

Ant versus Fungus versus Mutualism: Ant-Cultivar Conflict and the Deconstruction of the Attine Ant-Fungus Symbiosis

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ABSTRACT: A century of research on fungus-growing ants (Attini, Formicidae) has ignored the cultivated fungi as passive domesticates and viewed the attine fungicultural symbiosis as an integrated unit dominated by the evolutionary interests of the ant farmers. This article takes a different perspective and explores first the evolutionary interests and leverages of the fungal cultivars, then dissects eight potential evolutionary conflicts between ants and cultivars. Three types of ant-cultivar conflict are examined in depth. First, ant-cultivar conflict over the ant sex ratio is predicted because the cultivars are dispersed by female foundresses but not by males; cultivars thus may be selected to bias the ant sex ratio toward females. Second, ant-cultivar conflict over fungal sexual reproduction exists if the fungi are able to escape from the symbiosis and live independently, as is implied by phylogenetic analyses of the fungi; this conflict is exacerbated in colonies that experience queen death or senescence. A literature review reveals that sexual fruiting of attine cultivars is more common than has been traditionally realized and often occurs in moribund colonies. Third, the routine transplanting of fungal mycelium by ants could generate, through sensory-biased symbiont choice, selection favoring fungal features that increase the likelihood of transplantation within nests (symbiont drive) but that are detrimental to the survival of the whole colony. A balanced perspective incorporating both ant and fungal interests emerges as a more appropriate framework than the traditional myrmicocentric perspective. Indeed, the attine symbiosis offers unique experimental opportunities (cultivar switch experiments) to unravel the evolutionary dynamics of conflict and cooperation between ant and fungal partners.

Keywords: attine ants, conflict, cooperation, fungiculture, mutualism, symbiosis.

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The study of the Attini and other fungus-growing insects has only just begun, and further advance in this fascinating subject will be more difficult for the mycologist than for the entomologist. (William Morton Wheeler 1910, p. 338.)

Very few animals have evolved the ability to grow their own food. The best-known examples include the fungus-growing ants (tribe Attini: Formicidae: Hymenoptera; Weber 1972; Hölldobler and Wilson 1990), the fungus-growing termites (Batra and Batra 1979), the bark and ambrosia beetles (Paine et al. 1997; Farrell et al. 2001), and, of course, humans. But unlike human agriculture, which originated about 10,000 years ago (Cowan and Watson 1992; Diamond 1997, 1998), insect agriculture is ancient. Fungus-growing ants, for example, originated in the early Tertiary and thus predated human agriculture by about 50–60 million years (Wilson 1971; Mueller et al. [2001] date the origin conservatively between 45 and 80 million years ago and narrowly between 50 and 65 million years ago). Because of this ancient coevolutionary history, attine ant-fungus interactions can be expected to be very derived, and probably more complex, than younger coevolutionary associations like the ones between humans and their cultivars.

This article explores some of the complexities of ant-fungus interactions in the attine symbiosis, particularly the evolutionary conflicts that may have persisted between ants and fungi since the origin of this symbiosis, as well as the conflicts that may have arisen subsequently, during the course of the 60 million years of coevolutionary association. Any ant-cultivar conflict impacts the overall efficiency of the mutualism and needs to be incorporated into a comprehensive analysis of ant-cultivar coevolution. The article thus delimits first the areas of overlapping and incongruent evolutionary interests between the ant and fungal partners and then explores how the fungi could manipulate and take advantage of the ants, when such manipulations are most beneficial to the fungi, and whether the ants could evolve (or fail to evolve) counteradaptations against the manipulations of their own cultivars.

Table 1: Ant-cultivar conflicts in the attine ant-fungus symbiosis

Ant-cultivar conflict	Explanation
Conflicts under strict vertical cultivar transmission:	
Ant sex ratio	Cultivar induces female-biased sex ratios in the ants to maximize the number of reproductives that disperse the cultivar (see "Conflict over the Ant Sex Ratio")
Life-history trade-offs (colony growth vs. reproduction)	Cultivar manipulates life-history characteristics of the ants to maximize output of female reproductives (see "Conflict over Life-History Trade-Offs")
Symbiont drive under symbiont choice	Cultivar evolves traits that increase its subculturing chances within nests but that also compromise colony fitness (see "Symbiont Choice and Sensory Exploitation of Ants by the Cultivar")
Ant mating incompatibility	Cultivar induces mating incompatibilities in ants to eliminate competitor cultivars from the population (see "Conflict over Mating Incompatibility in Ants")
Conflicts emerging from horizontal cultivar transmission:	
Cultivar sexual reproduction	Cultivar diverts colony resources into its own sexual reproduction, while compromising colony fitness (see "Conflict over Cultivar Sexuality")
Cultivar escape	Cultivar diverts colony resources to escape from a colony and enter a free-living existence (see "Conflict over Cultivar Escape")
Cultivar replacement	Cultivar prevents import of a novel cultivar that the ants prefer over the existing cultivar (see "Conflict over Cultivar Replacement and Import of Novel Fungal Strains")
Cultivar competition	Cultivar competes with other cultivar strains that the ants prefer to copropagate in a colony (see "Conflict over Cultivar Competition within Gardens")

Note: No research into ant-cultivar conflicts has been conducted in attine ants to date, and conflicts are therefore listed here as untested hypotheses. See "Ant-Cultivar Conflicts in the Attine Symbiosis" for possible tests of each of these hypotheses.

The premise of ant-cultivar conflict contrasts with the premise of ant control (domination of the fungus by the ants) that has permeated attine research since the first description of this symbiosis over a century ago (Belt 1874; Möller 1893). The traditional view conceptualized the attine symbiosis as an integrated unit designed to serve the evolutionary interests of the ants. This myrmecocentric view undoubtedly stems from appealing analogies between ant fungiculture and human agriculture (Batra and Batra 1967; Rindos 1984; Mueller et al. 1998): because the ants play the behaviorally active role and physically manipulate their cultivated fungi, it seems intuitively compelling that the ants domesticated their fungi and control them for their own purposes. Consequently, the sessile, ant-manipulated fungi have been traditionally ignored as "passive domesticates," subservient to the ants.

Despite this prevailing view of complete ant control, two reasons justify a reanalysis within the framework of conflict-of-interest theory. First, even if the traditional assumption is correct in that the attine ant-fungus symbiosis originated with a true domestication event under complete ant control, the long coevolutionary history may have eventually selected for cultivar traits that benefit the fungi but not necessarily the ants and the overall mutualism, or the fungi may have retained ancestral traits that are undesirable to the ants. Second and more important, many

microorganisms are known to have evolved sophisticated mechanisms to manipulate the physiology and behavior of coevolving animals (Ewald 1994). Fungi in particular have been implicated in adaptive manipulation of arthropod-host behavior (Whisler 1979), including ant behavior (Marikovskiy 1962; Loos-Frank and Zimmermann 1976; Evans and Samson 1984; Oi and Pereira 1993; Schmid-Hempel 1998). Because of these precedents, it is not implausible that attine cultivars have evolved chemical and physiological schemes that alter ant behavior to serve the fungus' reproductive interests, possibly even compromising the reproductive interests of the ant hosts.

Application of conflict-of-interest theory will be a necessary first step in delimiting potential and actualized conflicts in the attine symbiosis (table 1 lists potential ant-cultivar conflicts). Relevant theory is briefly reviewed therefore in the section "Theory of Mutualism, Antagonism, and Alignment of Reproductive Interests." Conflict-of-interest theory will help define novel research dimensions that so far have remained unexplored because of the century-long preconception of ant control over their cultivated fungi. Readers familiar with the theory of mutualistic and antagonistic evolution may skip this theoretical section and move on to "Evolutionary History of Attine Ants and Their Fungi," which summarizes the natural history of the attine ant-fungus symbiosis.

Theory of Mutualism, Antagonism, and Alignment of Reproductive Interests

The Mutualism-Antagonism Continuum

Antagonistic (parasitic, pathogenic) and mutualistic associations between species are best viewed as extreme endpoints along a continuum of possible interspecific interactions (Ewald 1987, 1994; Bronstein 1994; Herre et al. 1999). The continuum ranges from one extreme of incongruent reproductive interests (antagonism) to the other extreme of complete alignment of reproductive interests (mutualism). With the exception of highly derived mutualisms (Moran and Wernegreen 2000), a particular species interaction is not fixed permanently at a point on this continuum but can be shifted by selection toward one or the other extreme over *evolutionary time*. Moreover, the interaction may shift facultatively along the continuum (e.g., from antagonism toward mutualism) over *ecological time* as environmental conditions change, then shift back as the original conditions are restored. As an extreme case, species interactions that are antagonistic at one point may readily flip into mutualism, or vice versa, but changes along the continuum can also be more gradual, subtle, and thus difficult to detect empirically.

It is furthermore helpful to view species interactions as multidimensional. At any given point in time, interactants within a symbiosis may be under selection to enhance or promote the same features of a particular symbiosis (i.e., the interactants' reproductive interests agree and are aligned with respect to this feature). With respect to other features of a symbiosis, interactants may be under selection to shape them in distinct or even opposite ways (i.e., the interactants' reproductive interests disagree and are incongruent). For example, in the case of a sexually transmitted disease, the host and the pathogen have incongruent reproductive interests with respect to the host's life-history resource allocation, but both host and pathogen agree that sexual contact should occur (though they may disagree over the frequency of contact). At the other extreme, in the case of an endosymbiotic microorganismal mutualist, both host and mutualist may agree over host resource expenditure to optimize host growth and host reproduction, but they may disagree over the host sex ratio if the mutualist is transmitted between generations by only one sex of the host species. (The other, nontransmitting sex is a resource waste from the perspective of the mutualist.)

Vertical versus Horizontal Transmission

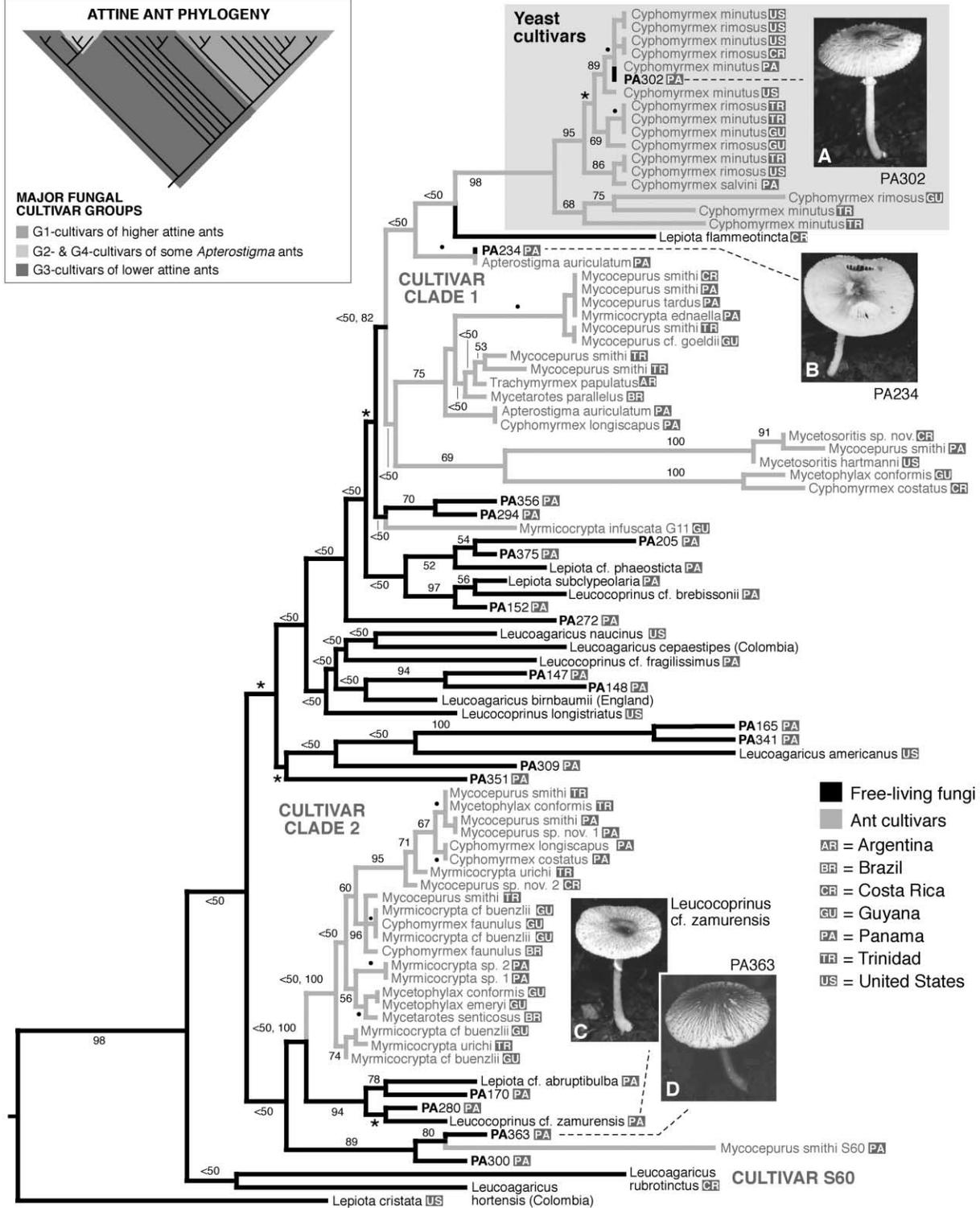
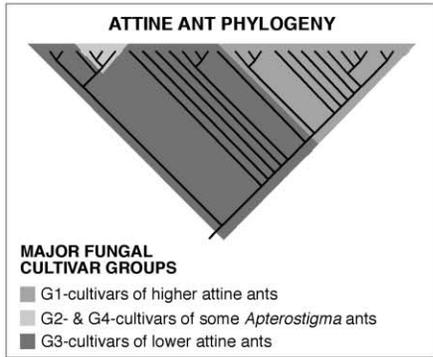
One of the key factors modulating the alignment of reproductive interests between different lineages (e.g., species) is the joining of evolutionary fates through strong links of reproductive interdependency. One common

mechanism linking reproductive lineages is vertical reproductive transmission, the transmission of one of the interactants (the so-called symbiont) vertically within the lineage of the other interactant (the host). Vertical transmission tightly connects selective processes and generates selective codependencies: what is selectively advantageous for one interactant (e.g., the host) most likely is also advantageous for the partner (e.g., the symbiont).

Vertical transmission does not necessarily result in complete overlap of reproductive interests, but it purges, or at least diminishes, many conflicts that exist under horizontal transmission. Horizontal transmission reshuffles reproductive lineages and erodes selective interdependencies between symbiont and host. Most importantly, horizontal transmission introduces additional variables into the life-history equation of the symbiont: how frequently to transmit horizontally, how to balance horizontal versus vertical transmission, and whether or not to compromise host fitness to enhance horizontal transmission. Generally, horizontal and vertical transmission trade off against each other, such that an increase in the efficiency of horizontal transmission compromises vertical transmission of the symbiont. Under such trade-offs, even low levels of horizontal transmission can result in complete erosion of overlapping reproductive interests between host and symbiont (Frank 1996b, 1997) and, depending on the conditions, can select for symbiont traits that are extremely harmful (virulent) to the host.

Symbiont Competition

Vertical transmission is a critical factor modulating alignment of reproductive interests, but it is not the only one. Several additional factors can generate reproductive conflict even in mutualistic systems with strict vertical transmission. The first such factor is competition between different symbiont types (or genetic variants of the same symbiont) that reproduce within the host lineage. Such competition can generate selection for symbiont traits that enhance competitive ability but that harm the host (Frank 1996a). Genetic variation among symbionts thus can lead to reduced host fitness. Hosts, in turn, may be selected to minimize genetic variation among associated symbionts, either by greatly curtailing horizontal transmission that could result in acquisition of (infection with) several symbionts (Frank 1996a; Bot et al. 2001) or by passaging the symbiont occasionally through a population bottleneck (e.g., allowing only a few symbionts, or only a single symbiont lineage, to associate with each host gamete; Frank 1996b; Grosberg and Strathmann 1998). Passaging the symbiont through a bottleneck curtails (or even purges) genetic variation that may arise because of horizontal transfer (multiple symbiont acquisitions, multiple infec-



tions) or because of mutation in the symbiont while associated with the host.

Symbiont Choice and Other Factors

Other factors modulating host-symbiont conflict are the relative generation times, relative population size, and the relative mutation rates (J. L. Sachs, J. J. Bull, U. G. Mueller, and T. Wilcox, unpublished manuscript); the relative genome complexity and genome competency (Moran and Wernegreen 2000); and the presence/absence of sexual recombination in the symbiont and in the host (Law and Lewis 1983; Moran 1996; Herre et al. 1999). These factors influence the pace of evolution progressing in symbiont versus host, potentially allowing the symbiont to evolve within the lineage of a host, while the host remains stationary relative to the evolution occurring in the symbiont lineage. Symbionts reproducing within, or in close association with, a single host lineage thus can potentially evolve at a substantially faster pace and stay ahead in any coevolutionary races between symbiont and host. This faster evolution does not necessarily favor the symbiont to win every conflict with its host for the following reasons. First, the host may retain control over critical resources that are impossible to manipulate for the symbiont. Second, the host may decelerate and restructure symbiont evolution by enforcing coreplication and alignment of reproductive interests (enforcing “partner fidelity”; Bull and Rice 1991; J. L. Sachs, J. J. Bull, U. G. Mueller, and T. Wilcox, unpublished manuscript). And finally, the host may choose between different symbiont variants, eliminate deleterious and less profitable variants, and thus engage and/or retain only the most beneficial symbionts (“partner choice”; Bull and Rice 1991; J. L. Sachs, J. J. Bull, U. G. Mueller, and T. Wilcox, unpublished manuscript). In mu-

tualistic systems with partners differing greatly in generation times, population sizes, and mutation rates (such as the mutualism between fungal cultivars and ant farmers), cooperation is likely to be stabilized evolutionarily by a partner-choice mechanism. For such cases, J. L. Sachs, J. J. Bull, U. G. Mueller, and T. Wilcox (unpublished manuscript) predict that the partner with the evolutionary handicap (longer generation time, smaller population size, and lower mutation rates) is the choosing partner (i.e., the ants would be the choosing partner in the case of the attine ant-cultivar symbiosis).

Application to the Attine Ant-Cultivar Symbiosis

The picture emerging from these cursory theoretical considerations reveals, first, that host-symbiont conflicts can evolve and persist even under strict vertical symbiont transmission and, second, that the evolutionary dynamics of conflicts are generally very complex, extremely system-specific, and thus impossible to predict a priori without a detailed knowledge of the natural history of a host-symbiont system, as well as knowledge of the partner fidelity and partner choice elements operating in a given system. Details of the natural history of the attine ant-fungus symbiosis, as well as inherent partner fidelity and partner choice elements, are therefore summarized in “Evolutionary History of Attine Ants and Their Fungi” and are followed by sections discussing specific dimensions of ant-fungus cooperation and conflict. Unfortunately, some of the key parameters that are expected to modulate ant-fungus conflict, such as any cultivar diversity within gardens, still remain unknown. In the following sections, therefore, I also summarize what is known, and what still remains to be determined and quantified, in order to understand ant-fungus conflict in the attine symbiosis.

Figure 1: Phylogeny for 57 attine cultivars and 36 free-living fungi in the tribe Leucocoprineae (Lepiotaceae, Basidiomycota), adapted from Mueller et al. (1998). All cultivars belong to a group grown only by lower attines (see insert and insert legend below), basal attine ant lineages that are most likely to have retained the ancestral states of ant-fungus associations that existed early in the history of this 60-million-year-old symbiosis. Cultivated fungi are marked in gray by their respective ant host species. Free-living fungi are marked in black; many of these are undescribed species and are denoted by their collection IDs. Two free-living counterparts of ant-cultivated fungi (*A*, *B*) show identical sequences with those of two cultivated fungi, thus revealing two recent domestications of cultivars by ants. Statistically distinct cultivar clades (Clade 1, Clade 2, *Mycocarpus smithi* S60) provide evidence for a minimum of three additional domestications. Almost all ant species (as currently recognized) 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 1,422 bp sequence of two nuclear rDNA regions (ITS, 25S) and resembles in all important details the tree found using maximum likelihood. Bootstrap values appear as numbers above branches. Asterisks indicate branches not present in the strict consensus of 180 equally parsimonious trees found in the unweighted analysis before successive weighting; bullets indicate branches above which a single representative taxon was included in the analyses. *Insert*, Phylogenetic relationships of attine ants, with the three major cultivar groups superimposed. The insert summarizes information from Schultz and Meier (1995) and Chapela et al. (1994). Three major ant groups are distinguished, corresponding to defined cultivar clades: the monophyletic, G1-cultivating higher attines (including the leaf-cutter ants); the monophyletic, G2- and G4-cultivating *Apterostigma* species (a monophyletic ant subgroup within the genus *Apterostigma*); and the basal G3-cultivating lower attines (a paraphyletic group with respect to the other two ant-cultivar groups). The prevalence of G3 cultivation among the basal attine lineages indicates that this type of fungiculture most likely represents the ancestral attine condition and that both G2 and G4 *Apterostigma* fungiculture and G1 higher-attine fungiculture are derived systems that arose from G3 fungicultural systems.

Evolutionary History of Attine Ants and Their Fungi

Attine fungiculture originated about 50–60 million years ago, most likely in a rain forest environment of the South American continent, as indicated by the strict Neotropical distribution of the tribe Attini with a highest species concentration in Amazonian Brazil (Mueller et al. 2001). Initially, reliance on fungal food by the ancestral attines was probably facultative, but it eventually evolved into an association of obligate dependence. All extant attines obligately depend on fungal food as the exclusive diet for their larvae and as a partial diet for adults (Littleddyke and Cherrett 1976; Stradling 1978; Quinlan and Cherrett 1979; Cherrett et al. 1989).

Most attine ants, including the most basal extant ant lineages, cultivate fungi in the tribe Leucocoprineae (Leptotaceae, Agaricales, Basidiomycota; fig. 1 insert; see table 2 for a glossary of key terms). The most parsimonious interpretation of this fungicultural pattern is that attine fungiculture probably originated with some ancestral attine-leucocoprineous association (Chapela et al. 1994; Mueller et al. 1998). Leucocoprineae are specialist litter-decomposing saprophytes that are particularly abundant in the moist Neotropics; the ancestral attine thus likely began to associate with fungi in the litter of rain forest floors. The exact nature of this ancestral association, and the exact evolutionary steps taken during the formation of this original association, are difficult to infer given the massive time and diversification since the fungicultural origin. But a series of hypotheses have been suggested in the literature for the sequential coevolutionary modifications during the attine origin (Mueller et al. 2001). These hypotheses will be discussed in detail in “Assumption of a Domestication Origin,” which examines whether attine fungiculture arose through a true domestication process serving the evolutionary interests of the ants or whether fungiculture may have arisen out of ancestral ant-fungus associations that originally served the evolutionary interests of the fungi.

The attine origin is obscured by the fact that all extant attines are obligate fungivores and that there exist no extant facultative attine-fungus associations that may reflect the very first, primitive stages of ant-fungus interaction. Any such primordial forms were transitory and lost in time. Even the closest known relatives of the Attini do not appear to exhibit obvious facultative associations with fungi (Mueller et al. 2001; but see Diniz et al. 1998). The absence of facultative attine-fungus associations suggests evolutionary transience and instability of nonobligate ant-fungus associations and possibly a rapid evolutionary transition from an ancestral hunter-gatherer ant to the derived fungicultural ant.

Attine-fungus associations have diversified substantially

since the attine origin. More than 200 ant species in 13 genera are currently recognized in the monophyletic tribe Attini (Schultz and Meier 1995; Brandão and Mayhé-Nunes 2001). Traditionally, the tribe Attini is subdivided into a derived monophyletic group called the “higher attines” (including the well-known leaf-cutter ants and three closely related genera) and a paraphyletic group called the “lower attines” (including all the more basal attine lineages), from which the higher attines arose (see insert in fig. 1). The more basal extant attine lineages are primitive, first, in that their colony sizes are relatively small (generally fewer than 100 workers) and, second, in that they manure their gardens with the type of substrate on which free-living leucocoprineous fungi are specialized: dead vegetable matter that the ants collect on the forest floor (e.g., plant detritus, flower parts, seeds, but also arthropod feces).

From such primitive ant-fungus associations evolved several derived fungicultural forms, such as the well-known leaf-cutter ants. Leaf-cutter ants (genera *Atta* and *Acromyrmex*) manure their gardens largely with fresh leaf material, and their colony sizes may reach several hundred thousand (in *Acromyrmex*) or even 5–10 million workers (in *Atta*; Weber 1972; Hölldobler and Wilson 1990). Leaf-cutter ants are herbivores, have substantial ecological impact because of their large colony sizes, and are generally considered major pests in areas of human agriculture.

Other attine ant-fungus systems are much less conspicuous than the one of leaf-cutter ants, but they are nevertheless highly derived forms of the basic attine ant-fungus design. For example, ants in one monophyletic group in the genus *Cyphomyrmex* cultivate their fungi not in the mycelial (multicelled) state typical for most attines, but in a yeast state (single celled). The *Cyphomyrmex* yeasts form a monophyletic clade within a larger mycelial cultivar clade (fig. 1; Mueller et al. 1998), indicating that these fungi derived from mycelial ancestors and switched to a single-celled growth form when they became associated with the *Cyphomyrmex* yeast specialists. The yeast-growing *Cyphomyrmex* ants may even have evolved the ability to manipulate the growth form of their cultivars and maintain them in a yeast state for some unknown purposes.

A second notable example of derived ant-fungus association is a monophyletic group in the ant genus *Apterostigma* that does not cultivate a leucocoprineous fungus but, instead, a distantly related fungus currently placed near some free-living, possibly wood-decomposing fungi in the family Tricholomataceae (fig. 1 insert; Chapela et al. 1994; P. Villesen, U. G. Mueller, T. R. Schultz, R. M. M. Adams, and A. C. Bouck, unpublished manuscript). This is the only exception to leucocoprineous fungiculture and represents an evolutionary unique switch to a fungus outside the group of leucocoprineous fungi.

The tricholomataceous *Apterostigma*-fungus associa-

Table 2: Glossary of mycological and myrmicological terms

Term	Definition
Agaricales	Order of basidiomycete fungi, including the two families Lepiotaceae and Tricholomataceae to which attine cultivars belong. The prototypical mushrooms are all agaric basidiocarps, generally with a stem and a gilled cap.
Attini	Neotropical tribe of about 210 described fungus-growing ant species in the ant subfamily Myrmicinae. The tribe is monophyletic, and all known attines are obligately dependent on fungiculture. Fungiculture arose only once in attine evolution, about 50–60 million years ago, probably in Amazonian rain forests of the South American continent. Attine ants are often incorrectly equated with leaf-cutter ants; however, not all attine ants cut leaves, and only about 25% of all attine species are leaf-cutters.
Basidiocarp	Fruiting structure (sporocarp) of fungi in the Basidiomycota, producing spores through meiosis. The prototypical mushroom is one type of basidiocarp.
Basidiomycota	One of the main divisions of higher fungi, characterized by specialized sexual structures (basidia) for spore production.
<i>Escovopsis</i>	Genus of ascomycete fungus, parasitic in gardens of all attine ants. Attine ants have evolved complex behavioral and antibiotic defenses against <i>Escovopsis</i> parasites, including a mutualistic association with actinomycete bacteria that the ants are cultivating on their own bodies and from which the ants derive antibiotic chemicals specifically targeted at <i>Escovopsis</i> parasites.
G1, G2, G3, and G4 fungi	Groupings of attine cultivars originally based on morphological differences but corresponding to clear phylogenetic cultivar groups associated with defined subsets of attine ants. G1 fungi are cultivated only by higher attine ants, G2 and G4 fungi only by reciprocally monophyletic clades within the primitive attine genus <i>Apterostigma</i> , and G3 fungi by the remaining lower-attine genera. G2 and G4 fungi are sister clades in the family Tricholomataceae. G1 and G3 fungi are in the family Lepiotaceae. The monophyletic G1 fungi arose from G3-like ancestors. Only G1 fungi produce gongylidia and staphylae.
Gongylidia	Hyphal tip swellings produced by the cultivars of higher attine ants. The swellings are filled with vacuoles rich in nutrients. Gongylidia are produced in clusters (called staphylae) that can be harvested easily by the ants.
Higher-attine vs. lower-attine ants	Attine ants are traditionally subdivided into the monophyletic higher attines and the more basal (primitive) attine lineages jointly referred to as lower attines. Lower attines are paraphyletic with respect to the monophyletic higher attines. Lower attine ants are generally smaller in size, have smaller and less complex nests, and do not develop caste polymorphism.
Leaf-cutter ants	A monophyletic clade within the higher Attini, including fungus growers in the genera <i>Atta</i> and <i>Acromyrmex</i> . Leaf-cutter ants typically manure their cultivars with fresh leaves and flowers that they cut from live plants. All other attine ants do not cut fresh vegetation (or do so minimally in only a few attine genera) and instead use dead vegetable debris, seeds, or arthropod feces as substrate for fungiculture. The leaf-cutter clade is part of the higher attines, and leaf-cutting behavior is a derived condition that arose late in attine evolution (possibly no more than 10–20 million years ago).
Lepiotaceae	Mushroom family in the order Agaricales.
Leucocoprineae	Tribe in the family Lepiotaceae, comprised of the mushroom genera <i>Leucocoprinus</i> and <i>Leucoagaricus</i> . The great majority of attine cultivars (G1 and G3 fungi) fall within this single tribe. Leucocoprineous fungi are particularly abundant in the Tropics and are specialized litter decomposers, suggesting that the original attine ant-cultivar interactions evolved in a litter microhabitat.
Staphylae	Clusters of gongylidia.
Tricholomataceae	Mushroom family in the order Agaricales, distantly related to the Lepiotaceae.

tion, though unique, reveals that at least one switch away from the ancestral leucocoprineous association must have occurred during attine evolution and that, therefore, fungal transmission is not always vertical. Indeed, phylogenetic and population genetic analyses (Mueller et al. 1998; fig. 1) of attine ants and their cultivars revealed the following: first, that many more such switches and substitutions must have occurred; second, that attine ants cultivate at least four phylogenetically independent fungal lineages (three lepiotaceous and one tricholomataceous cultivar lineage), each independently acquired; and finally, that cultivars are regularly exchanged between attine lineages through lateral transfer. These complications of the attine ant-fungus symbiosis will be discussed in "Assumption of Vertical Cultivar Transmission," which examines processes of vertical versus horizontal cultivar transmission.

Ant-Cultivar Interactions

Attine ants are true farmers, and many of their fungicultural practices have parallels in human agriculture. Most obvious, the ants are capable of physically manipulating the cultivars, and they provide substrate to optimize fungal growth. Physical manipulation occurs most noticeably during the routine, vegetative transplanting of the fungus from older gardens to newly prepared gardens. Workers excise small fungal inocula from older gardens and then transplant these cuttings into new gardens that the ants prepare through manuring and partial sterilization. About four to eight small tufts of fungal inocula are thus planted onto each square millimeter of new garden (Weber 1972, p. 95), carefully spaced out so the fungus can quickly overgrow and penetrate the prepared substrate. The ants furthermore optimize fungal growth through regular application of fecal manure (Martin 1987; Cherrett et al. 1989), grooming (combing through the mycelium to extract contaminant spores; Currie and Stuart 2001), and weeding (excision of garden fragments that are infested with fungal parasites, thus preventing parasite spread; Currie and Stuart 2001).

The newly planted fungus is meticulously tended and manured by workers for several weeks before the sprouting mycelium is harvested. At maturity, cultivars of the higher Attini produce conspicuous nodules called staphylae, clusters of hyphal-tip swellings comparable to miniaturized bunches of grapes. The hyphal-tip swellings, called gongylidia, are vacuolized packages rich in nutrients (Quinlan and Cherrett 1979; Martin 1987; Cherrett et al. 1989). Staphylae are picked by workers from gardens for consumption. Because only higher-attine cultivars produce true staphylae, cultivars of the lower attines appear to be harvested as mycelium (though conspicuous nodules also

exist in the interior of many lower-attine gardens; U. G. Mueller, personal observation). Staphylae are preferred by higher-attine workers over hyphae as food (Quinlan and Cherrett 1978; Angeli-Papa and Eymé 1985), and staphylae are fed to larvae by workers (Murakami 1998; Murakami et al. 2000). Larvae have higher growth rates when feeding on staphylae than on hyphae (Quinlan and Cherrett 1979), which is assumed to be due to the special nutrient complement of vacuoles that develop in gongylidia (Angeli-Papa and Eymé 1985).

The cultivated fungi generally exhaust the substrate within 3–4 mo after planting (Weber 1972, p. 92), at which point the exhausted substrate and the senescing cultivar are moved to a midden (dump) by the ants. Some time before complete substrate exhaustion, however, workers take vegetative inocula from matured gardens and use them for the planting of new gardens. The time interval between planting and subculturing measures one asexual cultivar generation, which is an important parameter for understanding the coevolutionary dynamics between cultivars and ants (see "Symbiont Choice and Other Factors"). Details of the relative generation times of cultivars and ants are therefore summarized here for the first time.

For leaf-cutter ants in the genus *Atta*, the time interval between original planting and maturation for the first subculturing has been estimated to be about 7 wk by Weber (1972, p. 92) and <5–6 wk by Fisher et al. (1994a, p. 884). Therefore, 5–7 wk is the current best minimum estimate for completion of an asexual fungal generation when propagated by ants within a nest. The maximum time interval between subculturing intervals is given by the maximum age that a garden fragment reaches before complete exhaustion of its substrate. Weber (1972) estimated a maximum age of about 3–4 mo, and Fisher et al. (1994a) of no more than 5–6 wk. Extrapolating from these values, the fungus completes about four to eight generations per year (about 10 generations when using Fisher et al.'s estimate) and, depending on nest longevity of a given ant species, at least 10–50 generations during the life span of an ant colony (20–100 generations using Fisher et al.'s estimate). The number of generations of a cultivar propagated clonally within a single ant generation (within the lifetime of a colony), therefore, greatly outpaces that of the ants. Given sufficient genetic variation, this difference in generation time implies that the cultivar could evolve substantially faster than the ants when engaged in an ant-cultivar arms race within the symbiosis, as will be discussed in "Symbiont Choice and Sensory Exploitation of Ants by the Cultivar."

As far as is known, transplanting of the cultivar within nests is a strict vegetative process. The cultivar biomass within a single nest, even when subdivided into different gardens in different chambers, therefore has long been

assumed to consist of a single asexual clone, descended asexually from the same ancestral clone that the foundress queen brought with her from her natal nest. Before leaving her natal nest, the foundress queen stores a small, pellet-shaped fungal inoculum in a specialized pouch (called the infrabuccal pocket) in the bottom of her mouth. She carries this inoculum with her during her mating flight and then expels it to start an incipient garden after locating and constructing a suitable nest site. Only female reproductives carry the fungus from parent to offspring nest (von Ihering 1898; Huber 1905; Wheeler 1907). Males do not transmit the fungus because they die shortly after mating (and thus they never reach any newly founded nests) because males do not have an infrabuccal pocket designed for fungal transmission (the pocket is very small; Huber 1905) and because the infrabuccal pockets of males do not contain fungus during the mating flight (see footnote 3 on p. 610 in Huber 1905 for details of the infrabuccal pocket of *Atta* males).

Because of the vegetative transfer between nests via the mechanism of the infrabuccal pocket and because the fungi generally do not produce sexual structures in gardens (but see “Assumption of Cultivar Incompetence for Sexual Reproduction”), it has long been assumed that fungal propagation within nests and between ant generations is strictly asexual and that cultivars are strictly vertically transmitted as asexual clones over long evolutionary time. These assumptions led to the traditional hypothesis that vertical cultivar transmission may have occurred uninterrupted since the origin of attine fungiculture 50–60 million years ago. Most recently, for example, Chapela et al. (1994) argued that the cultivars of some attine lineages may be at least 23-million-year-old clones, propagated vertically (and asexually) for this entire time in tight association within their ant lineages. However, recent phylogenetic and population genetic information questions the conclusions of Chapela et al. (1994) and suggests a much more complex scenario of frequent fungal substitution within ant lineages and possibly also occasional genetic recombination in cultivated fungi through sexual or parasexual processes (see “Assumption of Cultivar Incompetence for Sexual Reproduction” and “Conflict over Cultivar Sexuality”).

Ant-Cultivar Cooperation and Integration

Attine ant-cultivar associations are highly integrated units, characterized by nutritional, physiological, and antibiotic codependencies. The existence of complex mechanisms of integration indicates a long history of coevolutionary modification: ants and fungi appear optimized to reciprocate benefits while minimizing detrimental effects on each other. In addition, synergistic interactions were probably selected to increase fitness returns (mutualism effi-

ciency) of ant-cultivar associations as cooperating wholes. The most prominent features of the attine ant-cultivar associations, as, for example, the nutritional interdependencies, suggest unadulterated cooperation between ants and fungi.

Nutritional Cooperation

In all attine lineages, the cultivated fungi are an essential source of nutrients for the ants, and larval development depends exclusively on consumption of the cultivated fungi for food (Weber 1972; Quinlan and Cherrett 1979). Workers can subsist and thrive on an exclusive cultivar diet in the lab, but the worker diet is more variable under natural conditions and includes, in addition to cultivar tissue, a variety of plant juices, such as sugary liquid from floral and extrafloral nectaries (Murakami and Higashi 1997) or leaf juices that workers imbibe when cutting and processing fresh leaves (Quinlan and Cherrett 1979; Bass and Cherrett 1995).

Estimates of the caloric content of consumed cultivar tissue had suggested that the cultivated fungi comprise only a minor portion of the diet of *Atta* workers. Attine workers are therefore assumed to gain most nutrients (more than 90%) from other sources such as the plant juices mentioned above (Quinlan and Cherrett 1979; Bass and Cherrett 1995). This estimate of >90% plant-derived calories is surprisingly high; it was derived as a default value that remains after subtracting, from the estimated total caloric requirement of workers, the calories covered by the consumed fungal tissue (about 5%–8%). However, the default value of >90% plant-derived calories appears to be inflated, maybe substantially so, because a new mode of feeding was recently discovered in attine workers. Workers were observed to obtain nutrients from larvae via anal trophallaxis, a process whereby workers solicit and then imbibe nutrient droplets from the anus of larvae (Schneider 2000). Thus workers appear to obtain fungal nutrients also indirectly via the larvae, after fungal tissue has been processed by larvae. Fungus-derived nutrients therefore may be more important to workers (and plant-derived nutrients less important) than previously thought (Quinlan and Cherrett 1979; Hölldobler and Wilson 1990). This could imply that, in addition to larvae, workers too may be highly dependent on cultivated fungi for food.

Because ants can be maintained in the laboratory for years on a fungal diet, the cultivated fungi appear to provide the ants with an adequate nutrient complement. Crude protein, carbohydrates, and lipids (including essential steroids), comprise, respectively, about 24%, 2%, and 27% of the dry weight of the various cultivar tissues (Mueller et al. 2001 summarizes data from several studies). These values rank average in comparison to other fungi

(Mueller et al. 2001) and do not indicate any obvious evolutionary improvement in nutritional quality as food for ants. However, cultivars may well possess other unknown dietary qualities that could have evolved to serve the dietary needs of the ants, such as specific essential amino acids or steroid precursors. Detailed chemical analyses of cultivated and free-living leucocoprineous fungi, combined with detailed analyses of the nutrient requirement of the ants, could greatly advance the understanding of the nutritional dependencies of attine ants on their cultivars.

The one striking evolutionary modification of the higher-attine cultivars are the gongylidia mentioned above. Such clusters of hyphal-tip swellings are not found in free-living leucocoprineous fungi; they thus appear to have evolved specifically to facilitate harvesting and consumption by ants. The clustering of gongylidia not only facilitates easy harvesting but also permits easy transport of these nutrient packets by workers. Gongylidia are easily plucked, like a bunch of grapes, and then consumed directly by workers or, in the case of the higher attines, fed to larvae. (Lower attines do not feed their larvae directly; rather, because lower-attine fungi do not produce gongylidia, lower-attine workers place larvae into the garden for hyphal grazing [Murakami 1998; Murakami et al. 2000].)

Whereas the cultivated fungi provide the ants with an adequate nutrient complement, attine ants in turn provide their cultivars with a continuous supply of substrate and an optimized growth environment. First, the ants' foraging decisions are fine-tuned to avoid the harvesting of vegetable material with antifungal or other noxious compounds (Wiemer 1985; Howard and Wiemer 1986; Howard 1987, 1988). Second, the ants regulate growth conditions within the nest to provide the fungus with stable temperature, humidity, and adequate ventilation (Kleineidamm and Roces 2000, 2001). And finally, the weeding and grooming activities of the ants protect the fungus from parasites and pathogens, essentially creating an environment with minimal competition against other fungi (garden weeds; Currie 2001a, 2001b; Currie and Stuart 2001). Because of this constant caring by innumerable workers, life as a cultivar within the mutualism seems extremely pampered.

Physiological Cooperation

Fungi generally secrete digestive enzymes into their environment to degrade organic matter into small, absorbable compounds. The attine fungi too digest externally by secreting enzymes onto the substrate provided by the ant. However, in addition to the more passive fungal secretion, the ants actively distribute the enzymes through a unique mechanism integrating gardening behavior and digestive

physiology. Fungal enzymes that are ingested by the workers during consumption of cultivar tissue pass unharmed through the ant digestive system (Martin 1987). These enzymes are subsequently deposited in fecal droplets on substrate during manuring of the gardens. The ant-distributed enzymes prime the substrate in advance of the actual planting of the fungus on the substrate, significantly accelerating fungal growth in new gardens.

Fungus and ant thus have evolved an integrated enzyme physiology, and fungal enzymes are shunted through complex pathways within the ant-fungus symbiosis. In the most direct route, fungal enzymes move from cultivar to ant gut and then back to fungus as manure. The recent discovery of anal trophallaxis between larvae and workers (Schneider 2000) suggests that larvae may also be important intermediary stages for, or even concentrators of, fungal enzymes. Enzymes ingested by larvae may pass on to workers via anal trophallaxis and then onto garden substrate when workers manure gardens with their feces.

To fully appreciate the behavioral-physiological integration between ants and fungi, it may help to interpret the complex enzyme pathways from both the ant and the fungal perspective. From the ant perspective, the ants take advantage of secreted enzymes and efficiently disseminate them within the gardens. From the fungal perspective, the fungus takes advantage of the ants and exploits ant behavior to ensure optimal enzyme distribution. These two views are not mutually exclusive, and both may help to provide an understanding of the independent selective processes that shaped the tight physiological cooperation between ants and fungi.

Sanitary Cooperation

Attine ants are known to produce antibiotics in a specialized thoracic gland (the so-called metapleural gland). These antibiotics prevent germination of alien spores in their gardens (Maschwitz et al. 1970; Schildknecht and Koob 1970, 1971; Martin 1987; Jaffe et al. 1994; do Nascimento et al. 1996; Ortius-Lechner et al. 2000). In addition, the ants regulate the growth of actinomycete bacteria on their bodies in order to derive antibiotic compounds from these bacteria that suppress the growth of specialized garden parasites in the microfungus *Escovopsis* (Currie et al. 1999a, 1999b; Currie 2001a, 2001b; Poulsen et al. 2002; see table 2). The ants therefore provide significant antibiotic benefits to the cultivated fungi, either directly through metapleural gland secretions or indirectly through the management of bacterial communities on their bodies (Currie 2001a, 2001b) and possibly also in the garden (Craven et al. 1970; van Borm et al. 2002). Antibiotic effects of the cultivated fungi on ant pathogens/parasites are unknown but have never been researched.

Attine cultivars are known to produce antibacterial and antifungal compounds (Hervey and Nair 1979; Nair and Hervey 1979; Angeli-Papa 1984; Kermarrec et al. 1986; Wang et al. 1999), which undoubtedly improve the health of gardens, but these fungal-derived antibiotics may also have important sanitary effects on the ants. Future investigations of the antibiotic effects of cultivars on ant pathogens (e.g., entomopathogenic fungi) or on parasites (e.g., mites, nematodes) may prove fruitful in elucidating the antibiotic cooperation between attine ants and their fungi.

Genetic Cooperation

The rich synergisms between attine ants and their cultivars suggest complex mechanisms of genetic integration between ant and fungal genomes. Nothing is known about such integrated genome regulation, but this area may prove to generate exciting results in the future. For example, the existence of messaging molecules and integrated signal transduction systems from ant to cultivar, and vice versa, are predicted to have evolved for integration of ant and fungal genomes. Analyses of such signal transduction systems, as well as the underlying gene regulation, should reveal much about the manipulative power that the ants and cultivars can exert over each other and thus should elucidate the mechanistic bases of any ant-cultivar conflicts.

Deconstructing the Premises of Ant-Cultivar Cooperation and Reproductive-Interest Alignment

As documented in “Ant-Cultivar Cooperation and Integration,” the attine ant-cultivar symbiosis has been shaped by a long coevolutionary history amplifying beneficial effects that the ants and the cultivars have on each other. Although the ants hold the behaviorally active part of the mutualism, the cultivated fungi are also active participants of the symbiosis, providing the ants with a series of nutritional and chemical benefits. Active participation implies, however, that the fungi may be selected to take advantage of the ants using the leverages of their sessile, chemically mediated ways of life. Specifically, despite the advantage of behavioral flexibility of the ants over their cultivated fungi, selection may enable the cultivars to overcome their sessile handicaps with chemical manipulations.

The following sections will begin to explore this line of reasoning by deconstructing five traditional assumptions—first, that the attine ant-fungus symbiosis originated as a true domestication event under ant control; second, that the ants completely dominate their cultivars; third, that alignment of reproductive interests of ants and fungal partners is assured by vertical cultivar transmission; fourth, that the cultivated fungi are incapable of an independent existence outside ant gardens and are therefore

locked permanently into a mutualistic life; and fifth, that the cultivars are incapable of sexual reproduction and are kept in a permanent asexual state by the ants.

Assumption of a Domestication Origin

Two main models have been proposed for the evolutionary origin of the attine ant-fungus symbiosis. The traditional and widely accepted model, detailed first by Weber (1958), postulates an initial stage in which nonsymbiotic fungi grew accidentally in the walls of ant nests and gradually became part of the ant diet. Subsequently the ants evolved behavioral repertoires to promote fungal growth through substrate addition and finally evolved the ability to transmit fungi between nests via the mechanism of the infrabuccal pocket. An alternative model, outlined first by Bailey (1920; see also Mueller et al. 2001), reorders these evolutionary modifications and postulates an initial stage in which the ants did not use fungi for food, but in which the fungi used the ants, specifically the infrabuccal pellets, as vectors for dispersal (pellets are expelled by workers on dumps outside the nest or at random places during foraging). Subsequent to this myrmecochorous (ant vectoring) stage, the fungi became part of the ant diet, and finally the ants evolved fungicultural habits.

The key difference between these two alternative models is the timing of the evolutionary origin of fungal transmission relative to the origin of consumption and cultivation, specifically whether fungal transmission evolved after fungal consumption (the traditional consumption-first model) or before consumption because ants accidentally served as vectors for fungi (the alternative transmission-first model; Mueller et al. 2001). Specifically, under the transmission-first model, even before a true fungicultural stage, the ancestors of the cultivars-to-be may have evolved adaptations to promote their own dispersal—first, by an enhanced ability to survive the passage through the infrabuccal pocket and, second, by presenting attractants, such as enriched fungal tissue that matched the nutritional needs of ants, to ant foragers and thus ensuring ingestion and incorporation in the infrabuccal pellet. Whereas the fungi are viewed as passive domesticates under the consumption-first model (the attine ant-fungus symbiosis emerges as a modification of ancestral ant feeding habits), the fungi are viewed as active initiators of the ant-fungus association under the transmission-first model (the attine ant-fungus symbiosis emerges as a modification of an ancestral system of fungal myrmecochory).

There exists no hard evidence favoring either the consumption-first or the transmission-first model; both are mere theoretical possibilities in need of testing (see Mueller et al. 2001 for possible tests of the transmission-first model). However, the possibility that the attine ant-fungus

symbiosis may have arisen out of a myrmecochorous system, designed originally to serve the evolutionary interests of the fungi, undermines the traditional assumption that attine fungiculture originated with a true domestication event under complete ant control. If the fungi indeed had an active role in associating with ants before a fungicultural stage, then evolution during the early stages of fungiculture may also have been influenced, at least in part, by the evolutionary interests of the cultivars-to-be. This suggests the distinct possibility that the cultivars never surrendered reproductive control completely to the ants and that at least some feature of extant ant-cultivar associations serve the interests of the cultivars rather than the ant farmers.

Assumption of Complete Ant Control

Attine ants undoubtedly control many aspects of their cultivars' biology and thus greatly constrain the evolutionary options of the cultivars. Most obviously and as summarized above, the ants provision the fungi with substrate and optimize the growth environment. In addition, the ants appear to prevent fungal escape from gardens and even attempt to sterilize their cultivars by destroying the fruiting structures necessary for sexual reproduction of the cultivars (see "Assumption of Cultivar Incompetence for Sexual Reproduction" for details on cultivar fruiting). The traditional and intuitive inference from these observations is that the locus of control of the ant-fungus symbiosis resides exclusively with the ants. (That is, we humans do not think of ourselves as being controlled by our crops; by analogy, the ants too must be understood as masters controlling their fungal domesticates.)

Because of this prevailing preconception of complete ant control over domesticated fungi, virtually no work has been done on potential controls that the cultivars may have over the ants. The only exception hinting at fungal control is a recent study of leaf-cutter foraging behavior by Ridley et al. (1996), who analyzed foraging behavior from the fungal perspective and suggested that the fungus uses chemical manipulation to "subvert a colony of ants into providing it with a healthy diet" (p. 634). Specifically, foraging experiments implicated a fungal semiochemical that "regulates the selection of plant material by foragers" (Ridley et al. 1996, p. 631; see also North et al. 1999).

There are two ways to interpret Ridley et al.'s (1996) observed regulatory effects that the cultivar exerts on its host. First, signaling of the fungus to the ants could be an honest message from the fungus to shut down supply of suboptimal or toxic substrate that the ants may unknowingly provide for their fungus. Alternatively, the observed fungal messaging may imply partial manipulative power over ant foraging. Any honest signaling system could potentially be subverted by the fungus and thus could set

the evolutionary stage initiating selection for exploitation by the fungus. Despite counterselection on the ants in an evolutionary arms race with their cultivars (Futuyma and Slatkin 1983; Herre et al. 1999), even minor, incipient exploitation by the fungus could be modified eventually through selection into more elaborate fungal control mechanisms where "the fungus can select its substrate by controlling the foraging behavior of the ants" (North et al. 1997, p. 386). Beyond the single investigation by Ridley et al. (1996), no other study tested for the possible manipulative powers that the fungus may have over the ant hosts or even considered chemical mechanisms of fungal control (even though psychotropic effects of chemicals on ant behavior are known; see review by Kermarrec and Mauleon 1986). It appears that the prevailing assumption of complete ant control has precluded theoretical and empirical inquiry into any more complex chemical dynamics between attine ants and their fungi.

Assumption of Vertical Cultivar Transmission

Reproductive-interest alignment between ants and cultivars has traditionally been assumed because of apparent vertical cultivar transmission, as explained above. Two kinds of evidence, behavioral and molecular, support vertical transmission of attine cultivars. Behavioral observations of foundress queens documented vertical cultivar transmission through the mechanism of the infrabuccal pocket in the higher-attine genera *Atta*, *Acromyrmex*, and *Trachymyrmex* (Weber 1966, 1972), as well as in the lower-attine genus *Cyphomyrmex* (U. G. Mueller and B. Ferster, unpublished data). Because of a general lack of observations on the inconspicuous lower attines, the existence (or nonexistence) of vertical cultivar transmission is unknown for any other attine genus. However, molecular population-genetic analyses of cultivars provide additional, yet indirect, evidence for vertical cultivar transmission in lower and higher attines. Specifically, genetic-fingerprinting analyses of cultivars, with either amplified fragment length polymorphism (AFLP) markers (Mueller et al. 1996, 1998; Bot et al. 2001; Green et al. 2002; S. A. Rehner, personal communication) or microsatellite markers (M. Kweskin, unpublished data), revealed clear evidence of clonality in population samples of cultivars isolated from the same ant species. Consistent with the behavioral observations, cultivars in different nests of the same ant species may show identical genetic fingerprints, indicating that they must have been clonally derived from a common ancestor.

The same molecular analyses that supported vertical transmission of cultivar clones also revealed significant levels of lateral cultivar transfer between attine species, in some cases even between attine genera. Thus, vertical cul-

tivar transmission is not strict, and there exists latitude for occasional lateral cultivar transfer. As mentioned above, some lateral cultivar transfers are implicated by topological incongruence of ant and fungal phylogenies (fig. 1; Chapela et al. 1994; Mueller et al. 1998), but the best evidence of lateral cultivar transfer comes from detailed population-genetic analyses of cultivars propagated by different sympatric species of ants (Bot et al. 2001; Green et al. 2002). In the most comprehensively analyzed case (fig. 2; Green et al. 2002), at least six recent cultivar transfers could be inferred from the phylogenetic relationships of a cultivar group propagated by the sympatric lower attines *Cyphomyrmex costatus* and *Cyphomyrmex muelleri*. These two ant species are specialized on a narrow group of closely related cultivars (probably a single cultivar species), and exchanges of cultivars in this group occur only between *C. costatus* and *C. muelleri*, but apparently not with other sympatric attine species (Mueller and Wcislo 1998; Green et al. 2002; Schultz et al. 2002). Thus cultivar exchanges are constrained in the *costatus-muelleri*

system, most likely by ant preferences for their own cultivar group (Mueller et al., in press) or by strong selection against transitions to cultivars outside the narrow group of native cultivars (N. J. Mehdiabadi and U. G. Mueller, unpublished data). In any event, cultivar exchange between *C. costatus* and *C. muelleri* is an ongoing and frequent process, and more comprehensive population-genetic analyses than the one in Green et al. (2002; fig. 2) are likely to reveal even higher levels of cultivar sharing between the two ant species.

Apart from the well-studied *Cyphomyrmex costatus-muelleri* system, lateral transfers of cultivar clones between attine species have also been documented for three additional cases involving the closely related congeneric ant species pairs *Acromyrmex octospinosus* and *Acromyrmex echinator* (Bot et al. 2001), *Mycocepurus smithi* and *Mycocepurus tardus*, and *Cyphomyrmex rimosus* and *Cyphomyrmex minutus* (Mueller et al. 1998). Lateral cultivar exchange can also occur between ant species of different ant genera, as, for example, in the single documented case

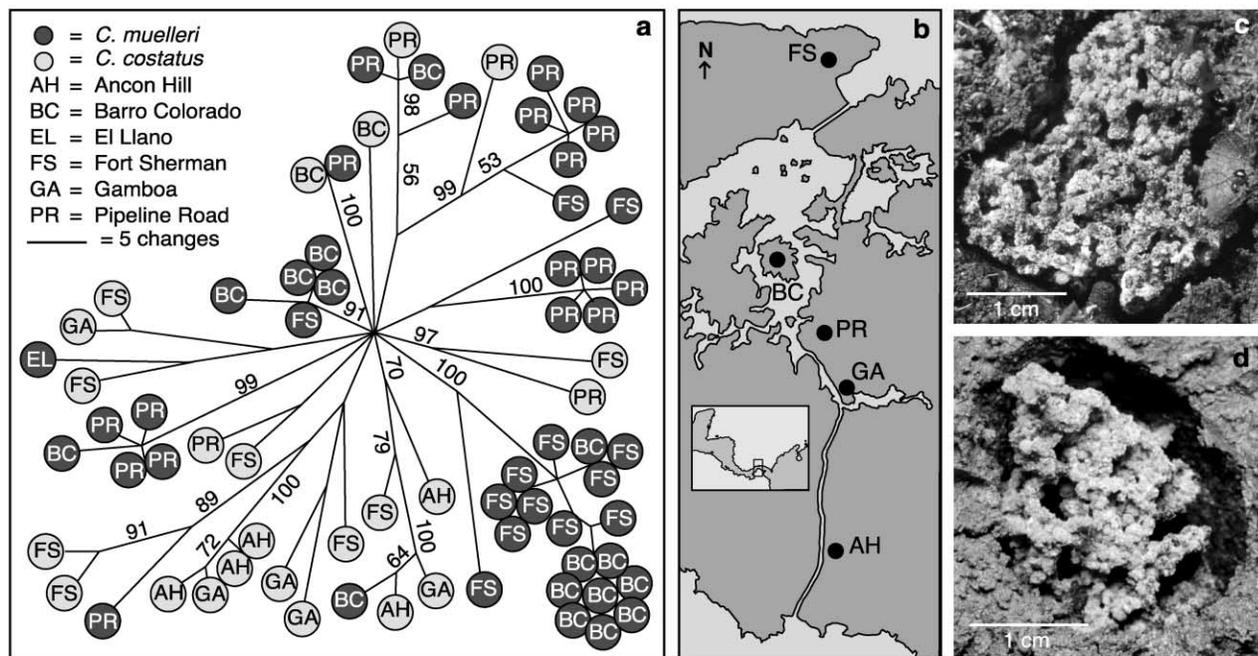


Figure 2: Exchange of fungal cultivars between the two sympatric fungus-growing ant species *Cyphomyrmex costatus* and *Cyphomyrmex muelleri*. *a*, Phylogenetic relationships between 72 cultivars propagated by *C. costatus* (light circles) and *C. muelleri* (dark circles) as inferred under the parsimony criterion from 127 informative amplified fragment length polymorphism (AFLP) markers. The phylogenetic relationships indicate at least six cases of recent cultivar transfers (grouping of cultivars from both ant species into the same clade); five of these transfers have bootstrap support of about 90% or higher. The asterisk marks two cultivars (one each from the two ant species) that are identical across all 127 AFLP markers; these two cultivars are clonally identical to each other, indicating a very recent transfer from one to the other species. *b*, Collecting sites (FA, BC, PR, GA, and AH) along the Panama Canal across the Isthmus of Panama (insert); El Llano (EL) is located about 80 km east of the Panama Canal and therefore is not indicated on the map. *c*, Garden of *C. costatus* under a log on the rain forest floor. *d*, Garden of *C. muelleri* in a shallow cavity in a clay embankment. Figure adapted from Green et al. (2002).

involving *Apterostigma auriculatum* and *Cyphomyrmex longiscapus* (Mueller et al. 1998). The case of the laterally transferred cultivars between the ant species *C. rimosus* and *C. minutus* is particularly instructive. Both species are currently sympatric in Florida, but *C. rimosus* is thought to have been introduced to Florida during this century (Snelling and Longino 1992; J. Longino, personal communication). The fact that *C. rimosus* presently shares a genetically identical cultivar with the indigenous *C. minutus* indicates that lateral cultivar exchanges occur on an ecological time scale and thus frequently punctuate vertical cultivar transmission.

Because the above cultivar-exchange systems (one higher-attine and four lower-attine systems) are the only cases for which cultivars have been analyzed with sufficiently powerful molecular techniques, and because lateral cultivar exchange has been documented in each case, it is possible that most or even all attine species regularly exchange cultivars with other sympatric species. In addition, because these molecular studies only tested for cultivar exchange between ant species, it is also possible that cultivar exchange between conspecific nests within the same ant species are even more rampant than the already surprisingly high levels of between-species exchange. This latter possibility is strengthened by laboratory experiments testing for horizontal transfer of cultivars as replacements after garden loss (e.g., as a result pathogens or as a result of garden usurpation by agropredatory ants in the genus *Megalomyrmex*; Adams et al. 2000a, 2000b): under garden loss experimentally induced in the laboratory, cultivar exchanges between nests of the same ant species were indeed four times more likely than cultivar exchanges between nests of different, closely related ant species. Under natural conditions, therefore, cultivar exchanges between nests of the same ant species may be rampant, and future research should aim at estimating precise levels of cultivar transfers in the field. Future research should also determine whether cultivar transfers are largely *direct*, between nests through exchange of cultivar clones, or *indirect*, following a cycle of cultivar escape, temporary free-living existence, and occasional reimport of free-living mycelium into ant cultivation (see "Assumption of Cultivar Incompetence for Free-Living Existence" for details on possible free-living cultivar existence).

Frequent cultivar exchange implies that a single ant lineage interacts with different cultivar clones in short evolutionary succession (or, vice versa, a single cultivar lineage may be propagated in succession by several ant lineages). Single ant lineages are therefore not locked permanently in a tight coevolutionary process with single cultivar lineages. Rather, because cultivar clones are passed around on a local level between different attine species, ant-cultivar coevolution appears substantially more diffuse (Futuyma

and Slatkin 1983) than previously thought (Weber 1972; Chapela et al. 1994).

Cultivar exchange drastically alters the evolutionary dynamics between ants and fungi. Most importantly, even low levels of horizontal transmission intensify selection for host-fitness-reducing, selfish ("virulent" sensu Frank 1996a, 1996b, 1997) cultivar traits (see above section on "Vertical versus Horizontal Transmission"). Specifically, cultivars may well be selected to compromise garden productivity (and thus the fitness of the ant hosts), yet compensate for their reduced likelihood of vertical transmission by ants through increased horizontal cultivar dispersal to other ant nests. Such cultivar selfishness may be minimal under normal symbiotic conditions, but may become facultatively expressed under conditions when vertical transmission is no longer possible for the cultivar and when escape from the current symbiosis represents the cultivar's only option. A facultative switch in cultivar life-history strategy could occur, for example, at a time when massive worker mortality endangers colony survival, or at time when natural queen death terminates the production of female reproductives (effectively terminating the life of the cultivar within this specific ant lineage). If the cultivar can indeed switch facultatively from a benign (cooperative) to a virulent (selfish) reproductive strategy, massive worker mortality and queen death are the kind of conditions that should result in destabilization of ant-fungus cooperation (see further details in "Conflict over Cultivar Sexuality").

Horizontal transmission not only selects for selfish cultivars but may also lead to cultivar mixing within gardens and thus may set the stage for competitive interactions between coexisting cultivar strains (see "Symbiont Competition"). Consequently, cultivars are selected to retain competitive traits (e.g., vegetative incompatibility mechanisms) that originated before their import into the symbiosis, and these traits may even become elaborated through selection within gardens (Bot et al. 2001). A cultivar life-history strategy involving investment of resources into between-cultivar competition is likely to compromise the overall efficiency of the ant-fungus mutualism and thus reduces the fitness of the ants. The ant hosts therefore should be selected to prevent between-cultivar competition by one of three means—first, by enforcing cultivation of a single cultivar strain (or accepting only compatible, non-competitive strains) throughout the entire nest (Bot et al. 2001); second, by managing cultivar diversity within gardens such that incompatible strains are allocated to different garden chambers, each with a single cultivar strain; or, finally, by curtailing the expression of competition between strains to keep frictions (loss of resources) at an acceptable minimum by, for example, inhibiting the expression of vegetative incompatibility interactions between cultivars. All of these competition-inhibiting mechanisms

are not mutually exclusive (see also “Conflict over Cultivar Replacement and Import of Novel Fungal Strains” and “Conflict over Cultivar Competition within Gardens”).

*Assumption of Cultivar Incompetence for
Free-Living Existence*

Two observations have led to the long-standing assumption that attine cultivars are obligately dependent on their hosts and cannot escape from the symbiosis to lead an independent, nonsymbiotic existence: laboratory gardens generally deteriorate when abandoned by ant hosts (Goetsch and Stoppel 1940; Muchovej and Della Lucia 1990; Currie et al. 1999a; Currie 2001a), and cultivars are outcompeted by most contaminant fungi and bacteria when grown in artificial culture in the laboratory (Weber 1955, 1972; Stradling and Powell 1986; C. R. Currie, personal communication; U. G. Mueller, personal observation). The cultivars' apparent inability to survive independently has traditionally been ascribed to their long coevolutionary association with ants, an association that, over time, has led to loss of crucial fungal adaptations for a free-living life. The view of cultivar frailty outside the symbiosis has been particularly promulgated by Weber, who argued repeatedly (Weber 1938, 1955, 1966, 1972, 1982) that “the fungi are clearly unable to maintain themselves and do not grow except under the care of the ants” (Weber 1955, p. 109).

While it is true that garden deterioration and abandonment by the ants are frequently linked, Weber's conclusion of cultivar dependency needs to be qualified. First, the causal relationship between abandonment by the ants and garden deterioration in the lab is often unclear, and it may be that the ants abandon the garden because deterioration has already progressed to an unrecoverable stage (e.g., the garden is hopelessly invaded by the parasite *Escovopsis*, which can cause immediate abandonment by the ants). Garden deterioration therefore may not be the consequence of, but rather the cause for, ant abandonment, at least in some instances. Second, while experimental removal of ants can trigger garden deterioration in the lab (Weber 1972; Currie et al. 1999a; U. G. Mueller, personal observation), a decline of the garden in the absence of ants is not inevitable. For example, ant gardens in the lab can persist for months after their ant hosts die off, and such gardens eventually perish because of desiccation, not because they are overwhelmed by alien microorganisms (T. R. Schultz, personal communication; U. G. Mueller, personal observation). Third, cultivar devastation by alien fungi under competition in a garden environment does not imply that the cultivar would predictably fail in a free-living existence. That is, the cultivar may be perfectly capable of independent survival under

certain ecological conditions but may be unable to do so in a garden environment without the help of the ants. An analogous argument applies to human-cultivated mushrooms, which can easily be outcompeted when left alone in artificial, human-manipulated growth environments (which are designed for high mushroom yield and easy harvesting by humans) but which do perfectly well under the ecological conditions to which they are naturally adapted. Fourth, and following the same kind of reasoning of the preceding point, most basidiomycetes are quickly overwhelmed on artificial culture plates by fast-growing contaminant ascomycete fungi, but these basidiomycetes are clearly capable of a free-living existence. Consequently, the widely cited observation that attine cultivars are generally overwhelmed by alien fungi when grown under the artificial conditions of a culture plate is meaningless and has no bearing on the cultivars' competitive ability in a free-living state under ecological conditions to which they may be adapted.

The strongest evidence undermining the assumption that cultivars cannot lead a free-living existence comes from a recent phylogenetic analysis of lower-attine cultivars and their free-living leucocoprineous relatives (Mueller et al. 1998; fig. 1). An analysis of a fast-evolving gene region (ITS) identified two free-living counterparts that were identical to cultivars propagated by attine ants (fig. 1A, 1B). This indicated that either the counterparts had recently escaped from the symbiosis with ants and had reentered a free-living existence, or that the ants had recently acquired a cultivar from free-living populations (of which the counterparts were representatives). Under the latter scenario, the recency of the ant-fungus association may well permit the cultivar to escape from the symbiosis because the adaptations necessary for independent existence may not have been lost during the evolutionarily short association with ants.

It is important to stress that the identification of free-living counterparts in Mueller et al.'s (1998) survey was based on broad collections of sympatric free-living and symbiotic leucocoprineous fungi in central Panama, and that this was the first such survey ever conducted (fig. 1). This suggests that a more exhaustive survey, including collections from Amazonian South America (the putative location of the attine origin; Mueller et al. 2001), may reveal many additional counterparts of other attine cultivars. Indeed, at this point one cannot exclude the possibility that most, if not all, attine cultivars have free-living counterparts and that import of novel cultivars into the symbiosis is an ongoing process occurring in all attine lineages, with the possible exception of some highly derived attine systems (e.g., the leaf-cutter ants; see “Conflict over Cultivar Sexuality”). Additional surveys of free-living leucocoprineous populations clearly will help to resolve these issues.

Such surveys can now be conducted efficiently because the known taxonomic placement of the attine cultivars permits targeted collecting within the Leucocoprineae.

Assumption of Cultivar Incompetence for Sexual Reproduction

Conceptually closely related to the issue of cultivar escape from the mutualism is the issue of cultivar competence for sexual reproduction. Three facts have traditionally been cited to support the view that cultivars are nearly (or even completely) incapable of sexual reproduction. First, the ants clearly propagate their cultivars clonally over many generations (see "Assumption of Vertical Cultivar Transmission"), leading to the expectation that, over long evolutionary time, at least some genes necessary for basidiocarp (sexual structure) development degenerate through accumulation of deleterious mutations (Mueller et al. 1998). Second, the development of cultivar basidiocarps has been observed so infrequently that it is thought to be more of an atavistic anomaly, but not an integral part of the cultivar life cycle. Third, in the few cases where basidiocarps developed in active gardens, the ants reacted adversely and began destroying the fruiting structures (Autuori 1940; Stahel 1943; Muchovej et al. 1991; Cruz and Batista Filho 1993b; Dörfelt and Creutzburg 1994; Fisher et al. 1994a, 1994b), thus essentially sterilizing the cultivar. These facts led Weber to conclude that, "through phylogenetic repression of the sporophore through countless generations, the power of the mycelium to develop this stage has been largely lost" (Weber 1938, p. 265). Wilson (1971) echoed this view of the "near inability of the fungus to form sporophores" (p. 43), and Chapela et al. (1994) even argued that cultivars of the higher-attine ants are ancient asexuals that have been propagated strictly clonally by the ants for at least 23 million years.

An exhaustive review of the literature on attine basidiocarps is actually remarkably inconsistent with the prevailing assumption of cultivar incompetence for sexual reproduction. The most surprising fact emerging from such a literature review (table 3) is that basidiocarps have been grown from cultivars of almost all known attine genera, including all of the seven lower-attine genera and three of the five fungus-cultivating, higher-attine genera (the two exceptions are the infrequently collected genus *Sericomyrmex* and the recently discovered new genus *Mycetagroicus*; Brandão and Mayhé-Nunes 2001). The accumulated evidence, summarized in table 3 for the first time, does not support the traditional view that attine basidiocarp production is a mere atavism.

Most importantly, spores from several attine basidiocarps have been shown to be viable, germinate, and develop into healthy mycelium in artificial culture. Not all

attempts at spore germination have been successful (Autuori 1940; Fisher 1994a, 1994b), which may be attributable to the known difficulty of germinating basidiomycete spores in artificial culture (Pegler and Young 1971). Because of these technical difficulties, even the few successful germination attempts of cultivar spores, including spores of higher-attine basidiocarps (Möller 1893) and lower-attine basidiocarps (M. Bacci, personal communication; A. G. Himler and T. Vo, personal communication), are highly significant. Spore production and spore viability refute the assumption of complete sexual incompetence of cultivars.

While the frequency of spore production, germination, and successful mating under natural conditions is still unknown for any attine cultivar, the facts of basidiocarp formation and spore viability indicate that these faculties have not degraded under the presumed long-term clonal propagation by the ants: either the cultivars recently entered the symbiosis (such that the genetic architecture underlying basidiocarp and spore formation has yet to degrade under long-term clonality) or sexual competence has been maintained through selection under regular outbreeding or other unknown processes. In either case, attine cultivars do not appear to be million-year-old clones, as hypothesized by Chapela et al. (1994).

Table 3 reveals an additional interesting fact that so far appears to have gone unnoticed. Higher-attine basidiocarps have been found exclusively in active gardens, but they have never been grown from pure cultures in the lab (i.e., in the absence of the ants). In contrast, lower-attine basidiocarps have never been found in active gardens, but they can be grown from axenic (pure) lab cultures in the absence of ants. The fruiting tendency of some lower-attine cultivars in artificial culture is actually surprisingly strong and does not require any special treatment, whereas none of the many higher-attine cultivars that have been propagated as artificial cultures during 120 years of research have ever yielded a basidiocarp. The absence of higher-attine basidiocarps grown from artificial cultures is puzzling, considering that, for development of pest control strategies, substantially more mycological research has focused on understanding the fungi of the higher attines (e.g., the leaf-cutters) than on understanding those of the lower attines.

It is unclear why higher-attine cultivars appear more prone than lower-attine fungi to fruit in association with ants (in natural gardens or on ant mounds). Because of their more derived coevolutionary relationship in the higher Attini (indicated, e.g., by derived features such as gongylidia), it may be that the higher attine cultivars are tied closely to the biology of their ant hosts, whereas the lower-attine cultivars may be more loosely integrated. For example, lower-attine cultivars could have retained strong

ties to free-living (sexually outcrossing) cultivar populations, and they may even move regularly between a symbiotic and a nonsymbiotic existence. This latter scenario would explain the free-living, lower-attine cultivar counterparts discovered in Mueller et al.'s (1998) free-living fungal survey (note that this survey did not yield a free-living counterpart of any higher-attine cultivar).

Like free-living mutualist populations known from other mutualisms (Douglas 1995; Herre et al. 1999), a free-living cultivar population could consist of feralized cultivar clones that are embedded into a larger, sexually recombining fungal population (Diamond 1998). The observed cultivar competence for basidiocarp and spore production (table 3) is consistent with this view. Additional evidence for the hypothetical free-living cultivar populations emerged from a recent phylogenetic reconstruction of a narrow group of cultivars propagated by the two sympatric lower-attines *C. costatus* and *C. muelleri* (see above, "Assumption of Vertical Cultivar Transmission"). The starlike evolutionary relationships (fig. 2) between the *C. costatus-muelleri* cultivars are peculiar. The star pattern suggests that the basal polytomy, which remains unresolvable even with substantial phylogenetic information, reflects a cohesive population undergoing sexual recombination (breaking up character correlations). In contrast, the resolvable radii of the phylogenetic star reflect the strict clonal propagation and independent evolutionary histories of asexual cultivars (accumulating character correlations over time). This interpretation implies that the *C. costatus-muelleri* ant-cultivar association, and possibly many lower-attine ant-fungus associations, may not be fundamentally different from other specialized mutualisms with connections to free-living populations, such as lichens (fungus and algae), endophytic fungi (fungus and plant), and corals (polyp and algae; Douglas 1995; Herre et al. 1999; Kane and Mueller 2002).

Ant-Cultivar Conflicts in the Attine Symbiosis

Table 1 lists a series of possible ant-cultivar conflicts that could exist in the attine symbiosis. Several of these (e.g., sex-ratio conflict, symbiont drive) exist even under strict vertical cultivar transmission, whereas others (e.g., cultivar competition) emerge only under significant levels of lateral cultivar transmission. The list is not exhaustive, but it summarizes the most obvious ant-cultivar conflicts.

Conflict over the Ant Sex Ratio

Because cultivars are propagated only by female reproductives from one generation to the next, cultivars are under strong selection to bias the sex ratio toward females, against the sex-ratio interests of the ants. Possible mech-

anisms of sex-ratio biasing employed by the cultivars could include, first, elimination of males through selective digestion of brood and physiological recycling of these resources; second, conversion of worker brood into female reproductive brood through hormonal manipulation of developmental programs; or third, production of fungal nourishment that is optimized for female development but inferior for male development. Because in most species the brood is kept within the gardens and because the brood is dependent on a cultivar diet (thus the brood cannot easily evade any chemical manipulation), cultivars may be in a unique position to apply biasing mechanisms throughout brood development. Indeed, the thick mycelial coat on brood (present in some but not all ant species; U. G. Mueller, unpublished data) or the intimate integration of the brood into the garden matrix may provide the cultivars with sex-ratio biasing leverages that workers do not possess. In extreme cases and analogous to other microbes transmitted solely through females (Hurst 1993; O'Neill et al. 1997; Wenseleers et al. 1998; Wenseleers 2001), cultivars could potentially induce parthenogenesis in the ants or eliminate males.

Bourke and Franks (1995) argued that "symbionts borne by queens might act as colony-level sex ratio distorters" (p. 63) but concluded that "workers should assert their sex-ratio preferences over the symbionts' in a conflict that—as a community of interest with perhaps greater power—workers might usually win" (p. 63). The outcome of ant-cultivar conflict over the sex ratio is indeed dependent on relative power (efficiency with which the ants vs. the cultivar can convert colony resources into a shift toward their respective optimal sex ratio; H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript); however, the overall dynamics is actually quite complex because workers and the queen differ in their respective optimum sex ratios. The overall conflict therefore involves three parties with differing sex-ratio interests. Fungal cultivars are selected to bias sex ratios toward 100% investment in females (males are useless to the cultivar; but see "Conflict over Mating Incompatibility in Ants"). In contrast, under the typical conditions of single mating in all attines but the leaf-cutters (Villesen et al. 1999; Villesen et al. 2002), workers and the queen prefer, respectively, 75% and 50% investment in females, following theoretical expectations derived originally by Trivers and Hare (1976; Crozier and Pamilo 1996; Mehdiabadi et al., in press; see footnote b in table 4 for complications of sex-ratio predictions pertaining to the leaf-cutter ants).

Tug-of-war models derived from optimal skew theory specify possible outcomes of this three-party, queen-worker-cultivar conflict (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript). First, the greater the power of a party (defined as the efficiency in

Table 3: Cultivar basidiocarps (mushrooms) growing from attine gardens or artificial cultures

Ant species	Basidiocarp species ^a	Remarks	References
Higher-attine ants (G1 cultivators):			
<i>Atta sexdens rubropilosa</i>	<i>Leucocoprinus gongylophorus</i>	Fully formed basidiocarps developed on two occasions in active gardens of two different lab nests; tissue culturing or spore germination not attempted	Bononi et al. 1981
<i>Atta sexdens rubropilosa</i>	<i>Leucoagaricus weberi</i>	Single basidiocarp developed from active garden in lab colony; subculturing from basidiocarp tissue yielded mycelium with staphylae; spore germination not attempted	Muchovej et al. 1991
<i>Atta sexdens rubropilosa</i>	<i>Leucocoprinus gongylophorus</i>	Two basidiocarps developed from gardens in active lab nest; tissue culturing or spore germination not attempted	Cruz and Batista Filho 1993a, 1993b
<i>Atta cephalotes</i>	Unidentified	Developing basidiocarps found in five of 30 active chambers in single nest during excavation; subculturing from developing basidiocarp yielded mycelium with staphylae; spore germination not attempted	Stahel 1938
<i>Atta cephalotes</i>	Unidentified	Developing basidiocarps grew from active gardens found during nest excavation; tissue culturing or spore germination not attempted	Stahel and Geijskes 1939, 1940
<i>Atta cephalotes</i>	<i>Rozites gongylophora</i>	Three developing basidiocarps found in single active garden during nest excavation in field; subculturing from basidiocarp tissue yielded mycelium with staphylae; spore germination not attempted	Stahel and Geijskes 1941
<i>Atta cephalotes</i>	<i>Rozites gongylophora</i>	Basidiocarp developed in soil after partial nest excavation; a mycelial connection was traceable from basidiocarp to intact garden; subculturing from basidiocarp tissue yielded mycelium with staphylae; spore germination not attempted	Stahel and Geijskes 1941
<i>Atta cephalotes</i>	<i>Leucocoprinus gongylophorus</i>	Repeated fruiting events in an active garden of a lab colony; subculturing from basidiocarp tissue yielded mycelium with characteristic staphylae; spore germination unsuccessfully attempted	Fisher et al. 1994a, 1994b
<i>Atta colombica</i>	<i>Leucocoprinus</i> cf. <i>gongylophorus</i>	Basidiocarp grew on external dump of active nest; tissue culturing unsuccessful; spore germination not attempted	Collection PA-236, U. G. Mueller, unpublished data
<i>Acromyrmex disciger</i>	<i>Leucocoprinus gongylophorus</i> ^b	Basidiocarps found on four occasions growing on thatched nest mounds; a mycelial connection was traceable from one basidiocarp to active attine garden; subculturing from basidiocarp tissue yielded mycelium with staphylae; spore germination successful	Möller 1893; Heim 1957
<i>Acromyrmex crassispinus</i> ^c	<i>Rozites gongylophora</i>	Small group of basidiocarps found on mound of active nest; tissue culturing or spore germination not attempted	Luederwaldt 1926; Gonçalves 1961
<i>Acromyrmex hispidus atratus</i>	<i>Rozites gongylophora</i>	Two developed and several expanding basidiocarps grew from garden in active lab colony; tissue culturing and spore germination unsuccessfully attempted	Autuori 1940
<i>Acromyrmex hispidus fallax</i>	<i>Rozites gongylophora</i>	Basidiocarp grew on thatched nest mound	Gonçalves 1961

<i>Acromyrmex hispidus fallax</i>	<i>Leucoagaricus gongylophorus</i>	Basidiocarps repeatedly grew on thatched nest mound; sub-culturing from basidiocarp tissue yielded mycelium with staphylae; DNA fingerprinting established genetic identity between basidiocarp tissue and garden cultivar	Pagnocca et al. 2001
<i>Acromyrmex octospinosus</i>	<i>Leucocoprinus gongylophorus</i>	Basidiocarp grew from garden of lab colony; tissue culturing or spore germination not attempted	Dörfelt and Creutzburg 1994
<i>Trachymyrmex bugnioni</i>	<i>Leucocoprinus</i> sp.	Basidiocarps grew on two separate occasions from garden in active laboratory colony; tissue culturing or spore germination not attempted	T. R. Schultz, personal communication
Lower-attine ants (G3 cultivators):			
<i>Cyphomyrmex costatus</i>	<i>Leucoagaricus wichanskyi</i>	Basidiocarp developed from pure cultivar isolate in lab	Heim 1957; Weber 1957; Singer 1975
<i>Cyphomyrmex costatus</i>	<i>Leucocoprinus (Lepiota)</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	Hervey et al. 1977
<i>Mycetosoritis hartmanni</i>	<i>Leucocoprinus</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	I. H. Chapela, T. R. Schultz, and U. G. Mueller, unpublished data; A. G. Himler, personal communication
<i>Mycetophylax conformis</i>	<i>Leucocoprinus (Lepiota)</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	Hervey et al. 1977
<i>Mycetarotes parallelus</i>		Basidiocarp developed from pure cultivar isolate in lab	M. Bacci, personal communication
<i>Apterostigma auriculatum</i>	<i>Leucocoprinus (Lepiota)</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	Hervey et al. 1977
<i>Myocepurus smithi</i>	<i>Leucocoprinus</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	A. G. Himler, personal communication
<i>Myocepurus smithi</i>	<i>Leucocoprinus</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	U. G. Mueller, unpublished data
<i>Myocepurus tardus</i>	<i>Leucocoprinus</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	A. G. Himler, personal communication
<i>Myrmicocrypta buenzlii</i>	<i>Leucocoprinus (Lepiota)</i> sp.	Basidiocarp developed from pure cultivar isolate in lab	Hervey et al. 1977
<i>Myrmicocrypta ednaella</i>	Unidentified	Basidiocarp developed from pure cultivar isolate in lab	Weber 1966, 1972, 1982

Note: Because attine cultivars are now known to be members of only two fungal families (Lepiotaceae and Tricholomataceae), the information summarized here ignores the vast number of fungi (contaminants, parasites) outside these two families that have been isolated from attine gardens or that were found as accidental associates on attine nest mounds.

^a The taxonomy of attine cultivars is in need of revision; genus or species assignments indicated here for the cultivars, therefore, are provisional. Basidiocarp names are listed exactly as published in the original report, or, if applicable, under the revised name if a collection was redescribed later. Möller's (1893) very first description of *Rozites gongylophora* from *Acromyrmex disciger* was redescribed by Heim (1957) as *Leucocoprinus gongylophorus*. Many researchers have followed this reassignment into the genus *Leucocoprinus* to name cultivars obtained from other leaf-cutter ant species. Other researchers have followed Singer (1975, 1986), who believed the leaf-cutter basidiocarps to be in the closely related genus *Leucoagaricus*. The generic boundaries between *Leucocoprinus* and *Leucoagaricus* are unclear, and *Leucoagaricus* even appears to be paraphyletic with respect to *Leucocoprinus* (Johnson 1999). Thus any final assignment of attine cultivars to either *Leucocoprinus* or *Leucoagaricus* will have to await a thorough taxonomic revision; however, the placement of the lepiotaceous attine cultivars into the clearly monophyletic tribe Leucocoprineae (comprised of *Leucocoprinus* and *Leucoagaricus*) appears certain (Mueller et al. 1998; Johnson 1999). Much of the taxonomic confusion and uncertainty over the fruiting competence of attine cultivars appears to be due to the fact that reports were published in half a dozen languages in sometimes obscure journals and often involved only cursory descriptions that could easily be missed without careful perusal. This constellation of factors, in addition to the prevailing myrmicocentric biases of researchers, may have hampered a comprehensive review of the cultivar-fruiting literature, which is actually quite extensive.

^b *Leucocoprinus gongylophorus* was originally described as *Rozites gongylophora* by Möller (1893) but was redescribed by Heim (1957).

^c Originally reported as *Acromyrmex nigra* by Luederwaldt (1926), but believed by Gonçalves (1961) to be *Acromyrmex crassispinus*.

Table 4: Investment sex ratios of lower- and higher-attine ant species

Ant species	References	Number of colonies	Investment sex ratio (% male)		
			Queenright colonies (%)	Queenless colonies (%)	Overall sex ratio (%)
Lower-attine ants, G2 cultivators:					
<i>Apterostigma dentigerum</i>	Forsyth 1981	53	37.4	100	50.9
<i>Apterostigma mayri</i>	Murakami et al. 2000	9			41.1
<i>Apterostigma mayri</i>	Weber 1946	1			81.3
G2 cultivator sex-ratio average ^a					48.0
Lower-attine ants, G3 cultivators:					
<i>Mycetosoritis hartmanni</i>	A. G. Himler and U. G. Mueller, unpublished data	24	51.5	100	58.9
<i>Cyphomyrmex muelleri</i>	U. G. Mueller, unpublished data	37	44.1	100	46.7
<i>Cyphomyrmex longiscapus</i>	U. G. Mueller, unpublished data	103	46.9	100	49.1
<i>Cyphomyrmex rimosus</i> , Panama	Murakami et al. 2000	27			30.4
<i>Cyphomyrmex rimosus</i> , Venezuela	Weber 1947	1			38.2
<i>Cyphomyrmex rimosus</i> , British Guyana	Weber 1946	1			12.2
<i>Cyphomyrmex costatus</i>	Murakami et al. 2000	16			25.4
G3 cultivator sex-ratio average ^a					48.3
Higher-attine ants, G1 cultivators: ^b					
<i>Atta bisphaerica</i>	Autuori 1950, 1956	5			25.1
<i>Atta laevigata</i>	Autuori 1950, 1956	6			25.5
<i>Atta sexdens</i>	Autuori 1950, 1956	7			39.0
<i>Atta sexdens</i>	Autuori 1942	1			48.5
<i>Atta sexdens</i>	Fowler et al. 1986	1			39.2
<i>Atta sexdens</i>	Geijskes 1953	1			42.3
<i>Atta colombica</i>	Murakami et al. 2000	5			69.1
<i>Atta cephalotes</i>	Geijskes 1953	1			55.7
<i>Atta cephalotes</i>	Fowler et al. 1986	1			65.9
<i>Atta cephalotes</i>	Fjeringstad 1994; E. J. Fjeringstad, personal communication	2			26.7
<i>Atta vollenweideri</i>	Jonkman 1980	2			59.0
<i>Acromyrmex octospinosus</i>	Weber 1945	1			56.4
<i>Atta octospinosus</i>	Murakami et al. 2000	5			69.2

<i>Atta octospinosus</i>	Lewis 1975	10	26.5
<i>Acromyrmex coronatus</i>	Pereira-da-Silva et al. 1981	1	39.3
<i>Acromyrmex heyeri</i>	Diehl-Fleig 1993	6	31.3
<i>Acromyrmex landolti</i>	Fowler et al. 1986	1	39.4
<i>Acromyrmex striatus</i>	Fowler et al. 1986	1	44.4
<i>Trachymyrmex isthmicus</i>	Murakami et al. 2000	17	15.8
<i>Trachymyrmex septentrionalis</i> , New York	S. N. Beshers, personal communication	34	60.0
<i>Trachymyrmex septentrionalis</i> , Florida	S. N. Beshers, personal communication	41	32.4
<i>Trachymyrmex septentrionalis</i> , Mississippi	Cole 1939	50	23.7
<i>Trachymyrmex ruthae</i>	Weber 1945	1	61.7
<i>Sericomyrmex amabilis</i>	Murakami et al. 2000	8	44.3
G1 cultivator sex-ratio average ^a			41.3 ^b

Note: Investment sex ratios were calculated as either percent male dry weight or percent male wet weight, depending on the original study. Estimates from dry weights generally overestimate the female investment (Boomsma 1989), particularly in species with strong sexual dimorphism (e.g., *Atta*), but this error should be negligible in the lower attines, which show very little sexual dimorphism. The table lists only sex-ratio data that were derived from colony excavations/collections in the field; sex ratio data from mating swarms or from laboratory colonies are not included here. A more exhaustive literature review of all attine sex-ratio information will be published elsewhere (U. G. Mueller, unpublished manuscript). The table also lists only ratios that were reported originally as investment ratios or ratios derived from reported numerical ratios by using a female : male weight conversion factor from the same or a very closely related ant species. The following conversion factors were used to derive investment from numerical ratios: for *Apterostigma mayri*, female : male weight ratio = 1.732 from Murakami et al. (2000); for *Cyphomyrmex rimosus*, 2.268 from Murakami et al. (2000); for *Atta bisphaerica*, 8.00 from Trivers and Hare (1976); for *Atta laevigata*, 8.37 from Trivers and Hare (1976); for *Atta sexdens*, 7.67 from Trivers and Hare (1976); for *Atta cephalotes*, 4.311 from Fjerdingstad (1994; E. J. Fjerdingstad, personal communication); for *Atta vollenweideri*, 7.67 from *A. sexdens* in Trivers and Hare (1976; *vollenweideri* was formerly recognized as a subspecies of *sexdens*); for all *Acromyrmex* species, 2.74 from *Acromyrmex octospinosus* in Trivers and Hare (1976), except 1.284 remained for Murakami et al.'s (2000) own estimate for *A. octospinosus*, but this latter ratio could be an underestimate because females seem substantially larger than males (U. G. Mueller, personal observation); for *Trachymyrmex septentrionalis* Mississippi (Cole 1939), 1.421 from *T. septentrionalis* Florida in Beshers and Traniello (1994); for *Trachymyrmex ruthae* (Weber 1945), 2.042 from *T. isthmicus* in Murakami et al. (2000).

^a Averages were calculated as follows: first, a weighted average was calculated for each species (weighing by the number of colonies used for calculations of sex-ratio averages in each published report); next, a genus average was calculated; last, sex ratios were averaged across genera within each of the three cultivator groups (G1, G2, and G3 cultivators). Calculations of averages therefore ignore phylogenetic relationships within and between genera; that is, species nested within genera, and the genera themselves, are treated as independent data points. Calculations also ignore the fact that several attines currently recognized as a single species (e.g., *Cyphomyrmex rimosus*, *Atta sexdens*, *Acromyrmex octospinosus*) probably represent cryptic species complexes (J. Longino, personal communication; J. J. Boomsma, personal communication; U. G. Mueller and T. R. Schultz, unpublished data).

^b Sex-ratio predictions for the G1-cultivating higher attines are complicated by the fact that the leaf-cutter genera (*Atta*, *Acromyrmex*) have multiply mated queens, whereas the basal higher attines (*Trachymyrmex* and *Sericomyrmex*) have singly mated queens (retaining the plesiomorphic single-mated condition found in all lower attines; Villesen et al. 1999, 2002). Under worker control, sex ratios thus should be closer to the queen-preferred sex ratio of 50% female investment in the two leaf-cutter genera (multiple mating) but more female biased in the two basal higher-attine genera (single mating; Crozier and Pamilo 1996). Because the average investment sex ratios do not differ as predicted between *Trachymyrmex* and *Sericomyrmex* (sex-ratio average = 39.6%), *Acromyrmex* (sex-ratio average = 39.2%), and *Atta* (sex-ratio average = 43.8%), worker control does not seem to be a major factor dominating other influences, such as cultivar control, queen control, or other unknown factors. Overall within-genus sex-ratio variances are too large to permit the conclusion that the average investment sex ratio of about 40% observed across the higher-attine lineages represents a significant (cultivar-induced?) deviation from the 50% sex ratio predicted under pure queen control.

converting colony resources into a shift toward the party's optimal sex ratio), the closer the actual (realized) colony sex ratio to the party's optimal sex ratio. Second and somewhat counterintuitive, when there is a three-party tug-of-war, such as queen-worker-cultivar conflict, one of the opponents always drops out (i.e., invests no resources in the tug-of-war); the realized sex ratio then is an outcome of the tug-of-war between the remaining two parties. The reason for why one party always drops out of the tug-of-war is essentially that this party relies on one of the remaining two parties to bring the sex ratio closer to its own optimal sex ratio. Third, the greater the disparity in the optimal sex ratios of the two conflicting parties, the greater the combined investment by the two parties into the tug-of-war and, consequently, the greater the decrement in colony fitness (output of reproductives). Tug-of-war theory thus makes precise predictions as to the dynamics of the three-party, queen-worker-cultivar conflict over the ant sex ratio (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript).

Empirically, attine sex-ratio data from the literature show little indication of overall female-biased sex ratios (table 4), even though species, populations, and colonies differ widely in sex ratio. If cultivars are indeed successful at inducing a female bias, the overall effect appears to be small and is difficult to distinguish from estimation biases (see table 4 footnotes; Boomsma 1989) or the multitude of other factors that can select for female-biased sex ratios (Crozier and Pamilo 1996; Mehdiabadi et al., in press). Second, some of the best-studied species (*Apterostigma dentigerum*, *Cyphomyrmex longiscapus*, and *Cyphomyrmex muelleri*) show sex-ratio compensation, a population-level phenomenon where queenright nests produce female-biased sex ratios to compensate for the male-biased reproductive output of queenless nests (unmated workers are able to produce sons in queenless nests). In these species (table 4), sex-ratio compensation equilibrates population sex ratios closely at 50% male investment, suggesting queen control, but clearly not cultivar control. Third, to date no parthenogenetic species has been discovered in the Attini, though parthenogenesis is suspected for the lower attine *Mycocetopus smithi* (Fernández-Marín 2000). The available sex-ratio information therefore does not lend support for any obvious influence of the cultivar over the ant sex ratio. Wenseleers and Billen (2000) and Keller et al. (2001) recently documented a similar absence of any overt sex-ratio biasing by *Wolbachia* bacteria existing endosymbiotically in ants (but see van Borm et al. 2001 for circumstantial evidence that the *Wolbachia* found in *Atta* and *Acromyrmex* may be partial "male killers").

The observation of relatively unbiased sex ratios in the Attini (table 4) does not imply that the cultivar and the ants are definitively not engaged in a tug-of-war over the

sex ratio. Ants and cultivars may well have experienced coevolutionary arms races over the sex ratio, but the ants appear to have won the tug-of-war, as predicted by Bourke and Franks (1995; see above). Specifically, the cultivars may have evolved mechanisms to bias the sex ratio, but the ants responded by evolving countermeasures to undo the cultivars' manipulations. Both cultivars and ants thus may be locked in a coevolved state of mutual undoing, yet the overt sex ratio does not show any obvious deviation from optimum values of the ants.

To uncover covert coevolutionary arms races over the sex ratio, it is necessary to dissociate currently associated cultivars from their ant hosts and then to determine whether the dissociated ants, released from the manipulations of their native cultivar, show a shift toward more male-biased sex ratios. Such a shift is predicted because the dissociated ant host will presumably continue to apply pull toward male bias, as if still engaged in a tug-of-war with its native cultivar. (This experimental design is analogous to antibiotic treatment eliminating sex-ratio-biasing endosymbiotic bacteria from their insect hosts, which results in the predicted shift to a male-biased sex ratio or, in parthenogenetic lineages, even to restoration of the male sex [Werren 1997].) Complete experimental dissociation from any cultivar is not possible for attine hosts because ants without a cultivar will die, but it is possible to switch ants to novel cultivars. These novel cultivars presumably will not have coevolved with the experimental ant lineage and thus will not possess effective mechanisms to bias the sex ratio toward females. Consequently, the experimentally switched ant host will be engaged in a tug-of-war with an impotent (novel) cultivar. This should result in a shift toward male-biased sex ratios if the ants continue to express the counteradaptations that evolved during the arms race with their native cultivar. Cultivar switch experiments of this kind may be a choice paradigm for investigating sex-ratio conflict between attine ants and their cultivars.

Conflict over Life-History Trade-Offs

Because large and small ant colonies tend to produce, respectively, female-biased versus male-biased sex ratios (Nonacs 1986; Boomsma 1989; Herbers et al. 2001), cultivars are under selection to delay ant reproduction until sufficient resources have been invested in colony growth and until colony size guarantees female-biased reproductive outputs. Cultivars thus could temporarily sterilize small colonies, shift life-history allocations to accelerate colony growth, and thus bias the sex ratio indirectly toward females. Paralleling sex-ratio conflict, the conflict dynamics over life-history allocations consequently involves three parties (worker, queen, cultivar) with incongruent interests, and three-party, tug-of-war models should provide

the appropriate analytical tools to make predictions about this life-history conflict (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript). The traditional view, summarized in Bourke and Franks (1995), Crozier and Pamilo (1996), and Herbers et al. (2001), has been that sex ratio is dependent on colony size because of resource constraints, with resource-constrained, smaller colonies investing into the energetically cheaper male sex. In contrast, tug-of-war models (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript) propose an alternative view in which the dependency of the sex ratio on colony size is not an outcome of resource constraints, but is an outcome of the changing power interplay of the three conflicting parties, starting with more queen-controlled sex ratios (male bias) when colonies are small, then gradually shifting to more worker-controlled or cultivar-controlled sex ratios (female bias) as worker number or garden size increases and as queen power gradually fades relative to the power of the two other parties. The recent development of novel modeling approaches (Herbers et al. 2001; Mehdiabadi et al., in press; H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript) makes such life-history conflicts a particularly promising area for future empirical work.

Symbiont Choice and Sensory Exploitation of Ants by the Cultivar

The routine transplanting of fungal mycelium by ants from old to new garden beds could generate, through sensory-biased symbiont choice, selection favoring cultivar traits that increase the likelihood of transplantation within nests but that are detrimental to the survival of the whole colony. Cultivars are subcultured by workers for 10–100 times in succession during the lifetime of a colony (see “Ant-Cultivar Interactions”); under these conditions, even minor subculturing biases (symbiont choice) favoring certain cultivar mutants will lead to the spread of the favored cultivar in the colony. This process is somewhat analogous to artificial selection in humans, except that, in extreme cases, mutant cultivars could exploit the sensory biases of ants and sweep through gardens to fixation, even though the driving cultivar may replace cultivar genotypes that are more beneficial to the ants (and the overall mutualism). Ultimately, the population-wide prevalence of such driving cultivars will be checked by selection against colonies with cultivar-dependent low productivity, but at least some level of cultivar drivers can be expected, depending, first, on the rate at which driving mutants arise and, second, on the strength of within-colony processes favoring cultivar drivers relative to the strength of colony-level processes favoring alternative cultivar types.

Subculturing biases of ants are presumably under se-

lection to maximize colony fitness, but because the ants in a colony are evolutionarily stagnant relative to the subcultured cultivars, the cultivars can be expected to evolve faster (see above, “Ant-Cultivar Interactions”). Sensory exploitation by cultivars thus could be highly effective. Symbiont choice in queens selecting cultivars for transfer to new nests generates further potential for cultivar drive, adding to the drive inherent in the subculturing choices of workers during the lifetime of a colony. The eventual invasion of driving cultivars almost seems inevitable.

In the few attine species tested, workers possess an acute discriminatory power to differentiate between closely related cultivars (Mueller et al., in press; U. G. Mueller, unpublished manuscript). The neurological mechanisms for symbiont drive and sensory exploitation therefore appear to be in place in attine ants. Acute discriminatory powers may have evolved in attine ants for several adaptive reasons that are unrelated to cultivar drive. The first reason is the prevention of accidental import of a novel cultivar and thus maintenance of an association with a coevolved cultivar with which the ants form a genetically and physiologically integrated mutualism (Martin 1987; Bot et al. 2001). The advantage of maintaining an association with a performance-optimized cultivar is obvious. A second reason is the detection of parasitic fungi (weeds) that may be specialized to invade gardens by mimicking the cultivated fungi. The ability to discern a desired cultivar from invading parasites has clear advantages because it allows workers to weed out infested parts of the garden, a behavior that was recently described by Currie and Stuart (2001). A third reason is the differentiation between cultivar strains that coexist in the same garden and that may compete with each other for garden space (see “Conflict over Cultivar Competition within Gardens”). Acute differentiation may allow workers to maintain gardens as monocultures, thus preventing the evolution of undesired cultivar traits that increase a cultivar’s competitive edge over other coexisting cultivars but that compromise the productivity of a garden. Imposing monoculture through symbiont choice (“partner choice”; Bull and Rice 1991) thus could prevent the invasion of selfish (virulent) competitor strains of the cultivar (Bot et al. 2001).

Symbiont choice has obvious parallels with mate choice (Ryan 1990; Endler and Basolo 1998); both are cases of partner choice operating within a reproductive enterprise, and many concepts generated in the context of mate choice (e.g., good genes, honest indicators, sensory drive) may also be applicable to symbiont choice. Whereas symbiont choice can select for cultivar traits if these are used by the ants as indicators of desirable cultivar properties (nutrient levels, growth rate; see Stradling and Powell 1986), symbiont choice may also be exploited by mutant cultivars. Specifically, cultivars may be exhibiting the equivalent of

peacock feathers, features which enhance a cultivar's success of being chosen by the ants for the planting of new gardens but which incur undesirable side effects (costs) to both the cultivar and the ants because of reduced garden productivity or viability. Such exploitative cultivars could still spread to fixation in gardens if the sensory biases of ants are strong relative to the disadvantages of reduced garden fitness. Symbiont choice thus is not only the equivalent of artificial selection but also the between-species equivalent of mate choice, and both symbiont choice and mate choice emerge as specific examples of a more general mechanism of partner choice (Bull and Rice 1991) that can operate at both within-species and between-species levels.

Conflict over Mating Incompatibility in Ants

Cultivars could evolve mechanisms to induce ant mating incompatibilities in a manner analogous to cytoplasmic incompatibility induced by *Wolbachia* bacteria in many arthropods (Turelli 1994; Hoffmann and Turelli 1997; Werren 1997; Hurst and Schilthuisen 1998), including ant species in the myrmicine genus *Leptothorax* (Wenseleers 2001) and possibly the leaf-cutter *Acromyrmex insinuator* (van Borm et al. 2001). Because males do not propagate the cultivars from one generation to the next, the cultivars could use the males to sterilize females from other colonies that carry a different cultivar type. Converting males into such "kamikaze maters" eliminates other cultivar types from the population and, in the competition between cultivars for host occupation in a population, thus increases the fitness of the incompatibility-inducing cultivar. If cultivar-induced mating incompatibility parallels the case of *Wolbachia*, matings between reproductives derived from nests with the same cultivar would be fertile, whereas matings between nests with incompatible cultivars would produce inviable female zygotes. (Note that incompatibly mated queens can still produce unfertilized eggs that develop into males; hence these queens are not truly sterile, but they cannot produce any workers; consequently, these queens are effectively sterile because their colonies never reach the reproductive stage.)

Mating incompatibility requires complex interactions between paternal and maternal factors in the zygote; for example, the cultivar imprints the sperm and renders it sterile unless it is rescued by a maternal factor in the zygote (Hoffmann and Turelli 1997). Such a modification-rescue system, hypothesized for *Wolbachia* (but speculative at this point for attine cultivars), may be difficult to evolve. If existing as bidirectional incompatibility (Werren 1997), however, mating incompatibility may contribute to attine speciation because colonies switched to a new cultivar become reproductively isolated through a postzygotic iso-

lation mechanism. Such symbiont-switch-triggered speciation can occur sympatrically but is most likely under allopatry and secondary contact (Turelli 1994). If any attine species can be mated in the laboratory, cultivar switch experiments (in conjunction with observations on mating and colony founding) could serve again as a choice paradigm for testing the hypothesized mating-incompatibility conflict between cultivars and attine ants.

Conflict over Cultivar Sexuality

Resource investment into structures (e.g., basidiocarps) for sexual reproduction presumably reduces investment elsewhere in the colony, as, for example, in colony growth and output of ant reproductives. Because of such trade-offs, ants are under selection to suppress cultivar fruiting or to keep it at a minimal necessary level. Cultivation regimes that the ants impose on their cultivar may even be designed to inhibit fruiting tendencies. For example, limiting the biomass (garden size) of the cultivar could inhibit fruiting because many fungi require a minimum mycelial biomass to support a fruiting structure. In addition, the physical disturbance inherent in the continuous reworking of the garden by the ants may prevent a developmental switch to sexual reproduction in the cultivar.

The observation that developing fruiting structures are actively destroyed by the ants is consistent with this view of ant-suppressed fruiting (Autuori 1940; Stahel and Geijskes 1941; Stahel 1943; Bononi et al. 1981; Muchovej et al. 1991; Cruz and Batista Filho 1993b; Dörfelt and Creutzburg 1994; Fisher et al. 1994a, 1994b). Autuori (1940) even believed that the development of cultivar fruiting structures causes a fundamental change in ant behavior throughout the colony, with the ants aggressively attempting to eradicate the formation of basidiocarp primordia (tuberousities growing on hardened garden surfaces; see also Stahel 1938; Stahel and Geijskes 1939, 1941). Cruz and Batista Filho (1993b) also noted a qualitative change in behavior ("atypical movement") during the time of basidiocarp development. Stahel (1943, p. 252) aptly describes the expression of this ant-cultivar conflict over colony resources: "Gardens may be found to be covered with a meaty, compact fungus tissue. From this crust eventually a mushroom may grow out, called *Rozites gongylophora*. The ants suppress these fruit bodies with so much success that only by great exception is a mushroom formed. Even the crust may be carried away by ants of the medium-size class, as they normally do with the exhausted and useless parts of the garden. Some food ... is lost in this manner."

Thus cultivar fruiting is costly to the colony. However, the traditional assumption that all sexual reproduction in the cultivars is detrimental to the ants needs to be reexamined because genetic recombination of the cultivars may

benefit the ants under some circumstances (e.g., genetic diversity may increase overall disease resistance). Occasional cultivar sexuality may also ameliorate the costs associated with long-term asexuality (slower evolutionary rates, accumulation of deleterious mutations). Indeed, the observation that the cultivars of the higher attines fruit only in the presence of the ants (see “Assumption of Cultivar Incompetence for Free-Living Existence”) could imply that occasional fruiting is an integral feature of the higher-attine symbiosis. This symbiosis thus may resemble somewhat the one between termites and their cultivated fungi, which are believed to undergo sexual recombination regularly (Batra and Batra 1979). This novel interpretation of the higher-attine symbiosis, though tantalizing, would need to be tested through rigorous field observation on higher attines, experimental work in the laboratory, and genetic analyses testing for genetic signals of recombination.

To investigate whether cultivar fruiting is an integral phenomenon of cultivar life, it will be necessary to devise reliable methods to induce the development of cultivar basidiocarps in active ant colonies. Four experimental approaches are suggested by a review of the conditions that may have triggered the fruiting events reported in the literature (table 3).

Several studies reported that cultivar fruiting occurred shortly after drastic declines of the number of workers (Autuori 1940; Stahel and Geijskes 1941; Bononi et al. 1981; Cruz and Batista Filho 1993*a*, 1993*b*; Dörfelt and Creutzburg 1994; Fisher et al. 1994*a*, 1994*b*). Precipitous reduction in the work force may shift the power balance in favor of the cultivar (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript), allowing the cultivar to develop sexual structures that normally are suppressed by the workers. Experimental elimination of workers from healthy gardens could test this hypothesis, following, for example, the methods of Autuori (1940).

Decline in worker number was associated with queen death in two reports (Bononi et al. 1981; Fisher et al. 1994*a*, 1994*b*). Loss of the queen has major implications for life-history strategies of the cultivar because queenless colonies will soon cease to produce the female sexuals that vector the cultivar to new locations (in many attines, colonies will produce worker-laid males for some time following queen death, but never females), and these colonies will die out eventually as no new workers are produced. Imminent termination of the ant lineage thus implies that cultivar escape from the symbiosis, either through spore production or through vegetative escape (see “Conflict over Cultivar Escape”), may be the only reproductive option left for the cultivar. This suggests the hypothesis that cultivars may respond to queen death by initiating the development of sexual structures. If queen death is indeed related to cultivar fruiting, it appears to be a sufficient

condition, not a necessary condition, because cultivar fruiting in the case reported by Cruz and Batista Filho (1993*b*) apparently occurred in the presence of a live queen (in a colony experiencing high worker mortality). Carefully designed laboratory experiments should be able to determine whether queen loss or decline in worker number (or both) can trigger cultivar fruiting.

In one case of cultivar fruiting in *Atta*, a colony was given gardens from other colonies before cultivar fruiting occurred (Fisher et al. 1994*a*, 1994*b*). This could have led to mixing of cultivar lineages, possible genetic exchanges, and eventually triggered development of fruiting structures. The relevance of cultivar mixing for basidiocarp production can be easily tested by combining gardens from different colonies in the laboratory (but see Bot et al. 2001 for incompatibility constraints on such mixing experiments).

Stahel and Geijskes (1939, 1941; see also Stahel 1943) hypothesized that increased nest ventilation and humidity fluctuations could lead to cultivar fruiting in *Atta* nests. This hypothesis was based on two observations. First, fruiting attempts were discovered by Stahel and Geijskes primarily in the most superficial gardens, which were assumed to be subject to greater humidity fluctuations than gardens situated deeper in the ground. Consistent with this hypothesis, fruiting from superficial garden chambers was also true in the cases reported by Möller (1893), Autuori (1940), and Pagnocca et al. (2001). Second, fruiting attempts in *Atta* gardens were discovered only during the dry season (Stahel and Geijskes 1941; Stahel 1943). Stahel and Geijskes’ hypothesis can probably be tested in the laboratory through manipulation of humidity; however, Stahel and Geijskes’ careful natural history observations may be more useful in focusing field work on drier seasons in order to collect leaf-cutter basidiocarps. Interestingly, most of Möller’s (1893), Pagnocca et al.’s (2001), and U. G. Mueller’s (unpublished data; table 3) observations of leaf-cutter–cultivar fruiting were also made during the drier parts of the year (or during the transition from wet to dry season).

Additional factors conducive for cultivar fruiting could be pathogen infestations of gardens (Dörfelt and Creutzburg 1994) or an exceptionally large garden biomass permitting nutritive support of a fruiting structure.

Conflict over Cultivar Escape

Basidiocarp and spore production not only permit sexual recombination of the cultivar but possibly also cultivar dispersal via spores. The ant-cultivar conflict over cultivar sexuality is therefore tied intimately to conflict over cultivar escape. Escape conflict has several dimensions, however, because escape can occur sexually via spore dispersal but also vegetatively via mycelial growth into the sur-

rounding soil or leaf litter. The nesting habits of many attines (in the top soil layer, in leaf litter, under logs) actually facilitates easy vegetative escape, and the ants therefore should be selected to prevent their cultivars from using colony resources to grow into the surrounding environment. Indeed, attine ants appear to sequester their cultivars in gardens of variable architectural design. For example, suspending gardens from roots in cavities, or limiting garden contact only to the cavity ceiling (hanging gardens) or floor (sessile gardens) minimizes contact with nest walls. These architectural features of gardens could have evolved to minimize cultivar escape, but alternative explanations are also possible, as, for example, minimization of contact with contaminant fungi or arthropod fungivores that could endanger the health of gardens.

No gardens with strong hyphal connections to mycelium outside the nest have been found for attine colonies, with the exception of a single *Apterostigma* garden in leaf litter in Amazonian rain forest (Reserva Ducke, Manaus, Brazil; U. G. Mueller, unpublished data). This single garden (G2 fungus) was constructed between leaves in thick leaf litter on the ground, and the mycelial veil that covered the garden merged into a large whitish and fluffy mycelium that penetrated the surrounding litter (encompassing an area of about 10 cm × 7 cm and 2–5 cm depth, hidden about 7 cm deep in the moist litter). The *Apterostigma* cultivar thus may have been in the process of vegetative escape from the symbiosis. Alternatively, the ants/cultivar may have taken advantage of this large external mycelial network to acquire additional resources (similar to mycorrhizae that ferry resources to their plant hosts). Clearly, the single *Apterostigma* collection does not allow any general conclusions, but it points to the type of field observations needed to determine whether vegetative cultivar escape is a potential source of ant-cultivar conflict (i.e., whether cultivar growth into the nest surroundings drains garden resources or whether it is beneficial because of the additional nutrients ferried to the colony).

Conflict over Cultivar Replacement and Import of Novel Fungal Strains

Cultivar substitution is an obvious manifestation of ant-cultivar conflict. Substitution is ipso facto fatal for the replaced cultivar, while termination of an inferior cultivar and replacement with a superior cultivar is advantageous for the ants. For example, Mueller et al. (1998, p. 2037) argued that ants may occasionally replace cultivars in order 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.” Thus, whereas the ants may have evolved the ability to monitor the productivity of cultivars and respond facul-

tatively with cultivar substitution, cultivars may be selected to prevent such host switches. (Preventing the ants from switching to a novel cultivar is the cultivar’s analog of preventing escape of the ants from an established symbiosis.) A recent report on ant-cultivar incompatibilities (Bot et al. 2001) suggests how cultivars could prevent host switches: fungus-derived factors that the ants routinely distribute through gardens via their feces cause degradation of novel-cultivar gardens, but these factors are compatible with native-cultivar gardens. Such a mechanism obviously impedes ant switches to novel cultivars. In other words, cultivars may have retained the ancestral vegetative incompatibility mechanisms, typical for any free-living fungus, to lock ants into a partnership, in essence sabotaging cultivar substitution attempts through chemical warfare against novel cultivars.

Conflict over Cultivar Competition within Gardens

Bot et al. (2001) persuasively argued that attine ants should prevent the import of novel cultivar strains in gardens because the coexistence of several cultivar strains (in a colony or even within single gardens) sets the stage for cultivar competition and thus selects for virulent cultivar traits (Frank 1996a; see also above, “Symbiont Competition”). Cultivar mixing thus could initiate a dangerous evolutionary trajectory that eventually harms the overall symbiosis. However, there may be conditions under which it could be advantageous for the ants to mix cultivars, at least temporarily (i.e., for durations too short to allow significant cultivar evolution toward virulence). For example, cultivar competition could enable the ants to identify and select against weaker cultivar strains that had accumulated deleterious mutations. Or, cultivar mixing could benefit the ants because of the advantages inherent in an intercropped cultivation regime (e.g., slower evolution of resistance in pathogens/parasites against antibiotic defenses in a complex intercropped environment; greater overall resilience against parasites/pathogens because of genetic diversity of cultivars in gardens). Thus, while cultivar mixing eventually risks the evolution of virulent cultivar traits (Frank 1996a; Bot et al. 2001), there may be short-term advantages of cultivar mixing that can partially offset this inherent disadvantage.

There exists at this point no information on whether several cultivar strains can coexist in a single attine garden or colony. The previously unsuspected high rate of horizontal cultivar transmissions between colonies (Mueller et al. 1998; Bot et al. 2001; Green et al. 2002) implies that cultivar mixing in single colonies is not implausible for any attine species, but it is unclear whether such chimeric gardens are transitory (the garden quickly returns to a cultivar monoculture) or whether several cultivars can co-

exist indefinitely (barring competition). If chimeric gardens are typical, any coexisting strains must be very closely related because multiple isolations from single attine gardens have so far yielded only one cultivar morphotype per colony (U. G. Mueller, unpublished data; C. R. Currie, unpublished data). This observation is consistent with the traditional assumption that single gardens are comprised of only one cultivar genotype, but this assumption will have to be verified eventually through careful mycological work and genetic fingerprinting analyses. In addition, experimental work on artificially created chimeric gardens (with mixed cultivars of known genotype) may generate the critical insights necessary for understanding cultivar competition per se as well as the ant-cultivar conflict over cultivar competition.

Synthesis and Future Research Directions

Almost a century ago, William Morton Wheeler predicted that “the study of the Attini ... has only just begun, and further advance in this fascinating subject will be more difficult for the mycologist than for the entomologist” (Wheeler 1910, p. 338). This statement has proven prophetic for two reasons. Mycological work on fungus-growing ants is logistically more difficult because it presupposes, in addition to microbiological proficiency, substantial entomological background for collection and maintenance of live attine colonies. More importantly, however, significant advances on the mycological side appear to have been precluded by analyzing the attine symbiosis from only a myrmicocentric perspective and thus essentially ignoring the evolutionary complexities and leverages of the cultivars.

This article contends that a conceptual shift from the traditional myrmicocentric view to a more mycocentric view of the attine ant-cultivar mutualism will be essential for a comprehensive appraisal of this 60-million-year-old symbiosis. Specifically, observations that appear aberrant when viewed from the myrmicocentric perspective may well emerge as logical outcomes of evolutionary conflicts between the ants and their cultivars. Analysis within the framework of tug-of-war theory (H. K. Reeve, N. J. Mehdiabadi, and U. G. Mueller, unpublished manuscript) appears to generate particularly promising predictions. What is needed at this point more than theory, however, are new empirical approaches that put to a test the traditional preconceptions, whether or not the attine symbiosis operates under an “extreme asymmetry in manipulative power between the host and the cultivar” (Bot et al. 2001, p. 1988) and, consequently, whether or not superiority in manipulative power is evolutionary stable such that the ants win all coevolutionary conflicts with their cultivars. Indeed, the attine ant-fungus symbiosis offers unique experimental opportunities because few other mutualistic systems per-

mit the experimental manipulations possible for the attine ant-cultivar mutualism. Cultivar switch experiments, such as those outlined in this article, may emerge as a major research tool for unraveling the evolutionary dynamics of conflict and cooperation within the attine symbiosis.

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