

THE EVOLUTION OF AGRICULTURE IN INSECTS

Ulrich G. Mueller,^{1,2} Nicole M. Gerardo,^{1,2,3}
Duur K. Aanen,⁴ Diana L. Six,⁵ and Ted R. Schultz⁶

¹*Section of Integrative Biology, University of Texas at Austin, Austin, Texas 78712;*
email: umueller@mail.utexas.edu

²*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama*

³*Department of Ecology and Evolutionary Biology, University of Arizona, Tucson,*
Arizona 85721-0088; email: ngerardo@email.arizona.edu

⁴*Department of Population Biology, Biological Institute, University of Copenhagen,*
2100 Copenhagen, Denmark; email: dkaanen@bi.ku.dk

⁵*Department of Ecosystem and Conservation Sciences, University of Montana,*
Missoula, Montana 59812; email: diana.six@cfc.unt.edu

⁶*Department of Entomology, National Museum of Natural History,*
Smithsonian Institution, Washington, District of Columbia 20013-7012;
email: schultz@lab.si.edu

Key Words Attini, Macrotermitinae, mutualism, symbiosis, Xyleborini

■ **Abstract** Agriculture has evolved independently in three insect orders: once in ants, once in termites, and seven times in ambrosia beetles. Although these insect farmers are in some ways quite different from each other, in many more ways they are remarkably similar, suggesting convergent evolution. All propagate their cultivars as clonal monocultures within their nests and, in most cases, clonally across many farmer generations as well. Long-term clonal monoculture presents special problems for disease control, but insect farmers have evolved a combination of strategies to manage crop diseases: They (*a*) sequester their gardens from the environment; (*b*) monitor gardens intensively, controlling pathogens early in disease outbreaks; (*c*) occasionally access population-level reservoirs of genetically variable cultivars, even while propagating clonal monocultures across many farmer generations; and (*d*) manage, in addition to the primary cultivars, an array of “auxiliary” microbes providing disease suppression and other services. Rather than growing a single cultivar solely for nutrition, insect farmers appear to cultivate, and possibly “artificially select” for, integrated crop-microbe consortia. Indeed, crop domestication in the context of coevolving and codomesticated microbial consortia may explain the 50-million year old agricultural success of insect farmers.

1. INTRODUCTION

The cultivation of crops for nourishment has evolved only a few times in the animal kingdom. The most prominent and unambiguous examples include the fungus-growing ants, the fungus-growing termites, the ambrosia beetles and, of

course, humans. For humans, who started the transition from an ancestral hunter-gatherer existence to farming only about 10,000 years ago (Diamond 1997, Smith 1998), sustainable, high-yield agriculture has become critical for survival in a global economy with projected food shortages, and diverse research programs are currently devoted to the optimization of agricultural productivity in the context of growing environmental challenges (Green et al. 2005). Agricultural progress has been achieved by humans through a combination of insight, creative planning, and a fair share of contingency and luck (Diamond 1997, Schultz et al. 2005, Smith 1998). However, humans have so far not examined nonhuman agricultural systems, such as the fungus-growing insects, for possible insights to improve agricultural strategies.

This lack of an applied interest in insect agriculture probably derives from a general perception that human agricultural systems (based largely on plant cultivation) function in a fundamentally different manner than insect systems (all based on fungus cultivation). However, humans have learned much of practical value through the close examination of adaptive features of other organisms (including insects), and comparable problems such as crop diseases affect all farmers regardless of their phylogenetic positions or those of their crops (plant, fungus, or otherwise). Because of the universality of crop diseases in both human and insect agriculture, it may be fruitful to examine the short-term and long-term solutions that have evolved convergently in insect agriculture for possible application to human agriculture (Denison et al. 2003). Such a synthesis is the goal of this review.

1.1. Behavioral and Nutritional Elements

Defining Agriculture

Insect fungiculture and human farming share the defining features of agriculture (see Table 1): (a) *habitual planting* (“inoculation”) of sessile (nonmobile) cultivars in particular habitats or on particular substrates, including the seeding of new gardens with crop propagules (seeds, cuttings, or inocula) that are selected by the farmers from mature (“ripe”) gardens and transferred to novel gardens; (b) *cultivation* aimed at the improvement of growth conditions for the crop (e.g., manuring; regulation of temperature, moisture, or humidity), or protection of the crop against herbivores/fungivores, parasites, or diseases; (c) *harvesting* of the cultivar for food; and (d) obligate (in insects) or effectively obligate (in humans) *nutritional dependency* on the crop. Obligate dependencies of the insect farmers can be readily demonstrated by experimental removal of their cultivated crops, resulting in reduced reproductive output, increased mortality, or even the certain death of the cultivar-deprived insect (Francke-Grosmann 1967, Grassé 1959, Norris 1972, Sands 1956, Weber 1972). Our definition of agriculture does not require conscious intent in planting and harvesting. Conscious planning, learning, and teaching have clearly accelerated the development of complex agriculture in humans, but presumably not in insects (Schultz et al. 2005).

TABLE 1 Agricultural behaviors of farming ants, termites, beetles, and humans

Agricultural behavior	Agriculture in:			
	Attine ants	Macrotermitine termites	Xyleborine ambrosia beetles	Humans
Dependency on crop for food	Obligate	Obligate	Obligate	Facultative
Engineering of optimal growth conditions for crop (e.g., substrate preparation; moisture or humidity regulation)	Present	Present	Present	Present
Planting of crop on improved substrate	Present	Present	Present	Present
Intensive, continuous monitoring of growth and disease status of all crops	Present	Present	Present	Absent
Sustainable harvesting of crop for food	Present	Present	Present	Present
Protection of crop from diseases and consumers	Present	Present	Present	Present
Weeding of alien organisms invading the garden	Present	Present	Unknown	Present
Use of chemical herbicides to combat pests	Present	Unknown	Unknown	Present
Use of microbial symbionts for nutrient procurement for the crop	Present	Unknown	Unknown	Present
Use of disease-suppressant microbes for biological pest control	Present	Unknown	Unknown	Absent ¹
Sociality	Strictly eusocial	Strictly eusocial	Subsocial or communal ²	Social
Task partitioning in agricultural processes	Present	Unknown	Present	Present
Application of artificial selection for crop improvement	Unknown, but ants exert symbiont choice	Unknown	Unknown	Present
Learning and cultural transmission of agricultural innovations	Absent	Absent	Absent	Present

¹ See Section 7 for some recently discovered microbes with potential disease-suppressant properties.² One ambrosia beetle species is eusocial, all other species appear to be subsocial or communal (see text).

We restrict our review to ant, termite, and beetle fungiculturists. Cases analogous to human animal husbandry, such as the tending by ants of hemipteran insects (e.g., aphids, treehoppers; Hölldobler & Wilson 1990), are beyond the scope of this review. We also exclude cases that fail to meet all four of the requirements of agriculture as defined above, including, e.g., the ant *Lasius fuliginosus*, which promotes fungal growth in the walls of its nest, because the fungus is apparently not grown for food but instead for strengthening the walls (Maschwitz & Hölldobler 1970) or for antibiotic protection of the walls (Mueller 2002). On the same grounds, we exclude a number of possible cases of incipient agriculture. For example, *Littoria* snails may “protofarm” fungi by creating plant wounds that become infected with fungal growth that is part of the snails’ diet, but the snails do not actively inoculate the plant wounds or otherwise garden the fungi (Silliman et al. 2003). Many more such protofarming species probably await discovery, particularly among invertebrates, and all of the known insect agriculturists (fungus-growing ants, termites, and beetles) probably originated from comparable protoagricultural ancestors (Mueller et al. 2001, Schultz et al. 2005). Comparison of these protofarming insects with “primitive” human agriculture exceeds the scope of this review.

1.2. A Coevolutionary Approach to Understanding Agriculture

We will analyze agriculture as a type of strong coevolutionary interaction, defined by the nutritional and behavioral criteria summarized above, in which natural selection acts upon both farmers and crops as reciprocally interdependent lineages (Futuyma & Slatkin 1983, Rindos 1984). Our coevolutionary approach to agriculture considers not only the interactions between a specific farming insect and a single cultivated crop, but also its interactions with other pathogenic and mutualistic microbes that have recently been discovered in insect gardens. Like the cultivars, some of these microbes are also managed by the insect farmers for specific purposes (Figure 1). In other words, an insect garden is not a pure monoculture, but a sequestered and engineered ecological community consisting of several interacting microbes, some beneficial and others detrimental to the farmers. To gain a comprehensive understanding of the principles of insect agriculture, it will therefore be necessary to examine the nature of insect-microbe interactions in gardens, the evolutionary origins of these interactions, and the convergent and divergent evolutionary trajectories that culminated in the extant agricultural systems of insects.

2. THE THREE INSECT-AGRICULTURE SYSTEMS

Behaviorally complex systems of insect agriculture are known from only three groups of insects: ant, termites, and beetles.

2.1. Ant Fungiculture

The fungus-growing ants are a monophyletic group of about 220 described and many more undescribed species in the tribe Attini (subfamily Myrmicinae) (Price et al. 2003, Schultz & Meier 1995). Attine ants occur only in the New World (Argentina to the southern United States) and attain their greatest diversity in the wet forests of equatorial South America, the region of their presumed evolutionary origin (Mueller et al. 2001). Attine ants are obligate agriculturists; their cultivated fungi are the sole source of food for the larvae and an important source of food for the adults. Although adults are able to supplement their diets by feeding on plant juices (Bass & Cherrett 1995, Murakami & Higashi 1997), the cultivated fungi are nutritionally sufficient to support the ants even in the absence of additional nutrients (Mueller 2002, Mueller et al. 2001). Garden fungi are transmitted vertically across generations when daughter queens transport small pellets of natal-nest mycelium within their infrabuccal pockets, pouches present in the mouthparts of all ants (Fernández-Marín et al. 2004, Huber 1905, Mueller 2002). In the derived leafcutter ants, the workers are divided into a remarkable range of differently sized morphological castes, each specialized on a different task (Hart et al. 2002, Weber 1972) (Figure 2*d*).

Different attine ant lineages cultivate their fungi on different substrates. The ancestral gardening substrate, still used by the so-called lower attines, consists of flower parts, arthropod frass, seeds, wood fragments, or other similar plant debris, whereas the leafcutting genera *Atta* and *Acromyrmex* primarily use freshly cut leaves and flowers. Despite these distinct substrate specializations, all attine systems contain at least four symbionts: (a) the fungus-growing ants; (b) their fungal cultivars (basidiomycetes in the mushroom families Lepiotaceae and Pterulaceae; Mueller et al. 1998, Munkacsí et al. 2004); (c) mutualistic antibiotic-producing actinomycete bacteria (family Pseudonocardiaceae; Currie et al. 1999b); and (d) garden parasites in the ascomycete fungal genus *Escovopsis* (Currie et al. 1999a, Currie et al. 2003b). Additional bacteria and yeasts also occur in attine gardens and may function as mutualists, e.g., by secreting digestive enzymes or antibiotics (Carreiro et al. 1997, Craven et al. 1970, Santos et al. 2004).

2.2. Termite Fungiculture

Of the more than 2600 described termite species, about 330 species in the subfamily Macrotermitinae cultivate a specialized fungus, genus *Termitomyces*, for food. Nests are generally founded by a single pair of reproductives, the future queen and king. They seal themselves permanently in a cell of hard clay (the so-called royal chamber) where they rear the first brood of sterile workers. In most termite species, a new colony acquires a fungal strain from wind-dispersed sexual *Termitomyces* spores shortly after nest founding and begins construction of the first gardens (De Fine Licht et al. 2005). These spores come from fruiting bodies (mushrooms) that arise from mature termite colonies. The fruiting of the fungus appears to be roughly synchronized to the period when the first foraging workers emerge from a

new nest, a few months after the nest-founding stage. Termite gardens are grown on dead plant material that is only partially decomposed, such as leaf litter, dead grass, dead wood, or dry leaves.

Termite gardens are built from spore-containing fecal pellets in chambers that the termites construct either inside a mound or dispersed in the soil. Fecal pellets are added continuously to the top of the comb and fungal mycelium rapidly permeates the new substrate (Figure 2*e*). After a few weeks, the fungus starts to produce vegetative nodules that are consumed by the termites. These nodules are a rich source of nitrogen, sugars, and enzymes. The nodules are also covered with indigestible asexual spores (conidia), so that consumption serves the additional function of inoculating the feces with spores, which pass through the gut unharmed and are then planted in new comb with the deposition of feces (Leuthold et al. 1989). Mature comb is also consumed (Darlington 1994), but it is nutritionally inferior to the nodules.

2.3. Beetle Fungiculture

Ambrosia beetles make up around 3400 of the 7500 species in the weevil subfamily Scolytinae (the bark and ambrosia beetles, including the traditionally separate Platypodinae; Farrell et al. 2001, Harrington 2005, Wood 1982). Most ambrosia beetles construct tunnel systems (galleries; Figure 2*f*) in woody tissues of trees (typically in weakened or recently dead trees or, more rarely, in vigorous hosts), although some species are specialized to colonize pith, large seeds, fruits, and leaf petioles (Harrington 2005, Wood 1982). The term ambrosia refers to the fungi cultivated by the beetles on gallery walls, upon which they feed as an exclusive, or near exclusive, food source. The beetles are obligately dependent upon the fungi, from which they acquire essential vitamins, amino acids, and sterols (Beaver 1989, Kok et al. 1970).

The most advanced fungiculturists among the ambrosia beetles occur in the Xyleborini, a large monophyletic tribe of about 1300 species (Farrell et al. 2001, Jordal 2002). It is this group of ambrosia beetles that we primarily focus on in this review. Although life histories among the Xyleborini vary considerably, most share a number of fungicultural characteristics. There is a sexual division of labor in the Xyleborini; only females perform gardening tasks, whereas males are short-lived and flightless (Norris 1979). After mating, females disperse to new host substrate, carrying the fungi in specialized pockets termed mycangia. Once within a new host, founding females “plant” the fungi on the walls of the excavated tunnels, lay eggs, and tend the resulting garden and brood (Norris 1979). In ways not fully understood, they are able to control the growth of the fungal crop, as well as, to a degree, the composition of its multiple fungal species (Beaver 1989, French & Roeper 1972, Kingsolver & Norris 1977, Roeper et al. 1980). If the female dies, the garden is quickly overrun by contaminating fungi and bacteria, which ultimately results in the death of the brood (Borden 1988, Norris 1979).

The ambrosia gardens of xyleborine beetles are not pure monocultures as was once believed, but are typically composed of an assemblage of mycelial fungi,

yeasts, and bacteria (Batra 1966, Haanstadt & Norris 1985). These assemblages were termed multi-species complexes by Norris (1965), who suggested that it is a complex as a whole, rather than any one individual microbe, that allows the beetles to exploit nutrient-poor substrates such as wood. However, most subsequent work has revealed that one “primary” fungus always dominates in beetle gardens (Baker 1963, Batra 1966, Gebhardt et al. 2004, Kinuura 1995). Furthermore, the beetles typically carry only the primary fungus in the mycangium (although secondary fungi are sometimes also isolated from mycangia), and the cultivation efforts of female beetles tend to favor the primary fungus, which imparts the greatest nutritional benefit (Francke-Grosmann 1967, Gebhardt et al. 2004, Morelet 1998, Norris 1979). Some auxiliary fungi also support beetle development, but survival on the auxiliary fungi alone is often greatly reduced (Norris 1979). These observations implicate the primary fungus as the intended crop, whereas the secondary fungi, yeasts, and bacteria may be contaminant “weeds” or may play additional auxiliary roles in the gardens, paralleling the hypothesized roles of the auxiliary bacteria and yeasts in attine gardens (see above).

3. EVOLUTIONARY ORIGINS OF INSECT AGRICULTURE

Phylogenetic analyses reveal nine independent origins of insect agriculture (Figure 2; Table 2). In ants, fungal cultivation arose only once, probably 45–65 Mya in the Amazon rainforest (Mueller et al. 2001; Schultz & Meier 1995). In termites, fungiculture likewise had a single origin, approximately 24–34 Mya in the African rainforest (Aanen et al. 2002; D.K. Aanen & P. Eggleton, submitted). In ambrosia beetles, however, agriculture arose independently seven times between 20–60 Mya, six times in various nonxyleborine lineages, and once in the ancestor of the Xyleborini about 30–40 Mya (Farrell et al. 2001). Whereas the common ancestors of the macrotermitines and of the xyleborines each domesticated a single, specific primary cultivar clade to which their descendants have adhered throughout subsequent evolution (Figure 2, Table 2), attine ants maintain associations with multiple independently domesticated cultivar lineages (which are for the most part very closely related; Mueller et al. 1998; Munkacsı et al. 2004; Table 2). Interestingly, there are no known cases of reversal from agricultural to nonagricultural life in any of the nine agricultural insect lineages (Figure 2*a*, 2*b*, and 2*c*), suggesting that the transition to fungiculture is a drastic and possibly irreversible change that greatly constrains subsequent evolution.

Two main models have been suggested for the independent evolutionary transitions to agriculture in insects, the “consumption-first” versus the “transmission-first” models (Mueller et al. 2001). In the consumption-first model (the likely model for the termites), an insect lineage initially begins to incorporate fungi into its more generalist diet, then becomes a specialized fungivore, and finally evolves adaptations for cultivating fungi. In the transmission-first model (the likely model for the beetles), the insect lineage begins its association with a fungus by serving

TABLE 2 Crop ecology and evolution in ant, termite, beetle, and human agriculture

Crop ecology & evolution	Agriculture in:			Humans
	Atine ants	Macrotermite termites	Xyleborine ambrosia beetles	
Number of inferred evolutionary origins of agricultural behavior	Single origin of agriculture in the Atitini No other ant practices true agriculture, but some ants allow fungi to grow in their nest walls for structural or antibiotic purposes.	Single origin of agriculture in the Macrotermitini No other termite practices true agriculture, but the distantly related termite <i>Sphaerotermes sphaerotherax</i> promotes the growth of bacteria in food stores.	Single origin of agriculture in the Xyleborini Agriculture originated independently in six additional ambrosia beetle lineages; these other beetle systems are less well understood (see text).	Multiple independent origins of agriculture
Estimated date and region of origin of agricultural behavior	45–65 Mya in Amazonian rainforests	24–34 Mya in African rainforests	30–40 Mya in xyleborine beetles; region of origin unknown 21–60 Mya in the other six ambrosia beetle lineages	10,000 years ago for the earliest known origins of human agriculture; multiple regions of origin
Number of crop clades cultivated	Multiple cultivar clades One cultivar clade belongs to the Perulaceae; at least three additional polyphyletic cultivar clades belong to the Lepiotaceae (tribe Leucocoprineae).	Single cultivar clade All termite cultivars belong to the genus <i>Termitomyces</i> .	Multiple cultivar clades The ambrosia cultivars <i>Ambrosiella</i> and <i>Raffiella</i> have a polyphyletic origin within the genera <i>Ophiotoma</i> and <i>Ceratocystis</i> .	Multiple crop clades from diverse lineages of plants and fungi

Crop transmission

Vertical cultivar inheritance from parent to offspring (i.e., cultivar transfer between generations)	Present Vertical cultivar inheritance is the rule in all attine ant lineages.	Variable Vertical cultivar inheritance is typical for two independently derived macrotermite lineages; all other macrotermittines acquire cultivars horizontally via wind-dispersed spores from other colonies.	Present Vertical cultivar inheritance is the rule in all xyleborine lineages.	Present
--	--	--	--	---------

Specialization on crops

Specialization at higher phylogenetic levels	Clade-clade correspondence Defined clades of ant species are specialized in that they only grow fungi typical for their own ant-specific cultivar clade.	Clade-clade correspondence Defined clades of termite species are specialized in that they only grow fungi typical for their own termite-specific cultivar clade.	No strict clade-clade correspondence The fungal lineages associated with xyleborine ambrosia beetles are only distantly related to each other, and all are also associated with some bark beetles.	Not applicable
--	---	---	---	----------------

Specialization at level of farmer species

Specialization at level of farmer species	Present In all cases studied in detail, single species of ants grow only a single, phylogenetically narrow group of fungi (i.e., a single, phylogenetically defined "species" of fungus).	Present Single termite species generally associate with multiple fungal lineages, but within the limits of specific cultivar clades (see above); however, some termite species are very specialized on a particular fungus.	Present Single beetle species generally associate with multiple, distantly related fungal lineages; the primary cultivar is typically a single fungal species, but secondary cultivars vary and often come from different fungal lineages than the primary-cultivar lineage.	Not applicable Specialization on crops is historically manifested by different human lineages (reflecting separate domestication events), but no single human lineage is nearly as specialized on a single crop as are the insect farmers.
---	--	--	---	---

(Continued)

Table 2 (Continued)

Crop ecology & evolution	Agriculture in:			
	Attine ants	Macrotermite termites	Xyleborine ambrosia beetles	Humans
Crop sharing and crop exchange				
Lateral exchange of cultivars within the same farmer species (i.e., exchange within same farmer generation)	Present Within a species of ant, cultivars are probably occasionally exchanged between colonies (e.g., after cultivar loss when cultivar-deprived colonies may acquire replacement cultivars from neighboring colonies).	Unknown	Unknown Lateral cultivar exchange is probably rare, but may occur when galleries containing brood of two or more foundress females, each carrying a different cultivar (e.g., different species of fungi), occur in close proximity in the same tree.	Present Lateral exchange of cultivars between farmer societies is a prominent feature of human agriculture.
Cultivar exchange between farmer species	Present Horizontal cultivar exchange occurs between sympatric ant species if they are specialized on the same cultivar lineage.	Present Horizontal cultivar exchange (through wind-dispersed spores) is typical for most termite lineages.	Rare Horizontal cultivar exchange occurs at least occasionally.	Not applicable
Genetic exchange between domesticated and wild populations (e.g., genetic interbreeding between	Variable Primitive attine ants probably import novel cultivar genotypes	Absent Wild populations are unknown for termite cultivars.	Absent Wild populations are unknown for ambrosia beetle cultivars.	Absent Some traditional cultivation regimes (e.g., potato farming in the

<p>domesticated and wild populations; or import of new domesticates from wild populations)</p>	<p>regularly from wild populations; cultivars of the derived higher attine ants do not appear to have free-living populations.</p>	<p>Present All available data indicate that only a single cultivar is grown within a nest.</p>	<p>Present All available data indicate that only a single cultivar is grown within a nest.</p>	<p>Andes) are based on regular genetic exchange between domesticated and wild populations, but most modern cultivars are highly derived and do not readily outcross with wild, ancestral populations.</p>
<p>Crop monoculture</p>	<p>Present All available data indicate that only a single cultivar is grown within a nest.</p>	<p>Present Most beetle species appear to be associated with a single primary cultivar and one or more secondary cultivars; it is unclear whether secondary cultivars are truly cultivars, weeds, or switch between these roles.</p>	<p>Variable Monoculture predominates agriculture in many but not all regions.</p>	<p>Variable Monoculture predominates agriculture in many but not all regions.</p>
<p>Crop sexuality</p>	<p>Variable, but sexual recombination is rare Predominant clonal cultivar propagation within nests and between nests is punctuated occasionally by genetic exchanges, outbreeding, or other events generating recombinants.</p>	<p>Variable, but sexual recombination is probably rare Asexuality is typical for both primary and secondary cultivars; some secondary cultivars are sexual.</p>	<p>Variable, but most cultivars are propagated sexually The great majority of human cultivars are sexually propagated, but some cultivars are largely clonally (e.g., potato) or strictly clonally propagated (e.g., banana).</p>	<p>Variable, but most cultivars are propagated sexually The great majority of human cultivars are sexually propagated, but some cultivars are largely clonally (e.g., potato) or strictly clonally propagated (e.g., banana).</p>

as a vector of that fungus, then begins to derive nutrition from it, and finally becomes a fungus cultivator. In a third possibility, an insect-fungus association evolves because the insects originally use fungi as a source of antibiotics, as for example in the lower termite *Reticulotermes speratus* that derives antibiotic protection from fungal sclerotia mixed into egg piles (Matsuura et al. 2000). Lastly, insect-associated fungi may have undergone even more complicated evolutionary histories, originating from the exploitation by one insect lineage (e.g., the ancestor of attine ants) of a preexisting insect-fungus association (the fungi ancestrally associating with beetles) when it encounters these insect-adapted fungi in a shared nest environment (e.g., decaying wood; Sanchez-Peña 2005). This latter hypothesis, however, is not supported by the phylogenetic relationships between beetle and ant fungi and is inconsistent with the estimated dates of origin of these insect-fungal associations (i.e., attine agriculture probably arose well before ambrosia beetle agriculture; see Table 2).

For attine ants, it is unclear whether agriculture arose from a state of ancestral fungivory, antibiotic acquisition, or fungal vectoring (Mueller et al. 2001). Termite agriculture most likely originated via the consumption-first route, because many nonfarming termite species are attracted to and feed on fungus-infested wood, which suggests that the nonfarming ancestors of the farming termites may have fed on fungi as well (Batra & Batra 1979, Rouland-Lefevre 2000). The nonfarming ancestors of the fungus-growing beetles appear to have associated with fungi even before the origin of fungiculture, because many of the more primitive nongardening scolytines act as fungal vectors without apparent dependence on their fungal associates (Harrington 2005, Malloch & Blackwell 1993, Six 2003, Six & Klepzig 2004). This suggests non-nutritional dependencies on fungi that predate the origins of fungiculture in the various ambrosia beetle lineages (Six 2003). However, many nonambrosial scolytines carry fungi in mycangia and feed as larvae on ungardened mycelium that colonizes host plants and feed as new adults on spore layers lining pupal chambers (Ayres et al. 2000, Barras 1973, Six & Klepzig 2004, Six & Paine 1998; also A. Adams & D.L. Six, unpublished data), suggesting a stage of nutritional dependency predating the origin of fungiculture. Thus, some of the seven agricultural origins in beetles appear to have followed the transmission-first route, whereas others followed the consumption-first route.

Insect agriculture is restricted to the cultivation of fungi rather than plants, which predominate in human agriculture. Although it is true that some insects are specialized on host plants that they protect from other herbivores (e.g., *Pseudomyrmex* ants protect acacia trees in exchange for shelter and nutritional benefits; Janzen 1966, Hölldobler & Wilson 1990), none of these insect-plant mutualisms possesses all four of the components of agriculture listed above. One could therefore ask what factors have predisposed insects to evolve fungal rather than plant agriculture. Indeed, there are several advantages of fungal agriculture over plant agriculture, and several characteristics of plants may even preclude their easy cultivation. First, unlike fungi, plants typically have stringent light and space requirements, excluding them from cultivation in the subterranean or otherwise enclosed

nests of insects. Such nesting habits may facilitate fungiculture by shielding fungal crops from unwanted consumers (i.e., other fungivores) and wind-dispersed diseases. Furthermore, unlike plants, which usually require regular pollination for long-term cultivation, fungi can be maintained indefinitely in a nonsexual mycelial state, yielding a more consistent food source. Thus, although seeds and plant material can be readily harvested, fungi are likely more cultivatable, explaining the predominance of fungal rather than plant agriculture among insects.

Ant, termite, and most beetle agriculturists are social. All ants and termites are eusocial (characterized by reproductive division of labor, cooperative brood care, and overlap of generations; Hölldobler & Wilson 1990). Only one ambrosia beetle (*Austroplatypus incompertus*) is known to be eusocial (Kent & Simpson 1992); the remainder are subsocial, in which a single female cares for her brood, or communal, in which several reproductive females cooperate in brood care and gardening (Kirkendall et al. 1997). Sociality may have facilitated the evolution of agriculture because of the inherent advantage to agriculture of division of labor, which enables the partitioning of agricultural tasks and augments agricultural efficiency (Hölldobler & Wilson 1990, Hart et al. 2002). In ant and termite farmers, for example, agricultural tasks are partitioned in a conveyor-belt-like series between different worker castes, each specialized on one main task: foraging; processing and cleaning of substrate before incorporation into the garden; planting of mycelium onto new substrate; monitoring and weeding of the garden; or disposal of diseased or senescent garden (Bot et al. 2001a, Hart et al. 2002, Traniello & Leuthold 2000). Task partitioning has so far not been investigated in the ambrosia beetles because of the logistical difficulties of studying beetle behavior in their concealed tunnels. Task partitioning likely facilitates great efficiency in defense against nest and garden robbers (Adams et al. 2000a,b; LaPolla et al. 2002), in monitoring gardens for diseases, and in modulating optimal environmental conditions for crop growth.

4. AGRICULTURAL EVOLUTION AND ECOLOGY

A series of convergent and divergent features of agricultural evolution emerge from a comparative analysis of ant, termite, and beetle fungiculture (summarized in Tables 1–3).

4.1. Cultivar Transmission Between Farmer Generations

In attine ant and xyleborine beetle agriculture, fungal cultivars are transmitted vertically by trophophoresy from parent to offspring generations (Fernández-Marín et al. 2004, Francke-Grosman 1967, Haanstad & Norris 1985, Huber 1905). Female reproductive ants and beetles acquire inocula from their natal gardens, carry these inocula with them in specialized pockets during dispersal flights early in life, and use these inocula as starter cultures for their new gardens. Trophophoretic

TABLE 3 Disease ecology and evolution in ant, termite, beetle, and human agriculture

Disease ecology and evolution	Agriculture in:			
	Attine ants	Macrotermite termites	Xyleborine ambrosia beetles	Humans
1. Disease and pest types	Crops are attacked by fungal and bacterial pests, including specialized (<i>Escovopsis</i>) and unspecialized (<i>Trichoderma</i>) fungal pathogens. Microbial "weeds" (e.g., <i>Xylaria</i> fungi) and fungivorous arthropods (e.g., mites) can also inhabit gardens.	Specialized crop parasites have not been documented, but some species of <i>Xylaria</i> are specialized as weeds of fungus gardens. Common soil fungi are present in gardens as well.	Most ambrosial gardens consist of a complex of mycelial fungi, yeasts, and bacteria. At least some of these are likely to cause disease or act as pests.	Crops are infected by fungal, bacterial, and viral diseases and are attacked by invertebrate and vertebrate herbivores. Weeds are an additional problem in human agriculture.
2. Disease prevalence	Infection of gardens by the crop parasite <i>Escovopsis</i> is frequent and sometimes devastating.	Unknown	Unknown	Ubiquitous and diverse diseases cause immense loss in crop harvests.
3. Defense mechanisms by farmers against crop disease				
3a. Protection from disease				
-Sequestration, sheltering	Present	Present	Present	Absent ^a
-Substrate sterilization or cleaning	Present	Present	Unknown	Uncommon
-Guarding or protecting against disease vectors	Present	Present	Present	Uncommon
-Partitioning of disease-removal tasks to minimize contact between contaminated workers and healthy crop	Present	Present	Unknown	Uncommon
	An uncontaminated worker-caste specializes on gardening: a contaminated worker-caste specializes on disease removal.	Foraging and gardening are performed by different worker castes in many species.		

3b. Eradication of disease	Present	Present	Present	Very rare
-Continuous, intensive monitoring of all crop	Present	Present	Unknown	Present
-Physical weeding	Ant gardeners continuously "weed" and "groom" their gardens, excising infected garden fragments.	Termite gardeners groom garden to eliminate some alien microbes (e.g., <i>Xylaria stromata</i>).	Female beetles continually tend crop gardens but whether directed weeding is part of this activity is unknown.	Physical weeding is more integral in primitive agricultural systems, but less so in modern monoculture systems.
-Application of chemical herbicides	Present	Present	Unknown	Present
	Metapleural and mandibular gland secretions have general antibiotic properties; antibiotics derived from actinomycete bacteria have specific antibiotic effects against <i>Excovopsis</i> parasites.	Salivary gland secretions have antibiotic properties; defensive secretions of soldiers have antibiotic properties, but their sanitary function in the garden is unknown.		Chemical control of diseases and herbivores is an essential element of most modern monoculture systems.
-Routine use of disease-suppressant microbes	Present	Unknown	Unknown	Absent ^b
4. Defense mechanisms of crop against crop diseases	Unknown	Unknown	Unknown	Present
4a. Induced resistance of crop	Present	Unknown	Unknown	Present
4b. Constitutive resistance of crop	Cultivars produce antibiotics, which appear to mediate resistance of some cultivar genotypes to some <i>Excovopsis</i> genotypes.			

^aGreenhouses provide a sequestered environment for some human crops, but greenhouse farming is costly and contributes only a small fraction (about 0.02%, Paulitz & Bélanger 2001) to the total productivity of human agriculture; the bulk of human agriculture operates in open landscapes that expose crops to environmental stresses (fluctuations in moisture and temperature; wind-borne pathogens; migratory herbivores; etc.).

^bSome recently developed uses of disease-suppressant microbes in agricultural pest control are discussed in the text; application of disease-suppressant microbes has yet to become an integral part of mainstream human agriculture (but see Morrissey et al. 2004).

vertical transmission also occurs in two macrotermite groups, except that in one of these two groups the fungal cultivar is transmitted via the king (the single species *Macrotermes bellicosus*), whereas in the other group (the genus *Microtermes*) the fungus is transmitted via the queen. In the few cases where fungal transmission has been studied in the remaining macrotermitines (Johnson 1981, Johnson et al. 1981, Korb & Aanen 2003, Sieber 1983), these termites rely on horizontal acquisition of fungal crops from the environment in each generation.

4.2. Higher-Level Specialization (Clade-Clade Congruence) Between Farmers and Crops

Vertical transmission of cultivars leads to the expectation of clade-clade correspondences and topological congruence between the phylogenies of insect farmers and those of their cultivars. Indeed, in all insect farming systems, major groups of farmers (large clades or paraphyletic grades, e.g., the lower attine ants) strictly specialize on major groups of corresponding fungal cultivars (Figure 2). The expected farmer-cultivar congruence therefore does occur at higher (i.e., broad) phylogenetic levels, possibly because of ancient evolutionary codependencies (e.g., physiological/nutritional requirements of the farmers, cultivation requirements of the fungi, etc.) that strictly preclude switches by farmers to cultivars outside of their specialized major cultivar groups.

Phylogenetic patterns (Figure 2) indicate, however, that within these strictly constrained major cultivar groups, insect-farmer species occasionally switch between fungal species or strains. This combination of lower-level, within-group switching and higher-level major-group specialization in insect farmers would be analogous, in humans, to defined clades of specialized wheat-farmers, rice-farmers, potato-farmers, bean-farmers, etc., each of which is able to switch between varieties within their area of specialization (e.g., between varieties of wheat and to closely related species such as barley), but which cannot switch across major groups (e.g., from wheat to beans). Among insect farmers, switches to novel major cultivar groups have been exceedingly rare evolutionary events (Villesen et al. 2004).

4.3. Lower-Level Specialization on Cultivars

Though low-level switching between cultivar species and strains within major cultivar groups occurs occasionally over evolutionary time, over ecological spans of time most insect farmer species associate with only a very narrow subgroup of cultivars (species or strains). For example, every attine ant species surveyed to date cultivates only a phylogenetically narrow set of cultivars (e.g., a single species of fungus), implicating species specificity between ants and cultivars at very recent levels of evolutionary diversification (Bot et al. 2001b, Green et al. 2002, Schultz et al. 2002). In ambrosia beetles, like ants, only one primary cultivar is associated with a particular beetle species within a particular geographic region (Gebhardt et al. 2004, Batra 1967). However, although most beetles are associated with a species-specific, primary fungus across their entire geographic

ranges, some beetle species associate with different primary cultivars in different geographic regions (Baker 1963, von Arx & Hennebert 1965). Among macrotermite species, there exists considerable variation in cultivar specialization: Some species are limited to a single, unique cultivar, whereas other species cultivate a great diversity of fungal cultivars, which they sometimes share with other, usually closely related, macrotermite species (Kato et al. 2002; D.K. Aanen & P. Eggleton, submitted). The factors underlying variation in termite specialization are unknown, but different cultivars may serve different primary functions, providing specific, termite-adapted enzymes in some cases (leading to termite-cultivar specialization), while providing generalized food in other cases (permitting exchange between termite species; D.K. Aanen, V.I. Ros, H.H. de Fine Licht, C. Roulant-LeFèvre, J. Mitchel, et al., in review).

4.4. Cultivar Sharing and Exchange Between Farmer Species

Even though each attine ant species is specialized on a single cultivar species, a given cultivar species may be cultivated by several sympatric species of ants, and these sympatric ant species may not necessarily be closely related to one another (e.g., they may represent different ant genera) (Bot et al. 2001b, Green et al. 2002, Villesen et al. 2004). Cultivar transfer between ant species may occur via direct or indirect avenues. Direct avenues may include raiding of neighboring colonies (Adams et al. 2000a, Rissing et al. 1989) or, in polygynous species, cofounding of colonies by multiple queens that exchange cultivars or recombine them in the cofounded garden. Indirect avenues may include cultivar escapes from gardens, followed by a free-living (feral) existence and subsequent reincorporation into a symbiosis when a different attine colony imports the free-living strain into its nest (Mueller et al. 1998).

For ambrosia beetles, the available phylogenetic evidence points to cultivar sharing between different sympatric beetle species (Farrell et al. 2001, Gebhardt et al. 2004) but few investigations have addressed this question. Distantly related ambrosia beetle species are sometimes associated with the same cultivar (Gebhardt et al. 2004), implicating fungal exchange, either direct or indirect, as explained above for the ants. Cultivar exchange between and within beetle species may occur when different female beetles colonize the same tree and the fungal associates cross-contaminate adjacent galleries.

In contrast to attine ants and ambrosia beetles that all transmit their cultivars vertically between generations, most macrotermite species acquire their fungi horizontally each generation. This implies that new termite-cultivar combinations arise each generation, which should facilitate cultivar exchange between species, as well as between lineages of the same species. Cultivar surveys of sympatric macrotermite communities indeed indicate that cultivars are generally shared between closely related species via interspecific cultivar exchanges (Aanen et al. 2002; but see the exceptions mentioned above in Section 4.3). Intra-specific cultivar exchanges have so far not been investigated in macrotermitines.

4.5. Propagation of Sexual Versus Asexual Cultivars and Links to Free-Living Cultivar Populations

All vertically transmitted insect cultivars, including the cultivars of attine ants, ambrosia beetles, and termites in the genus *Microtermes* and the species *Macrotermes bellicosus*, seem to be asexually propagated by their insect farmers across multiple farmer generations. In contrast, the horizontally transmitted termite cultivars (propagated by all other macrotermitine genera) undergo regular meiosis and sexual recombination (see above).

Evidence for cultivar asexuality in attine ants comes from DNA fingerprinting studies that indicate that all gardens of a single leafcutter colony contain a single cultivar clone (monoculture) (Kweskin 2003, Poulsen & Boomsma 2005; J. Scott & U.G. Mueller, unpublished data); that identical cultivar clones occur in different colonies of the same geographically widespread attine ant species (Bot et al. 2001b, Green et al. 2002, Mueller et al. 1996); and that different sympatric ant species occasionally share genetically identical cultivar clones (see above; Bot et al. 2001b, Green et al. 2002, Mueller et al. 1998). Contrary to previous suggestions (Chapela et al. 1994), however, attine cultivar clones are not ancient. Although attine cultivars are clonally propagated across many ant generations (Mueller 2002), this clonality is punctuated by occasional recombination events, involving either sexual (meiosis, mating) or parasexual (e.g., mitotic recombination, exchange of haploid nuclei) processes. Evidence for occasional recombination includes: (a) fruiting structures (mushrooms), which are known for cultivars of nearly all genera of attine ants, contradicting the expectation of loss of fruiting ability under strict clonality spanning millions of years (Mueller 2002); (b) rates of allele sequence divergence in attine cultivars that are similar to those of closely related, sexually reproducing fungi (Mueller et al. 1998; S.A. Rehner, unpublished data); and (c) cultivars of the lower attine ants that have close genetic links to free-living fungal populations (Green et al. 2002, Mueller et al. 1998), suggesting that these fungi are capable of moving in and out of the symbiosis, that cultivar lineages may regularly interbreed with wild lineages, or both. Taken together, the genetic and natural-history information suggest predominantly asexual cultivar propagation within ant nests and across many ant generations, punctuated by occasional genetic recombination events.

As in attine ants, a single cultivar monoculture is grown in a single termite colony (Aanen et al. 2002, Katoh et al. 2002). Within termite nests, the *Termitomyces* cultivar is propagated asexually by inoculating fresh garden substrate with asexual spores (Leuthold et al. 1989), and probably also by transplanting mycelium from older to younger gardens. Although *Termitomyces* species have no known free-living populations existing entirely independent of the termite farmers, they have nonetheless retained the ancestral (presymbiotic) condition of regular sexual reproduction, and most *Termitomyces* cultivars are spread from one termite nest to another horizontally via sexual spores produced by fruiting bodies (mushrooms) growing on the external surfaces of mature nests. The *Termitomyces* cultivar of the

termite *Macrotermes natalensis*, for example, has an outcrossing mating system (De Fine Licht et al. 2005). Asexual cultivar propagation spanning several termite generations only occurs in those species with vertical uniparental propagation. Phylogenetic patterns implicate occasional horizontal cultivar exchange between nests of the same and different termite species (Aanen et al. 2002), but whether such horizontal exchange is associated with cultivar sexual reproduction remains unknown.

In xyleborine beetles, the primary fungi are strictly asexual (Jones & Blackwell 1998, Rollins et al. 2001), whereas the less specific, auxiliary fungi are often sexual (Francke-Grosman 1967). A preponderance of asexual reproduction in fungal cultivars also occurs in the primary fungi of all other non-xyleborine ambrosia beetles, whereas, again, the more incidental fungi are often sexual (Six 2003, Six & Paine 1999), suggesting that this may have been the ancestral condition at the origin of the xyleborine beetle-fungus symbiosis.

4.6. Coevolutionary Modifications

Farmer-cultivar specialization enhances the potential for coadaptation, in which evolutionary modification in one of the partners causes a reciprocal coevolutionary modification in the other partner (Futuyma & Slatkin 1983). It is relatively easy to identify evolutionary modifications in the farmer species, such as specialized morphological structures for the trophophoretic transport of cultivars by females during the dispersal flight (e.g., mycangia in the beetles, infrabuccal pocket in the ants), modifications of mandibles and guts of beetle and ant larvae for fungus-feeding (Browne 1961, Schultz & Meier 1995), or the suite of behavioral, glandular, or physiological modifications that form the basis of insect farming. Examples of evolutionary modifications in the cultivars have been more difficult to identify, however, because the cultivated fungi are inherently more difficult to study.

The clearest examples of cultivar modifications are the hyphal-tip swellings (gongylidia) produced by the cultivars of the higher attines and the analogous nodules produced by macrotermitine cultivars. Both gongylidia and nodules are nutrient-rich structures designed for easy harvesting by the farmers, ingesting, and feeding to the larvae or nymphs. Nutrient-rich structures are not known for beetle gardens, although the ambrosia morphology of the beetle cultivars suggests evolutionary modification designed specifically for efficient consumption and digestion by the beetle larvae. Ambrosial growth consists of tightly packed conidiophores with copious spores and is only formed in the presence of the beetles (French & Roeper 1972). Ambrosia formation has not been reported from nonsymbiotic fungus species. Interestingly, two of the major genera of fungi associated with ambrosia beetles (*Ambrosiella*, *Raffaelea*) are each polyphyletic, and the multiple lineages within each genus have converged on the same ambrosial morphology (Blackwell & Jones 1997, Jones & Blackwell 1998), suggesting evolutionary convergence due to selection. Other likely coevolutionary modifications that have

yet to be investigated include predominantly asexual reproduction in the insect cultivars while under cultivation and the cultivars' capacity to survive storage in the dispersal pockets of the beetles and ants, or the passage through the alimentary canal of the termites.

4.7. Symbiont Choice and "Artificial Selection" of Cultivars

From an evolutionary perspective, insect agriculture represents a case of cooperative interaction between farmer and cultivar lineages, each exploiting the other for its own reproductive purposes (Herre et al. 1999, Mueller 2002). Such cooperative interactions are frequently unstable and can erode over evolutionary time, for example, when mutant overexploiters arise (so-called cheater cultivars) and invade a mutualism. A series of additional farmer-cultivar conflicts are predicted that could destabilize the mutualism (Mueller 2002, Aanen & Boomsma 2005, Schultz et al. 2005), but at least two evolutionary mechanisms preserve the cooperative nature of the farmer-cultivar association: First, partner feedback, inherent in vertical cultivar transmission, is an automatic feedback mechanism in which an uncooperative partner reduces the other partner's fitness to the extent that it reduces its own fitness as well; and, second, partner (symbiont) choice in which farmers favor associations with productive cultivars and discriminate against inferior cultivars in specific choice situations (e.g., a choice between cultivar strains that may coexist in a garden or in proximate gardens, exercised either by workers during the planting of new gardens or by reproductives when choosing a cultivar strain for dispersal). In cases where the evolutionary rates differ between two cooperating partners, partner choice is a particularly important mechanism (Sachs et al. 2004). The slower-evolving partner (e.g., the insect farmer) is expected to exert the choice between variants of the faster evolving partner (e.g., the fungal cultivar), and thus the slower-evolving farmer imposes selection favoring beneficial symbiont variants (e.g., productive cultivars) and prevents the spread of nonbeneficial cultivar mutants (e.g., degenerate or suboptimal cultivars; Mueller 2002, Sachs et al. 2004). Symbiont choice has yet to be investigated for termite and beetle farmers, but ant farmers are able to discern surprisingly fine genotypic differences between cultivars (Mueller et al. 2004; also N.K. Advani & U.G. Mueller, submitted), suggesting that cultivar diversity in ant gardens, arising, for example, through mutation in a garden or through the import of novel strains, may evolve under an analog of "artificial selection."

5. ROLE OF DISEASE IN INSECT AGRICULTURE

"Weedy" fungi frequently invade the gardens of ants, termites, and beetles, and may coexist at low or manageable levels along with the crop. If the gardening insects are removed or if they abandon their nests, the garden is quickly overrun by these weeds (Batra & Batra 1979, Norris 1979). One such group of weeds, wood-degrading

fungi in the endophytic genus *Xylaria*, is found in most gardens of fungus-growing ants and termites, probably because it is introduced with garden substrate (Fisher et al. 1995; V.I. Ros, A.J. Debets, T. Læssøe, D.K. Aanen, submitted; N.M. Gerardo & U.G. Mueller, personal observation). Though weeds like *Xylaria* do not directly attack the cultivar, they compete with it for nutrients and thus decrease crop yield (V.I. Ros, A.J. Debets, T. Læssøe, D.K. Aanen, submitted). *Escovopsis* species, ascomycete fungi found in colonies of fungus-growing ants, are specialized parasites that subsist directly on the cultivars and reduce the nutrients available to the ants (Currie 2001a, Currie et al. 2003b). Weed fungi and bacteria are also known in termite and beetle agriculture, but have yet to be studied in detail (Six 2003; V.I. Ros, A.J. Debets, T. Læssøe, D.K. Aanen, submitted; D.K. Aanen, unpublished data).

Escovopsis infections reduce garden productivity, which in turn reduces ant colony growth and the likelihood of colony survival (Currie 2001a, 2001b, Currie et al. 1999a). *Escovopsis* is geographically widespread and taxonomically diverse. The parasite has been isolated from colonies of every attine genus throughout their geographic ranges, and particular *Escovopsis* lineages are specialized to parasitize particular cultivar lineages. This high degree of host specificity suggests a long history of host-parasite coevolution in *Escovopsis*, in which the cultivars, the ants, and their mutualistic bacteria have likely coadapted to defend against *Escovopsis* attack and in which each *Escovopsis* species has become narrowly specialized to overcome the defenses of some hosts but not others (Gerardo et al. 2004).

6. DISEASE AND MICROBIAL MANAGEMENT STRATEGIES

In response to the persistent selection pressure imposed by weeds and pathogens, fungus-farming insects have evolved an arsenal of strategies for preventing and suppressing infection (Table 3).

6.1. Sequestration of Gardens

All insect agriculturists sequester and separate their gardens from the surrounding environment, e.g., by growing their gardens in underground chambers or galleries in wood, or by covering them with a protective mycelial veil in the case of some *Apterostigma* ants (Villesen et al. 2004). Although sequestered nests are the ancestral condition in fungus-growing ants, termites, and beetles, and although sequestered nests may serve other agricultural functions such as the regulation of temperature and humidity, sequestration no doubt also buffers the garden against fungivores, wind-borne pathogens, and arthropod vectors of diseases (e.g., mites, collembolans).

6.2. Maintenance of Cultivar Genetic Variability

Although attine ants clonally propagate their cultivars across generations over short evolutionary time spans, no attine cultivar has been found to be an ancient clone. Instead, the evidence indicates that the lower attines occasionally acquire new cultivars from wild (free-living), sexually reproducing fungal populations and that both lower and higher attines occasionally acquire new cultivars from the nests of other attines. The cultivars of higher attines, which are not known to have free-living populations (Mueller 2002), nonetheless retain the ability to fruit (Mueller 2002) and demonstrate patterns of DNA-sequence diversity that suggest occasional genetic recombination through self-mating (S.A. Rehner, personal communication) or through true intercrossing between different cultivar strains (M. Bacci, personal communication). Thus, whereas the crop employed by any attine ant colony at any given time is a clonally propagated monoculture, the genetic variability and resilience necessary for long-term disease management resides in the fungal population external to the nest. As already noted, sexual reproduction is the norm in the cultivars of those termites that reacquire their cultivars horizontally each generation, and sexual reproduction may also occur in the fungi of those termites that transmit their cultivars vertically across generations. Whether the primary cultivars of the ambrosia beetles occasionally reproduce sexually remains unknown. At least for the termites and ants, then, and possibly for the beetles as well, access to a population-level reservoir of cultivar genetic variability is a consistent feature of insect agriculture that may provide alternative crops for dealing with disease.

6.3. Intensive Monitoring of Gardens, Weeding, and Herbicide Application

All insect agriculturists constantly inspect their gardens, and no part of the garden is left untended for periods of time sufficient to allow the establishment and spread of diseases and fungivores (Batra & Batra 1979, Currie & Stuart 2001). Insect agriculturists, particularly the ants and termites, are able to invest in such intensive monitoring because their societies possess a nonreproducing worker caste, a large portion of which is dedicated to garden care. In the beetles, the relatively small size of the garden allows for intensive monitoring by a single female or by a small family of females. Intensive monitoring ensures that diseases are discovered and eradicated in the early stages of infection before they are able to spread and cause significant crop loss. Early detection is an effective defense against novel disease mutants that might evolve greater virulence if left untreated, because in the early stages of infection these strains can more readily be controlled with standard treatments.

Garden treatment in attine ants includes the use of secretions from their metapleural and mandibular glands to clean substrate as it is brought into the nest, probably removing some or most weeds and pathogens from the surface of the substrate before it is added to the fungus garden (Maschwitz et al. 1970, Ortius-Lechner et al. 2000). Although antibiotic-producing glands have not been studied in fungus-growing termites, some secretions of nonfungus-growing termites have

antibiotic properties (Rosengaus et al. 1998, 2004). Antimicrobial glands in ambrosia beetles remain unknown and unstudied. In addition to glandular secretions, attine ants have another antimicrobial defense. Some or all of their integuments are covered with actinomycete bacteria. These bacteria are known to inhibit *Escovopsis* growth (Currie et al. 1999b), and experimental reduction of actinomycetes in colonies increases *Escovopsis* infection (Currie et al. 2003a). Garden bacteria in the genus *Burkholderia* (Santos et al. 2004) also provide antibiotics that provide protection against the garden parasite *Escovopsis* and against entomopathogenic diseases of the ants themselves. Termite gardens contain actinomycetes and other bacteria (Batra & Batra 1979), and beetle gardens contain a great diversity of bacterial secondary symbionts; however, the exact roles of these bacterial associates remain unknown.

6.4. Microbial Buffering

Beyond the known antibiotic-producing, disease-suppressing bacteria in attine colonies (Currie 2001a, Currie et al. 1999b, Santos et al. 2004), other secondary bacteria and fungi occur in insect gardens (Carreiro et al. 1997, Craven et al. 1970; C. Wang & U.G. Mueller, unpublished data), but their roles remain largely unknown. Although some of these secondary microbes may be neutral or detrimental to garden health and productivity, others may provide disease-modulating effects through competitive exclusion, antibiotic suppression of disease-causing microbes, resistance induction, or other mechanisms of microbial interaction. Competitive exclusion, disease suppression, and resistance induction have been demonstrated in both experimental and natural microbial systems (Hood 2003, Paulitz & Bélanger 2001, Wille et al. 2001). Some secondary microbes may even facultatively switch between beneficial and detrimental roles, depending on garden growth conditions, seasonal factors, or interactions with the insects or other microbes. For example, although the detrimental effects of *Escovopsis* are obvious in natural garden outbreaks and in interactions with cultivars in vitro (Currie 2001a,b; Currie et al. 1999a; Gerardo et al. 2004), it remains an untested possibility that *Escovopsis* may provide beneficial effects when present at low levels in the garden matrix.

Secondary microbes in termite gardens remain uninvestigated, but the possible significance of a secondary microbial flora in beetle gardens has been recognized for some time (Norris 1965). Norris (1965) suggested that it is the microbial complex as a whole (filamentous fungi, yeasts, and bacteria), rather than the dominant ambrosia fungus per se, that allows the beetles to exploit nutrient-poor substrates such as wood. Norris did not speculate on any additional roles for the secondary microbes, such as suppressing diseases, but such auxiliary roles deserve further study.

6.5. Management of Crop-Associated Microbial Consortia

If, as recent evidence indicates, secondary microbes serve ancillary functions in gardens by buffering against disease organisms or by producing antibiotics,

enzymes, and metabolites, it is possible that insect farmers have evolved the capacity to manage these microbial consortia. Such microbial management strategies by insect farmers could include the following three methods.

6.5.1. STERILIZATION OF SUBSTRATE BEFORE INCORPORATION INTO THE GARDEN (ANTS, TERMITES) OR USE OF ESSENTIALLY STERILE SUBSTRATE FROM THE OUTSET (BEETLES) In termites, the passage of substrate through the gut before incorporation into the garden probably eliminates many unwanted microbes and may increase the abundance of desired microbes (Figure 1). In attine ants, the considerable effort spent cleaning substrate surfaces appears to partially sterilize the substrate (Weber 1972). In the case of the ambrosia beetles, gardening occurs in a closed system because the beetles do not need to leave the nest to forage and because galleries are excavated in what is essentially a sterile medium, the sapwood or heartwood of living or recently killed trees, which are generally free of endophytic fungi and other microbes. This closed system greatly reduces the potential for accidental introduction of unwanted microbes and likely facilitates the management of desired microbes in beetle gardens.

6.5.2. SPATIALLY STRUCTURED GARDEN MATRIX Structuring of gardens allows insect farmers to assess properties of particular, localized crop-microbe consortia. Any unwanted mutant genotypes, arising locally under particular microbe-microbe competitions, thus can be identified indirectly through the detection of their detrimental effects on the properties of the subgarden, and that subgarden piece can then be excised. Conversely, novel microbial mutants with beneficial effects can be identified indirectly by their beneficial effects on the subgarden, and preferentially subcultured and propagated across the rest of the garden (Figure 1). Such “symbiont-community choice” is possible only because of the fixed garden matrix, enabling farmers to assess properties of local consortia.

6.5.3. CONTINUOUS CO-OCCURRENCE OF GARDENS OF ALL AGES IN CLOSE PROXIMITY, RANGING FROM “UNPLANTED” TO MATURE GARDENS Coexistence of gardens at different developmental stages is inherent in the vertical structuring of attine and termite gardens into younger subgardens at the top and older subgardens at the bottom. A range of differently-aged gardens allows farmers to efficiently practice one-way, selective transfer of only beneficial crop-microbe consortia from mature to younger gardens. Age-structuring also delays the spread of mutant microbes from older garden material to younger, more sterile gardens and thus prevents deterioration of the symbiont-community.

6.6. Multipartner Coevolution and Coevolving Antibiotic Defenses

One hypothetical advantage of secondary mutualistic microbes is that, unlike the insect farmers, microbes can potentially evolve at the same rate as the coevolving

garden pests, enabling mutualistic insect-microbe systems to respond rapidly to the emergence of novel disease genotypes (Currie 2001a, Mueller & Gerardo 2002). Although such rapid microbial antibiotic defenses would obviously confer clear advantages, their evolutionary maintenance remains unclear. One possibility is that any single farmer society may have access to a diverse array of microbes from which it can select particular, desired types as needed. This scenario raises the question of how the insect farmers could maintain such a diverse array in their colonies in the face of both competition between microbes and the regular bottlenecks of the entire microbial “library” that presumably occurs at the founding of every new insect colony. Alternatively, the associated secondary microbes may be inherently fast-mutating, so that novel beneficial genotypes can rapidly arise to muster an appropriate defensive response. This scenario raises the question of how the most beneficial genotypes are recognized by the insect farmers and chosen for selective “amplification” against particular pathogens. The lack of clear evolutionary mechanisms for maintaining functional associations with coevolving, mutualistic microbes is not trivial, and future research needs to assess not only the diversity of microbial genotypes within single farmer colonies, but also to identify the mechanisms underlying adaptive symbiont-choice selection of beneficial, novel microbial genotypes. Future research also needs to address whether the coevolution of several, mutualistically-aligned partners (i.e., a “multidimensional alliance” of ants, cultivar, and auxiliary microbes), each mustering its own defense, provides for a more evolutionarily stable disease-management strategy compared to a strategy in which the insect farmers act alone in a coevolutionary arms race against particular pathogens.

7. PRINCIPLES OF INSECT AGRICULTURE: LESSONS FOR HUMAN AGRICULTURE?

Perhaps the most striking feature of insect agriculture is the long-term cultivation of clonal monocultures. Monoculture increases agricultural efficiency through an economy of scale (Wolfe 1985), and clonality preserves the desirable properties of the crop by eliminating sexual recombination, but these advantages come at two costs: (a) increased vulnerability to the rapid spread of disease mutants (Barrett 1981, Shipton 1977, Mitchell et al. 2002, Mundt 2002, Peacock et al. 2001, Piper et al. 1996, Wolfe 1985), and (b) decreased resistance to fast-evolving diseases due to decreased genetic variability in the crop (Barrett 1981, Gustafson et al. 2003, Hamilton et al. 1990, Jaenike 1978, Zhu et al. 2000). These economic trade-offs (i.e., monoculture/clonality efficiency versus disease vulnerability) apply to both human and insect farmers.

The insect farmers’ solution to the monoculture-disease problem appears to be not a single, “magic bullet” strategy (e.g., sole reliance on pesticides), but rather a combination of several strategies consisting of (a) crop sequestration, (b) intensive monitoring of crops for diseases, (c) access to a population-level reservoir of crop

genetic variability, and (*d*) management of disease-suppressant microbes associated with the crop (Table 3). Of these strategies, large-scale crop sequestration is the least feasible in human agriculture because human crops need exposure to sunlight and because greenhouse cultivation is costly (Paulitz & Bélanger 2001). Intensive (e.g., daily) monitoring of every single crop plant for diseases may be feasible for some crops (e.g., in greenhouse environments); however, hourly monitoring of the kind implemented in insect agriculture seems cost-prohibitive for human agriculture at large.

A more novel approach is to design human agricultural systems that more efficiently take advantage of the microbial consortia that are known to play beneficial roles in crop nutrient uptake and disease resistance (Morrissey et al. 2004, Paulitz & Bélanger 2001, Wardle et al. 2004). Microbes of the rhizosphere (e.g., nitrogen-fixing bacteria and mycorrhizal fungi) have long been managed as critical associates of certain crops and trees (Finlay 2004, Johansson et al. 2004). More recently, disease-suppressant bacteria have been discovered that live on the root exudates of crops and produce antibiotics that protect the crop against pathogens (Haas & Keel 2003, Mazzola 2004, Morrissey et al. 2004, Weller et al. 2002, Whipps 2001). Disease-suppressant effects on crop plants have also been documented for phyllosphere microbes (Lindow & Brandl 2003) and endophytic microbes (Narisawa et al. 2002, Sturz et al. 2000). Agricultural research on rhizosphere, phyllosphere, and endophyte microbes of human crops is a very new field, however, and many beneficial microbes remain to be discovered and put to use (e.g., inoculation of crops with phyllosphere microbes to deter herbivores or to suppress airborne diseases).

Two problems commonly encountered in human agricultural experiments with beneficial microbial consortia are, first, that the composition of microbial species is difficult to manage and stabilize (Garbeva et al. 2004, Mazzola 2004), and, second, that beneficial microbes can rapidly evolve into detrimental ones (Alves et al. 2003, Morrissey et al. 2002). The farming insects' solution to these problems appears to consist of (*a*) selection on spatially limited microbial consortia (i.e., high-resolution, spatial separation of evolutionary processes, preventing the uncontrolled spread of microbes from inferior consortia); (*b*) propagation of crops with fast generational turnovers, thus minimizing the time for the evolution of any deleterious traits in the microbes; and (*c*) partial or complete sterilization of the substrate prior to planting, thus minimizing the influx of microbial contaminants into a largely closed agricultural system.

Perhaps it is from strategies such as these that humans have the most to learn from insect farmers, certainly if disease-suppressant microbes are ever to be managed in human agriculture (Morrissey et al. 2004). In developing these strategies, agriculturists would need to keep in mind that, during the domestication process, current human crops were not necessarily selected for capacities to interact with auxiliary microbes, i.e., the alleles in the wild ancestors optimally mediating such interactions may have been lost during the domestication process. Thus, a full evaluation of the potential uses of auxiliary microbes in human agriculture may

require the study of the microbial consortia associated with the wild populations from which human-domesticated crops were originally derived. Such domestication within the context of coevolving and codomesticated microbial consortia may well be the key element explaining the 50-million year old agricultural success of the insect farmers.

ACKNOWLEDGMENTS

This review synthesizes work supported by the National Science Foundation (awards DEB-9707209 and IRCEB DEB-0110073 to U.G.M. & T.R.S.; CAREER DEB-9983879 to U.G.M.; DEB-0308757 to N.M.G.; INT-0434171 to D.L.S.), the Smithsonian Tropical Research Institute (to U.G.M., N.M.G. & D.K.A.), the Smithsonian Institution (to T.R.S.), and the Danish Natural Science Research Council (to D.K.A.). Some of the ideas presented here evolved out of discussions with P. Abbot, L. Ancel-Meyers, M. Bacci, K. Boomsma, J. Bull, Y. Carriere, C. Currie, A. Herre, A. Himler, N. Mehdiabadi, A. Mikheyev, T. Murakami, F. Pagnocca, S. Rehner, J. Sachs, R. Samuels, J. Scott, S. Solomon, L. Thomashow, and B. Wcislo. Special thanks to P. Abbot, R. Adams, K. Boomsma, S. Brady, S. Bruschi, E. Caldera, H. De Fine Licht, M. Dijkstra, A. Green, A. Himler, D. Kronauer, J. LaPolla, C. Marshall, A. Mikheyev, J. Miller, A. Mosegaard, D. Nash, J. Pedersen, M. Poulsen, C. Rabeling, J. Scott, B. Slippers, S. Solomon, J. Sosa-Calvo, and one anonymous reviewer for constructive comments on the manuscript; and to M. Bacci, A. Himler, C. Currie, and S. Rehner for unpublished information. We are most grateful to Greg Dimijian, Susanne Kühnholz, and Karen Machielsen for photos in Figure 2; and especially to Barrett Klein for producing Figure 1.

The *Annual Review of Ecology, Evolution, and Systematics* is online at <http://ecolsys.annualreviews.org>

LITERATURE CITED

- Aanen DK, Boomsma JJ. 2005. Evolutionary dynamics of the mutualistic symbiosis between fungus-growing termites and *Termitomyces* fungi. See Vega & Blackwell 2005, pp. 191–210
- Aanen DK, Eggleton P, Rouland-Lefèvre C, Guldberg-Frøsløv T, Rosendahl S, Boomsma JJ. 2002. The evolution of fungus-growing termites and their mutualistic fungal symbionts. *Proc. Natl. Acad. Sci. USA* 99:14887–92
- Abe T, Bignell DE, Higashi M, eds. 2000. *Termites: Evolution, Sociality, Symbiosis, Ecology*. Dordrecht: Kluwer Acad. 488 pp.
- Adams RMM, Mueller UG, Green AM, Narozniak JM. 2000a. Garden sharing and garden stealing in fungus-growing ants. *Naturwissenschaften* 87:491–93
- Adams RMM, Mueller UG, Schultz TR, Norden B. 2000b. Agro-predation: usurpation of attine fungus gardens by *Megalomyrmex* ants. *Naturwissenschaften* 87:549–54
- Alves BJR, Boddey RM, Urquiaga S. 2003. The success of BNF in soybean in Brazil. *Plant Soil* 252:1–9
- Ayres MP, Wilkens RT, Ruel JJ, Lombardero MJ, Vallery E. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi (Coleoptera: Scolytidae). *Ecology* 81:2198–10

- Baker JM. 1963. Ambrosia beetles and their fungi, with particular reference to *Platypus cylindricus* Fab. *Symp. Soc. Gen. Microbiol.* 13:232–65
- Barras SJ. 1973. Reduction of progeny and development in the southern pine beetles following removal of symbiotic fungi. *Can. Entomol.* 105:1295–99
- Barrett JA. 1981. The evolutionary consequences of monocultures. In *Genetic Consequences of Man-Made Change*, ed. JA Bishop, LM Cook, pp. 209–48. London: Academic. 409 pp.
- Bass M, Cherrett JM. 1995. Fungal hyphae as a source of nutrients for the leaf-cutting ant *Atta sexdens*. *Physiol. Entomol.* 20:1–6
- Batra LR. 1966. Ambrosia fungi: extent of specificity to ambrosia beetles. *Science* 153:193–95
- Batra LR. 1967. Ambrosia fungi: a taxonomic revision and nutritional studies of some species. *Mycologia* 59:976–1017
- Batra LR. 1979. *Insect-Fungus Symbiosis*. Monclair: Allanheld, Osmun & Co. 288 pp.
- Batra LR, Batra SWT. 1979. Termite-fungus mutualism. See Batra 1979, pp. 117–63
- Beaver RA. 1989. Insect-fungus relationships in the bark and ambrosia beetles. In *Insect-fungus Interactions, 14th Symp. R. Entomol. Soc. London*, ed. N Wilding, NM Collins, PM Hammond, JF Webber, pp. 121–43. London: Academic
- Blackwell M, Jones K. 1997. Taxonomic diversity and interactions of insect-associated ascomycetes. *Biodivers. Conserv.* 6:689–99
- Borden JH. 1988. The striped ambrosia beetles. In *Dynamics of Forest Insect Populations*, ed. AA Berryman, pp. 579–96. New York: Plenum. 624 pp.
- Bot ANM, Currie CR, Hart AG, Boomsma JJ. 2001a. Waste management in leaf-cutting ants. *Ethol. Ecol. Behav.* 13:225–37
- Bot ANM, Rehner SA, Boomsma JJ. 2001b. Partial incompatibility between ants and symbiotic fungi in two sympatric species of *Acromyrmex* leaf-cutting ants. *Evolution* 55:1980–91
- Browne F. 1961. The biology of Malayan Scolytidae and Platypodidae. *Malay. For. Rec.* 22:1–255
- Carreiro SC, Pagnocca FC, Bueno OC, Bacci M, Hebling MJA, de Silva OA. 1997. Yeasts associated with the nests of the leaf-cutting ant *Atta sexdens rubropilosa* Forel, 1908. *Antonie van Leeuwenhoek* 71:243–48
- Chapela IH, Rehner SA, Schultz TR, Mueller UG. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266:1691–94
- Craven SE, Dix MD, Michaels GE. 1970. Attine fungus gardens contain yeasts. *Science* 169:184–86
- Currie CR. 2001a. A community of ants, fungi, and bacteria: a multilateral approach to studying symbiosis. *Annu. Rev. Microbiol.* 55:357–80
- Currie CR. 2001b. Prevalence and impact of a virulent parasite on a tripartite mutualism. *Oecologia* 128:99–106
- Currie CR, Bot ANM, Boomsma JJ. 2003a. Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. *Oikos* 101:91–102
- Currie CR, Mueller UG, Malloch D. 1999a. The agricultural pathology of ant fungus gardens. *Proc. Natl. Acad. Sci. USA* 96:7998–8002
- Currie CR, Scott JA, Summerbell RC, Malloch D. 1999b. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature* 398:701–4
- Currie CR, Stuart AE. 2001. Weeding and grooming of pathogens in agriculture by ants. *Proc. R. Soc. London Ser. B* 268:1033–39
- Currie CR, Wong B, Stuart AE, Schultz TR, Rehner SA, et al. 2003b. Ancient tripartite coevolution in the attine ant-microbe symbiosis. *Science* 299:386–88
- Darlington JEC. 1994. Nutrition and evolution in fungus-growing termites. In *Nourishment and Evolution in Insect Societies*, ed. JH Hunt, CA Nalepa, pp. 105–30. Boulder, CO: Westview. 449 pp.
- de Fine Licht HH, Andersen A, Aanen DK. 2005. *Termitomyces* sp. associated with the termite *Macrotermes natalensis* has a

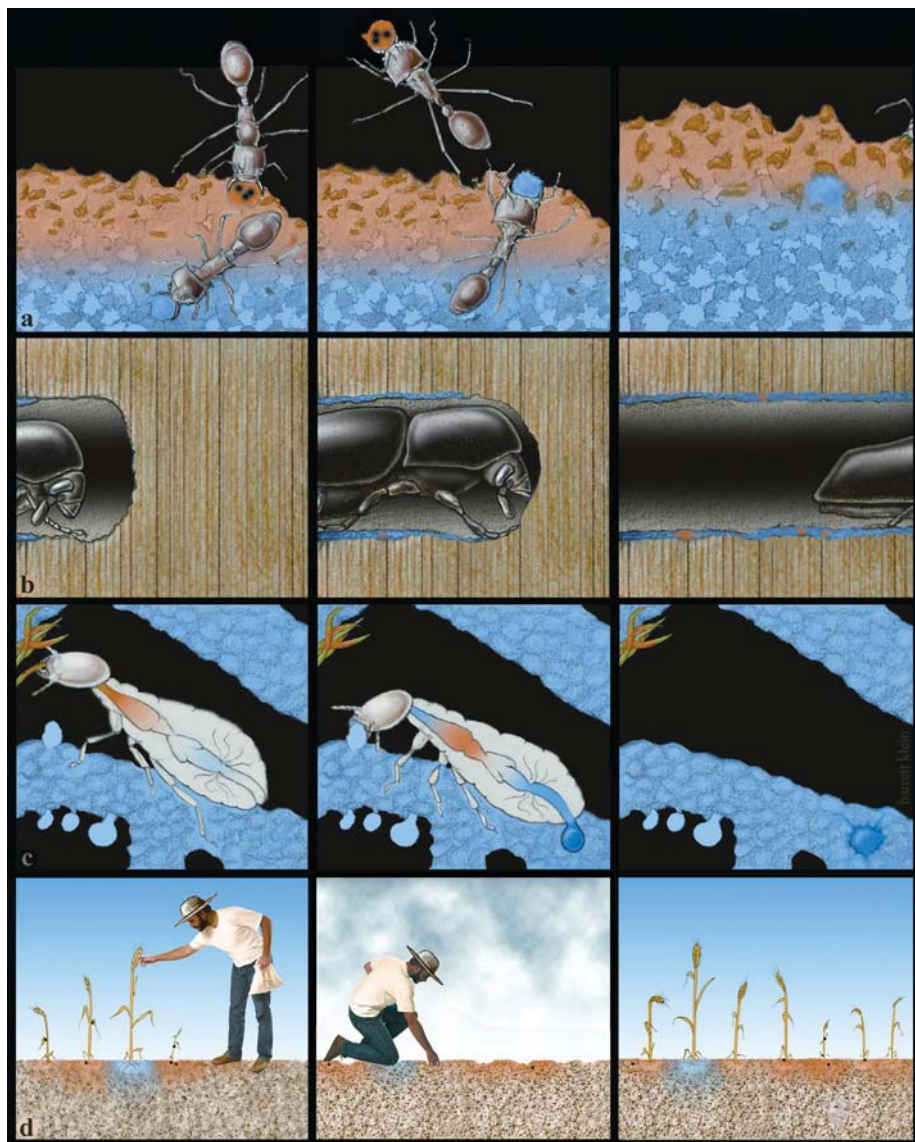
- heterothallic mating system and multinucleate cells. *Mycol. Res.* In press
- Denison RF, Kiers ET, West SA. 2003. Darwinian agriculture: When can humans find solutions beyond the reach of natural selection? *Q. Rev. Biol.* 78:145–68
- Diamond J. 1997. *Guns, Germs, and Steel: the Fates of Human Societies*. New York: Norton. 480 pp.
- Farrell BD, Sesqueira AS, O'Meara BC, Normark BB, Chung JH, et al. 2001. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). *Evolution* 55:2011–27
- Fernández-Marín H, Zimmerman JK, Wcislo WT. 2004. Ecological traits and evolutionary sequences of nest establishment in fungus-growing ants (Hymenoptera, Formicidae, Attini). *Biol. J. Linn. Soc.* 81:39–48
- Finlay RD. 2004. Mycorrhizal fungi and their multifunctional roles. *Mycologist* 18:91–96
- Fisher PJ, Stradling DJ, Sutton BC, Petrini LE. 1995. Microfungi in the fungus gardens of the leaf-cutting ant *Atta cephalotes*: a preliminary study. *Mycol. Res.* 100:541–46
- Francke-Grosman H. 1967. Ectosymbiosis in wood-inhabiting insects. In *Symbiosis*, ed. SM Henry, 2:141–205. New York: Academic. 443 pp.
- French JRJ, Roeper RA. 1972. Interactions of the ambrosia beetle *Xyleborus dispar* (Coleoptera: Scolytidae) with its symbiotic fungus, *Ambrosiella hartigii* (Fungi Imperfecti). *Can. Entomol.* 104:1635–41
- Futuyma DJ, Slatkin M. 1983. *Coevolution*. Sunderland, MA: Sinauer. 555 pp.
- Garbeva P, van Veen JA, van Elsas JD. 2004. Microbial diversity in soil: selection of microbial populations by plants and soil type and implications for disease suppressiveness. *Annu. Rev. Phytopathol.* 42:243–70
- Gebhardt H, Bergerow D, Oberwinkler F. 2004. Identification of the ambrosia fungus of *Xyleborus monographus* and *X. dryographus* (Curculionidae, Scolytinae). *Mycol. Prog.* 3:95–102
- Gerardo NM, Mueller UG, Price SL, Currie CR. 2004. Exploitation of a mutualism: specialization of fungal parasites on cultivars in the attine ant symbiosis. *Proc. R. Soc. London Ser. B* 271:1791–98
- Grassé PP. 1959. Une nouveau type de symbiose: La meule alimentaire des termites champignonnistes. *Nature* 3293:385–89
- Green AM, Adams RM, Mueller UG. 2002. Extensive exchange of fungal cultivars between two sympatric species of fungus-growing ants. *Mol. Ecol.* 11:191–95
- Green RE, Cornell SJ, Scharlemann JPW, Balmford A. 2005. Farming and the fate of wild nature. *Science* 307:550–55
- Gustafson DM, Boe A, Jin Y. 2003. Genetic variation for *Puccinia emaculata* infection in switchgrass. *Crop Sci.* 43:755–59
- Haanstad JO, Norris DM. 1985. Microbial symbionts of the ambrosia beetle *Xyloterinus politus*. *Microb. Ecol.* 11:267–76
- Haas D, Keel C. 2003. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu. Rev. Phytopathol.* 41:117–53
- Hamilton WD, Axelrod R, Tanese R. 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl. Acad. Sci. USA* 87:3566–73
- Harrington TC. 2005. Ecology and evolution of mycophagous bark beetles and their fungal partners. See Vega & Blackwell 2005, pp. 257–91
- Hart AG, Anderson C, Ratnieks FL. 2002. Task partitioning in leafcutting ants. *Acta Ethol.* 5:1–11
- Herre EA, Knowlton N, Mueller UG, Rehner SA. 1999. The evolution of mutualisms: exploring the paths between conflict and cooperation. *Trends Ecol. Evol.* 14:49–53
- Hölldobler B, Wilson EO. 1990. *The Ants*. Cambridge: Harvard Univ. Press. 732 pp.
- Hood ME. 2003. Dynamics of multiple infection and within-host competition by the anther-smut pathogen. *Am. Nat.* 162:122–33
- Huber J. 1905. Über die Koloniegründung bei *Atta sexdens*. *Biol. Cent.* 25:606–19, 625–35
- Jaenike J. 1978. A hypothesis to account for the

- maintenance of sex within populations. *Evol. Theory* 3:191–94
- Janzen DH. 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20:249–75
- Johansson JF, Paul LR, Finlay RD. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS Microbiol. Ecol.* 48:1–13
- Johnson RA. 1981. Colony development and establishment of the fungus comb in *Macrotermes* sp. nr. *Usambaricus* (Isoptera, Macrotermitinae) from Nigeria. *Insect. Soc.* 28:3–12
- Johnson RA, Thomas RJ, Wood TG, Swift MJ. 1981. The inoculation of the fungus comb in newly founded colonies of the Macrotermitinae (Isoptera). *J. Nat. Hist.* 15:751–56
- Jones KG, Blackwell M. 1998. Phylogenetic analysis of ambrosial species in the genus *Raffaelea* based on 18S rDNA sequences. *Mycol. Res.* 102:661–65
- Jordal BH. 2002. Elongation factor 1 resolves the monophyly of the haploid ambrosia beetles Xyleborini (Coleoptera: Curculionidae). *Insect Mol. Biol.* 11:453–65
- Katoh H, Miura T, Maekawi K, Shinzato N, Matsumoto T. 2002. Genetic variation of symbiotic fungi cultivated by the macrotermite *Odontotermes formosanus* (Isoptera: Termitidae) in the Ryukyu Archipelago. *Mol. Ecol.* 11:1565–72
- Kent DS, Simpson JS. 1992. Eusociality in the beetle *Austroplatypus incomperatus* (Coleoptera: Curculionidae). *Naturwissenschaften* 79:86–87
- Kingsolver JG, Norris DM. 1977. External morphology of *Xyleborus ferrugineus* (Fabr.) (Coleoptera: Scolytidae) I. Head and prothorax of adult male and females. *J. Morphol.* 154:147–56
- Kinuura H. 1995. Symbiotic fungi associated with ambrosia beetles. *Jpn. Agric. Res. Q.* 29:57–63
- Kirkendall LR, Kent DS, Raffa KF. 1997. Interactions among males, females, and offspring in bark and ambrosia beetles: the significance of living in tunnels for the evolution of social behavior. In *Evolution of Social Behavior in Insects and Arachnids*, ed. JC Choe, BJ Crespi, pp. 181–215. Cambridge, UK: Cambridge Univ. Press. 561 pp.
- Kok LT, Norris DM, Chu HM. 1970. Sterol metabolism as a basis for a mutualistic symbiosis. *Nature* 225:661–62
- Korb J, Aanen DK. 2003. The evolution of uniparental transmission of fungal symbionts in fungus-growing termites (Macrotermitinae). *Behav. Ecol. Sociobiol.* 53:65–71
- Kweskin M. 2003. *Molecular and behavioral ecology of fungus-growing ants and their fungi*. MA thesis. Univ. Tex., Austin. 79 pp.
- LaPolla JS, Mueller UG, Seid M, Cover SP. 2002. Predation by the army ant *Neivamyrmex rugulosus* on the fungus-growing ant *Trachymyrmex arizonensis*. *Insect. Soc.* 49:251–56
- Leuthold RH, Badertscher S, Imboden H. 1989. The inoculation of newly formed fungus comb with *Termitomyces* in *Macrotermes* colonies (Isoptera, Macrotermitinae). *Insect. Soc.* 36:328–38
- Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Appl. Environ. Microbiol.* 69:1875–83
- Malloch D, Blackwell M. 1993. Dispersal biology of the ophiostomatoid fungi. In *Ceratocystis and Ophiostoma: Taxonomy, Ecology and Pathogenicity*, ed. MJ Wingfield, KA Seifert, J Webber, pp. 195–206. St. Paul: Am. Phytopathol. Soc. 304 pp.
- Maschwitz U, Hölldobler B. 1970. Der Kartonbau bei *Lasius fuliginosus* Latr. (Hymenoptera: Formicidae). *Z. Vgl. Physiol.* 66:176–89
- Maschwitz U, Koob K, Schildknecht H. 1970. Ein Beitrag zur Funktion der Metathorakdrüse der Ameisen. *J. Insect Physiol.* 16:387–404
- Matsuura K, Tanaka C, Nishida T. 2000. Symbiosis of a termite and a sclerotium-forming fungus: *Sclerotia mimic* termite eggs. *Ecol. Res.* 15:405–14
- Mazzola M. 2004. Assessment and management of soil microbial community structure for disease suppression. *Annu. Rev. Phytopathol.* 42:35–59

- Mitchell CE, Tilman D, Groth JV. 2002. Effects of grassland plant species diversity, abundance, and composition on foliar fungal disease. *Ecology* 83:1713–26
- Morelet M. 1998. Une espece nouvelle de *Raffaëlea*, isolee de *Platypus cylindrus*, coleoptere xylomycetophage des chenues. *Extr. Ann. Soc. Sci. Nat. Archeol. Toulon Var* 50:185–93
- Morrissey JP, Dow JM, Mark GL, O’Gara F. 2004. Are microbes at the root of a solution to world food production? *EMBO Rep.* 10:922–26
- Morrissey JP, Walsh UF, O’Donnell A, Moëgne-Lococo Y, O’Gara F. 2002. Exploitation of genetically modified inoculants for industrial ecology applications. *Antonie van Leeuwenhoek* 81:599–606
- Mueller UG. 2002. Ant versus fungus versus mutualism: Ant-cultivar conflict and the deconstruction of the attine ant-fungus symbiosis. *Am. Nat.* 160(Suppl.):S67–98
- Mueller UG, Gerardo N. 2002. Fungus-farming insects: Multiple origins and diverse evolutionary histories. *Proc. Natl. Acad. Sci. USA* 99:15247–49
- Mueller UG, Lipari SE, Milgroom MG. 1996. Amplified Fragment Length Polymorphism (AFLP) fingerprinting of fungi cultured by the fungus-growing ant *Cyphomyrmex minutus*. *Mol. Ecol.* 5:119–22
- Mueller UG, Poulin J, Adams RMM. 2004. Symbiont choice in a fungus-growing ant (Attini, Formicidae). *Behav. Ecol.* 15:357–64
- Mueller UG, Rehner SA, Schultz TR. 1998. The evolution of agriculture in ants. *Science* 281:2034–38
- Mueller UG, Schultz TR, Currie CR, Adams RMM, Malloch D. 2001. The origin of the attine ant-fungus mutualism. *Q. Rev. Biol.* 76:169–97
- Mundt CC. 2002. Use of multiline cultivars and cultivar mixtures for disease management. *Annu. Rev. Phytopathol.* 40:381–410
- Munkacsı AB, Pan JJ, Villesen P, Mueller UG, Blackwell M, et al. 2004 Convergent coevolution in the domestication of coral mushrooms by fungus-growing ants. *Proc. R. Soc. London Ser. B* 271:1777–82
- Murakami T, Higashi S. 1997. Social organization in two primitive attine ants, *Cyphomyrmex rimosus* and *Myrmicocrypta ednaella*, with reference to their fungus substrates and food sources. *J. Ethol.* 15:17–25
- Narisawa K, Kawamata H, Currah RS, Hashiba T. 2002. Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. *Eur. J. Plant Pathol.* 108:103–9
- Norris DM. 1965. The complex of fungi essential to growth and development of *Xyleborus sharpi* in wood. *Mater. Org. Beih.* 1:523–29
- Norris DM. 1972. Dependence of fertility and progeny development of *Xyleborus ferrugineus* upon chemicals from its symbiotes. In *Insect and Mite Nutrition*, ed. JC Rodriguez, pp. 299–310. North-Holland: Amsterdam. 702 pp.
- Norris DM. 1979. The mutualistic fungi of Xyleborini beetles. See Batra 1979, pp. 53–65
- Ortius-Lechner D, Maile R, Morgan ED, Boomsma JJ. 2000. Metapleural gland secretions of the leaf-cutter ant *Acromyrmex octospinosus*: New compounds and their functional significance. *J. Chem. Ecol.* 26:1667–83
- Paulitz TC, Bélanger RR. 2001. Biological control in greenhouse systems. *Annu. Rev. Phytopathol.* 39:103–33
- Peacock L, Hunter T, Turner H, Brain P. 2001. Does host genotype diversity affect the distribution of insect and disease damage in willow cropping systems? *J. Appl. Ecol.* 38:1070–81
- Piper JK, Handley MK, Kulakow PA. 1996. Incidence and severity of viral disease symptoms on eastern gamagrass in monoculture and polycultures. *Agric. Ecosyst. Environ.* 59:139–47
- Poulsen M, Boomsma JJ. 2005. Mutualistic fungi control crop-diversity in fungus-growing ants. *Science* 307:741–44
- Price SL, Murakami T, Mueller UG, Schultz TR, Currie CR. 2003. Recent findings in

- fungus-growing ants: Evolution, ecology, and behavior of a complex microbial symbiosis. In *Genes, Behavior, and Evolution in Social Insects*, ed. M Kikuchi, S Higashi, pp. 255–80. Sapporo: Hokkaido Univ. Press. 314 pp.
- Rindos D. 1984. *The Origins of Agriculture*. Orlando: Academic. 325 pp.
- Rissing SW, Pollock GB, Higgins MR, Hagen RH, Smith DR. 1989. Foraging specialization without relatedness or dominance among co-founding ant queens. *Nature* 338: 420–22
- Roeper RA, Treeful LM, O'Brien KM, Foote RA, Bunce MA. 1980. Life history of the ambrosia beetle *Xyleborus affinis* (Coleoptera: Scolytidae) from in vitro culture. *Great Lakes Entomol.* 13:141–44
- Rollins F, Jones KG, Krokene P, Solheim H, Blackwell M. 2001. Phylogeny of asexual fungi associated with bark and ambrosia beetles. *Mycologia* 93:991–96
- Rosengaus RB, Guldin MR, Traniello JFA. 1998. Inhibitory effect of termite fecal pellets on fungal spore germination. *J. Chem. Ecol.* 24:1697–706
- Rosengaus RB, Traniello JFA, Lefebvre ML, Maxmen AB. 2004. Fungistatic activity of the sternal gland secretion of the dampwood termite *Zootermopsis angusticollis*. *Insect. Soc.* 51:259–64
- Rouland-Lefevre C. 2000. Symbiosis with fungi. See Abe et al. 2000, pp. 289–306
- Sachs J, Mueller UG, Wilcox TP, Bull JJ. 2004. The evolution of cooperation. *Q. Rev. Biol.* 79:135–60
- Sanchez-Peña, SR. 2005. New view on origin of attine ant-fungus mutualism: exploitation of a preexisting insect-fungus symbiosis (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* 98:151–64
- Sands WA. 1956. Some factors affecting the survival of *Odontotermes badius*. *Insect. Soc.* 3:531–36
- Santos AV, Dillon RJ, Dillon VM, Reynolds SE, Samuels RI. 2004. Occurrence of the antibiotic producing bacterium *Burkholderia* sp. in colonies of the leaf-cutting ant *Atta sexdens rubropilosa*. *FEMS Microbiol. Lett.* 239:319–23
- Schultz TR, Meier R. 1995. A phylogenetic analysis of the fungus-growing ants (Formicidae: Attini) based on morphological characters of the larvae. *Syst. Entomol.* 20:337–70
- Schultz TR, Mueller UG, Currie CR, Rehner SA. 2005. Reciprocal illumination: A comparison of agriculture in humans and ants. See Vega & Blackwell 2005, pp. 149–90
- Schultz TR, Solomon SA, Mueller UG, Villeseen P, Boomsma JJ, et al. 2002. Cryptic speciation in the fungus-growing ants *Cyphomyrmex longiscapus* Weber and *Cyphomyrmex muelleri* Schultz and Solomon, new species (Formicidae, Attini). *Insect. Soc.* 49: 331–43
- Shipton PJ. 1977. Monoculture and soil borne pathogens. *Annu. Rev. Phytopathol.* 15:387–407
- Sieber R. 1983. Establishment of fungus comb in laboratory colonies of *Macrotermes michaelseni* and *Odontotermes montanus* (Isoptera, Macrotermitinae). *Insect. Soc.* 30:204–9
- Silliman BR, Newell SY. 2003. Fungus farming in snails. *Proc. Natl. Acad. Sci. USA* 100: 15643–48
- Six DL. 2003. Bark beetle-fungus symbiosis. In *Insect Symbiosis*, ed. T Miller, K Kourtzis, pp. 99–116. Boca Raton, FL: CRC. 368 pp.
- Six DL, Klepzig KD. 2004. *Dendroctonus* bark beetles as model systems for studies on symbiosis. *Symbiosis* 37:207–32
- Six DL, Paine TD. 1998. Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Environ. Entomol.* 27:1393–1401
- Six DL, Paine TD. 1999. Allozyme diversity and gene flow in *Ophiostoma clavigerum* (Ophiostomatales: Ophiostomataceae), the mycangial fungus of the Jeffrey pine beetle, *Dendroctonus jeffreyi* (Coleoptera: Scolytidae). *Can. J. For. Res.* 29:324–31
- Smith BD. 1998. *The Emergence of Agriculture*. New York: Sci. Am. Libr. 230 pp.
- Sturz AV, Christie BR, Nowak J. 2000.

- Bacterial endophytes: Potential role in developing sustainable systems of crop production. *Crit. Rev. Plant Sci.* 19:1–30
- Traniello JFA, Leuthold RH. 2000. The behavioral ecology of foraging in termites. See Abe et al. 2000, pp. 141–68
- Vega F, Blackwell M, eds. 2005. *Insect-Fungal Associations: Ecology and Evolution*. Oxford: Oxford Univ. Press. 333 pp.
- Villesen P, Mueller UG, Schultz TR, Adams RMM, Bouck MC. 2004. Evolution of ant-cultivar specialization and cultivar switching in *Apterostigma* fungus-growing ants. *Evolution* 58:2252–65
- von Arx JA, Hennebert GL. 1965. Deux champignons ambrosia. *Mycopathol. Mycol. Appl.* 25:309–15
- Wardle DA, Bardgett RD, Klironomos JN, Setälä H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. *Science* 304:1629–33
- Weber NA. 1972. *Gardening Ants: The Attines*. Philadelphia: Am. Philos. Soc. 146 pp.
- Weller DM, Raaijmakers JM, Gardener BB, Thomashow LS. 2002. Microbial population responses for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* 40: 309–48
- Whipps JM. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52: 487–511
- Wille P, Boller T, Kaltz O. 2001. Mixed inoculation alters infection success of strains of the endophyte *Epichloa bromicola* on its grass host *Bromus erectus*. *Proc. R. Soc. London Ser. B* 269:397–402
- Wolfe MS. 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annu. Rev. Phytopathol.* 23:251–73
- Wood SL. 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Great Basin Nat. Mem.* 6:1–1359
- Zhu Z, Chen H, Fan J, Wang Y, Li Y, Chen J, et al. 2000. Genetic diversity and disease control in rice. *Nature* 406:718–22



See legend on next page

Figure 1 Comparison of agriculture in attine ants, xyleborine beetles, macrotermite termites, and humans. The time series (*left to right*) highlight the roles of beneficial auxiliary microbes (*blue shading*) that suppress diseases (*black dots*) or aid in buffering against contaminant microbes (*orange shading*). **(a) Ant agriculture.** Ants attempt to clean contaminant microbes from garden substrate (not shown) and remove garden diseases (*black dots*) through active weeding (*top ant*). The ants (*bottom ant*) then plant a crop-microbe consortium (*crop plus beneficial auxiliary blue microbes*) onto the prepared substrate, spreading beneficial microbes through the garden matrix. **(b) Beetle agriculture.** Primary fungus (crop) lining the tunnel grows intermixed with secondary microbes (*blue shading*) and occasional contaminant microbes (*orange dots*). No disease microbes (*black dots*) are indicated because they very rarely occur in young gardens near a tunnel head. The exact roles of the secondary microbes in beetle fungiculture are still unknown. **(c) Termite agriculture.** Hypothetical passage of a mixture of crop spores, auxiliary microbes, and substrate (ingested plant material) through the gut of a termite, followed by defecation of the substrate-crop-microbe consortium in fecal pellets that the termite adds to new garden. Other (external) modes of crop-microbe copropagation may exist in termite farmers, paralleling the planting of crop-microbe consortia in attine ants. Selective passage of microbes through the alimentary canals of attine ants and ambrosia beetles is unknown, but has never been investigated. No disease microbes (*black dots*) are indicated because no specialized pathogens have yet been identified in the fungus-growing termite system. **(d) Human agriculture** (wheat). A seed, fortuitously planted in soil enriched in antibiotic-secreting rhizosphere bacteria (*blue-shaded soil*), grows into a vigorous, disease-resilient crop plant (Weller et al. 2002). However, crops are often planted in microbially suboptimal soil (*orange-shaded soil*), leading to higher disease loads (*black dots*) on such plants. Traditional human planting schemes passage crops through a seed stage without copropagating disease-suppressant rhizosphere microbes. Illustrations by Barrett Klein.

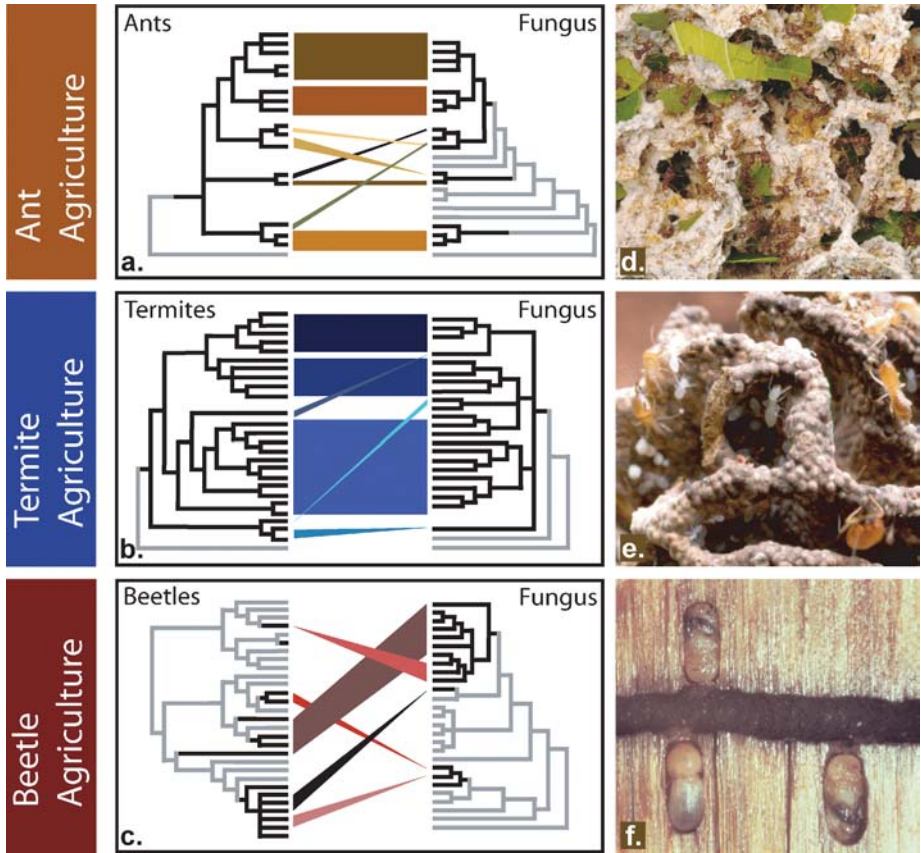


Figure 2 Evolutionary histories of insect agriculture. (a–c) Comparison of the patterns of evolutionary diversification in the insect farmers (left cladograms) and their cultivated fungi (right cladograms). In the left cladograms, farmer lineages are black and nonfarmer relatives are gray, whereas in the right cladograms, cultivated fungal lineages (cultivars) are black and noncultivated feral fungal lineages are gray. Independent origins of agricultural behavior are indicated for each farmer clade in the left cladograms, and independently domesticated fungal lineages appear as separate cultivar lineages in the right cladograms. (d) Garden of the fungus-growing ant *Atta texana* (photo by Greg Dimijian). The workers are cleaning and shredding leaf cuttings before expanding new gardens through the addition of leaf material. (e) Garden of the fungus-growing termite *Macrotermes bellicosus* (photo by Karen Machielsen). The fungus is grown on fecal pellets that are stacked into lamellar walls of the fungus garden (comb). (f) Gallery of the ambrosia beetle *Trypodendron lineatum* (photo by Susanne Kühnholz) with ambrosia fungus (black) lining the main gallery and beetle brood developing in niches adjacent to the gallery. Galleries are constantly patrolled by adult beetles (not shown). Figure adapted from Mueller & Gerardo 2002.

CONTENTS

THE GENETICS AND EVOLUTION OF FLUCTUATING ASYMMETRY, <i>Larry J. Leamy and Christian Peter Klingenberg</i>	1
LIFE-HISTORY EVOLUTION IN REPTILES, <i>Richard Shine</i>	23
THE EVOLUTIONARY ENIGMA OF MIXED MATING SYSTEMS IN PLANTS: OCCURRENCE, THEORETICAL EXPLANATIONS, AND EMPIRICAL EVIDENCE, <i>Carol Goodwillie, Susan Kalisz, and Christopher G. Eckert</i>	47
INDIRECT INTERACTION WEBS: HERBIVORE-INDUCED EFFECTS THROUGH TRAIT CHANGE IN PLANTS, <i>Takayuki Ohgushi</i>	81
EVOLUTIONARY HISTORY OF POALES, <i>H. Peter Linder and Paula J. Rudall</i>	107
THE EVOLUTION OF POLYANDRY: SPERM COMPETITION, SPERM SELECTION, AND OFFSPRING VIABILITY, <i>Leigh W. Simmons</i>	125
INDIVIDUAL-BASED MODELING OF ECOLOGICAL AND EVOLUTIONARY PROCESSES, <i>Donald L. DeAngelis and Wolf M. Mooij</i>	147
THE INFLUENCE OF PLANT SECONDARY METABOLITES ON THE NUTRITIONAL ECOLOGY OF HERBIVOROUS TERRESTRIAL VERTEBRATES, <i>M. Denise Dearing, William J. Foley, and Stuart McLean</i>	169
BIODIVERSITY AND LITTER DECOMPOSITION IN TERRESTRIAL ECOSYSTEMS, <i>Stephan Hättenschwiler, Alexei V. Tiunov, and Stefan Scheu</i>	191
THE FUNCTIONAL SIGNIFICANCE OF RIBOSOMAL (R)DNA VARIATION: IMPACTS ON THE EVOLUTIONARY ECOLOGY OF ORGANISMS, <i>Lawrence J. Weider, James J. Elser, Teresa J. Crease, Mariana Mateos, James B. Cotner, and Therese A. Markow</i>	219
EVOLUTIONARY ECOLOGY OF PLANT ADAPTATION TO SERPENTINE SOILS, <i>Kristy U. Brady, Arthur R. Kruckeberg, and H.D. Bradshaw Jr.</i>	243
BIODIVERSITY-ECOSYSTEM FUNCTION RESEARCH: IS IT RELEVANT TO CONSERVATION? <i>Diane S. Srivastava and Mark Vellend</i>	267
CONSEQUENCES OF THE CRETACEOUS/PALEOGENE MASS EXTINCTION FOR MARINE ECOSYSTEMS, <i>Steven D'Hondt</i>	295
LANDSCAPE ECOLOGY: WHAT IS THE STATE OF THE SCIENCE? <i>Monica G. Turner</i>	319
ECOLOGY AND EVOLUTION OF APHID-ANT INTERACTIONS, <i>Bernhard Stadler and Anthony F.G. Dixon</i>	345

EVOLUTIONARY CAUSES AND CONSEQUENCES OF IMMUNOPATHOLOGY, <i>Andrea L. Graham, Judith E. Allen, and Andrew F. Read</i>	373
THE EVOLUTIONARY ECOLOGY OF GYNOGENESIS, <i>Ingo Schlupp</i>	399
MEASUREMENT OF INTERACTION STRENGTH IN NATURE, <i>J. Timothy Wootton and Mark Emmerson</i>	419
MODEL SELECTION IN PHYLOGENETICS, <i>Jack Sullivan and Paul Joyce</i>	445
POLLEN LIMITATION OF PLANT REPRODUCTION: PATTERN AND PROCESS, <i>Tiffany M. Knight, Janette A. Steets, Jana C. Vamosi, Susan J. Mazer, Martin Burd, Diane R. Campbell, Michele R. Dudash, Mark O. Johnston, Randall J. Mitchell, and Tia-Lynn Ashman</i>	467
EVOLVING THE PSYCHOLOGICAL MECHANISMS FOR COOPERATION, <i>Jeffrey R. Stevens, Fiery A. Cushman, and Marc D. Hauser</i>	499
NICHE CONSERVATISM: INTEGRATING EVOLUTION, ECOLOGY, AND CONSERVATION BIOLOGY, <i>John J. Wiens and Catherine H. Graham</i>	519
PHYLOGENOMICS, <i>Hervé Philippe, Frédéric Delsuc, Henner Brinkmann, and Nicolas Lartillot</i>	541
THE EVOLUTION OF AGRICULTURE IN INSECTS, <i>Ulrich G. Mueller, Nicole M. Gerardo, Duur K. Aanen, Diana L. Six, and Ted R. Schultz</i>	563
INSECTS ON PLANTS: DIVERSITY OF HERBIVORE ASSEMBLAGES REVISITED, <i>Thomas M. Lewinsohn, Vojtech Novotny, and Yves Basset</i>	597
THE POPULATION BIOLOGY OF MITOCHONDRIAL DNA AND ITS PHYLOGENETIC IMPLICATIONS, <i>J. William O. Ballard and David M. Rand</i>	621
INTRODUCTION OF NON-NATIVE OYSTERS: ECOSYSTEM EFFECTS AND RESTORATION IMPLICATIONS, <i>Jennifer L. Ruesink, Hunter S. Lenihan, Alan C. Trimble, Kimberly W. Heiman, Fiorenza Micheli, James E. Byers, and Matthew C. Kay</i>	643
INDEXES	
Subject Index	691
Cumulative Index of Contributing Authors, Volumes 32–36	707
Cumulative Index of Chapter Titles, Volumes 32–36	710

ERRATA

An online log of corrections to *Annual Review of Ecology,
Evolution, and Systematics* chapters may be found at
<http://ecolsys.annualreviews.org/errata.shtml>