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Ecology and Evolution of Insect–Fungus Mutualisms

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Keywords

attine ants, termites, ambrosia beetles, cooperation, symbiosis, insect agriculture

Abstract

The evolution of a mutualism requires reciprocal interactions whereby one species provides a service that the other species cannot perform or performs less efficiently. Services exchanged in insect–fungus mutualisms include nutrition, protection, and dispersal. In ectosymbioses, which are the focus of this review, fungi can be consumed by insects or can degrade plant polymers or defensive compounds, thereby making a substrate available to insects. They can also protect against environmental factors and produce compounds antagonistic to microbial competitors. Insects disperse fungi and can also provide fungal growth substrates and protection. Insect–fungus mutualisms can transition from facultative to obligate, whereby each partner is no longer viable on its own. Obligate dependency has (*a*) resulted in the evolution of morphological adaptations in insects and fungi, (*b*) driven the evolution of social behaviors in some groups of insects, and (*c*) led to the loss of sexuality in some fungal mutualists.

> If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection.
>
> Charles Darwin (1859, p. 201)

> Recalling that Schmidberger already in 1836 called the "crumbly substance" found in wood-boring bark beetles "Ambrosia," which is used as food for the beetles, it should be obvious to call all of the fungi in similar symbiotic relationships with animals Ambrosia fungi.
>
> Franz W. Neger (1908, p. 737; translated from German)

INTRODUCTION

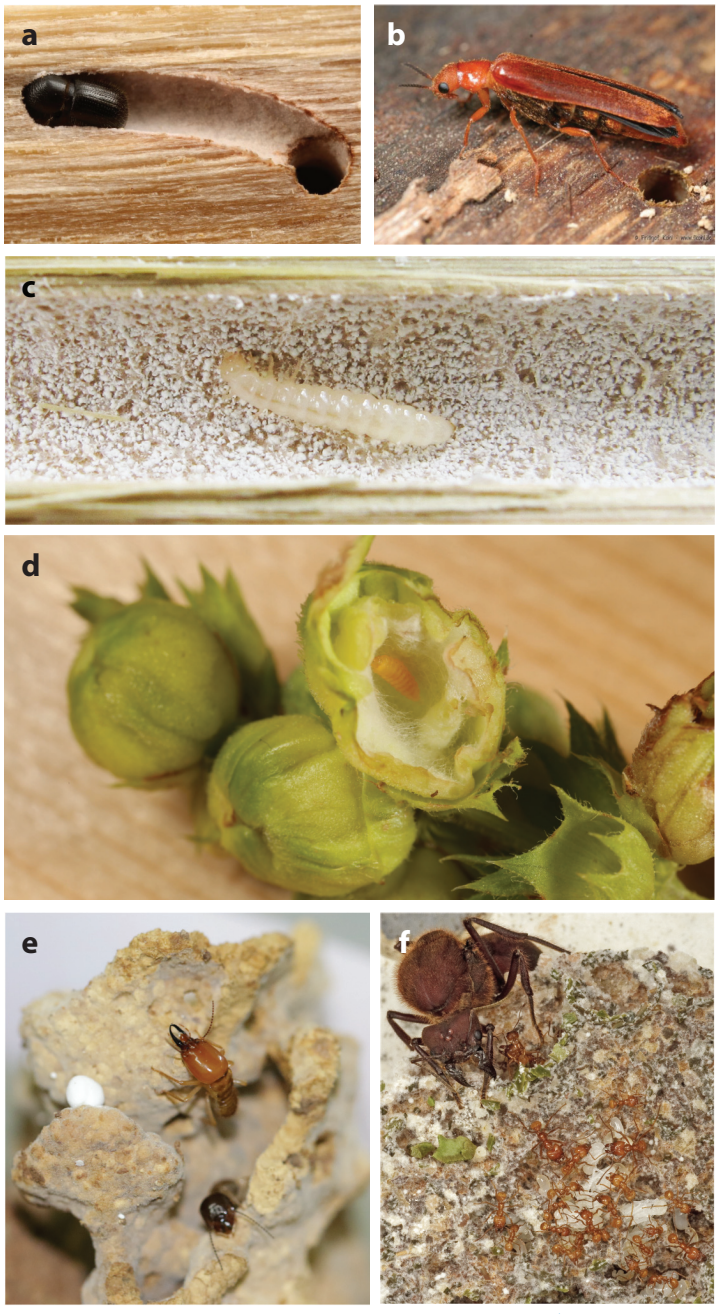
Insects and fungi are among the most speciose groups of organisms, with estimates of up to 5.5 and 2.2–3.8 million species (140), respectively. Both groups are abundant in many environments and have cohabited for >400 million years (98, 129). Therefore, insects and fungi often interact in ways that are positive for both (mutualism), positive for one and neutral for the other (commensalism), positive for one and negative for the other (predation, parasitism), or negative for both (competition) (23).

The best-known mutualisms involve dispersal (e.g., pollination), protection (e.g., ant–plant protection), and nutrition (e.g., fungal cultivation by some insects) (23). The classical and best-studied insect–fungus mutualisms include fungal cultivation (fungiculture) by bark and ambrosia beetles (Coleoptera: Curculionidae: Scolytinae and Platypodinae), fungus-farming ants (Hymenoptera: Formicidae: Myrmicinae: Attini: Attina), and fungus-farming termites (Blattodea: Termitidae: Macrotermitinae) (2, 3, 47, 63, 100, 101, 103) (**Figure 1**). Other nonclassical insect–fungus mutualisms in the Coleoptera, Hymenoptera, Diptera, Lepidoptera, and Hemiptera have received scant attention (9, 24, 30, 42, 55, 59, 79, 85, 96, 109, 118, 125, 135, 144, 147, 148). One goal of this review is to bring these to the attention of readers (**Figure 1**, **Tables 1–3**).

Examining the nonclassical insect–fungus mutualisms is not only interesting from a natural history point of view, but also essential for a comparative approach to elucidating the ecological factors driving the evolution of insect–fungus mutualisms. Compared to intraspecific cooperation, for which the ecological factors that facilitate group formation have been established (77), there has been little systematic effort to define the ecological drivers of insect–fungus mutualisms (17, 19). Despite the fact that both insects and fungi are heterotrophs (i.e., unable to synthesize their own food), they differ fundamentally in many aspects of their life histories, most importantly with regard to nutrition, mobility, and defense against natural enemies. Insects digest food in the gut, are highly mobile, and have an immune system (54). In contrast, fungi secrete enzymes for extracellular digestion, are typically sedentary, and produce secondary metabolites for defense (149). Some of these characteristics are complementary and serve as pre-adaptations for interactions that may evolve to be mutually beneficial, as we outline below.

Our analysis of all currently known ectosymbiotic mutualisms between insects and filamentous fungi and yeasts revealed fungal mutualisms with either Ascomycota (21 origins) or Basidiomycota (12 origins) (**Figure 2**) in six orders of insects: Coleoptera (16 origins), Hymenoptera (7 origins), Diptera (>3 origins), Hemiptera (4 origins), Blattodea (1 origin) and Lepidoptera (1 origin) (**Tables 1–3**). We briefly describe the ecologies of each mutualistic system and outline evolutionary scenarios to explain how they might have developed. We also show how insect–fungus mutualisms can be classified in relation to the benefits for each partner and clearly define the terms advanced and primitive fungiculture, the uses of which have been chaotic in the literature (96, 144, 148). Finally, we present shortcomings in the conceptual assessment of insect–fungus mutualisms

and propose areas that need to be elucidated to define ecological factors that consistently facilitate the evolution of insect–fungus mutualisms. Endosymbiotic or gut-associated insect–fungus mutualisms (139) are not included in this review; nor are mycetophagous insects (i.e., fungi consumers) that more or less passively vector spores of their fungal hosts [e.g., Coleoptera feeding on Polyporales (Basidiomycota)] (15).



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Some insects involved in mutualisms with fungi. (*a*) Gallery with the ambrosia beetle *Anisandrus dispar* (Coleoptera: Curculionidae: Scolytinae) and its mutualistic fungus *Ambrosiella hartigii* (Ascomycota: Microscales) in the xylem of *Juglans regia* (Juglandaceae). (*b*) The ship-timber beetle, *Elateroides dermestoides* (Coleoptera: Lymexylidae), exhibits a mutualistic association with *Alloascoidea hylecoeti* (Ascomycota: Saccharomycetales) in the xylem of *Fagus sylvatica* (Fagaceae). (*c*) Larva of a lizard beetle, *Doubledaya bucculenta* (Coleoptera: Erotylidae: Languriinae), feeding on *Wickerhamomyces anomalus* (Ascomycota: Saccharomycetales) in a bamboo (Poaceae) internode. (*d*) Gall midge larva, *Asphondylia stachydis* (Diptera: Cecidomyiidae), mutualistic with *Botryosphaeria dothidea* (Ascomycota: Botryosphaeriales) in a gall within a flower bud of *Stachys recta* (Lamiaceae). (*e*) *Macrotermes bellicosus* (Blattodea: Termitidae: Macrotermitinae) on a fungus comb with *Termitomyces* (white growth; Basidiomycota: Agaricales). (*f*) Queen and workers of the leafcutter ant *Atta cephalotes* (Hymenoptera: Formicidae: Myrmicinae: Attini: Attina) on a fungus garden (note leaves with fungal growth of *Leucocoprinus gongylophorus*; Basidiomycota: Agaricales). Photos provided by (*a*) Gernot Kunz, (*b*) Frithjof Kohl, (*c*) Wataru Toki, (*d*) Roland Spohn, (*e*) Michael Poulsen, and (*f*) Karie Darrow.

HISTORY OF MUTUALISMS

The oldest estimated ages of extant insect groups associated with fungi are woodwasps [Hymenoptera: Siricidae; 150–200 million years ago (My)], gall midges (Diptera: Cecidomyiidae; approximately 150 My), and ship-timber beetles (Coleoptera: Lymexylidae; approximately 150 My) (53). Not all of the extant species in these groups are fungus growers (25, 105), so it is unclear when the fungal mutualisms originated. The oldest evidence for the origin of an insect–fungus mutualism comes from molecular data for wood-boring weevils in the ambrosia beetle subfamily Platypodinae and their fungal mutualists (Ascomycota: Ophiostomatales). Estimated ages are approximately 96 My (beetles) and approximately 86 My (fungi) (146), which are supported by a 97–110 My beetle–fungus fossil (111, 112). Regardless of when exactly this mutualism originated, it coincided with the diversification of Eudicotidae, novel plant hosts within the angiosperms; this event is believed to have led to diversification in both weevils (Curculionidae) and Ophiostomatales (146). This corroborates our contention that ecological factors are critical for the natural selection of mutualisms, an argument to which we repeatedly return in this article. Fungiculture in the second major group of ambrosia beetles in the Scolytinae (Curculionidae) originated repeatedly but much later, i.e., between 1–58 My, depending on the lineage (66, 110), and initially through farming of cultivars that had been domesticated by ambrosia beetles in the Platypodinae (146) (**Tables 1–3**). Fungus-growing in the Attina has been hypothesized to have emerged between 55 and 60 My (21) and in termites between 37 and 55 My (18) (**Tables 1–3**). No hypotheses on the origins of other insect–fungus mutualisms are available.

ECOLOGY OF MUTUALISMS

Mutualisms have traditionally been conceptualized as exchanges of services, and we group them into four types relative to their benefits for one or both partners (15, 16, 23, 74). (*a*) The first is nutrition, i.e., supply of nutrients by a partner, directly (as by fungi) or indirectly by substrate provisioning (as by insects), breakdown of indigestible compounds (e.g., cellulose), or detoxification of a food source (e.g., plant material) (as by fungi). (*b*) The second is protection, i.e., insects or fungi defend their partners against environmental variation, competitors, and/or natural enemies, including microorganisms. This comprises insects regulating nest climate for optimal fungal growth, ant soldier behavior, and weeding of fungal gardens, as well as fungi stabilizing insect-nest structure and producing repellents or antimicrobial metabolites. (*c*) The third is dispersal, i.e., insect dispersal of spores or other fungal propagules. All of these benefits are nonexclusive, and a

Table 1 Outline of various aspects related to the evolution and ecology of insect–fungus mutualisms that have evolved in the order Coleoptera

						
	Ambrosia beetles (Curculionidae: Platypodinae)	**Bark and ambrosia beetles (Curculionidae: Scolytinae)**	**Leaf-rolling weevils (Curculionidae: Attelabinae)**	**Ship-timber beetles (Lymexylidae)**	**Lizard beetles (Erotylidae)**	**Burying beetle yeast farming (Silphidae)**
Groups of primary mutualistic fungi	Ascomycota: Ophiostomatales, Saccharomycetales	Ascomycota: Ophiostomatales, Microascales, Hypocreales, Saccharomycetales; Basidiomycota: Russulales, Polyporales	Ascomycota: Eurotiales	Ascomycota: Saccharomycetales	Ascomycota: Saccharomycetales	Ascomycota: Saccharomycetales
Evolution						
Age	90–110 My	1–58 My	?	?	?	?
Number of independent origins in insects/fungi	1/3	11/7	1/1	1/1	1/1	1/1
Suggested route to mutualism	Dispersal of fungi → consumption by insects → cultivation by insects	Consumption by insects → cultivation by insects → dispersal of fungi	Dispersal of fungi → usage by insects	Dispersal of fungi → consumption by insects → cultivation by insects	Dispersal of fungi → consumption by insects → cultivation by insects	Dispersal of fungi → consumption by insects → cultivation by insects
Consequences of mutualism in insects	Morphology, social behavior	Morphology, social behavior	Morphology	Morphology	Morphology	Social behavior?
Consequences of mutualism in fungi	Morphology, asexuality	Morphology, asexuality	None	Morphology	None	None
Ecology						
Mode of fungal transmission	Mostly vertical	Mostly vertical	Vertical	Vertical	Vertical	Vertical

(*Continued*)

Table 1 (*Continued*)

						
	Ambrosia beetles (Curculionidae: Platypodinae)	**Bark and ambrosia beetles (Curculionidae: Scolytinae)**	**Leaf-rolling weevils (Curculionidae: Attelabinae)**	**Ship-timber beetles (Lymexylidae)**	**Lizard beetles (Erotylidae)**	**Burying beetle yeast farming (Silphidae)**
Level of domestication of fungi by insects	Advanced farming mutualism	Advanced farming mutualism	Dispersal mutualism	Primitive farming mutualism	Dispersal mutualism	Primitive farming mutualism
Substrate for fungus	Living or freshly dead xylem	Living or freshly dead xylem or phloem	Fresh leaves	Freshly dead xylem	Bamboo internode	Freshly dead carrion
Benefit to fungi (in order of suggested importance)	Dispersal, nutrition, physical and antimicrobial defense	Dispersal, nutrition, physical and antimicrobial defense	Dispersal	Dispersal, nutrition	Dispersal	Dispersal, antimicrobial defense, nutrition
Benefit to insect (in order of suggested importance)	Nutrition, antimicrobial defense	Nutrition, antimicrobial defense	Mechanical and antimicrobial defense, nutrition of larvae?	Nutrition of larvae, antimicrobial defense?	Nutrition of larvae	Nutrition, antimicrobial defense
Brief description	Fungus grown within tunnel systems in xylem of living or freshly dead trees, where adults and larvae feed	Fungus grown within tunnel systems in phloem or xylem of living or freshly dead trees, where adults and larvae feed	Fungus grown at the outside of a rolled-up leaf in which a larva develops	Fungus grown within a tunnel system in xylem of a freshly dead tree, where a solitary larva develops	Fungus grown within the internode cavity of a freshly dead bamboo, where solitary larvae develop	Yeast grown on a fresh cadaver maintained by adults for larval food (within a cavity in the soil)
Literature						
Most important references	Hulcr & Stelinski 2017, Kirkendall et al. 2015	Hulcr & Stelinski 2017, Kirkendall et al. 2015	Wang et al. 2015, Li et al. 2012 (References 84 and 85)	Francke-Grosmann 1967, Batra & Francke-Grosmann 1961	Toki et al. 2012	Shukla et al. 2018

Table 2 Outline of various aspects related to the evolution and ecology of insect–fungus mutualisms that have evolved in the orders Blattodea, Lepidoptera, Diptera, and Hemiptera

	Blattodea	Lepidoptera	Diptera		Hemiptera	
						
	Fungus-growing termites (Macrotermitinae)	**Moth fungus mutualism (Tortricidae)**	**Gall midges (Cecidomyiidae)**	**Various flies–yeast mutualisms (Drosophilidae, Muscidae, etc.)**	**Aphid fungus shields (Aphididae)**	**Scale insect fungus shields (Diaspididae, Pseudococcidae)**
Groups of primary mutualistic fungi	Basidiomycota: Agaricales	Ascomycota: Helotiales	Ascomycota: Botryosphaeriales	Ascomycota: Saccharomycetales	Basidiomycota: Boletales	Ascomycota: Capnodiales; Basidiomycota: Boletales, Septobasidiales
Evolution						
Age	37–55 My	?	?	?	?	?
Number of independent origins in insects/fungi	1/1	1/1	3/1	?/?	2/2	2/3
Suggested route to mutualism	Consumption by insects → cultivation by insects → dispersal of fungi	Dispersal of fungi → consumption by insects	Consumption and usage by insects → cultivation by insects → dispersal of fungi	Dispersal of fungi → consumption by insects	Nutrition of fungus → encasement of insect	Nutrition of fungus → encasement of insect
Consequences of mutualism in insects	Social behavior	None	Morphology, social behavior?	None	None	None
Consequences of mutualism in fungi	Morphology	None	Morphology	None	None	None
Ecology						
Mode of fungal transmission	Mostly horizontal	Mixed	Vertical	Mixed	Horizontal	?
Level of domestication of fungi by insects	Advanced farming mutualism	Dispersal mutualism	Dispersal mutualism	Dispersal mutualism	None	None

(*Continued*)

Table 2 (*Continued*)

	Blattodea	Lepidoptera	Diptera		Hemiptera	
						
	Fungus-growing termites (Macrotermitinae)	**Moth fungus mutualism (Tortricidae)**	**Gall midges (Cecidomyiidae)**	**Various flies–yeast mutualisms (Drosophilidae, Muscidae, etc.)**	**Aphid fungus shields (Aphididae)**	**Scale insect fungus shields (Diaspididae, Pseudococcidae)**
Substrate for fungus	Partially digested plant material	Fruits	Fresh plant tissue	Living or freshly dead organic matter (plant, fruit, mushroom, carcass)	Soil and honeydew excretions of aphids	Bark, honeydew excretions and bodies of scale insects
Benefit to fungi (in order of suggested importance)	Dispersal, mechanical and antimicrobial defense, nutrition	Dispersal, help in colonization of substrate	Dispersal, help in colonization of plant	Dispersal, help in colonization of substrate, antimicrobial defense?	Nutrition	Nutrition
Benefit to insect (in order of suggested importance)	Nutrition, antimicrobial defense?	Nutrition, antimicrobial defense of larvae	Nutrition, mechanical and antimicrobial protection of larvae, help in colonization of the plant	Nutrition and antimicrobial defense of larvae, defense against parasitoids	Mechanical and antimicrobial protection	Mechanical and antimicrobial protection (but examples of parasitism)
Brief description	Fungus inoculated into various worker-collected substrates, resulting in fungus comb, which is fed upon by all developing stages	Adult moths infect fruits with fungus that secures habitat against other microbes and supplements larval diet	Insect–fungus complex induces galls in stems, leaves, or flower buds of herbaceous plants or shrubs, in which the fungus lines the walls and nourishes and defends the larva(e)	Adult flies infect dead organic matter with yeasts that secure habitat against other microbes, detoxify plant compounds, and supplement the diet of the flies' offspring	Insects attack roots and become encased by protective fungus tissue (often associated with ants)	Insects attack roots or above-ground surfaces of plants and become encased by protective fungus tissue (sometimes associated with ants)
Literature						
Most important references	Aanen et al. 2009, Aanen & Boomsma 2006	Fermaud & Menn 1989	Heath et al. 2018, Rohfritsch 2008	Günther & Goddard 2018, Anagnostou et al. 2010, Starmer & Fogleman 1986	Brundrett & Kendrick 1987, Krzywiec 1964	Gavrilov-Zimin 2017, Gonçalves 1940, Couch 1938

Table 3 Outline of various aspects related to the evolution and ecology of insect–fungus mutualisms that have evolved in the order Hymenoptera

					
	Fungus-growing ants (Formicidae)	**Carton nests of ants (Formicidae)**	**Ant–plant–fungus associations (Formicidae)**	**Woodwasps (Siricidae and Xiphydriidae)**	**Stingless bee (Apidae)**
Groups of primary mutualistic fungi	Basidiomycota: Agaricales	Ascomycota: Capnodiales, Chaetothyriales	Ascomycota: Chaetothyriales	Ascomycota: Xylariales; Basidiomycota: Russulales, Polyporales	Ascomycota: Saccharomycetales, Eurotiales
Age	55–60 My	?	?	?	?
Number of independent origins in insects/fungi	1/3	1/2	1/1	3/3	1/2
Suggested route to mutualism	Consumption by insects → cultivation by insects → dispersal of fungi	Usage by insects → dispersal of fungi	Usage by insects → dispersal of fungi	Usage by insects → dispersal of fungi	Dispersal of fungi → consumption by insects
Consequences of mutualism in insects	Morphology, social behavior	None	None	Morphology	None
Consequences of mutualism in fungi	Morphology, asexuality	None	None	None	None
Mode of fungal transmission	Vertical	Vertical?	Vertical?	Mostly vertical	?
Level of domestication of fungi by insects	Advanced farming mutualism	Primitive farming mutualism	Primitive farming mutualism	Dispersal mutualism	Primitive farming mutualism
Substrate for fungus	Partially digested (arthropod frass), decomposing, or fresh plant material	Carton made of bark, soil, and decomposing leaves	Dead plant material	Freshly dead xylem	Nectar and nest comb material
Benefit to fungi (in order of suggested importance)	Dispersal, nutrition, physical and antimicrobial defense	Dispersal, nutrition	Dispersal, nutrition, antimicrobial defense, mutualism with plant	Dispersal, nutrition (mucus deposited during oviposition stimulates fungal growth)	Dispersal, growth substrate

(*Continued*)

Table 3 (*Continued*)

					
	Fungus-growing ants (Formicidae)	**Carton nests of ants (Formicidae)**	**Ant–plant–fungus associations (Formicidae)**	**Woodwasps (Siricidae and Xiphydriidae)**	**Stingless bee (Apidae)**
Benefit to insect (in order of suggested importance)	Nutrition, antimicrobial defense (against ant and garden pathogens)	Mechanical and antimicrobial protection	Mechanical protection (reinforcement of covered galleries)	Nutrition for larvae (via wood degradation)	Nutrition for larvae, antimicrobial defense?
Brief description	Fungus inoculated into predigested plant material and fed upon by all developing stages	Carton nests, built out of soil particles and plant material, are inoculated with fungus and thus mechanically stabilized	Covered galleries built by ants using trichomes and oral regurgitate are inoculated with fungus for stabilization	Fungus inoculated into xylem, which is fed upon by solitarily developing larvae	Fungus grown inside the nectar within larval brood cells
Most important references	Mueller et al. 2001, 2018	Schlick-Steiner et al. 2008	Voglmayr et al. 2011	Slippers et al. 2011, Francke-Grosmann 1967	Paludo et al. 2018, Menezes et al. 2015

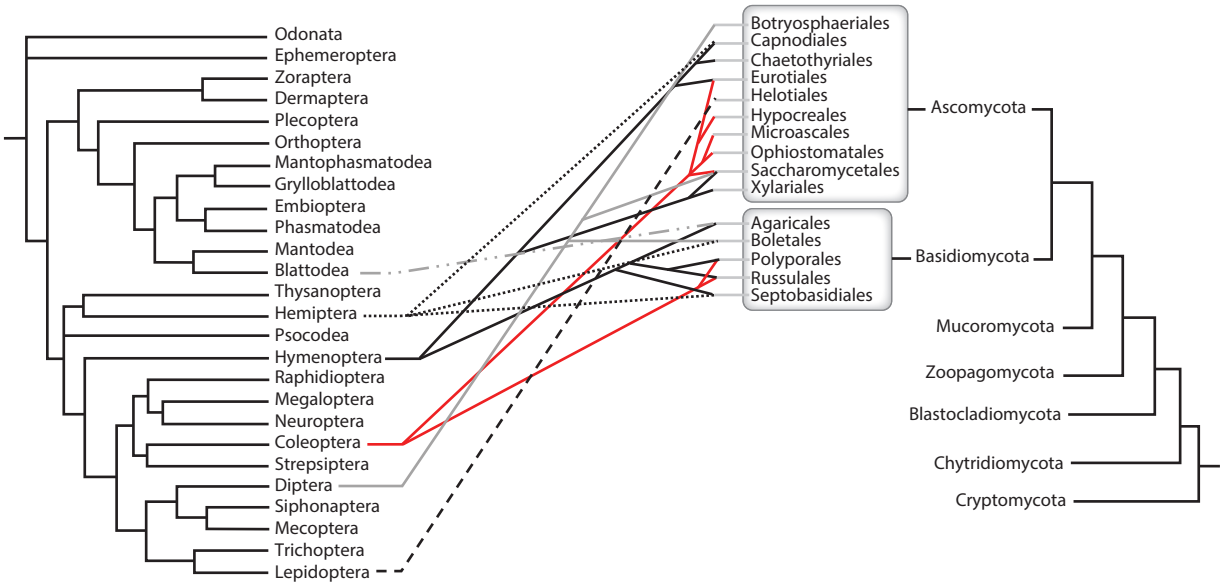


Figure 2

Diagrammatic representation of insect–fungal mutualisms with insect (*left*) and fungal (*right*) cladograms connected with respective mutualisms. Insect tree modified from Reference 73, and fungal tree modified from Reference 137.

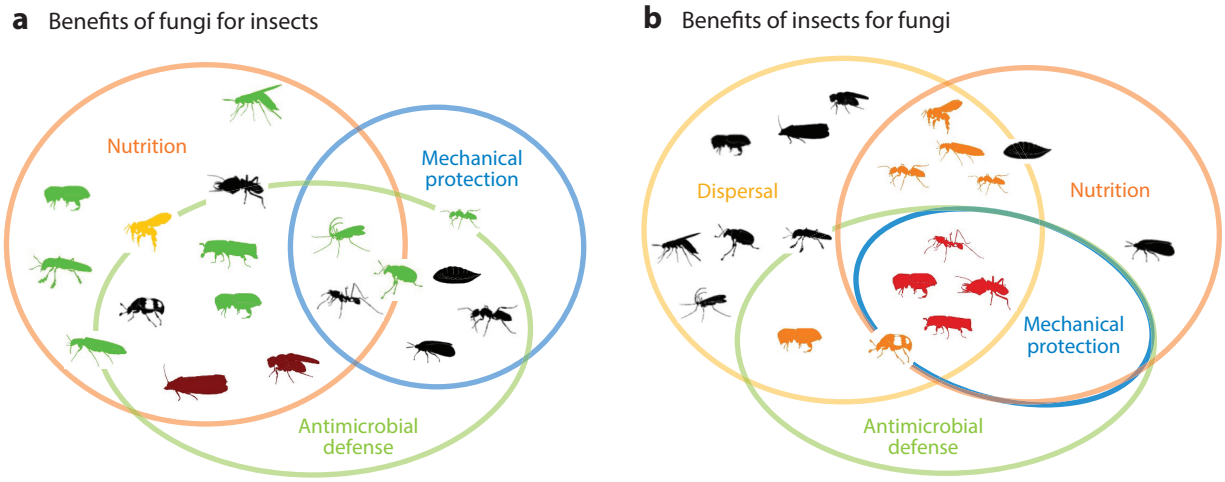


Figure 3

Overview of reciprocal benefits of all known ectosymbiotic insect–fungus mutualisms. (*a*) Benefits of fungi for insects in relation to the environment where the mutualism takes place: soil (*black*), living or freshly dead plants (*green*), fruits (*brown*), and nectar (*yellow*). (*b*) Benefits of fungi for insects in relation to the level of domestication of the fungi by the insects: advanced farming mutualism (*red*), primitive farming mutualism (*orange*), and simple dispersal or nutritional mutualism (*black*). Taxa for which a benefit has not been safely established are displayed on the borders of the circles. Taxa that are displayed repeatedly show within-taxon variation in the specific benefits.

typical mutualism involves many different services exchanged between the partners (**Figure 3**) (74, 100).

We define advanced fungiculture, as practiced by ambrosia beetles, attine ants, and termites (3, 72, 100, 101), as happening externally (outside the body of the insects) and comprising dispersal of fungal mutualists, provision of a nutritive substrate to the fungus, defense of the fungus against natural enemies, and physical protection of the fungus (**Tables 1–3**). It is hard to imagine a nonsocial organism providing all of these services (100). Primitive fungiculture includes dispersal of fungi and either defense against natural enemies, as expressed in burying beetles and some bark and ambrosia beetles (72, 130, 131), or progressive substrate provisioning, as expressed in a stingless bee, ship-timber beetles, and ants associated with nest-wall fungi (47, 96, 125, 147). All other insect–fungus mutualisms are monofactorial, with either only nutritional or only dispersal benefits for the fungi (**Tables 1–3**). Therefore, leaf-rolling weevils (148) and lizard beetles (144) should no longer be referred to as fungus farmers.

Nutritional Mutualisms

It has been hypothesized that nutritional mutualisms are particularly common between organisms from different kingdoms (22) and in marginal habitats (83). Both assumptions are related to nutrient acquisition. First, partners from different kingdoms often utilize complementary strategies to acquire nutrients. Insects are highly mobile and can locate rare and unequally distributed resources, and they have efficient methods for penetrating the physical barriers of plants or animals (e.g., bark or skin, respectively). In contrast, many fungi have a rich repertoire of enzymes that degrade polymeric materials (e.g., wood) whose entrapped nutrients are otherwise unobtainable to insects. Additionally, many plant-associated fungi can degrade plant defensive compounds that make plant substrates unpalatable to insects (37). Second, it has been suggested that mutualistic

associations have higher fitness on low-quality diets than either partner living separately because division of labor allows elimination of redundant metabolic pathways (17). Although complementary abilities to acquire nutrients play an early role in the formation of mutualistic partnerships (see below), the elimination of metabolic pathways is unlikely to happen before, but may possibly happen with, the transition to obligate dependency (i.e., the loss of metabolic pathways could be the cause of obligate dependency). Very few insect–fungus mutualisms have reached obligate dependency (**Tables 1–3**).

Our overview reveals that insect–fungus mutualisms evolved particularly often on nutritionally challenging substrates, most frequently in association with wood, phloem, or foliage (25 insect lineages and 24 fungal lineages), followed by association with fruits, nectar, or honeydew (7 insect lineages and 9 fungal lineages) and animal carcasses (2 insect lineages and 2 fungal lineages) (**Figure 3*a***, **Tables 1–3**). Except for carcasses, all of these substrates are rich in carbon (C) but extremely low in nitrogen (N), phosphorus (P), and other trace elements (K, Na, Mg, Fe, Zn, Cu), all of which are essential for insect growth and reproduction (43). Foliage and wood, for example, have N and P contents 10–20 times or 2,000–3,000 times lower (40), respectively, than required by the insects (43). Sap-, foliage-, and decayed wood–feeding insects often engage in bacterial mutualisms to alleviate these deficiencies (28, 57), whereas feeders of fresh and undecayed wood and phloem tissue rely more on fungal mutualists (25, 47, 132). An indicator of the relatively poor nutritional quality of wood is evidenced by wood-feeding insects that do not associate with fungi, which typically have developmental periods of several years (e.g., Buprestidae, Cerambycidae), whereas many mycetophagous or xylomycetophagous ambrosia beetles (i.e., feeding on xylem and fungi), as well as phloeomycetophagous bark beetles (i.e., feeding on phloem tissue and fungi), develop in half a year or less (56). The evolution of phloeomycetophagy allows for more efficient use of the phloem tissue, thus providing an advantage over nonfungus-associated phloem-feeding insects (e.g., Cerambycidae) (58).

Filamentous fungal mutualists are directly beneficial to feeding insects due to their ability to synthesize essential sterols; translocate nutrients; and concentrate N, P, and other trace elements at sites where the insects feed (15, 57, 133). However, on many plant substrates like wood, phloem, and foliage, their primary role is to degrade and detoxify lignocellulosic plant tissues enriched with defensive plant allelochemicals [e.g., terpenes, alkaloids, phenols (15, 37, 65)]. Fungi associated with bark and ambrosia beetles [Ascomycota: Ophiostomatales (33, 152)], fungus-growing ants [Basidiomycota: Agaricales (36, 65)], and yeasts of fruit flies on cacti (138) are important for detoxification of plant allelochemicals. Ship-timber beetles, lizard beetles, and woodwasps, as well as foliage-consuming termites and gall midges (**Tables 1–3**), are further candidates for having fungal mutualists with plant toxin–degrading capabilities. Degradation of cellulose, hemicellulose, and pectin by fungal enzymes is presumably important for all mutualisms on plant substrates (56, 88), even though it has only been studied in bark and ambrosia beetles (34, 61, 82), woodwasps (80, 143), termites (90), and fungus-growing ants (7, 35, 81). Many bark and ambrosia beetles, fungus-growing termites, and some woodwasps feed not only on fungal biomass, but also on plant tissue ingested along with it (34), and for the latter two groups, it has been shown that fungal cellulases and hemicellulases remain active in the insects' guts (80, 88, 90). In addition, fungus-growing ant larvae and adults apply fecal droplets containing active enzymes acquired from their fungal diet onto their gardens (89).

Animal carcasses colonized by burying beetles (Coleoptera: Silphidae) and their *Yarrowia* mutualists (Ascomycota: Saccharomycetales) supply a very different substrate because they are rich in protein (i.e., N) and easily degradable. The enzymatic capabilities of yeasts are of primary importance (130) for two reasons. First, the reduced protease activity in carcasses with symbiotic

yeasts could facilitate a gradual breakdown of proteins and thus avoid N toxicity caused by the accumulation of urea, uric acid, and ammonia (29). Second, the high lipase activity of symbiotic yeasts accumulates fatty acids with defensive antimicrobial properties against bacteria that would quickly spoil the cadaver with toxins (67, 130).

Protective Mutualisms

Insects protect their fungal mutualists through environmental manipulation and unique behaviors (12, 62, 97). In many ambrosia beetles, fungus-growing ants, and termites, the insects build more or less homeostatic habitats [e.g., ventilated nests, fungus chambers (100)], which reduce environmental fluctuations and exposure to and establishment of natural enemies of insects and their fungal mutualists (**Figure 3*b***) (62). These mutualists also possess protective traits that may also benefit the fungi [e.g., soldiers in ants and termites (77), tunnel blocking in ambrosia beetles (72)], even if these traits are not confined to fungus-associated taxa (77). Cooperative behavioral defenses (social immunity) against antagonistic microorganisms, such as grooming of nest mates, weeding of the fungal garden, and nest sanitation, are displayed not only in these systems (12, 100) but also in burying beetles and bark beetles (27, 72, 130) and, in rudimentary form, even in gregariously feeding larvae of fruit flies (12, 119, 120), for example.

Mechanical protection and antimicrobial defense by fungi are widespread in mutualisms with insects (72, 100), in particular for relatively vulnerable or exposed stages of insects on plants or in soil (**Figure 3*a***, **Tables 1–3**). For example, fungus coating of ants by nestmates has been hypothesized to protect against predators, parasites, diseases, and desiccation (6, 102) and in fruit flies against parasitoids (4). In addition, at least two aphid and two scale insect species are protected by fungal covers produced by various fungal species [Ascomycota: Capnodiales and Basidiomycota: Boletales, Septobasidiales (24, 51, 52, 69, 79)]. These covers are hypothesized to provide protection against parasitoids (30).

Larvae of gall midges develop in galls on herbaceous plants (**Figure 1**), and fungal mutualists (Ascomycota: Botryosphaeriales) strengthen the gall tissue, making it difficult for natural enemies (e.g., birds and parasitoids) to reach the larvae (69, 117, 118). Mechanical protection against environmental factors is also provided by carton-nest fungi (Ascomycota: Capnodiales, Chaetothyriales) in several groups of ants (106, 125, 147). With the exception of gall midges, there is clear separation between mutualistic fungi that provide nutritional benefits and those that provide mechanical protection (**Figure 3*a***), as the latter requires the production of a strong cell wall (i.e., chitin and other polysaccharides), and these polymers might trade off with palatability for the insects. Interestingly, there is no case of fungal mechanical protection within wood, suggesting that this habitat is well-protected against natural enemies and environmental factors.

Fungi also produce a wide array of volatile and nonvolatile secondary metabolites with antimicrobial properties (45, 149). Except for a few systems that have not yet been investigated (some bark and ambrosia beetles, lizard beetles, and woodwasps), all insect hosts profit from fungal antimicrobial defenses (**Figure 3*a***) (45). The full overlap between mechanical protection and antimicrobial defenses (**Figure 3*a***) suggests that these defenses complement each other. In contrast to a previous assumption that there should be selection against secondary metabolites in fungi that are nutritional mutualists of insects, due to the possible detrimental effects of such metabolites on the insects (104), there is an almost complete overlap between fungi that provide nutrition and those that provide antimicrobial defenses (**Figure 3*a***). The various antimicrobially active alcohols and terpenoids, for example, which are produced by many insect-associated fungi (e.g., 13, 68, 115), are apparently harmless to their hosts.

Dispersal Mutualisms

Fungi are sessile by nature, and it has been suggested that sessile organisms often engage in dispersal mutualisms (**Figure 3*b***) (19). The more specialized and difficult-to-access a niche is, the more benefit fungi derive from directed dispersal of their propagules. About half of the insect–fungus mutualisms were likely first initiated by insects passively dispersing fungi (**Tables 1–3**), followed by adaptations of fungal spores for external [e.g., hooks, sticky surfaces (58)] or internal [e.g., thick-walled spores, yeast-like growth (139)] dispersal by the insect (**Tables 1–3**). To retain the beneficial effects of the fungi, many insects evolved dedicated fungus spore–carrying organs (e.g., mycetangia) (47, 75, 84) or transmit their fungal symbiotes in the gut [common in Saccharomycetales (11, 130, 139)] (**Tables 1–3**). Our analysis of extant insect–fungus mutualisms shows that almost all fungi involved in mutualisms are dispersed by their hosts, with the exception of fungus-growing termites [most fungus-growing termite species collect their fungal mutualists from the environment (76)] and poorly studied scale insects and aphids (Hemiptera), which engage in fungal associations that have not yet been shown to involve dispersal (24, 30, 52, 79).

EVOLUTION OF MUTUALISMS

Ever since the publication of *On the Origin of Species* (31), we have known that natural selection will never lead to an organism that invests more in helping another organism than it gains in (possibly inclusive) fitness. Current evolutionary theory views mutualism, therefore, as reciprocal exploitation that provides net benefits to each partner (19, 23, 93, 122, 150). Nevertheless, ecological conditions can favor division of labor between species, and if the fitness interests of both partners become aligned, then selection may lead to reciprocal investments. Mutualisms based on such investments are prone to cheaters (i.e., individuals that reap the benefits of partnership but do not reciprocate) (142), which have to be controlled by the partnering species to maintain the partnership over long time scales (122, 150). If such a relationship managed to become stable and mutually beneficial, it could lead to a so-called major evolutionary transition to obligate dependence between species, i.e., a situation in which organisms cooperate to form a new, more complex superorganism by losing their abilities to survive independently (93).

To understand the evolution of obligate mutualisms, it is useful to divide their evolution into three steps: (*a*) the formation of a mutualistic group, (*b*) the maintenance of a mutualistic group, and (*c*) the transformation of a mutualistic group into obligate dependency (19, 150).

Formation of a Mutualistic Group

What are the factors that favor the formation of a mutualism? First, there need to be ecological or physiological benefits to both partners for engaging in cooperation. Individuals of different species, especially in different kingdoms, may start to interact if one partner can provide a service that the other partner cannot perform or performs less efficiently (150). Exchange of the major services provided by insects (dispersal, protection) and by fungi (nutrition, protection) allowed insect–fungus mutualists to inhabit previously unoccupied niches, like freshly dead wood in trees, which had been previously unsuitable for insects and inaccessible to fungi (15, 74, 132).

Second, there needs to be a mechanism for partner choice (i.e., for finding a beneficial partner), and this mechanism appears to be used by the more slowly evolving species to control the quickly evolving partner (122). Indeed, if partner choice occurs in insect–fungus mutualisms, insects always appear to be in control of choosing, whereas fungi attract their partners by the production of visual or, more typically, particular chemical signals in the volatilome (13, 26, 32, 68). Signaling is evolutionarily stable only if the signals cannot be faked (i.e., if they remain honest

indices of cooperative quality) or are significantly costly to fake (150). Chemical signals in insect–fungus mutualisms are poorly studied (13, 68), but we hypothesize that nutritional benefits provided by fungi are predisposed to being the source of signals because the nutritional state of a fungus is reflected by its volatile profile (78), and signals might therefore be difficult to fake. As an alternative to chemical signaling, insects may screen possible partners by creating a competitive market, i.e., an environment with nutrients and/or bioactive compounds in which only the mutualist prevails (5). Screening is hypothesized for some animal–microbe mutualisms (for theory and examples, see 124) and was recently described for fungus mutualisms of certain ambrosia beetles (115). We hypothesize that screening is likely to be found in defensive and protective insect–fungus mutualisms (e.g., in fruit flies or termites) because competition within microbial communities can be fueled by nourishment through the insects and thus favor the recruitment of bioactive fungal partners (13).

Maintenance of a Mutualistic Group

What factors maintain mutualistic partnerships and protect against cheaters? Cooperative groups persist when associations between mutualistic genotypes are formed and the benefits of helping are returned to the cooperator or related individuals (19). One possibility is partner-fidelity feedback generated by vertical transmission (parent to offspring) of closely related, often clonal symbiotes by hosts (5, 122, 134). Vertical transmission is common in insect–fungus mutualisms and often recognizable by the presence of spore-carrying organs (e.g., infrabuccal pockets, mycetangia) for the dispersal of their fungal mutualists (**Tables 1–3**), even though horizontal transmission of fungi between neighboring nests may occur (100, 134). As vertical transmission is a very strong tool for aligning the fitness interests of insects and fungi, the presence of selective spore-carrying organs is a strong indicator of reciprocal, mutually beneficial adaptations and in many cases of obligate dependencies. Genetic homogeneity can be further enforced by positive feedbacks between fungus productivity and relatedness of fungal strains [e.g., attine ants, termites (3, 113)]. Conversely, the loss of mycetangia in ambrosia beetles is associated with cheaters that steal fungal cultivars but do not provide dispersal in return (134).

An alternative mechanism for maintaining mutualistic partnerships is host sanctioning, whereby a symbiote is punished if it misbehaves [e.g., fungus does not deliver service, tries to reproduce sexually or disperse horizontally (48, 95, 150)]. Sanctioning has been described in plant–rhizobia mutualisms (70), although it may be uncommon in insect–fungus mutualism because most fungi seem to rely on dispersal by their hosts [although there are examples of free-living mutualists in Blattodea, Hemiptera, Diptera, and Lepidoptera (26, 42, 79)], which is correlated with insect fitness, and therefore, less beneficial fungi are automatically punished by decreased dispersal (3). However, although it has not been demonstrated, compartmentalization of the fungus in more or less enclosed chambers or tunnel systems (e.g., those of attine ants, termites, and ambrosia beetles) improves host control over fungi, possibly including sanctions (3, 95, 141). Sanctioning of insects by fungi might be present in the plant sap–sucking scale insect–*Septobasidium* association, in which the fungi often consume a few of the insect symbiotes that they normally just protect under their fungal shields on the surface of plants (30). Although this association has been proposed to be mutualistic (30), others claim it to be fungal parasitism (60, 64).

Transformation to Obligate Dependency

Given that ecological benefits for division of labor prevail, and cheaters are controlled (see above), dependencies between partners can increase over time. Thus, insect–fungus mutualisms

can transition from facultative to obligate, meaning that each partner becomes so specialized in helping the other that it is no longer viable on its own. Insect–fungus mutualisms in higher attine ants[1] (101, 128), fungus-farming termites, ambrosia beetles, ship-timber beetles, galling midges, and woodwasps have evolved one- or two-way obligate dependencies (**Figure 3**, **Tables 1–3**). Why have other mutualisms, such as most flies and yeasts [but contrast with fruit flies on cacti (138) or root-aphids and defensive fungi (24, 79)], not transitioned to obligate dependence? Crucial for the transition to take place is negligible conflict, and it turns out that, in some systems, conflict is almost impossible to minimize due to environmental acquisition of symbiotes. This horizontal transmission of symbiotes automatically increases their diversity, leading to conflict and selection for less cooperative symbiotes (150). This is most obvious in the high frequency of sexuality in horizontally transmitted fungal mutualists, whereas strictly vertically transmitted ones are commonly asexual (see below). Even though partner choice and sanctions are more important in systems with horizontal transmission (150), obligate dependencies rarely evolve in these systems. In some environments with high fungal diversity, like rotting fruits or soil, it is probably impossible for the hosts to exclusively vertically transmit fungal mutualists.

In contrast, living or freshly dead wood, which is nearly sterile (116), is inhabited by several insect–fungus mutualisms with vertical transmission (**Tables 1–3**). As outlined above, there will be no conflict between exclusively vertically transmitted symbiotes, which can be transmitted to more individuals only by increasing the reproductive success of their host. In theory, genetic drift in these small, asexual symbiote populations (tight bottleneck) can accelerate the evolution of mutual dependency (41, 71, 94), although gene loss may go unnoticed when functions are compensated for by the partner (39, 107). There is currently no evidence for genome reduction in ectosymbiotic fungal mutualists of insects, however (107, 114, 146). Overall, vertical transmission will be favored only if it has immediate benefits and not because lower conflict will select for more cooperative symbiotes in the future (48, 150). In the higher attine fungus mutualism, ants profit from higher productivity with decreasing symbiote-genotype diversity and reduce symbiote conflict by weeding out all but the resident fungal cultivar strain (113).

Another remarkable fact is that, in host–bacteria mutualisms under vertical transmission, mutualists providing nutrients are a stronger driver of host dependency than mutualists providing defensive benefits (44). The reason may be that nutrients are utilized in every generation, whereas defenses may be needed only in certain environments (44, 151). To some extent, this pattern also occurs in insect–fungus mutualisms, as reflected by the evolution of morphological structures, asexuality, and sociality, particularly under vertical transmission (see below; **Tables 1–3**).

Evolutionary Consequences of Mutualism

Coevolution between insects and fungi can lead to adaptations in one or both partners, which can relate to various life-history traits. In this section, we give three examples related to the morphology, mating system, and behavior of the organisms.

Evolution of morphological structures in both insects and fungi. Apart from physiological adaptations to the mutualism in both insects and fungi, as well as changes to the mouthparts of insects when feeding on fungi, two morphological adaptions in obligate insect–fungus mutualisms are striking (74).

[1]Five agricultural systems have been identified in attine ants: lower agriculture, coral fungus (Pterulaceae) agriculture, yeast agriculture, higher (domesticated) agriculture, and leaf-cutter agriculture (101, 128). The last two are referred to as higher attine.

Fungal spore-carrying organs [i.e., mycetangia (46, 47, 75, 84, 118) or infrabuccal pockets (86)] guarantee the vertical transmission of mutualists. Most advanced are the mycetangia of ambrosia beetles (46, 47, 63, 131), which are lined with glands that are active only during beetle dispersal (126, 127) and that are highly selective in transmitting only the mutualistic fungus and, to some degree, closely related species (20, 92, 134). The underlying mechanisms are unknown and might involve selective chemicals produced by the insects or nutrients that specifically benefit the mutualists through competitive exclusion [i.e., screening (134)].

In fungi, a characteristic feature of obligate mutualism with insects is their pleomorphism to produce swollen, nutrient- and enzyme-rich conidia, first termed kohlrabi-like structures in attine ants (99) and then ambrosia structures for all fungi-growing insects because of their convergent morphology (105). The term ambrosia structures is still in use for the fungi of ambrosia beetles, ship-timber beetles, and gall midges, whereas gongylidia and nodules are used for attine ant and termite fungal mutualisms, respectively (100). Ambrosia structures and gongylidia evolved from asexual spores (conidia) that developed their nutritional value over time. In ants and some ambrosia beetles, they are only present in fungus gardens tended by the insects (8, 10, 50). The mechanisms of induction remain enigmatic in these systems. Nodules are immature mushrooms that produce asexual conidia on their surface and are induced by depletion of particular nutrients in the substrate (35); the termites prevent them from reaching maturity (1, 3).

Evolution of asexuality and clonality in the fungal mutualists. Vertical transmission links the fitnesses of insects and fungi and is highly advantageous for the evolution of obligate mutualisms (see above; 49). Transmission of fungal propagules in spore-carrying organs results in severe bottlenecks and reduced mutualist population sizes, which in the long run produce clonal monocultures (1, 3, 76, 145). Clonal monocultures have the advantage that no resources are wasted for between-mutualist conflict within insect nests, which always arises if the mutualist's fitness depends more on its ability to outcompete other mutualists than on the overall success of the group (48). Despite some exceptions (38, 76, 91, 108), there is remarkably convergent evolution from sexuality in ancestors of the mutualistic fungi to asexuality (or suppressed sexuality) and clonal symbiote populations in the primary fungal mutualists of insects with vertical transmission, like ambrosia beetles, fungus-growing ants, and a few termite species (76, 100, 145). Infrequent mating and low within-nest diversity of mutualists can also be expected in some of the other insect–fungus mutualisms (e.g., lizard beetles, ship-timber beetles) (9).

Evolution of social behavior in the insect hosts. There is a striking pattern of social evolution in some insect–fungus mutualisms in which the insects profit nutritionally from the fungi. Social groups of insects are apparently often more effective in the maintenance of their fungal mutualists, including (*a*) vertical symbiote transmission from parents to offspring [e.g., by overlapping generations in the same nest, as seen in ambrosia beetles, burying beetles, attine ants, and some termites (72, 87, 100, 130)]; (*b*) provisioning of their fungal mutualists with new substrate [e.g., by foraging, as in ants and termites, or tunneling in wood, as in bark and ambrosia beetles (72, 100)]; and (*c*) regulation of the microclimate, physical protection, and antimicrobial defense of mutualists against natural enemies (12, 72, 97, 100). Therefore, it is no coincidence that two of the most advanced insect–fungus mutualisms (termites and attine ants) evolved in lineages that were already eusocial at the beginning of the association. These insects were predisposed to evolve specialized castes for substrate collection and provisioning, as well as tending and weeding of fungus gardens (100, 103).

In other systems with solitary or subsocial ancestors, social interactions—from simple gregarious feeding in fruit flies to complex division of labor in ambrosia beetles—evolved along with the

insect–fungus mutualism (12, 14, 120). Gregarious larval feeding, for example, is found in several groups of ambrosia beetles, galling midges, and fly larvae. In the latter, it is useful for spreading mutualistic yeasts and defending against antagonistic molds (119, 120). Parental care in burying beetles selectively promotes mutualistic *Yarrowia* yeasts over other carcass-degrading microbes (130). Finally, there is a striking coevolution in ambrosia beetles between the temporal maintenance of the fungus mutualism in beetle nests and the social system: Simple parental care and nests in the wood-boring ancestors have evolved into facultative eusociality in some Xyleborini or even obligate eusociality with sterile castes and nests active for more than 30 years in *Austroplatypus incompertus* (Platypodinae) (72, 136). Protection and defense against other microbes has probably been the major driver of sociality in all of these systems that evolved from non-eusocial ancestors (12). Corroborating this, related, non-fungus-farming bark beetles (Scolytinae) never evolved social structures more complex than parental care (72).

CONCLUSIONS

It remains unclear what ecological factors facilitate the formation and maintenance of a mutualistic group and its transformation to obligate dependency (19, 150). Insect–fungus mutualisms are ideal for studying these topics, as there are many independent origins of mutualism. Insect–fungus mutualisms evolved in at least 14 families of insects in six orders (Coleoptera, Blattodea, Lepidoptera, Hymenoptera, Diptera, and Hemiptera) and at least 15 orders of fungi in the Ascomycota and Basidiomycota (**Tables 1–3**). In several of these groups, mutualisms evolved repeatedly (ambrosia beetles, woodwasps, attine ants, gall midges). Of all insect–fungus mutualisms reported in the literature, only about a dozen have been studied in detail: some of the bark and ambrosia beetles, attine ants, termites, and woodwasps. Many others remain vastly underexplored, e.g., leaf-rolling weevils, ship-timber beetles, lizard beetles, burying beetles, stingless bees, and scale insects (**Tables 1–3**). Thus, there is a clear need for more studies of additional species in the well-known groups and more in-depth studies in the poorly studied species. In addition, it is likely that more mutualisms will be discovered, especially among specific groups such as pollinators and flies (11, 121) or in habitats that are still relatively unexplored, such as soil (16). These discoveries will be interesting not only from a natural history point of view, but also for understanding the evolutionary ecology of mutualisms.

In comparison to other mutualisms (e.g., insect–bacteria), insect–fungus mutualisms lend themselves to experimentation with aposymbiotic insects and the effects of separate fungal species on the mutualism. Microbiome studies and multi-omic approaches, including genomics, metabolomics, proteomics, and transcriptomics, will allow for a better understanding of insect–fungal mutualisms, as well as the microbiome associated with the insect. It is evident that insect–fungus mutualisms are currently not in the forefront of studies addressing questions related to the evolution of cooperation between species. Compared to within-species cooperation, not much is known about the ecological factors that facilitate the formation and maintenance of mutualisms (19, 150). The multiple independent origins of mutualisms, such as in the closely related lineages of ambrosia beetles, could be excellent models to study this. Conversely, it would be interesting to also determine if and how mutualisms can disappear, as documented for some insect mutualisms with bacteria (123), but not fungi. More specifically, it would be interesting to investigate partner choice mechanisms in the insects more closely: is it really the signaling of the fungi that maintains partnerships in the absence of vertical transmission or could newly theorized mechanisms such as screening (5, 124) be involved? Also, it is yet to be determined whether certain services that are exchanged in mutualisms trade-off with each other; in insect mutualisms, this could be the case for the protective and nutritional value of fungi, for example. Finally, from an applied

perspective, some of the advanced fungus-farming insects could also be comparative models for problems involving human agriculture, such as pathogen defense and evolution of resistances against pesticides. Therefore, studying insect–fungus mutualisms could elucidate important aspects in the evolution of cooperation and maintenance of agriculture.

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