two cultivars (Fig. 1, A and B) that, as argued above, represent two additional cases of recent domestication. This increases to five the minimum number of domestications of fungi by ants (*18*).

Only one ant cultivar, *Myco. smithi* S60 (Fig. 1), confidently falls outside of the two main cultivar clades, suggesting that lower attine agriculture is somewhat specialized in that domestication of fungi from outside of the two clades occurs infrequently, or that such associations are relatively short-lived, or both. This may indicate that cultivars derived from the two main clades are more suitable for cultivation, nutritionally or otherwise, than other leucocoprinoid fungi.

Whereas any single attine nest contains only a single cultivar, different nests of the same ant species may contain distantly related cultivars (Fig. 1). In an extreme case, *Myco. smithi* was found to cultivate four distinct fungal lineages in central Panama and four additional lineages in Trinidad (Fig. 1); even adjacent nests of this ant, sometimes separated by only a few centimeters, were found to contain distantly related cultivars. At the other extreme, all 34 nests of *Myrm. sp. 1* contained the same fungal lineage. Most species cultivate at least two distinct fungal lineages (*19*), sometimes drawn from both of the two main cultivar clades, such that most lower attine ants are generalists within or even across the two clades. The only exceptions are the yeast-cultivating *Cyphomyrmex* species, which specialize on a derived monophyletic group of cultivars (Fig. 1). Within this yeast group, however, the same ant species may cultivate a diverse set of fungi, a pattern consistent with both cultivar acquisition from free-living stocks [note the free-living PA-302 in the yeast clade (Fig. 1)] and with cultivar exchange between yeast-cultivating species (see below). Thus, although the yeast-cultivating *Cyphomyrmex* species are specialized on a narrowly defined fungal clade, there is no apparent specialization within that clade.

The same or very closely related fungi may be cultivated by distantly related ants. For example, in central Panama, *Myrmicocrypta ednaella*, *Mycocepurus tardus*, and *Myco. smithi* cultivate fungi with identical rDNA sequences; similarly, in Guyana, *Cyphomyrmex faunulus* and *Myrmicocrypta cf. buenzlii* share identical cultivars. Overall, cultivar sharing was observed seven times between two species from the same genus and four times between two or more species of different genera (Fig. 1). Such sharing obviously generates topological incongruence across ant and fungal phylogenies and indicates that different ants acquired cultivars from the same free-living stock, or that they acquired cultivars from each other, or both.

To determine whether some instances of this cultivar sharing were due specifically to cultivar transfers between ant species, we generated amplified fragment length poly-

U. G. Mueller, Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama, and Department of Biology, University of Maryland, College Park, MD 20742, USA. S. A. Rehner, Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama, and Department of Biology, University of Puerto Rico, Rio Piedras, San Juan 00931, Puerto Rico. T. R. Schultz, Department of Entomology MRC 165, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560, USA.

*To whom correspondence should be addressed at the University of Maryland (e-mail: um3@umail.umd.edu) or the Smithsonian Institution (e-mail: schultz@onyx.si.edu).

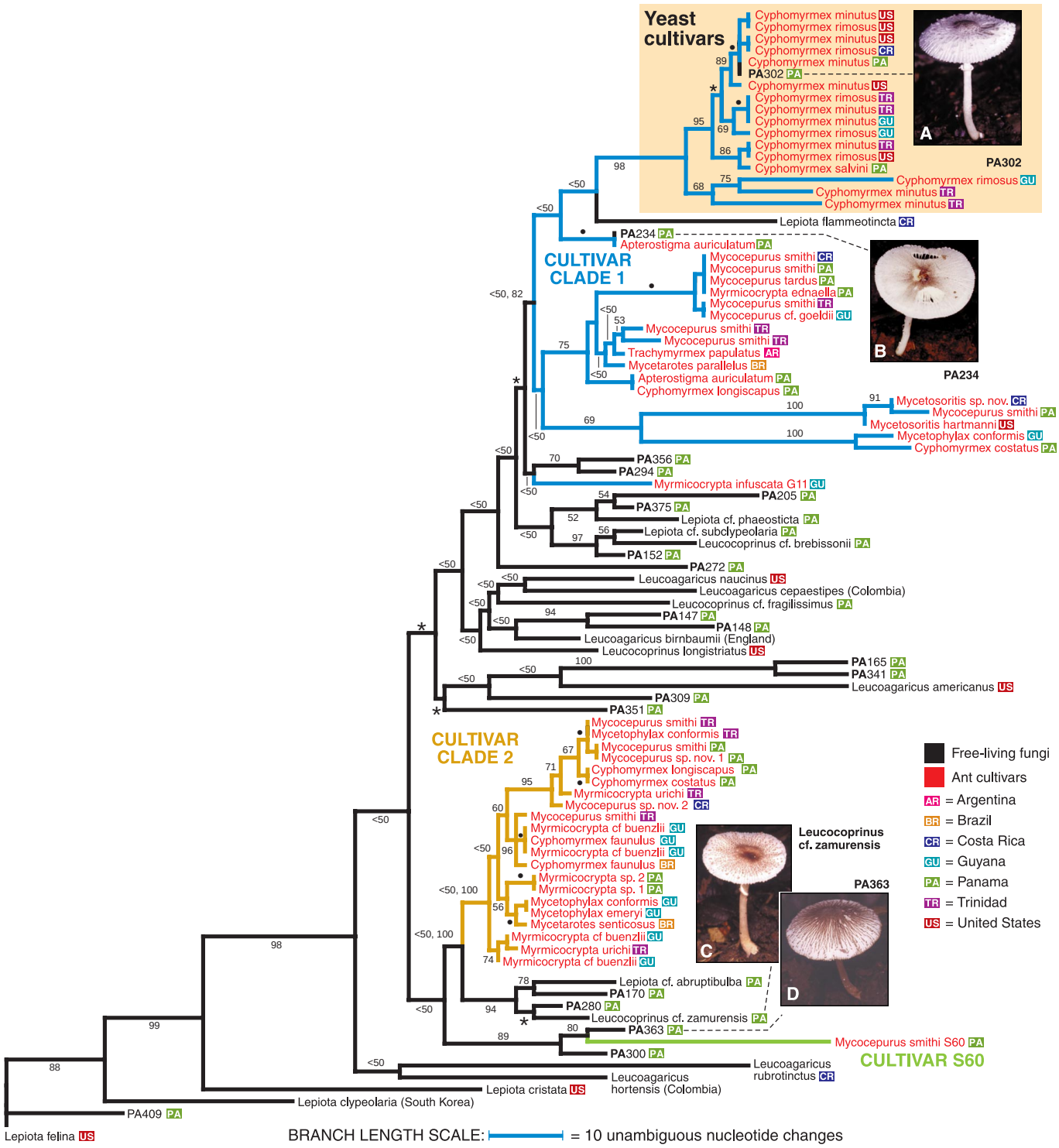

Fig. 1. Phylogeny for 57 fungal cultivars of the seven genera of "lower" fungus-growing ants and 36 free-living fungi in the family Lepiotaceae. All cultivars belong to a group grown only by "lower attines," ant lineages that are phylogenetically "primitive" and thus most likely to have retained ancestral farming behaviors that existed early in the history of this 50-million-year-old symbiosis. Two free-living counterparts of ant-cultivated fungi (**A** and **B**) reveal two recent domestications of cultivars by ants. Statistically distinct cultivar lineages, represented by colored branches, provide evidence for a minimum of three additional domestications (*18*). Cultivated fungi are indicated in red by their respective ant host species. Free-living fungi are indicated in black; some of these are undescribed species and are denoted by their collection IDs. Almost all ant species cultivate several phylogenetically distant cultivars. The tree shown is the strict consensus of five equally parsimonious trees obtained by parsimony analysis and successive approximations weighting of a 1422–base pair sequence of two nuclear rDNA regions (ITS and 25S), and resembles in all important details the tree found using maximum likelihood (*15*). Numbers on branches are bootstrap values from 500 pseudoreplicates; where multiple bootstrap values are indicated, the second value is derived from analyses of two phylogenetically restricted subsets of taxa in which additional characters, excluded from the global analysis because of alignment ambiguities, could be unambiguously aligned. Asterisks (*) indicate branches not present in the strict consensus of 180 equally parsimonious trees found in the analysis before successive weighting; bullets (•) indicate branches above which a single representative taxon was included in the analyses (*15*).

morphism (AFLP) fingerprints for cultivars that were identical in rDNA sequence and that were isolated from gardens of different ant species (*20*). Fingerprints revealed that some cultivars grown by different ant species are genetically identical and that they are asexual descendants of the same clone (Fig. 2). Cases of genetically identical, shared cultivars were detected in two species of *Cyphomyrmex* (*minutus* and *rimosus* from Florida), in two species of *Mycocepurus* (*smithi* and *tardus* from Panama), and in two species in widely separated genera, *Cyphomyrmex longiscapus* and *Apterostigma auriculatum* [from Panama (Fig. 2)]. Within each species pair, nests were collected over areas spanning hundreds of square kilometers (Fig. 2). Given this geographic scale, it is implausible that the ants acquired their cultivars from the same free-living fungal clone (*21*); instead, cultivars appear to be occasionally transferred across ant species and subsequently disperse with the ants. Such lateral transfer may occur fortuitously (for example, gardens from several nests are simultaneously disturbed and mixed) or after accidental cultivar loss that forces ants to obtain a replacement from a neighboring colony. The case of shared cultivars in *C. minutus* and *C. rimosus* from Florida is particularly instructive, because *C. rimosus* was introduced to Florida apparently during this century (*22*). The fact that *C. rimosus* presently shares a genetically identical cultivar with the indigenous *C. minutus* indicates that cultivar replacement may occur on an ecological time scale.

Two lines of evidence support the clonal nature of attine cultivars: the existence of widespread AFLP-fingerprint cultivar types (Fig. 2) (*23*), and behavioral observations of clonal cultivar transfer by foundress queens from parent to daughter nests (*2*, *5*). The absence of significant allele sequence divergence (ASD) (*24*) at the two sequenced regions indicates that these clones are not ancient. Specifically, ASD levels across the two rDNA genes are almost identical between sequenced cultivars and free-living Leucocoprini [0.00146 ± 0.00148 and 0.00109 ± 0.00163 heterozygosity per site (mean ± SD), respectively], as is expected if cultivars were acquired recently from sexually outcrossing stocks (*24*). Observed rDNA ASD levels therefore are inconsistent with the long-standing model of ancient cultivar clonality spanning millions of years (*2*).

Cultivar collections did not reveal phylogeographic patterns of cultivar usage along the transect Costa Rica–Panama–Trinidad–Guyana (Fig. 1). Cultivars are diverse at each location and largely shared across the entire transect. Thus cultivar lineages do not cluster by location. This suggests that ants disperse widely and mix cultivar lineages across locations, or that the free-living stocks from which cultivars are drawn are widespread and have overlapping ranges, or both.

In summary, phylogenetic patterns demonstrate that ants, like humans, succeeded at domesticating multiple cultivars during the history of their agricultural symbiosis, that the acquisition of novel cultivars is an ongoing process occurring in at least some extant ant species, and that cultivars are shared occasionally between ant lineages, probably by transfer between ant species. Domestication of free-living stocks therefore continued after the origin of fungus-growing and, along with cultivar exchange between ant species, may have persisted for 50 million years of ant evolution as a means to replace cultivars after accidental or pathogen-driven loss of gardens, to respond to environmental changes by acquiring cultivars with novel features, or to capitalize on strains that were improved while associated with other ant lineages. If exchanges occur frequently among ants, as population-genetic patterns suggest, the history of ant agriculture may have been characterized by the same rapid lateral spread of cultivars that has shaped the history of human agriculture (*3*). Given these parallels between ant and human agriculture, promising avenues for future attine research include the testing for ecological zoning (correlating cultivar types with ecological conditions) and, given the recent and repeated domestications of novel cultivars, testing for the gradual modification of cultivars by ants through a sensory bias–driven analog of artificial selection.

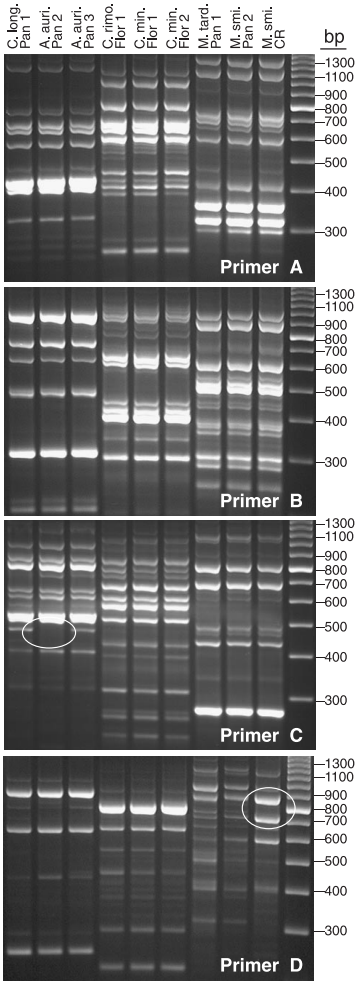

Fig. 2. AFLP fingerprints of three cultivar clones shared between different ant species. These three cultivar clones were found, respectively, in gardens of three species pairs: *Cyphomyrmex longiscapus–Apterostigma auriculatum*, *C. rimosus–C. minutus*, and *Mycocepurus tardus–Myco. smithi* (*20*). Three cultivars are shown for each clone: two isolated from geographically proximate nests of different species (shown as the two leftmost in each triplet; with distances of 9 km, 100 m, and 11 km, respectively, for the three species pairs), one from a distant nest of one of the two species (85, 110, and 360 km, respectively). AFLP fingerprints are shown for 4 of 12 different primer systems tested (*20*). Ten systems (only two are shown in the two top panels) failed to reveal any genetic variation within each cultivar clone, and only two (two bottom panels) revealed minor band differences (circles). The largest detected difference (2 of more than 100 bands total) involved two isolates from the geographically most distant nests (360 km; Panama–Costa Rica). Identity of banding patterns (amplifying more than 100 AFLP fragments that are dispersed randomly throughout the genome) indicates that the respective cultivars are genetically identical and therefore are asexual descendants of the same clone. The genetic identity of cultivars found in gardens of different ant species or genera, some of them separated by large geographic distances, suggests that these cultivars were transferred between ant nests and were subsequently dispersed by the ants to distant locations. Panama: Pan 1 = Pipeline Road, Pan 2 = Gamboa, Pan 3 = Nusagandi; Florida: Flor 1 = Archbold Biological Station, Flor 2 = Orlando; CR = Costa Rica.

References and Notes

1. E. O. Wilson, *The Insect Societies* (Belknap, Cambridge, MA, 1971).
2. N. Weber, *Gardening Ants* (American Philosophical Society, Philadelphia, PA, 1972).
3. D. Rindos, *The Origins of Agriculture* (Academic Press, Orlando, FL, 1984).
4. M. Martin, *Invertebrate-Microbial Interactions* (Cornell Univ. Press, Ithaca, NY, 1987).
5. B. Hölldobler and E. O. Wilson, *The Ants* (Belknap, Cambridge, MA, 1990).
6. A. Hervey, C. T. Rogerson, I. Long, *Brittonia* **29**, 226 (1977).
7. I. H. Chapela, S. A. Rehner, T. R. Schultz, U. G. Mueller, *Science* **266**, 1691 (1994).
8. Although the systematics of the family Lepiotaceae is poorly understood, the lepiotaceous tribe Leucocoprini (consisting of two genera, *Leucocoprinus* and *Leucoagaricus*, and some species still assigned to *Lepiota*) is monophyletic [J. Johnson, thesis, Duke University, Durham, NC (1997)].
9. The tribe Attini is divided into the derived "higher attine" genera (including leafcutter ants) and the phylogenetically basal "lower attine" genera [T. R. Schultz and R. Meier, *Syst. Entomol.* **20**, 337 (1995)]. Lower attine cultivars are largely unmodified and resemble free-living Leucocoprini, whereas higher attine cultivars are highly derived (*6*, *7*).
10. We excluded cultivars of two derived ant clades: the subgroup of *Apterostigma* that cultivates non-lepiotaceous fungi, and the higher attine clade with their highly derived lepiotaceous fungi (*9*). The single ex-

ception is that of *Trachymyrmex papulatus*. Of two nests examined, one cultivated a lower attine fungus (included in Fig. 1), the other a typical higher attine fungus. This is the only known case of a higher attine ant cultivating a lower attine fungus.
11. Cultivated and free-living *Lepiotaceae* were surveyed in central Panama in November 1995 through December 1996. Most of the free-living fungi appear to be undescribed species ("PA" collection IDs in Fig. 1); a few collections could be associated with published descriptions ("cf." notation).
12. Our Web site (www2.sel.barc.usda.gov/Schultz/agants.html) lists sample sizes by ant species of all 553 cultivar isolates, and additional information on phylogenetic analyses.
13. RFLPs were generated by restricting ITS polymerase chain reaction products [T. White, T. Bruns, S. Lee, J. Taylor, in *PCR Protocols*, M. Innis, D. Gelfand, J. Sninsky, T. White, Eds. (Academic Press, New York, 1990)] with Hae III or Taq I. Fungi were called the same type if RFLPs matched. For each ant species per collection locality, we sequenced at least one representative of each cultivated RFLP type.
14. Forward and reverse sequences were generated on an ABI 377 sequencer for the entire ITS region (680 to 740 bp) (*13*) and the first 610 bp of the 25*S* gene (*7*) (GenBank accession numbers AF079591-AF079754). Sequences of 11 Lepiotaceae were obtained from GenBank (U11921, U85281-U85283, U85287, U85288, U85291, U85292, U85295, U85296, U85306, U85315-U85318, U85321-U85323, U85326, U85327, U85330, U85331). We thank J. Johnson for posting these unpublished sequences in GenBank.
15. Alignments were generated in Megalign 1.1 DNASTAR). Regions of ambiguous alignment (306 of 811 sequence positions in ITS; 5 of 611 positions in 25*S*) were excluded. Phylogenetic analyses were carried out in PAUP* 4.0d61 [D. Swofford, unpublished test version]. Sequence data consisted of 1422 nucleotide sites (minus the 311 unalignable characters), 229 of which were parsimony-informative. Maximum parsimony (MP) analysis identified 180 trees (length = 915, CI = 0.352, RI = 0.663). Successive approximations weighting (SAW) identified five equally parsimonious trees (all members of the original set of 180), the strict consensus of which is presented in Fig. 1. Transition:transversion weighting schemes of 1:2 and 1:5 produced parsimony trees identical in all key features to the tree in Fig. 1. To render maximum-likelihood (ML) analysis computationally tractable, we reduced the data set to 51 taxa by retaining one representative of each set of taxa differing by fewer than five bases. Each of the five SAW trees was "pruned" to retain these 51 taxa, then evaluated under the ML criterion (estimating parameters from the data). The most parameter-rich model (general time-reversible + proportion of sites invariant + rate heterogeneity modeled as a gamma distribution with six rate categories) was significantly better fitting than the next best model. Starting with this model and the most likely of these trees, and using increasingly more efficient branch-swapping algorithms and successively readjusted parameter values, five iterative ML searches identified a single most likely tree, which resembles in all key features the parsimony tree (Fig. 1). See our Web site (*12*) for details of the analyses.
16. A scenario of single domestication 50 million years ago, followed by escapes, is implausible because it stipulates that all free-living fungi (including major Nearctic clades) that arose after the divergence of the most recent common ancestor of all cultivars (Fig. 1) must have descended from "escaped" cultivar ancestors; it is inconsistent with observed levels of allele sequence divergence (ASD); and it contradicts the theoretical prediction that ancient clones will not retain intact the multigene architecture for fruiting [M. Lynch, R. Burger, D. Butcher, *J. Hered.* **84**, 339 (1993)].
17. Tests contrasted unconstrained MP and ML trees with constraint trees, which were inferred using the same methods described above (*15*) for MP and ML, respectively. For MP, tests consisted of all possible pairwise comparisons of multiple equally parsimonious trees. Forcing cultivar monophyly (single domestication) significantly reduced goodness-of-fit [MP: Kishino-Hasegawa (KH), $P < 0.0006$; Templeton's Wilcoxon ranked sums (TWRS), $P < 0.0007$; winning sites (WS), $P < 0.0003$; ML: KH, $P = 0.0005$]. Ad hoc assumptions of two domestications involving (Clade 1 + *Myrm. infuscata* G11) and (Clade 2 + *Myco. smithi* S60) also failed (MP: KH, $P < 0.0122$; TWRS, $P < 0.0122$; WS, $P < 0.0080$; ML: KH, $P = 0.0355$). This failure was due to strong support for the monophyly of Clade 2 excluding *Myco. smithi* S60 (MP: KH, $P < 0.0160$; TWRS, $P < 0.0285$; WS, $P < 0.0428$; ML: KH, $P = 0.0363$). Monophyly of Clade 1 excluding *Myrm. infuscata* G11 was not supported (MP: KH, $P > 0.4398$; TWRS, $P > 0.4534$; WS, $P > 0.3877$; ML: KH, $P = 0.7095$). See our Web site (*12*) for details of the tests.
18. This firm refutation of a single domestication event suggests that further sampling may reveal additional domestications. Promising locations include Amazonian Brazil (putative center of attine origin) and peripheral populations existing under extreme ecological conditions that promote cultivar loss and prompt novel domestications.
19. It is possible that some cases of "intraspecific" cultivar diversity involve unrecognized cryptic species, each specialized on a distinct cultivar.
20. For each of six cases where different ant species farmed cultivars with identical rDNA sequences (*A. auriculatum–C. longiscapus*; *C. minutus–C. rimosus*; *Myco. smithi–Myco. tardus–Myrm. ednaella*; *C. costatus–C. longiscapus*; *Myco. smithi–Myco. sp. nov. 1*; *Myrm. cf. buenzlii–C. faunulus*) (Fig. 1), we fingerprinted the sequenced isolates, and additional isolates of the same RFLP types, with AFLP techniques [U. G. Mueller, S. E. Lipari, M. G. Milgroom, *Mol. Ecol.* **5**, 119 (1996)], permitting highly resolved subdifferentiation into AFLP fingerprint types.
21. Leucocoprinoid fungi use an ephemeral substrate (litter), are relatively short-lived, and are not known to form geographically widespread clones.
22. R. R. Snelling and J. T. Longino, in *Insects of Panama and Mesoamerica*, D. Quintero and A. Aniello, Eds. (Oxford Univ. Press, New York, 1992), pp. 481–494.
23. Asexual cultivar propagation was shown by AFLP fingerprint identities of cultivars from different nests of Floridan *C. minutus* (*20*) and was corroborated by AFLP surveys of lower-attine cultivars from Trinidad (S. A. Rehner and U. G. Mueller, unpublished data).
24. Theory predicts elevated levels of heterozygosity (ASD) for ancient asexual diploid organisms [O. Judson and B. Normark, *Trends Ecol. Evol.* **11**, 41 (1996)]. Without recombination, homologous alleles accumulate mutations independently and diverge with time. Heterozygosity therefore is a relative measure of the time since the origin of asexuality. Recombination (sexuality) purges ASD. Heterozygosities were scored from sequencing contigs as superimposed peaks each of about half intensity (present in forward and reverse sequences), and insertions or deletions (typically involving one base, causing a partial frame-shift at the same position, but in opposite directions, in forward and reverse sequence).
25. We thank the Smithsonian Tropical Research Institute, the National Geographic Society, the Biodiversity of the Guyanas Project, the Smithsonian Scholarly Studies Program, the Laboratory of Molecular Systematics (National Museum of Natural History), and the National Science Foundation (DEB-9707209) for funding; the Bermingham lab for sequencing support; the Instituto Nacional de Recursos Naturales Renovables (INRENARE) of Panama, the Kuna Comarca, the Office of the President of Guyana, the Ministerio de Recursos Naturales Energía y Minas (MIRENEM) and the Instituto Nacional de Biodiversidad (INBIO) of Costa Rica, the Forestry Division and Wildlife Section (FDWS) of Trinidad, and the Conselho Nacional de Pesquisas (CNPq) and the Instituto Nacional de Pesquisas da Amazónia (INPA) of Brazil for collecting and export permits; R. Adams, D. Agosti, V. Aswani, J. Boomsma, M. Braun, M. Chen, C. Currie, G. deAlba, Z. Falin, V. Funk, N. Gomez, J. Heacock, J. Huelsenbeck, J. Hunt, J. Narozniak, N. Knowlton, M. Leone, J. Longino, S. McCafferty, G. Maggiori, B. Norden, D. Piperno, I. Rubinoff, J. Sullivan, D. Swofford, J. Wilgenbusch, R. Wilson, T. Wright, and especially E. Bermingham, C. Delwiche, A. Herre, J. Kays, and B. Wcislo for logistical support, advice, and various other kinds of help. We dedicate this paper to the memory of William L. Brown Jr. (1922–1997).

13 April 1998; accepted 7 August 1998

Impaired Spatial Learning after Saturation of Long-Term Potentiation

Edvard I. Moser,* Kurt A. Krobert, May-Britt Moser, Richard G. M. Morris

If information is stored as activity-driven increases in synaptic weights in the hippocampal formation, saturation of hippocampal long-term potentiation (LTP) should impair learning. Here, rats in which one hippocampus had been lesioned were implanted with a multielectrode stimulating array across and into the angular bundle afferent to the other hippocampus. Repeated cross-bundle tetanization caused cumulative potentiation. Residual synaptic plasticity was assessed by tetanizing a naïve test electrode in the center of the bundle. Spatial learning was disrupted in animals with no residual LTP (<10 percent) but not in animals that were capable of further potentiation. Thus, saturation of hippocampal LTP impairs spatial learning.

An important prediction of the hypothesis that activity-dependent synaptic plasticity in the hippocampus (such as LTP) plays a critical role in certain kinds of learning (*1, 2*) is that physiological saturation of synaptic weights should disrupt new memory encoding. Saturation of an intrinsic pathway can be viewed as a neural state in which no further potentiation is feasible, at least for a period of time, at any site in the pathway (*3*). Repeated tetanization at a single site in the perforant path has been reported to block spatial learning when leading to cumulative LTP in the dentate gyrus (*4*), but this result has not been