THE ORIGIN OF THE ATTINE ANT-FUNGUS MUTUALISM

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ABSTRACT

Cultivation of fungus for food originated about 45–65 million years ago in the ancestor of fungus-growing ants (Formicidae, tribe Attini), representing an evolutionary transition from the life of a hunter-gatherer of arthropod prey, nectar, and other plant juices, to the life of a farmer subsisting on cultivated fungi. Seven hypotheses have been suggested for the origin of attine fungiculture, each differing with respect to the substrate used by the ancestral attine ants for fungal cultivation. Phylogenetic information on the cultivated fungi, in conjunction with information on the nesting biology of extant attine ants and their presumed closest relatives, reveal that the attine ancestors probably did not encounter their cultivars-to-be in seed stores (von Ihering 1894), in rotting wood (Forel 1902), as mycorrhizae (Garling 1979), on arthropod corpses (von Ihering 1894) or ant faeces in nest middens (Wheeler 1907). Rather, the attine ant-fungus mutualism probably arose from adventitious interactions with fungi that grew on walls of nests built in leaf litter (Emery 1899), or from a system of fungal myrmecochory in which specialized fungi relied on ants for dispersal (Bailey 1920) and in which the ants fortuitously vectored these fungi from parent to offspring nests prior to a true fungicultural stage.

Reliance on fungi as a dominant food source has evolved only twice in ants: first in the attine ants, and second in some ant species in the solenopsidine genus Megalomyrmex *that either coexist as trophic parasites in gardens of attine hosts or aggressively usurp gardens from them. All other known ant-fungus associations are either adventitious or have nonnutritional functions (e.g., strengthening of carton-walls in ant nests). There exist no unambiguous reports of facultative mycophagy in ants, but such trophic ant-fungus interactions would most likely occur underground or in leaf litter and thus escape easy observation. Indirect evidence of fungivory can be deduced from contents of the ant alimentary canal and particularly from the contents of the infrabuccal pocket, a pharyngeal device that filters out solids before liquids pass into the intestine. Infrabuccal pocket contents reveal that ants routinely ingest fungal spores and hyphal material. Infrabuccal contents are eventually expelled as a pellet on nest middens or away from the nest by foragers, suggesting that the pellet provides fungi with a means for the dispersal of spores and hyphae. Associations between such "buccophilous" fungi and ants may have originated multiple times and may have become elaborated and externalized in the case of the attine ant-fungus mutualism. Thus, contrary to the traditional model in which attine fungi are viewed as passive symbionts that happened to come under ant control, this alternative model of a myrmecochorous origin of the attine mutualism attributes an important role to evolutionary modifications of the fungi that preceded the ant transition from hunter-gatherer to fungus farmer.*

MYCOPHAGY, the consumption of fungi for food, has evolved multiple times among the insects, occurring in such diverse lineages as springtails, beetles, flies, moths, termites, wood wasps, and ants (Wheeler and Blackwell 1984; Martin 1987; Hammond and Lawrence 1989). In some of these groups, mycophagy is the dominant form of feeding; in others it is facultative, and fungi comprise only a portion of the diet. As far as is currently known, reliance on fungi as a dominant food source has evolved only twice in ants (Formicidae): (1) in fungus-growing ants (subfamily Myrmicinae, tribe Attini), a monophyletic group of 12 genera that includes over 200 described species, all of which are obligately dependent on the cultivation of a mutualistic fungus for food (Weber 1972a); and (2) in the *Megalomyrmex silvestrii* group of ants (Myrmicinae, tribe Solenopsidini), comprising eight known species that are social parasites of attine ants and that consume the fungus grown by their attine hosts (Wheeler 1925; Brandão 1990; Adams et al. 2000b). All other known ant-fungus associations do not demonstrably involve ant fungivory. For example, some ant species have been observed to forage occasionally for fungal tissue (Orr and Charles 1994), but it is unclear whether these ants consume the fungi or collect them for some other purpose (e.g., to prey on larvae living in fungal tissue; Lewis and Worthen 1992). There also exist rare cases of specialized, nonpathogenic associations between ants and fungi—for example, ants that maintain fungi in walls of

their nests (Lagerheim 1900; Maschwitz and Hölldobler 1970) or ants that harbor endosymbiotic fungi in their intestines (Caetano and da Cruz-Landim 1985)—but in none of these cases are the symbiotic fungi used for food. Thus the attine ant-fungus mutualism and the secondary exploitation of this fungicultural mutualism by *Megalomyrmex* ants appear to be the results of two independent and historically unique transitions from ancestral predatory lifestyles to derived mycophagous lifestyles.

Seven explicit hypotheses have been proposed for the origin of fungus growing in attine ants. Each of these hypotheses postulates a different substrate that the ancestral attine might have used for culturing fungi during the transition from hunter-gatherer to farmer ant: (a) stored seeds; (b) nest walls; (c) rotting wood; (d) mycorrhizae; (e) arthropod corpses; (f) ant faeces; and (g) infrabuccal pellets. This review will evaluate each of these hypotheses in light of recent findings regarding the phylogenies of the attine ant and fungal mutualists (Chapela et al. 1994; Schultz and Meier 1995; Mueller et al. 1998; Schultz 1998; Wetterer et al. 1998), as well as the ecologies of their closest ant and fungal relatives (Diniz et al. 1998; Mueller et al. 1998).

MODELS FOR THE ORIGIN OF ATTINE FUNGICULTURE

Theoretically, there are two principal models for successively ordering the putative evolutionary behavioral stages that culminated in

The Traditional "Consumption First" Model

CONSUMPTION   CULTIVATION   TRANSMISSION
Fungi are part of   Ants cultivate fungus   Fungal transmission between
the ant diet   by adding substrate   parent and offspring nests

The Alternative "Transmission First" Model

TRANSMISSION   CONSUMPTION   CULTIVATION
Specialized fungi are   Specialized fungi become   Ants cultivate fungus
dispersed by ants   part of the ant diet   by adding substrate

FIGURE 1.   THE TWO MAIN MODELS FOR THE SUCCESSIVE EVOLUTION OF THE BEHAVIORAL ELEMENTS
        OF ATTINE FUNGICULTURE.
    Each of the three stages in either model can be subdivided into substages that are not listed. The
Transmission stage of the "Transmission First" model, for example, probably involved an initial substage
where fungi were accidentally dispersed by ants, followed by a substage where the fungi developed special-
izations that increased the likelihood of dispersal, such as ant attractants (nutritive, psychotropic, or other-
wise) that stimulated contact or ingestion. The Consumption stage in either model could be subdivided
into a series of substages in which fungivory initially was facultative and fungi comprised only a minor part
of the ants' diet, followed by substages of increasingly greater reliance on fungi for food.

attine fungiculture (Figure 1). The traditional
and widely accepted model was outlined in de-
tail by Weber (1958, 1972a) and postulates an
initial stage in which unspecialized fungi,
growing perhaps accidentally in ant nests, be-
came part of the ant diet (Consumption); sub-
sequently the ants evolved the ability to pro-
mote fungal growth through the addition of
substrate (Cultivation), and finally evolved a
mechanism to transmit fungi between parent
and offspring nests (Transmission). An alter-
native scenario (Alternative Model) reorders
these stages and postulates an initial state in
which ants did not feed on fungi, but instead
were used by specialized fungi as vectors for
dispersal (Transmission); subsequently the
ants incorporated these specialized fungi into
their diet (Consumption), and finally evolved
the ability to cultivate these fungi (Cultiva-
tion). The key difference between these mod-
els is the timing of the evolutionary origin
of fungal transmission relative to the origins
of consumption and cultivation, specifically
whether fungal transmission by ants evolved
subsequent to fungal consumption (Tradi-
tional Model), or whether it evolved prior to
this stage (Alternative Model).
    The first six hypotheses for the origin of at-
tine fungiculture listed above (seeds, nest

walls, wood, leaf litter, mycorrhizae, arthro-
pod refuse) all conform to the Traditional
Model. These hypotheses share the underly-
ing assumption that the primary driving mech-
anism behind the origin of the attine ant-fun-
gus mutualism was the ants; i.e., that the ants
began cultivating fungi that had previously be-
come a part of their diet, and that those fungi
were therefore passively domesticated culti-
vars that the ants encountered on a variety of
substrates, depending on the particular hy-
pothesis. In marked contrast, the "infrabuccal
pellet" hypothesis assumes that the initial ant-
fungus association may have been largely un-
der the control of the fungi; i.e., the fungi may
have initially utilized the ants as agents for the
dispersal of spores or mycelium, and only sub-
sequently served as a component in the diet
of the ants (Bailey 1920). Unlike the first six
hypotheses, this latter hypothesis thus sug-
gests that the attine ant-fungus mutualism
arose from a system analogous to that of extant
myrmecochore plants that rely on ants to dis-
perse their seeds (Serenander 1906; Beattie
1985). We explore this alternative scenario in
Section 6 on "Fungal Myrmecochory" and out-
line some of the investigatory steps necessary
for testing the Alternative Model.

1. OBLIGATE ANT-FUNGUS ASSOCIATIONS

1A. THE ATTINE ANT-FUNGUS MUTUALISM

Fungus-growing ants in the strictly New World and largely Neotropical tribe Attini (Formicidae) comprise a monophyletic group of over 210 described species, all of which obligately depend on the cultivation of fungi for food (Weber 1972a). Fungal cultivars are grown in near axenic (pure) gardens (Craven et al. 1970; Currie et al. 1999a,b) and, depending on the ant species, are manured with a variety of fresh or dead plant matter. Two genera of so-called leafcutter ants with large colonies, *Acromyrmex* and *Atta*, culture their fungi on freshly cut foliage and flowers, making these ants major agricultural pests. Leafcutters are grouped with three additional genera into the derived, monophyletic group of "higher attines" (Schultz and Meier 1995), which comprise about one-half of the species diversity of the tribe. Ants in the remaining seven genera of "lower attines" are inconspicuous, frequently cryptic, and do not attack plants. Lower attines therefore have received little attention, and their phylogeny, ecologies, and life histories are largely unknown. The symbioses between lower attine ants and their fungi are diverse, with some species growing their fungi entirely on dead vegetable matter, some entirely on caterpillar frass, and others on a mixed substrate that may even include seeds (Weber 1972a).

A subset of species of fungus-growing ants in the "*rimosus*" group of the lower attine genus *Cyphomyrmex* are particularly unusual because they culture their fungus as a yeast (a single-celled growth form) rather than as mycelium (the filamentous, multicellular form typical of all other attines). Phylogenetic analyses indicate that yeast cultivation is a derived form of fungus farming that arose in the genus *Cyphomyrmex* from mycelium-cultivating ancestors (Schultz and Meier 1995; Meier and Schultz 1996; Schultz 1998), contradicting the prevailing opinion that yeast cultivation is the most primitive mode of attine fungiculture and that the cultivation of a multicellular, filamentous fungus is more complex and, thus, logically more derived (Weber 1972a; Hölldobler and Wilson 1990). In artificial culture, however, *Cyphomyrmex* yeast fungi revert to a filamentous phase; in addition, these fungi envelop

the *Cyphomyrmex* larvae in a mycelial blanket while simultaneously growing as yeast in the colony garden. Both these facts indicate that the *Cyphomyrmex* yeast cultivars are "pleomorphs" (i.e., fungi that are able to alter their growth form depending on growth conditions). *Cyphomyrmex* yeasts therefore are developmentally complex and, convergent with other known cases of pleomorphy among the basidiomycota, derived from an ancestral mycelial state (Mueller et al. 1998). The most basal attine lineages all cultivate hyphal fungi (Schultz and Meier 1995; Meier and Schultz 1996; Schultz 1998), further indicating that the ancestral attine ant cultivated hyphal rather than yeast gardens.

The great majority of attine fungi, including the yeast cultivars, belong to two genera, *Leucoagaricus* and *Leucocoprinus*, which together comprise the tribe Leucocoprineae in the family Lepiotaceae (Agaricales: Basidiomycota), a group of saprobic litter specialists (Möller 1893; Hervey et al. 1977; Chapela et al. 1994; Mueller et al. 1998; Johnson 1999). Because the most basal attine lineages cultivate leucocoprineous mutualists, attine fungiculture likely originated with the cultivation of leucocoprineous fungi. Domestication of these cultivars probably was facilitated by the particular abundance of the Leucocoprineae in the tropics, specialized as litter decomposers and occurring in the same microhabitats as leaf-litter dwelling ants. In a unique event in attine ant evolution, one group of ants in the genus *Apterostigma* has secondarily switched to nonlepiotaceous fungi that are distantly related to lepiotaceous cultivars and that belong to a phylogenetically narrow group within the family Tricholomataceae (Agaricales: Basidiomycota; Chapela et al. 1994), closely related to fungi in the genus *Gerronema* (Moncalvo et al. 2000). Because putatively basal *Apterostigma* species (Lattke 1997) cultivate lepiotaceous fungi (Mueller et al. 1998), the transition to the tricholomataceous cultivars probably occurred in a derived *Apterostigma* species long after the origin of the genus *Apterostigma*. Apart from these tricholomataceous mutualists, attine ants appear to be entirely specialized on a closely related group of leucocoprineous fungi. Thus, while ant lineages have repeatedly switched between cultivar lineages in the tribe Leucocoprineae (see

below), switches to fungi outside of this tribe have apparently occurred only once (in the case of the tricholomataceous-cultivating *Apterostigma* species), suggesting that leucocoprineous fungi may be more suitable for cultivation (nutritionally, morphologically, or otherwise) than are fungi outside of this group.

Fungal transmission in the Attini has long been thought to be strictly vertical (i.e., to remain within lineages of ants), because foundress queens of some Attini have been observed to carry fungi from parent to daughter nests (von Ihering 1898; Huber 1905a,b; Weber 1972b). Before departing from the natal nest, the foundress queen sequesters a small pellet of hyphae in a specialized pouch at the bottom of her mouth—the infrabuccal pocket, present in all ants (Janet 1899; Gotwald 1969) and also some wasps (Janet 1895; Duncan 1939)— where it remains viable for at least several days. After mating and selecting/excavating a suitable nest site, the foundress queen expels the contents of the infrabuccal pocket and uses it as an inoculum to start a new fungus garden. Such transgenerational transfer of cultivars from parent to offspring nest has been observed so far in three genera of higher attines (Weber 1972a,b) and in the derived lower attine genus *Cyphomyrmex* (Mueller, personal observation), but it is commonly assumed that attine queens of all species transmit their fungal mutualists via the mechanism of the infrabuccal pocket.

Vertical transmission of fungal cultivars from parent to offspring nests leads to the expectation that ancient, clonally propagated fungal lineages evolved in parallel with the lineages of their ant hosts. Apparent coevolutionary adaptations are present in some attine cultivars; for example, the fungi cultivated by the higher attine ants produce densely packed clusters of hyphal-tip swellings, called "gongylidia," that are both rich in nutrients and easily harvested by the ants. Morphological comparison of "lower attine" versus "higher attine" fungi indicates that gongylidia are an evolutionary innovation possibly derived from homologous structures in lower attine fungi (see Section 6), whereas lower attine fungi closely resemble their closest free-living leucocoprineous relatives. Indeed, the recent discovery of genetically identical, free-living counter-

parts of lower attine cultivars suggests that many if not most lower attine fungi are frequently domesticated from free-living populations and thus share only short coevolutionary histories (if any) with their host ants (Mueller et al. 1998). In addition to being recruited from free-living populations, lower attine fungi are also transferred laterally between different ant lineages, and such lateral transfers may even take place across nests of ant species in different attine genera (Mueller et al. 1998; Adams et al. 2000a). Thus, contrary to the longstanding assumption of strict clonal propagation of fungi since the origin of the attine ant-fungus mutualism, some (and probably most) lower attine fungal cultivars are not ancient clones; rather, they have been domesticated repeatedly and independently by different ant lineages. Clonal propagation from parent to daughter colonies may indeed be the general rule for most attine ant species over short-term evolutionary time spans (Mueller et al. 1996), but the long-term evolutionary histories of attine fungal lineages may be complex, involving both lateral transfer between distantly related ant lineages and repeated cycles of domestication of free-living fungi followed by a return to the free-living state (Mueller et al. 1998).

Attine ants have traditionally been thought to maintain their fungal gardens as pure (axenic) cultures in near isolation from other organisms, and the ant-fungus mutualism has been viewed as a bipartite mutualism. However, recent work has identified two additional critical attine symbionts: a microfungus in the genus *Escovopsis* (anamorphic Hypocreales, Ascomycota) that parasitizes attine gardens (Currie et al. 1999a; Currie 2001a), and an antibiotic-producing filamentous bacterium (actinomycete) in the genus *Streptomyces* that the ants carry on their bodies (Currie et al. 1999b). The antibiotics produced by the actinomycete specifically target the *Escovopsis* parasites, significantly suppressing their growth. The discovery of such evolutionarily derived and intricate microbial interactions in the attine ant-fungus mutualism suggests that additional bacteria or other microbes also participate in a multipartite symbiosis that is substantially more complex than previously known.

1B. NATURAL HISTORY OF THE CLOSEST ATTINE RELATIVES

Twelve lineages of myrmicine (and even nonmyrmicine!) ants have been variously proposed as the sister group to the attine ants (Schultz and Meier 1995). Recently, Moffett (1986) and Wheeler and Wheeler (1985) suggested the Malaysian *Proatta butelli* as a candidate for the attine sister group, based on morphological characters of adults and larvae that resemble those of some fungus-growing ant species. This sentiment echoes the original placement of *Proatta* within the Attini (Forel 1912, 1913; Emery 1913, 1922), based on its resemblance to ants in the primitive attine genus *Mycocepurus*. In contrast, phylogenetic analyses by Schultz and Meier (1995, based on larval morphology) and by Diniz and Mayhé-Nunes (Diniz et al. 1998, based on worker morphology) suggest that the South American genus *Blepharidatta* is the sister clade to the Attini, concurring with Wheeler's (1915) original placement of *Blepharidatta* in the Attini. However, molecular phylogenetic studies (Schultz 1998) as well as expert opinion (Brown 1953; Kempf 1975), suggest a sister group relationship between *Blepharidatta* and the New World genus *Wasmannia*. Thus, a hypothesis of a close relationship between *Blepharidatta* and the Attini is only tenable if (*Blepharidatta* + *Wasmannia*) is postulated to be the attine sister group. Unlike the morphological studies mentioned above, molecular studies are so far inconclusive regarding the sister group of the Attini (Schultz 1998; Wetterer et al. 1998).

To reconstruct the possible incipient states of ant-fungus association that preceded the suite of derived fungicultural behaviors present in all attine ants, Moffett (1986) conducted behavioral studies of *Proatta butelli* in Malaysia, concluding that fungi are frequently encountered on arthropod corpses that these ants accumulate near the nest entrance. Similarly, Diniz et al. (1998) studied a large population of *Blepharidatta conops* (79 nests) over a period of 4 months and found no direct evidence of fungiculture or fungivory. These authors noted that, unlike many other ant species, *B. conops* does not construct a special refuse chamber in their subterranean nests, but instead allow discarded arthropod carcasses to accumulate in the same chamber that houses their brood. Unidentified fungi consistently grow on these carcasses, and Diniz et al. (1998) speculate that this arrangement increases the chance that brood "might come into contact and eventually feed on fungi or on carcasses already partially digested by fungi," consistent with Moffett's (1986) hypothesis that the original attine fungus may have been derived from fungi growing adventitiously on accumulated arthropod corpses. This scenario is somewhat undermined, however, by the fact that leucocoprineous fungi are not known to utilize insect remains as a substrate, nor are they capable of outcompeting other fungi and microbes that are specialized on such substrates (see Section 5E). A possibly more promising exploration of the putative link between *Blepharidatta* and the Attini might focus on an undescribed species of *Blepharidatta* that constructs nests "in rolled leaves over the litter" (Diniz et al. 1998), or on *B. brasiliensis* that inhabit preexisting spaces in rainforest leaf litter or top soil levels (Mueller and Schultz, personal observation). Unlike the subterranean *B. conops* species, these two epigaeic species may be commonly in close contact with lepiotaceous fungi, and detailed behavioral studies of these species might provide clues to the ant-fungus association that preceded the origin of fungiculture in the Attini.

Ants in the genus *Wasmannia* are thought to be omnivorous generalist foragers, consuming a combination of arthropod prey, dead arthropods, honeydew, floral and extrafloral nectar, and other plant material such as seeds (Ulloa-Chacon and Cherix 1990; Oliveira and Brandão 1991; Tobin 1994). However, Wheeler (1901) published an interesting report that suggests a possible association of *W. auropunctata* with fungi. Wheeler describes a nest of *W. auropunctata* from Cuernavaca, Mexico, that "contained a small mass of the same peculiar fungus as *Cyphomyrmex rimosus*" (Wheeler 1901: 201), a yeast-cultivating fungus grower. Although tantalizing, Wheeler's isolated observation needs to be interpreted with caution. First, as discussed above, hyphal cultivation is the ancestral condition in attine ants. Yeast cultivation is highly derived, very likely having a single origin in a lineage within the attine genus *Cyphomyrmex* (Schultz and Meier 1995;

Meier and Schultz 1996; Schultz 1998). Second, Wheeler's unique observation has never been replicated, even though *W. auropunctata* is both abundant and conspicuous in its native Neotropics and a widespread, relatively well-studied pestiferous tramp species (Williams 1994; Jourdan 1997; McGlynn 1999). *W. auropunctata* colonies have been found nesting adjacent to nests of the yeast-growers *C. rimosus* (Weber 1947) and *C. minutus* (Mueller, personal observation). Because *W. auropunctata* preys on other ant species (McGlynn 1999), it is not implausible that colonies may occasionally overwhelm and usurp nests of yeast-cultivating attines, and Wheeler may have encountered the result of just such an occurrence.

IC. FUNGICULTURAL PARASITISM IN *MEGALOMYRMEX*

Ant species in the *Megalomyrmex silvestrii* group (Myrmicinae, tribe Solenopsidini) are social parasites of fungus-growing ants, feeding on the fungus gardens of their hosts (Wheeler 1925; Weber 1941; Kempf and Brown 1968; Brandão 1990; Adams et al. 2000b). In similar fashion to attine ants, workers of these parasitic *Megalomyrmex* species harvest fungal tissue by cropping the mycelium, masticating it, and ingesting the released liquids. Presumably, some of this processed mycelium is also fed by trophallaxis to larvae and queens, but queens are also capable of harvesting fungus directly. Despite their ability to consume fungi, workers of the *M. silvestrii* species group do not cultivate fungi by adding substrate to gardens, although they do tend the garden in other ways, for example, by readjusting its overall size and shape (Adams et al. 2000b). Ultimately, however, they are dependent on their attine "hosts" for garden maintenance.

The degree of dependency on the attine "host" varies between *M. silvestrii*-group species, which are so far known from nests of attine species in the genera *Apterostigma*, *Cyphomyrmex*, *Trachymyrmex*, and *Sericomyrmex* (Wheeler 1925; Kempf and Brown 1968; Brandão 1990; Adams et al. 2000b; W L Brown, unpublished label data). Some *M. silvestrii*-group species have been found in secondary cavities in attine nests, others in the fungus gardens cohabiting with their hosts, and some alone in the attine nest following garden usurpation and displacement of attine tenants. In the usurping species *Megalomyrmex sp. nov.* (to be described by Brandão, in preparation), workers eliminate the attine ants by expelling or killing them (Adams et al. 2000b). This species therefore is not a social parasite *sensu stricto*, but a true garden thief or garden "predator." Because *M. sp. nov.* workers are unable to maintain gardens independently, colonies of *M. sp. nov.* are forced periodically to abandon depleted gardens, locate unparasitized attine nests, and migrate to new gardens. *M. sp. nov.* therefore possesses an elaborate scouting and recruitment system that enables the ants to move between attine nests (Adams et al. 2000b). Thus there appear to be two distinct modes of *Megalomyrmex*-attine association: first, social parasitism wherein attine hosts maintain gardens and *Megalomyrmex* colonies coexist with the attine hosts in the same nest (first suggested by Wheeler 1925), and second, garden usurpation wherein *Megalomyrmex* workers eliminate the resident attine ants and depend on successively usurping new gardens (first suggested by Kempf and Brown 1968).

Very little is known about the diet of *Megalomyrmex* species in general, but some species are known to consume nonfungal foods such as membracid honeydew (Mann 1916) and tunafish bait (Brandão 1990). Seemingly healthy *M. silvestrii* nests have been collected unassociated with attine hosts (Kempf and Brown 1968; Brandão 1990). For example, an undescribed Panamanian species near *M. silvestrii* has two modes of existence: living either independently in the absence of an attine host, or as a garden parasite of a *Trachymyrmex* species (Adams, personal observation). Whether all *M. silvestrii*-group species can switch facultatively between nonparasitic and parasitic existences, or whether some species are obligately dependent on a permanent association with attines, is unknown. If, as suggested by Kempf and Brown's (1968) and Brandão's observations, some *M. silvestrii*-group species routinely switch between parasitic and free-living strategies, the required nutritional and biochemical adaptations for alternatively processing animal versus fungal food sources may be complex, more so than the corresponding adaptations in attine ants. Too little is known about the parasitic *Megalomyrmex* species to

evaluate whether consumption of fungi supplements a predominantly carnivorous diet, or whether it represents the main source of nutrition. *M. sp. nov.* workers prey on and consume attine larvae (Adams et al. 2000b), suggesting that *M. sp. nov.* is only partially fungivorous. Yet even in the absence of attine brood, *M. sp. nov.* ants can be maintained for months on laboratory gardens, and a *M. sp. nov.* colony does not migrate to a new garden until the currently occupied garden is exhausted. Thus, because workers of some *M. silvestrii*-group species can subsist on an exclusively fungal diet for an extended period (Adams et al. 2000b), and because nonparasitic *Megalomyrmex* are predators (Brandão 1990), the derived fungivory in the monophyletic *Megalomyrmex silvestrii* species group of ants represents a case of evolutionary convergence paralleling that of the Attini.

1D. FUNGI IN CARTON NESTS OF ANTS

Many ants construct "carton" nests from a mix of soil, sand, wood pulp, fibrous bark, dead leaves, rootlets, and humus, all cemented together with salivary secretions (Forel 1894, 1899; Santschi 1910; Wheeler 1910, 1922; Escherich 1917; Hölldobler and Wilson 1990). Several such carton-builder species in the genus *Lasius* (subfamily Formicinae; *fuliginosus, umbratus, rabaudi, emarginatus*) fill large preexisting hollows with spongy systems of irregular carton chambers and galleries, either in the rotting interior at the base of old trees or in the ground among roots, and maintain a fungal growth in their nest walls (Bönner 1915; Escherich 1917; Kutter 1969; Hölldobler and Wilson 1990). *Lasius fuliginosus*, which is most frequently found in the base of trees, constructs walls that are infiltrated and overgrown with the mycelium of the hyphomycete (anamorphic Ascomycota) *Cladosporium myrmecophilum* (Lagerheim 1900; Elliott 1915), such that the carton walls and the wood anchoring the carton are covered with a velvety tapetum of the fungus. A different fungus (*Hormiscium pithyophilum*) has been isolated from the carton of *L. umbratus*, which constructs small underground carton nests among the roots of trees (Elliott 1915). The fungi associated with *L. emarginatus* and *L. rabaudi* are unknown. According to Elliott

(1915), the fungal associates are specialized on the ants, with *H. pithyophilum* always in nests of *L. umbratus*, and *C. myrmecophilum* always in nests of *L. fuliginosus*. However, *L. fuliginosus* is a hyperparasite of *L. umbratus* (Kutter 1969), which could lead to the "inheritance" of the resident fungus when *L. fuliginosus* replaces *L. umbratus* in a nest.

The *Lasius* carton fungi grow as nearly pure monocultures in ant nests (Lagerheim 1900; Elliott 1915; Maidl 1934; Mueller, in preparation). The ants appear to weed out competing molds (Lagerheim 1900) and to promote the growth of their carton fungus. In *L. fuliginosus*, for example, the ants supply their fungus with a culture medium, a mixture of honeydew and other sugary plant juices that the ants apply to their carton walls (Maschwitz and Hölldobler 1970). Even though Lagerheim (1900) and Maidl (1934) reported that the ants crop the fungal "lawns" growing on their walls, and even though some *Cladosporium* fungi are known to be palatable to fungivorous arthropods (Shaw 1992), it is generally assumed that the mycelium is not consumed by the ants, or, if it is, that it plays a minor role in the ants' diet. Instead, the primary function of the hyphal growth in the carton walls appears to be the strengthening of the otherwise fragile carton (Elliott 1915; Escherich 1917; Maschwitz and Hölldobler 1970). This hypothesis is supported by the observation that fungus-free carton of other *Lasius* species is relatively fragile (Maschwitz and Hölldobler 1970). The recent discovery of antibacterial chemicals secreted by the fungus of *L. fuliginosus* suggests an additional, sanitary role for the carton fungi (Mueller, in preparation).

Cladosporium fungi are common saprophytic phylloplane fungi that are abundant in many habitats (Carrol and Wicklow 1992) as generalized decomposers of wood, leaf litter, and even mushrooms (Malloch, personal observation). Thus the association between *Cladosporium* and *Lasius* species, specialized to inhabit preexisting cavities in wood and to construct carton nests, is not surprising. An association between carton-nesting ants and wood-rotting fungi is not inevitable, however, because many other species of ants, including some in the genus *Lasius* (e.g., *L. niger*), construct carton structures that remain free of

fungal growth (Wasmann 1913; Wheeler 1922; Maschwitz and Hölldobler 1970). The absence of carton fungi in other species of *Lasius*, their consistent presence in the three specialized carton-building *Lasius* species, and particularly the ant behavior of encouraging fungal growth with a sugary medium, all suggest that the *Lasius* ant-fungus symbiosis has undergone substantial adaptive modification during a long coevolutionary history. Lagerheim (1900) and Maschwitz and Hölldobler (1970) even speculate that the carton-fungus of *L. fuliginosus* occurs exclusively in the nests of ants and does not have an independent existence.

Although many ant species construct carton nests (Wheeler 1910, 1922), fungal growth on the carton appears to be rare. Apart from the accounts of the four *Lasius* species mentioned above, there are only two additional records of ant-carton fungi. First, Farquharson (1914) described a xylariaceous fungus growing on "a shelter of chewed wood which the *Crematogaster* ants made over a species of coccid" (scale insect). The fungus formed fructifications and stroma on the shelter. Second, Santschi (1910) and subsequent authors (Forel 1916; Wheeler 1922) note the consistent presence of unidentified fungal hyphae in the arboreal carton nests of "*Macromishoides aculeatus*" (= *Tetramorium aculeatum*). According to Bolton (1980:354), "the nest, a mixture of silk, vegetable fragments, fungal hyphae and other debris, is constructed under or between leaves or in the branches of trees, commonly at the junction of two or more stems or twigs." Because the nest carton is "lined with a felt-work of very fine vegetable debris and of a mycelium bearing fructifications" (Santschi 1910, translated in Wheeler 1922:189), Santschi believed that "it would be interesting to study this fungus where it grows and to ascertain whether or not it is used habitually by the ants as food and is cultivated for that purpose." This early speculation of fungivory in *T. aculeatum* was incorrect. *T. aculeatum* is predaceous and occasionally honeydew feeding, rather than fungivorous (Room 1971; Leston 1973; Majer 1976), and the function (if any) of the mycelial lining in nests of *T. aculeatum* remains unclear.

1E. FUNGI IN ANT-PLANTS

Several ant species in the genus *Iridomyrmex* inhabit galleries inside hypocotyl swellings of epiphytic "ant-plants" in the genera *Myrmecodia* and *Hydnophytum* (Rubiaceae) of southern Asia and northern Australia. These ants deposit faecal material and other debris in their galleries, which are regularly overgrown by a fungus (Janzen 1974; Huxley 1978). The fungus appears to be a species of *Anthrocladium* (Moniliales) and is virtually always present in the galleries of *Myrmecodia* that are occupied by *I. cordatus* ants, but is less prevalent in galleries of *Hydnophytum*. It is unclear whether the fungal growth on the gallery debris is adventitious or is actively promoted by the ants. Huxley (1978) speculates that the ants may feed on the fungal conidia, or that the fungi may release nutrients from the debris and thus facilitate the nutrient uptake (particularly of nitrogen) by the plants through their highly absorptive hypocotyl galleries.

Some species of the African *Cuviera* (Rubiaceae) possess hollow swellings above the nodes of stems that are occupied by ants in the genera *Cataulacus*, *Crematogaster*, *Tetramorium*, and *Technomyrmex* (Bequaert 1922). Bailey (1920) reports that the inner walls of these internodal chambers are coated with a "luxuriant growth of fungi" (p 175), and that these fungi grow largely on the discarded infrabuccal pellets of the resident ants. Such fungal growth has also been reported from within hollow stems of South American *Hirtella* (Chrysobalanaceae) occupied by *Allomerus* ants (Dumpert 1981). Regarding *Cuviera*, Bailey (1920:177) believes that the "sporadic distribution of the hyphae in most of the myrmecodomatia suggests that the [fungi] are purely adventitious," rather than participating in a mutualistic association with the ants.

2. FACULTATIVE FUNGIVORY IN ANTS

2A. FUNGAL CROPPING AND INFRABUCCAL PELLETS

Foragers of diverse ant genera are occasionally seen on mushrooms (Orr and Charles 1994; Worthen et al. 1994; Rosciszewski 1995; Pfeiffer 1996), but it is unclear whether these foragers are collecting mushroom tissue for food or whether they are hunting for mush-

room-feeding arthropod prey (e.g., collembola, coleopteran, or dipteran larvae). Because ants are ubiquitous and abundant predators that forage in a wide diversity of environments, and because ant predation on fungivorous insects is widely documented (Courtney et al. 1990; Lewis and Worthen 1992; Worthen et al. 1993; Worthen et al. 1994), the mere presence of ant foragers on fungi can hardly serve as evidence of possible trophic ant-fungus interactions. The demonstration of trophic interactions requires documenting actual ingestion by ants of fungal mycelium, or the transport of fungal tissue by foragers to their nest.

The myrmicine ant *Pheidole bicornis*, a mutualistic inhabitant of hollow cavities in the leaf bases of *Piper* understory shrubs, gleans microbial lawns on the leaves of its host plant (Letourneau 1998). Similar gleaning has also been observed in various pseudomyrmicine ants (P Ward, personal communication) and in the Malaysian giant forest ant *Camponotus gigas* (Pfeiffer 1996). Transportation of fragments of free-living fungi by foragers occurs sporadically in leafcutter ants (e.g., workers cutting mushrooms growing on their nest mounds and transporting the fragments into their nest; Weber 1946), and is a conspicuous behavior in *C. gigas* (Orr and Charles 1994; Levy 1996). In *C. gigas*, fungus fragments comprise between 40–80% of all food items carried to the nest (Orr and Charles 1994; Levy 1996). Fungal fragments are sheared off by *C. gigas* workers from bracket fungi and "encrusting moulds," but the number of such observations is low (eleven observations total), and fungal-foraging breadth in *C. gigas*, including specialization on particular fungal groups, is therefore unclear. Similarly unclear is the function of fungal foraging (Pfeiffer and Linsenmair 1998), because no fungal tissue, or anything resembling fungal gardens, were found in the few *C. gigas* nests excavated (Orr and Charles 1994; Pfeiffer 1996).

If some ants do indeed utilize fungi as food—as a nitrogen supplement or as supplementary food for queens to survive the long period of solitary confinement during colony founding, as hypothesized by Malyshev (1968)—then the fungal tissue they most commonly consume is probably not the fruiting bodies (e.g., mushrooms), which are transient organs of reproduction, but rather the vegetative mycelium, the perennial somatic tissue that permeates soil, litter, or wood. Because such mycelial feeding by ants could be expected to occur in subterranean or leaf-litter environments, it may have so far escaped human observation. Indeed, the existence of ant-fungus interactions might be explored most efficiently by indirect means, for example, by examining contents of the ant alimentary canal. Given the terrestrial or subterrestrial nesting habits of most ants, encounters with fungal mycelium certainly must be an unavoidable feature of formicid life.

With the possible exception of ant species with arboreal or otherwise unusual, evolutionarily derived nesting habits (e.g., weaver ants), most ants are confronted at least occasionally with adventitious fungi growing in the various parts of their nests. For example, adventitious fungi have been found growing in nest walls (Emery 1899; Weber 1958), in refuse chambers (Weber 1958; Huxley 1978), in food-storage chambers (Went et al. 1972; Reichman and Rebar 1985; Crist and Friese 1993; Knoch et al. 1993), or on the ants themselves (Schmid-Hempel 1998). Ants possess elaborate behavioral and morphological adaptations for eliminating such fungi from the nest environment, including mechanical cropping and licking, and the secretion of antibiotic compounds (Maschwitz et al. 1970; Schildknecht and Koob 1970, 1971; Maschwitz 1974; Brough 1983; Beattie et al. 1986). Indeed, the antibiotic-producing exocrine metapleural gland is a synapomorphy for the Formicidae (Hölldobler and Wilson 1990; Grimaldi et al. 1997). Cropping of fungal growth generally results in the ingestion of fungal mycelium and spores, which are subsequently compacted into a pellet in the infrabuccal pocket, part of a complex filtering machinery that extracts larger solid particles prior to the passage of the food into the intestine (Janet 1899; Eisner 1957; Gotwald 1969; Febvay and Kermarrec 1981; Fowler et al. 1991). All ants regurgitate and discard these "infrabuccal pellets" at regular intervals, and expulsion of infrabuccal pellets either outside the nest or on intranidal refuse dumps has been observed in the leafcutter ants *Atta cephalotes* (Weber 1976) and *Acromyrmex octospinosus* (Quinlan and Cherrett 1978a).

Experiments with corundum particles of various sizes indicate that the infrabuccal pocket-proventricular valve filtering system of a wide diversity of ants is highly effective in filtering out particles between 100–200 μm in diameter and that such particles accumulate in the infrabuccal pocket (Eisner and Happ 1962). Particles larger than 200 μm diameter are expelled directly without accumulation in the infrabuccal pocket. Particles smaller than 100 μm diameter are filtered out with decreasing efficiency, and particles smaller than 10 μm may pass on into the intestine in some ants, but are retained with some efficiency in the pocket. This filtering efficiency holds for ants in general (Eisner and Happ 1962), but minima workers of leafcutter ants possess more efficient capacities. Leafcutter minims filter particles as small as 10 μm in diameter and possibly smaller, while maxima and minima workers routinely filter particles 30 μm in diameter (Quinlan and Cherrett 1978a), with decreasing efficiency for smaller particles. As an extreme case, workers of the ant *Solenopsis invicta*, which are about the size of the average lower attine ant, can filter out particles as small as 0.88 μm diameter (Glancey et al. 1981). These filtering capacities of infrabuccal pockets set approximate lower limits on the size of spores, hyphal fragments, and other cellular material that can accumulate in the infrabuccal pockets of ants.

The diameters of fungal spores (measuring between 2.5–150 μm; Dennis 1952; Pegler 1983) and those of fungal hyphae exceed the filtering threshold of the infrabuccal pocket (Fowler et al. 1991). Specifically, leucocoprineous spores measure 3–5 to 5–7 μm in diameter (Johnson 1999) and thus would be expected to be filtered out. In contrast, bacteria (typically with a maximum diameter of 1 μm) fall below the threshold and are almost completely absent from the infrabuccal pocket (Bailey 1920). Thus, fungal spores and hyphae—but not bacteria—accumulate in the infrabuccal pocket and could be vectored via infrabuccal pellets.

2B. FUNGI IN THE INFRABUCCAL POCKET OF ANTS

Of all microorganisms, fungal material seems to be particularly abundant in the infrabuccal pockets of ants (Bailey 1920). In *Pheidole bicornis*, for example, fungal and algal spores as well as hyphal fragments comprise over 96% of all particles in the alimentary canal anterior to the proventricular filter (Letourneau 1998). A careful examination of the gut contents of ants taken from over 2500 nest series (covering seven subfamilies, 35 genera, and 113 species) revealed no fungal material or spores in the gut posterior to the proventriculus, with the single exception of spores in the gut of a *Pheidole* species (Beckham et al. 1982), but these spores probably derived from a primary fungal infection of *Myrmicinosporidium* proliferating in the hemoplymph (Sanchez-Peña et al. 1993).

This absence of hyphae and fungal spores from ant guts underscores the filtering efficiency of the infrabuccal pocket and proventricular valve, and implies that virtually all ingested fungal material must accumulate in the infrabuccal pellet. This is confirmed by empirical studies. Bailey (1920) examined the infrabuccal contents of representatives of several ant genera and discovered that workers of most ants carry a mix of fungal spores, hyphal fragments, and bits of plant tissue in their infrabuccal pockets. In a second study that focused largely on pseudomyrmecine ants, a group of ants with the peculiar habit of nourishing their brood with regurgitated infrabuccal pellets (see Section 2d), Wheeler and Bailey (1920) reported the "constant occurrence of a variety of spores in the infrabuccal pockets" of ants. They further speculated that the plant and arthropod parts that are also present in the pellet could "serve as a substrate for the growth of the numerous fungus spores which are such surprisingly constant constituents of the pellets" (p 271). This suggested that ants "may be very important agents, or vectors in the distribution of many kinds of fungi in general" (p 272), particularly because "the ants may drop the pellets anywhere" (Wheeler 1914:165) in their foraging territory or other localities (e.g., middens) where the spores may readily germinate.

The observations and logic summarized above led Bailey (1920) to suggest that the filtering properties of the infrabuccal pocket makes it an effective organ for the selective extraction, enrichment (while in the infrabuccal pocket), and subsequent dispersal (after

expulsion) of fungal mycelium or spores. Thus, in addition to the general morphological features that facilitate fungal dispersal by terrestrial arthropods (e.g., the integument and gut; Malloch and Blackwell 1990), ants possess an additional structure, the infrabuccal pocket, with great potential for fungal dispersal. Such reasoning led Bailey (1920:184) to assert that "the Formicidae are active agents in the dissemination of many of the higher fungi, particularly in tropical regions where ants are so numerous and ubiquitous." Indeed, there may exist a guild of specialized "buccophilous" fungi that rely on dispersal via the infrabuccal pellets of ants (see Section 6 below). The dispersal distances afforded by such putative ant vectors would be directly dependent on the sizes of the ants' foraging territories, as well as on the ants' foraging habits (e.g., arboreal vs. terrestrial foraging). For most ants, foraging territories are probably less than 10 meters in diameter (Leal and Oliveira 2000), but may be substantially larger in ant species with specialized foraging habits (e.g., army and driver ants) or with extended colonies spanning multiple nests (Hölldobler and Wilson 1990).

The notion of fungi utilizing ants for dispersal is not entirely unprecedented. Ants are thought to disperse the spores of mycorrhizal fungi in the order Glomales, family Endogonaceae, including those of the genus *Glomus* (McIlveen and Cole 1976; Allen and MacMahon 1984; Friese and Allen 1988, 1993; Janos 1993). Since glomalean spores are large (50 to 800 microns in diameter) and rich in lipids, and since these spores are often found associated with roots in the chambers of soil-nesting ants, it has been suggested that some ants may consume them (D P Janos, personal communication). The nutrients in glomalean spores could only benefit ants, however, if the spores are at least partially crushed or malaxated, because glomalean spore size precludes their intact entry into the gut due to the filtering mechanism of the infrabuccal pocket, as discussed above. No detailed observations exist on how ants process glomalean spores, so the question of a possible role for these spores in the diet of ants remains open.

2C. FUNGI IN THE INFRABUCCAL PELLETS OF ATTINE ANTS

Bailey (1920:183) examined infrabuccal pellets from workers of the attine ants *Atta cephalotes* and *Trachymyrmex septentrionalis* and found that they are "composed largely of dirt, plant and animal hair, bits of epidermal and other plant tissues, fragments of hyphae, spores, pollen, etc. . . . but none of the pellets of the Attini . . . contain more than a few small bits of delicate, white hyphae [probably of the cultivar]" (p 183). But at least some hyphae and spores of noncultivar fungi are always imbedded in the infrabuccal matrix of attine ants (Weber 1972a; Quinlan and Cherrett 1978a; Febvay and Kermarrec 1981). Weber (1972a: 14) for example found "two types of fungal spores" in the infrabuccal pellets of *Atta sexdens* workers. Similarly, infrabuccal contents of the leafcutter ant *Acromyrmex octospinosus* include a diverse microflora (Febvay and Kermarrec 1981). These microbes probably do not aid in the digestion of organic material in the pocket because pellets are expelled too frequently (about 1 pellet/day, sometimes much more frequently; Febvay and Kermarrec 1981). The microflora appears to be diverse and very variable over time, which suggested to Febvay and Kermarrec (1981) that, apart from the fungal cultivars that are carried by virgin queens, there may be no other microorganisms that are adapted to a specialized existence in the pocket (see also Section 6).

Quinlan and Cherrett (1978a) observed in *A. octospinosus* that infrabuccal pellets are never expelled in the fungus gardens, but are either expelled on the refuse dump or "randomly outside the nest housing." Pellets streaked out on growth medium were all "grossly contaminated" with various microorganisms, and only 2 of 20 pellets yielded the mutualistic fungus as a subculture, consistent with Bailey's (1920) finding that pellets of workers contain little mycelium of the cultivar. Thus, as suggested by Quinlan and Cherrett (1978a), grooming concentrates within the infrabuccal pocket particles that accumulate on the cuticle through accidental contact. In this way fungal spores are mixed in the infrabuccal pocket with masses of potential nutrient substrates such as vegetable debris. When the infrabuccal pellets are expelled by workers, either

on the nest refuse pile or by foragers at locations distant from the nest, such spores may be dispersed by the ants over substantial distances, probably 1–10 meters in the case of the more primitive lower attines (Leal and Oliveira 2000) and up to several hundreds of meters in the case of the leafcutter ants (Weber 1972a).

2D. INFRABUCCAL PELLETS AS LARVAL FOOD

Infrabuccal pellets are fed directly to larvae in some groups of ants. This unusual method for nourishing larvae is the predominant mode in the subfamily Pseudomyrmecinae, but occurs also in the myrmicine genera *Cryptocerus*, *Leptothorax* (Wheeler and Bailey 1920), and *Solenopsis* (Petralia and Vinson 1980). Examination of pseudomyrmecine larval stomach contents revealed frequently intact, compacted pellets formed from spores, hyphae, pollen, dirt, and prey particles, all of which are consistent with infrabuccal pocket contents known from nonpseudomyrmecine ants. In the words of Wheeler and Bailey (1920), "the Pseudomyrminae combine the contents of the dust-bin and garbage-can and serve up the mixture as appropriate food for their young—a truly remarkable example of food-conservation" (p 260).

Wheeler and Bailey (1920) note that hyphae and a diversity of fungal spores are "surprisingly constant constituents of the pellets," the presence of which they attribute to the ants' "incessant patrolling of the surface of bushes and trees" (p 271). This patrolling leads to the accumulation of "great numbers of the most diverse fungus-germs" (p 266) on the integument, and a "most heterogenous collection of minute particles" that the ants eventually gather "in their infrabuccal pockets by licking with the tongue or using the strigils" (p 271). Accumulated integumental debris would explain the presence of fungal spores in the pellets, but not necessarily the presence of hyphae, suggesting that the ants must gather hyphal material in other ways. Indeed, Phil Ward (personal communication) has observed that *Pseudomyrmex* ants routinely glean the surfaces of their host plants' leaves while patrolling. This gleaning behavior probably results in the infrabuccal accumulation of epiphyllous microorganisms, lichens, and plant and fungal parts, thus accounting for Wheeler and Bailey's (1920) observations. The unusual feeding of infrabuccal pellets to larvae in this group prompts the question of whether *Pseudomyrmex* ants preferentially glean epiphyllous material that is of critical nutritional value for larval development.

3. NUTRITIONAL VALUE OF FREE-LIVING AND ANT-CULTIVATED FUNGI

Ants are derived from a wasp-like ancestor and, as in their wasp relatives, larvae of all ant subfamilies are fed with a protein-rich diet (Stradling 1978; Fowler et al. 1991). The adult ant diet is generally carbohydrate rich, but is highly variable across ant lineages, with adults of some species subsisting exclusively on arthropod prey, while adults of other species rely on plant-derived foods such as seeds, nectar, homopteran honeydew, or so-called "ant bodies" provided by plants to support mutualistic ants (Wheeler 1942; Wilson 1971; Stradling 1978; Beattie 1985; Fowler et al. 1991; Tobin 1994). Dietary habits are particularly diverse in the large ant subfamily Myrmicinae, and clear cases of fungivory have been observed only in that subfamily (Tobin 1994).

The transition to ant fungivory that culminated in attine fungiculture probably began with the typical formicid ancestral condition in which larvae were largely dependent on proteinaceous food in the form of arthropod prey and adults were dependent on a diversity of foods, including arthropod prey and sugary plant juices (Stradling 1978; Meier and Schultz 1996; Murakami and Higashi 1997). The predatory feeding habits of such putative close relatives of the Attini as *Blepharidatta* and *Wasmannia* (Schultz and Meier 1995; Diniz et al. 1998) are consistent with the plesiomorphic presence of this typical formicid adult diet in the attine ancestor. Sugary plant juices and sap have remained a dominant food source for all attine workers (Weber 1972a; Littledyke and Cherrett 1976; Murakami and Higashi 1997); thus, in the transition from carnivory to fungivory, the largest step in dietary evolution may have been taken by the larvae rather than by the workers, with the transition possibly occurring first in the larvae and later in the workers (Murakami 1998).

The carnivory-fungivory transition in the

ancestors of the Attini raises the question of whether fungi in general, or any fungal group in particular, represent a physiologically accessible substitute for arthropod prey and/or plant carbohydrate; i.e., whether at least some fungi can readily provide, at least in part, the nutritional requirements of a hypothetical predatory attine ancestor. If not, then the transition to fungivory in ants must have required major physiological adaptations necessary for utilizing this novel food source, and/or major shifts in nutritional profile must have occurred in a coevolving fungus as it adapted to the dietary requirements of the ants.

3A. NUTRITIONAL VALUES OF FREE-LIVING FUNGI

Fungal spores and hyphae are a valuable resource for insects, with nutritional values comparable to high-grade vegetables such as legumes (Crisan and Sands 1978; Buswell and Chang 1993). Filamentous fungi in particular are among the most important nutritional sources of nitrogen, a critical component of the insect diet (Mattson 1980; Martin 1987). It is therefore not surprising that insect fungivory has evolved repeatedly: insects from at least nine orders consume fungi as part of their diet (Wheeler and Blackwell 1984).

Insect-fungus associations span the full range of nutritional symbioses, from facultative to obligate fungivory. The most common form of fungivory in terrestrial arthropods (e.g., isopods, mites, springtails, thrips, beetles, and flies) is one in which detritivores supplement a nutrient-poor diet of plant detritus with occasional, opportunistic fungus feeding (Anderson and Healey 1972; Lindquist 1975; Martin and Kukor 1984; Martin 1987). The dietary importance of fungi to a specialized subset of these arthropods is underscored by the fact that some detritivores can more readily assimilate fungal polysaccharides than plant polysaccharides, implying a considerable degree of physiological adaptation for fungivory (Nielson 1962, 1963; Martin et al. 1980; Martin 1987). Fungi also supplement the diets of some dung-feeding insects, aquatic invertebrates, and insects that feed on fermenting fruit and plant exudates, all of which ingest fungi indirectly while feeding on a primary food source (Freeman 1967; Starmer 1981; Begon 1982). Facultative fungivory may also be common in om-

nivorous insects, with fungi being consumed both directly and indirectly.

Table 1 summarizes the average protein, lipid, and carbohydrate contents for 49 species in 25 genera of one ascomycete and fifteen basidiomycete families. These data include two *Lepiota* species (family Lepiotaceae), which are the closest free-living relatives of the ant-cultivated fungi for which data are available. The average crude protein content of the two *Lepiota* species (23.05% protein per dry weight) is only slightly higher than the average protein content of all examined fungi (21.4% ± 10.26%), but it is considerably lower than that of fungi in such "high protein" content genera as *Agaricus* (32.4%), *Volvariella* (28.9%), and the termite-cultivated *Termitomyces* (28.6.0%) (Crisan and Sands 1978). The available data therefore indicate that, whereas lepiotaceous fungi may have a slightly higher protein content than the average fungus, they are not outstanding in this regard. Reasoning from protein content alone (rather than, for example, amino acid complement), other fungi appear to be better suited for meeting the protein requirements of ants. Similarly, lipid and carbohydrate levels of the two *Lepiota* species are comparable to the fungal average (Table 1), and, again, other fungi seem to be superior sources of these nutrients.

Unfortunately, nutritional values of free-living fungi in the Leucocoprineae (the tribe containing the majority of attine cultivars) have never been analyzed, leaving open the possibility that leucocoprineous fungi may have unknown exceptional nutritive values. However, given that the available data (Table 1) indicate no obvious crude nutritional qualities that recommend lepiotaceous fungi as candidates for ant cultivation, we may speculate that perhaps instead it was some combination of qualities—for example, a well-balanced protein-lipid-carbohydrate profile—that favored the original domestication of a leucocoprineous fungi by the ancestral attine ant. In addition to the nutritional profile, nonnutritive factors may have played a critical role in preadapting leucocoprineous fungi for association with ants, including their saprophytic, litter-decomposing habits, their role as a dominant agaricales of Neotropical forests, and the resulting likelihood of their repeated contact with litter-inhabiting and soil-dwelling

TABLE 1

Average nutritional values of sixteen families of higher fungi, in comparison to insect-cultivated fungi

Family	Division/Family	% Protein	% Lipid	% Carbohydrate
Tremellaceae (n=1)	Basidiomycota	4.6	0.2	93.4
Schizophyllaceae (n=1)	Basidiomycota	5.6	3.0	67.4
Auriculariaceae (n=12)	Basidiomycota	8.6 (4.4–10.4)	1.7 (0.8–9.7)	64.4 (53.7–86.2)
Polyporaceae (n=1)	Basidiomycota	14.4	3.2	72.1
Tricholomataceae (n=24)	Basidiomycota	18.8 (4.7–28.8)	3.7 (0.6–8.0)	57.9 (49.3–75.8)
Russulaceae (n=4)	Basidiomycota	20.6 (18.2–25.8)	4.9 (3.0–7.1)	47.7 (35.5–63.6)
Clavariaceae (n=1)	Basidiomycota	21.4	4.7	no data available
Cantharellaceae (n=2)	Basidiomycota	21.5 (21.4–21.5)	4.9 (4.7–5.0)	53.7
Morchellaceae (n=3)	Ascomycota	22.2 (20.4–23.4)	5.5 (4.3–7.5)	52.1 (46.0–55.7)
Lepiotaceae (n=2)	Basidiomycota	23.1 (20.4–25.7)	2.9 (2.1–3.6)	56.3 (50.5–62.1)
Coprinaceae (n=3)	Basidiomycota	23.5 (20.5–25.4)	3.9 (3.1–5.7)	50.9 (50.3–51.5)
Strophariaceae (n=3)	Basidiomycota	24.3 (20.8–27.5)	4.3 (3.7–5.0)	54.8 (49.0–60.4)
Pluteaceae (n=29)	Basidiomycota	25.3 (8.7–43.0)	6.2 (1.0–20.6)	45.8 (19.0–65.3)
Boletaceae (n=1)	Basidiomycota	29.7	3.1	51.7
Agaricaceae (n=28)	Basidiomycota	32.4 (19.4–45.3)	4.0 (1.5–11.1)	49.1 (32.2–56.9)
Lycoperdaceae (n=1)	Basidiomycota	46.0	7.5	26.5
Average		21.37±10.26[c]	3.98±1.74	56.25±14.69
Ant-cultivated fungi				
Mycelium of *Atta colombica* cultivar (n=1)[a]	Lepiotaceae	13.0	0.2	27.2
Hyphae of *Atta sexdens* cultivar (n=1)[b]	Lepiotaceae	42.2	2.6	21.9
Staphylae of *Atta sexdens* cultivar (n=1)[b]	Lepiotaceae	16.6	4.1	31.6
Termite-cultivated fungi				
Termitomyces (n=6)[c]	Tricholomataceae[d]	28.6 (27.4–33.0)	3.9 (2.2–6.0)	49.9 (38.7–56.3)

Protein, lipid, and carbohydrate (excluding fiber) levels are given as percent dry weight (ranges are given in parentheses). The reported nutritional values may be inflated compared to natural conditions because most of the analyzed fungi were cultivated for human consumption and grown in artificial culture rather than collected in the wild; such cultivated fungi are generally fertilized (e.g., with sources of nitrogen) by mushroom growers to improve nutritional value and flavor (Manning 1985). These data may be further distorted by the fact that the tissues analyzed from human-cultivated fungi were the sporocarps (mushrooms) rather than the mycelium that would be encountered most frequently by ants. The potential variation in the nutritional values of different fungal tissues is demonstrated by the disparate values for the hyphae versus staphylae of the ant-cultivated fungi. The information summarized here for human-cultivated fungi is taken from Crisan and Sands (1978), Manning (1985), Quimio et al. (1990), Rajarathnam and Bano (1991), and the Standard Table of Food Composition in Japan (1982). Averages for the sixteen fungal families were derived by first averaging multiple values within fungal species (n=49), then within genera (n=25), and finally within families (n=16). The number of fungal strains analyzed for each family is given in the first column. Raw data from which the averages were calculated can be requested from UGM. [a]Martin et al. 1969; [b]Quinlan and Cherrett 1979; [c]Crisan and Sands 1978; [d]Moncalvo et al. 2000. [c]Note added after manuscript acceptance: Grönwall and Pehrson (1984) calculate a very similar average of 21.5% (SD = 6.32; range 14.8–35.2) for crude protein content (% dry weight) for fourteen fungi consumed by squirrels in Sweden.

ants such as the putative ancestral attine. Additional, unexplored factors such as the existence of ant-attractant fungal "elaiosome" analogues that favor dispersal by ants (see Section 6) may also have been, and may continue to be, critical in the repeated and possibly ongoing domestication by fungus-growing ants of a very restricted group of leucocoprineous fungi (Mueller et al. 1998). Understanding the relative importance of these factors in the origin of the attine mutualism requires a careful survey of the very poorly known, litter-inhabiting fungi in Neotropical habitats (i.e., those putatively available for domestication) and analyses of their respective nutritional values for ants.

3B. NUTRITIONAL VALUES OF ANT-CULTIVATED FUNGI

The only fungal cultivars that have been analyzed for nutritional composition are those of leafcutter ants in the genus *Atta* (Table 1). Nutritional data for comparison with free-living fungi are therefore lacking for the major-

ity of the attine cultivars, including the cultivars of most higher attines and of all of the lower attines. The fungal cultivars of *Atta* are clearly not representative for attine cultivars as a whole because, like other members of the clade comprising the higher attine cultivars, they possess a complex of highly derived morphological homologies (Chapela et al. 1994). For example, the fungi of *Atta* produce hyphal-tip swellings (called "gongylidia"), clusters of which ("staphylae") are directly consumed by workers or are harvested and fed to the brood. Staphylae are richer in lipids and carbohydrates, while hyphae are richer in proteins (Table 1; Quinlan and Cherrett 1979). Martin et al. (1969) found 20 amino acids in a cultivar of *Atta colombica*, together with a series of carbohydrates (trehalose, mannitol, arabitol, and glucose) and lipids (87% ergosterol, a sterol unique to fungi). Proteins (13%) and free amino acids (4.7%) make up almost 18% of the dry weight (Martin et al. 1969), compared with a 13% average for trees and shrubs (Cherret et al. 1989). It is worth noting, however, that although the higher attine fungi appear to provide a "rich and complete diet" for the ants (Stradling 1978), the data in Table 1 indicate that—with the notable exception of high protein levels in cultivar hyphae—the *Atta*-cultivated fungi are not nutritionally outstanding at the level of crude content. In fact, the staphylae of *Atta* cultivars are lower in lipid, protein, and sugar content compared to the two free-living lepiotaceous fungi and also the hyphae of *Atta* cultivars (Table 1). This is surprising because *Atta* workers greatly prefer staphylae over hyphae when given a choice (Quinlan and Cherrett 1978b, 1979; Angeli-Papa and Eymé 1985), and *Atta* workers live longer when feeding on staphylae than on hyphae (Bass and Cherrett 1995). Clearly, the nutritional value of hyphae versus staphyla cannot be understood from the crude analyses summarized in Table 1. The present data suggest, however, that it may not be the absolute levels of crude proteins, lipids, and carbohydrates that uniquely suit the attine cultivars to their hosts, but rather the particular combination of specific amino acids, lipids, carbohydrates, and other trace nutrients (e.g., vitamins and minerals).

3C. PALATABILITY AND TOXICITY OF LEPIOTACEOUS FUNGI

Basidiomycetes exhibit a rich secondary chemistry as constituent defenses against fungivores, and lepiotaceous fungi are probably no exception in this regard. Some (but not all) lepiotaceous fungi are toxic to humans (Ammirati et al. 1985) and therefore possibly toxic to other animals, including ants. For example, several *Lepiota* produce cyclic peptides that are toxic to animals (Griffin 1994), and at least two species in the genus *Leucoagaricus* produce a furanone (basidalin) with general cytotoxic activity (Iinuma et al. 1983; Huff and Kuball 1994). Thus it seems unlikely that the original cultivars were completely free of mycotoxins and, as in the case of human cultivars (Cowan and Watson 1992; Diamond 1997), they probably underwent selection for decreased toxicity to the ant hosts. It is possible that certain fungal groups like the Leucocoprineae are less toxic to ants even in a free-living condition and thus represent a more suitable fungal diet, but this supposition remains to be tested.

4. DATING THE ORIGIN OF THE ATTINE ANT-FUNGUS MUTUALISM

4A. FOSSIL RECORD AND ATTINE BIOGEOGRAPHY

The oldest known definitive ant fossils date from 90–94 million years ago (Mya) (Wilson et al. 1967; Wilson 1985, 1987; Grimaldi et al. 1997). Despite controversy over a substantially older date inferred from a molecular-clock analysis (Agosti et al. 1997; Crozier et al. 1997; Rust and Andersen 1999), most workers accept an early Cretaceous origin (about 110–130 Mya) for the ant family Formicidae and a late Cretaceous origin (about 80–90 Mya) for the subfamily Myrmicinae (Grimaldi et al. 1997). A major, explosive radiation of ants, which produced the extant diversity of tribes and genera, occurred in the early Tertiary (45–64 Mya) (Wilson 1985; Grimaldi et al. 1997; Rust and Andersen 1999). Because the tribe Attini is a highly derived group within the subfamily Myrmicinae, it seems reasonable to assume that it arose during this radiation, although there is no solid fossil evidence that absolutely precludes an attine origin as far in the past as 80 Mya.

The fossil record for both the tribe Attini and the subfamily Myrmicinae is poor. Fossils are known for only four genera of fungus-growing ants, including three from Dominican amber (about 20 Mya), *Trachymyrmex*, *Cyphomyrmex*, and *Apterostigma* (Baroni Urbani 1980; Wilson 1988), and one from Chiapas amber (also about 20 Mya), probably *Mycetosoritis* (Brown 1973). Because the genus *Trachymyrmex* is the most basal member of the derived higher attine clade, its presence in Dominican amber sets a lower limit of 20 Mya for the age of this clade. Furthermore, because the higher attines comprise somewhat less than 50% of the total generic and species diversity of the Attini, and because cladograms for the Attini imply greater branching evolution in the lower versus higher Attini (Schultz and Meier 1995; Wetterer et al. 1998), it seems reasonable to assume that an equivalent or somewhat greater time span was required for the evolution of the lower attines, setting the minimum date for the origin of the Attini in the neighborhood of about 45 Mya. If, as discussed above, the Attini arose during the radiation of modern ant genera during the early Tertiary, then the most plausible range for dating their origin might be 45–65 Mya, bracketing the estimate of 50 Mya by Wilson (1971). This range is consistent with an origin of fungus-growing ants on the South American continent following its separation from Africa in the mid to late Cretaceous (Parrish 1993; Pitman et al. 1993), which is indicated by the exclusively New World and overwhelmingly Neotropical distributions of the Attini and their most likely sister group, the Blepharidattini (*Blepharidatta* and *Wasmannia*).

4B. HISTORICAL ECOLOGY OF ATTINE FUNGICULTURE

Weber (1972a) suggested that fungiculture originated in the wet forests of the Amazon basin, based on the observation that the highest species density of fungus-growing ants occurs there. This hypothesis is bolstered by the additional observation that, of all South American habitats, wet Neotropical forests are home to the highest diversity and abundance of species in the fungal tribe Leucocoprineae from which the ancestral attine cultivars very likely arose (Chapela et al. 1994; Mueller et al. 1998).

In addition, most species of the Blepharidattini, the most likely sister group of the Attini, are denizens of wet Neotropical forests, as are most species of basal Attini.

In humans, the transition from hunting-gathering to farming is thought to have been triggered, in part, by major climatic changes which caused the disappearance of large game and an increased abundance of plants suitable for domestication (Cowan and Watson 1992; Diamond 1997). It is logical to ask whether a similar ecological upheaval might have coincided with the origin of attine fungiculture. Perhaps significantly, the time interval bracketing the attine origin discussed above, 45–65 Mya, includes the K-T extinction event that appears to have been triggered by a catastrophic asteroid impact. It is at least plausible that an association between ants and fungi would be favored during a postimpact period of catastrophic declines in typical ant food sources (e.g., arthropod prey, plant nectars, seeds) and of corresponding increases in the abundance of decomposers and detritivores. As pointed out by Janzen (1995) in connection with the K-T mass extinction: "What animals are most likely to survive a serious nuclear winter? Those whose food in some form does not directly depend on immediate photosynthesis. That is to say, those that eat dormant seeds and insects, those that eat decaying organic matter (especially nongreen plant parts), and those that eat these eaters. And especially those that are very good at finding small particulate bits of these resources, scattered and dwindling until sunlight again can penetrate the clouds in amounts sufficient for serious vegetation growth. That is to say, seed and detritus-eating invertebrates and the invertebrates and small vertebrates that eat them and each other" (p 785). To complete this speculation about a possible connection between the K-T mass extinction event and the origin of the fungus-growing ants, we simply note that there exists an exclusively Old World parallel to the exclusively New World Attini: the fungus-growing termites in the subfamily Macrotermitinae, which also cultivate a group of basidiomycetous decomposers. In contrast to the much greater age of other termite subfamilies, the Macrotermitinae are thought by some researchers to have arisen, like the Attini, during

the early Tertiary (Emerson 1955; Darlington 1994), although other researchers favor an older date (P Eggleton, personal communication; B Thorne, personal communication).

5. HYPOTHESES OF THE ORIGIN OF THE ATTINE MUTUALISM

There exist seven published hypotheses about the origin of attine fungiculture. These hypotheses differ with respect to the putative nutrient substrate on which the ancestral attine first encountered its fungal symbiont, and with which it presumably manured its first fungus gardens.

5A. STORED SEEDS

One of the oldest hypotheses for the origin of the attine ant-fungus mutualism is that of von Ihering (1894, 1898; see also Weber 1972a, 1979), who suggested that attines evolved from seed-harvesting ants, and that the ancestral attine first encountered its fungal symbiont as a contaminant ("unerwünschte Nebenerscheinung"; von Ihering 1898:238) in its granaries. Seed-harvesting myrmicine ants, which are especially abundant in drier habitats, include species of *Aphaenogaster, Messor, Monomorium, Pheidole, Pogonomyrmex, Solenopsis*, and others, but none of these genera are closely related to the attines (Meier and Schultz 1996; Schultz 1998). Although it is possible for leucocoprineous fungi—which are, in fact, specialized litter decomposers—to grow on seeds in an otherwise axenic state, in nature they are unlikely to outcompete seed-specialist fungi on this substrate, and thus are unlikely to occur on seeds under natural conditions. Indeed, leucocoprineous fungi have never been isolated from seed stores of ants or other animals; instead, fungal contaminants in such seed stores are mostly ascomycetous or zygomycetous fungi, including widely distributed soil fungi (e.g., *Penicillium, Mucor*), seed-pathogenic fungi (e.g., *Fusarium, Phoma*), or endophytic fungi (e.g., *Acremonium*) (Reichman and Rebar 1985; Crist and Friese 1993; Knoch et al. 1993). Went et al. (1972) documented the presence of fungi in nests of the desert harvester *Veromessor* (= *Messor*) *pergandei*, but these were found on refuse and discarded infrabuccal pellets rather than on seeds, and none were basidiomycetes. Finally,

the fungal substrates utilized by the basal Attini provide little support for a seed-harvesting origin; they include some seeds (Weber 1972a; Roberts and Heithaus 1986) but mostly insect frass, dead arthropods, and a variety of plant materials (flower stamens, small petals).

5B. ADVENTITIOUS FUNGI ON NEST WALLS

Virtually all ants select humid nest sites, and most regulate intranest humidity to levels much above the environment (Hölldobler and Wilson 1990). Many species of leaf-litter inhabiting ants locate their nests between layers of leaves on the forest floor or occupy shallow preformed or excavated cavities in the soil humus layer immediately adjacent to the leaf litter. Further, some ants construct and inhabit "carton nests" formed from soil and plant debris transported from the leaf litter for this purpose. Carton nests are commonly arboreal, although they may also occur in hollow logs or under stones (Santschi 1910; Wheeler 1910; Farquharson 1914; Black 1987). Conditions in such ant nests typically promote fungal growth, and indeed fungi are evident on the inner walls of many nests (Bailey 1920). As discussed above, during the course of their evolution, ants have acquired various morphological and behavioral adaptations for suppressing the adventitious growth of fungi in the nest environment, including the secretion of antimicrobial substances and the physical removal of hyphae through cropping (Bailey 1920; Schildknecht and Koob 1970, 1971; Schmid-Hempel 1998). Emery (1899), Santschi (1910), and Farquharson (1914) suggest that the attine ant-fungus mutualism may have arisen from such cropping of adventitious fungi growing on the walls of the attine ancestor's nest, because cropping may have resulted in the partial ingestion of nest-wall fungi and may have led to routine feeding on these adventitious fungi.

The "nest wall" hypothesis is rendered implausible if the attine ancestor inhabited nests excavated deep in the ground, because leucocoprineous fungi are unlikely to occur at such depths in pure soil. But this hypothesis becomes correspondingly more likely if the attine ancestor either inhabited the litter layer, or the soil just below the litter, or if the ancestor inhabited carton nests constructed from debris obtained from the litter layer and thus

was regularly exposed to litter-decomposing fungi such as the Leucocoprineae. Indeed, many of the lower attines live in the narrow leaf-litter stratum at the forest floor (Weber 1972a), and the recent discovery of an undescribed litter-inhabiting *Blepharidatta* by Diniz et al. (1998) and observation of *B. brasiliensis* in the field (Mueller and Schultz, personal observation) indicates that such nesting habits also obtain in the close relative of the attines. The Emery-Santschi-Farquharson hypothesis that the attine cultivars derived from adventitious fungi growing on the walls of nests is therefore plausible, particularly if the ancestral attine inhabited leaf litter.

5c. ROTTING WOOD

Forel (1891) suggested a sister-group relationship between the Dacetini and the Attini, and in 1902 proposed a behavioral grade linking the dacetine genus *Strumigenys* to the Attini. As part of this conjecture, Forel noted that *Strumigenys* nests are found in rotting wood and that the ancestral attine, living in a similar habitat, may have entered into an association with a wood-decomposing fungus. This hypothesis now appears unlikely, because the Leucocoprineae are specialized litter decomposers and, although they are capable of wood digestion in vitro (Martin and Weber 1969) or when cultivated by attine ants (Murakami and Higashi 1997), none are known to grow on wood in the wild (Dennis 1952; Singer 1986; Johnson 1999). It is true, however, that a small, possibly monophyletic group of species in the attine genus *Apterostigma* cultivates nonleucocoprineous fungi that may have been derived from wood-specialist fungi, and Weber (1979) noted that "rotted wood fragments may be used . . . especially by *Apterostigma*" (p. 86) species as a substrate for the cultivation of these fungi. Based on DNA sequence information (Moncalvo et al. 2000), the closest known relatives of these *Apterostigma* fungi are fungi in the genus *Gerronema* (Tricholomataceae, Agaricales), which are specialized wood rotters (Singer 1964, 1970, 1986). Forel's hypothesis may thus apply to the ancestral *Apterostigma* that switched to this nonlepiotaceous fungus, but it does not apply to the common ancestor of the tribe Attini, which most likely cultivated a lepiotaceous, litter-decomposing fungus.

5D. MYCORRHIZAE

Because soil nesting is the most common habit in all basal attine genera, as well as in most putative sister groups, the ancestral attine could plausibly have been a soil-nesting ant that routinely came into contact with mycorrhizal fungi. This led Garling (1979) to suggest that the ancestral attine encountered its fungal symbiont as mycorrhizae on subterranean plant rootlets. However, most mycorrhizae in the tropics are vesicular-arbuscular Zygomycota. In contrast, basidiomycetous mycorrhizae are rare and, like zygomycetous mycorrhizae, difficult to cultivate except in the presence of the associated plant roots. Most importantly, now that it has been established that the ancestral attine fungus was almost certainly leucocoprineous (Chapela et al. 1994; Mueller et al. 1998), the mycorrhizal hypothesis must be judged highly unlikely because no species in the Leucocoprineae are known to be mycorrhizal (Johnson 1999).

5E. ARTHROPOD CORPSES AND REFUSE PILES

Many lower attines incorporate arthropod exoskeletons and, reportedly, freshly collected arthropod corpses into their fungus gardens (Weber 1972a). Von Ihering (1894), Maidl (1934), and Moffett (1986) therefore suggested that the attine cultivars may have been derived from fungi growing on deceased workers or brood, or on discarded prey accumulating on refuse piles in the nest. Diniz et al. (1998) tested this hypothesis by examining the refuse piles of the close attine relative *Blepharidatta conops* and indeed observed fungi growing on refuse piles consisting of discarded arthropod prey. While Diniz et al. (1998) did not identify these fungi, it is unlikely that they are closely related to attine cultivars, because lepiotaceous fungi (and the Agaricales in general) do not exploit arthropod exoskeletons or tissue as a nutrient substrate. This is supported by the fact that, even though lower attines routinely incorporate dry arthropod parts into their gardens, and even though attine fungi secrete low levels of chitinolytic enzymes (Martin 1974), the cultivated fungi do not grow into these insect parts (Weber 1972a; Martin 1974); in fact, intact parts are eventually removed by the ants and discarded (Weber 1972a). The use of arthro-

pod parts as a primary substrate in the gardens therefore is highly unlikely, and an hypothesis of an arthropod-remains origin of the attine cultivars can be rejected.

5F. ANT FAECES

Forel (1902) proposed that attine ants evolved from predaceous ancestors that nested in rotting wood, then began feeding on coprophilous fungi that grew on the feces of woodboring insects. This hypothesis was based on the observation that attines incorporate insect feces into their gardens, and that some attine species, such as those in the genus *Apterostigma*, exclusively use caterpillar, beetle, or millipede excrement as manure. Wheeler (1907) modified this "insect excrement hypothesis" into an "ant excrement hypothesis," noting that "the method employed by the *Atta* queens in manuring their incipient fungus-gardens suggests that the food plants may have been originally grown on fecal substances" (p 796). Subsequent authors, most prominently Weber (1956, 1958), accepted the Wheeler scenario because it provided explanations for both the origin of the fungi and for the unusual manuring behavior of foundress queens. It is now known, however, that the purpose of fecal manuring is to redistribute proteolytic enzymes originating in the fungus and concentrated in the guts of the ants from ingested fungi (Martin 1984, 1987). Obviously, this ability is unlikely to have preceded the origin of attine fungiculture, but it remains possible, though improbable, that "manuring" without proteolytic enzymes represents the ancestral mode of nourishing the attine garden.

As with most of the other substrates suggested in the preceding hypotheses, it is possible that leucocoprineous fungi could grow on ant excrement in the absence of competitors. They are not, however, specialized for this substrate and, as specialized litter decomposers, they are consistently outcompeted in nature by specialized coprophilous fungi. Thus the Wheeler-Weber hypothesis of a coprophilous origin for the attine fungi is contradicted by the phylogenetic position of the attine cultivars within the litter-specializing Leucocoprineae.

5G. INFRABUCCAL PELLETS

As summarized above, Wheeler and Bailey (1920) and Bailey (1920) carefully examined the infrabuccal pellets of a wide diversity of ants to infer their feeding habits. They assumed that such an analysis would throw "considerable light not only on the nature of the food, but also on that of the environment in which the ants nest" (Wheeler and Bailey 1920:237). Examining over 1000 pellets from over 60 ant species (28 genera, 8 subfamilies), Bailey (1920) and Wheeler and Bailey (1920) found that the great majority of pellets contain a mixture of animal, plant, and fungal fragments, and, depending on the ants' nesting habits, also soil or wood debris. Surprisingly, bacteria were rarely found in the pellets, and "remains of insect food were by no means as abundant as would be expected" (Wheeler and Bailey 1920:246) from the predatory feeding habits of ants. In contrast, fungal spores or hyphal fragments "occur with surprising constancy, and are often numerous or very abundant, quite irrespective of the nesting habits" (Wheeler and Bailey 1920:246), and in no species were spores or hyphae entirely absent from the pellets. Several distinct sporal types were usually found per pellet, but occasionally all spores in a pellet were of the same type.

Bailey (1920) concluded that "there is a general tendency among ants . . . to take spores and fragments of mycelia . . . into their infrabuccal pockets" (p 181), and that therefore "it seems probable that the Formicidae are active agents in the dissemination of many of the higher fungi, particularly in tropical regions where ants are so numerous and ubiquitous" (p 185). This use of ants by fungi for the dissemination of spores or hyphal fragments may have led to the occasional growth of fungi on pellet debris piles in ant nests, and "if such mats of aerial hyphae were edible, it would be a simple matter for the ants to increase the volume of their primitive fungus-gardens by adding extraneous materials . . . to the original compost" (Bailey 1920:186). Under such a scenario, attine fungiculture may have arisen secondary to, and well after, the origin of a fungus-ant association where fungi utilized ants as vectors for dispersal.

This scenario is consistent with the findings

of Febvay and Kermarrec (1981), who examined the microflora in pellets of *Acromyrmex octospinosus* to determine whether this microflora could aid in the digestion and "recuperation of nutritive elements" from the pellets (p 447). They conclude that "digestion of organic material by the abundant microflora of the infrabuccal pocket can be disregarded with respect to the turnover speed (about 1 pellet/day) and the lack of a constant dominant microflora" (p 447). This high turnover also suggests that much of the microflora may remain viable throughout the process of compaction in the infrabuccal pocket. Consequently, fungi could be dispersed via infrabuccal pellets not only as spores, but also as hyphal masses.

In the genera *Leucocoprinus* and *Leucoagaricus*, containing the species most closely related to attine fungi (Mueller et al. 1998), spores measure about $5-7 \times 3-5$ μm and hyphae $2-15$ μm in diameter (Dennis 1952; Pegler 1983; Johnson 1999). The corresponding measures for attine fungi, taken from the few attine fungus sporocarps (i.e., mushrooms) reported in the literature, are $6-10 \times 4-5$ μm diameter for spores (Weber 1957; Hervey et al. 1977; Muchovej et al. 1991; Fisher et al. 1994), $2-6$ μm diameter for hyphae of lower attine fungi, and $6-10$ μm diameter for hyphae of leaf-cutter-ant fungi (Weber 1972a; Quinlan and Cherrett 1978a). These values suggest that both spores and hyphae of the original leucocoprineous cultivar could have been easily trapped in the infrabuccal pellet of the ancestral attine ant, and extant fungi may still be so trapped.

6. FUNGAL MYRMECOCHORY AND THE ORIGIN OF ATTINE FUNGICULTURE

To our knowledge, Bailey's hypothesis (1920) for the origin of the attine ant-fungus mutualism has not been cited in the subsequent literature. Although several studies stressed the importance of ants as dispersers of spores of hypogeous fungi (McIlveen and Cole 1976; Allen and MacMahon 1984; Friese and Allen 1988, 1993; Janos 1993), and although ants have been implicated in the dispersal of lichen soredia and spores of mosses and ferns (Plitt 1907; Bailey 1970; Paterson 1982), Bailey's hypothesis on the importance of infrabuccal pellets in fungal dispersal has been ignored and,

so far, failed to stimulate any empirical work. Because many insects are known to disperse ingested fungal spores (Rabatin and Stinner 1985; Janos 1993), and because ants are and have been one of the most abundant and ubiquitous insect groups for at least 45 million years (Fittkau and Klinge 1973; Hölldobler and Wilson 1990; Tobin 1991), Bailey's hypothesis of fungal dispersal due to the action of this ancient vector is eminently plausible.

If ants have been important agents in fungal dispersal for millions of years, it is not unlikely that fungi have evolved adaptations to promote and optimize dispersal by ants. In an analogy with plants that employ ant-attractant nutritive structures ("elaiosomes"; Beattie 1985), some fungi may produce specialized hyphal or sporal structures that serve the dual function of both offering a nutritious reward that attracts ants and forming a propagule for dispersal. Such specializations need not be obvious to human observation and thus may have been overlooked by both mycologists and myrmecologists. In the simplest cases, such specializations could resemble other simple structures known to serve a dispersal function in fungi, such as stromata that are moved by rain splashes and water currents (Malloch and Blackwell 1990).

The difficulty in explaining the origin of the attine ant-fungus mutualism has always been that a constellation of several modifications is required, and that these modifications must have arisen simultaneously or in some logical order. These include: (a) modification of the nutritional value of the fungi to meet the needs of the ants, or modification of the nutritional requirements of the ants to adjust to a fungal diet, or both; (b) behavioral modifications in the ants necessary for cultivating fungi; and (c) adaptations in both the ants and the fungi that guarantee fungal transfer via the infrabuccal pocket. Bailey's (1920) hypothesis proposes a scenario that partitions this complex set of modifications into two evolutionary events: first, fungi associated with and adapted to ants for fungal dispersal; subsequently the ants domesticated the fungi (see Alternate Model in Figure 1). Thus, unlike the first six hypotheses mentioned above (Sections 5A–5F), Bailey's hypothesis (Section 5G) reverses the order of events and proposes that

domestication by ants was secondary to an evo-
lutionary stage in which the fungal ancestors
of the eventual cultivars adapted to the ecol-
ogy of infrabuccal pocket transmission and to
the nutritional requirements of their future
hosts.

Bailey's hypothesis could potentially ex-
plain a series of unresolved questions about
the evolution of the attine ant-fungus mutu-
alism:

(1) Why is the fungus transmitted via the
infrabuccal pocket, rather than transported in
external integumental pockets common in
other known insect-fungus symbioses (Wheeler
and Blackwell 1984; Malloch and Blackwell
1990) or held in the mandibles (e.g., as in coc-
cid-rearing ants that disperse with their coc-
cids; Silvestri 1925; Roepke 1930; Bünzli 1935;
Wheeler 1935; Brown 1945; Buschinger et al.
1987)? If leucocoprineous fungi were "pre-
adapted" to an infrabuccal existence, the
transmission of the fungus by foundress
queens required little modification of the
ants' preexisting behavioral repertoire, and
infrabuccal transmission did not need to
evolve de novo at the origin of fungiculture.
A preexisting transmission mechanism via in-
frabuccal pellets thus renders the origin of at-
tine fungiculture substantially more likely.

(2) Given that free-living fungi are unlikely
to fulfill all nutritional requirements of carniv-
orous ants, how did ants make the complex
physiological transition from carnivore to fun-
givore? If ant-associated fungi utilized elaio-
some analogues to reward ants for dispersal,
such structures could have been high in nutri-
ent content and, through coevolutionary in-
teractions with the ants, may have gradually
become an adequate diet for them, thus en-
abling the switch from hunter-gatherer to fun-
gal farmer. That is, fungi that are specialized
for dispersal by ants are likely to evolve a simi-
lar nutritional profile to those plants that pro-
duce elaiosomes and other ant attractants
(Beattie 1985; Hölldobler and Wilson 1990).
This fungal preadaptation to serve as ant food
may have facilitated the evolution of fungivory
not only in the Attini, but also in the *Megalo
myrmex silvestrii* species group of ants, thus pro-
viding a single explanation for the unlikely
transition from carnivory to fungivory in two

independent ant lineages. To speculate fur-
ther, could gongylidia, the highly nutritious
hyphal swellings that are harvested by higher
attines, represent structures derived from these
ancestral ant-rewards? The fungi of lower at-
tines possess hyphal swellings (Weber 1972a)
that may be homologous to gongylidia (Weber
1979), suggesting that such modifications pre-
date the origin of the higher attines and may
date back to (or even predate) the origin of
the attine ant-fungus mutualism. In addition,
gongylidia may actually be modified cystidia
(J A Scott, personal communication), which
are sterile hyphal swellings located on the hy-
menium layer of fruiting bodies in many groups
of basidiomycetes. This hypothesis provides a
plausible starting point for a series of evolu-
tionary modifications from an ant-reward pre-
cursor in the ancestral fungus to its present
gongylidial form. To test this hypothesis on
the origin of gongylidia, it will be imperative
to study the mycelium of free-living leucoco-
prineous fungi in a natural environment, as
well as the gongylidia-like hyphal swellings in
the gardens of lower attines, with particular
emphasis on comparative histology and devel-
opment, and thus determine possible homolo-
gies between gongylidia of higher attine fungi,
the swellings of lower attine fungi, and the hy-
pothesized specialized structures of free-living
Leucocoprineae.

(3) Why were the repeated (and possibly on-
going) domestications of fungi by attine ants
restricted to a narrow group of Leucocopri-
neae (Mueller et al. 1998), and why were
switches to fungi outside of this group (by a
subgroup of ants in the genus *Apterostigma*) re-
stricted to a single, probably evolutionarily
unique occurrence? Under Bailey's (1920) hy-
pothesis, the reason may be that at least some
tropical Leucocoprineae are specialized on
ants for dispersal, are inherently attractive to
ants, and thus represent fungi that were and
still are most likely to be domesticated when
the ants recruit free-living cultivars into the
symbiosis.

If Bailey's hypothesis is correct, it seems un-
likely that leucocoprineous fungi are the only
group of fungi that are specialized to use ants
for dispersal. Instead, "buccophily" may have
evolved multiple times in the fungi. Indeed,

preliminary isolations from infrabuccal pellets of a temperate *Camponotus* species indicate that, although a diverse assemblage of fungi can be present within the pocket, often only a single, apparently specialized species of fungus (probably genus *Mortierella*; Mucorales: Zygomycota) remains viable in the pellet and germinates after pellet expulsion (Malloch, personal observation). Similar studies of infrabuccal contents of diverse attine and non-attine ants, in addition to studies of the ecological succession of microorganisms in discarded infrabuccal pellets, may reveal diverse fungi that are specialized to disperse via these pellets. As Wheeler and Bailey (1920) argued eighty years ago, "a cultivation of the pellets on artificial media will very probably show that the spores and pieces of hyphae are quite viable after their sojourn in the infrabuccal pocket . . . The fact that the pellets, even of other species of ants, are cast out by the workers somewhere in their environment, either in the kitchen middens of their nest or outside its precincts, i.e., in situations where the spores may readily germinate, is of no little ecological and economic significance, for it shows that ants may be very important agents, or vectors in the distribution of many kinds of fungi" (p 271).

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