

# Chapter 17

## Symbiotic Harmony Between Insects and Fungi: A Mutualistic Approach



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**Abstract** Microbes are known to interact with a variety of organisms belonging to different classes, genera, or species through their own diverse and specific pathways and mechanisms. Such an interaction, which exists between microbes and herbivores like insects, has become a topic of great importance for researchers far and wide. Since such interfaces occur in the form of mutual interactions, which in turn leads to the participating organisms achieving rich and important advantages that are necessary for their survival and development. Much of the research on reciprocal interactions between insects and microbes have focused on bacterial associations with insects, more or less ignoring the fact that interactions between insects and fungi are equally important which usually follow the same mechanisms and pathways as associations between insects and Bacteria.

This chapter deliberates the various aspects and properties of fungal interactions with mushroom growers such as leaf-cutters (*Attina* ants), termites, and ambrosia beetles. These interactions are based on a complex and interesting evolutionary line, which finally introduces the concept of mutuality into this insect community. The benefits of these interactions range from nutrition to the spore dispersal of fungi as well as protection from predators and competitors. The interaction between yeast and insects has also been discussed in ample detail, with our focus mainly on the areas in which each participant in the interaction benefits.

**Keywords** Fungi · Insects · Beetles · Ants · Termites · Yeast · Mutualism

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## 17.1 Introduction

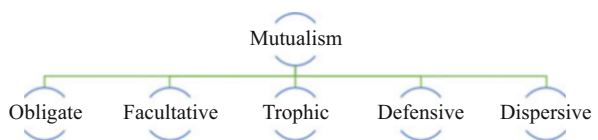
Nature has its own way of astonishing us at the intricate details found in each of its naturally occurring phenomena, as well as the beauty of the complexity that revolves around it. The harmony of the ecological interactions between different organisms of different species and the balance that it offers for the environment as a whole is such that, ecologists all over the world always leave with awe and wonder at the perfection of nature's creativity.

Ecological interactions, which indicate that certain organisms are primarily dependent on one another for their survival, are classified according to whether these interactions have neutral, advantageous, or harmful effects for one of the interacting organisms. Symbiotic interactions contribute to a main category of such interactions in which both interacting organisms are closely related. Depending on whether the interaction partners produce a positive, negative, or neutral effect, symbiotic relationships are divided into mutuality, parasitism, or commensalism (Molles 2015; Smith et al. 2012).

Mutualism as a positive category of symbiotic relationships is examined in more detail and investigated, which brings us closer to the complexity of mutual interactions between different species or organisms that cannot be taxonomically related, but thrive together and contribute to mutual survival in a competent and challenging environment. Although there are mutual relationships between almost all organisms on earth, including humans, this chapter mainly focuses on the characteristics and evolutionary history of such an interaction between different types of fungi and insects.

So what exactly is understood when the term “mutualism” is being referred to? Mutualism involves an interaction between two kinds of organisms, a host and a symbiont, belonging to different class, species, or taxa. It is different from other types of interactions as mentioned in Fig. 17.1, wherein both the organisms are equally benefitted from the interaction, in contrast to commensalism or parasitism in which either of the organisms gets no benefit, or they are caused harm by the other. In short, mutualism is a reciprocatory positive interaction between a pair of organisms (Lu et al. 2016). For example, the interaction between ants and aphids in which the ants rear aphids for their source of food which the aphids produce and in turn the aphids are protected by the ants from potential predators. Another example of mutualism, within the aquatic kingdom is that of clownfish and sea anemone. The clownfish lives within the poisonous tentacles of the anemone, being immune to its poison and act as a bait for luring other small sea animals close to the vicinity of the anemone's tentacles. The association of sharks with remora fish is another of the many wonders of mutualistic interaction in the sea world. Last, but not the least, we

**Fig. 17.1** Different Types of Mutual Associations



humans ourselves survive on the basis of a mutualistic relationship with microbes such as bacteria which thrive well in the intestinal microenvironment depending on the host's nutrition, and at the same time see to it that we remain protected against the harmful effects of ingested pathogenic microbes (Roossinck 2008).

## 17.2 Fungi and the Ecosystem

It is a well-known fact that many species belonging to kingdom fungi live in symbiotic associations with lower organisms such as cyanobacteria as well as higher organisms belonging to kingdom plants or animals. While some species may have a parasitic relationship with the associated organism wherein they derive food and thereby have harmful effects on the host, it is found that some other species survive through a mutually beneficial exchange of services between them and their associated partner. One such relationship that is observed, is between fungi and plant roots. Mycorrhiza is the term used to refer to such an alliance in which the fungus is benefitted by the food accessed from the plant and in return the fungi mycelia help to absorb water and aid nutrition thereby providing nourishment to the plant.

The mutual association between fungi and photosynthetic organisms, usually cyanobacteria or green algae, is called lichen. The fungus grows around its host and absorbs food prepared by photosynthesis in exchange for water and nutrients (<https://www.Ck12.Org/Biology/Fungi-Symbiosis/Lesson/Symbiotic-Relationships-Of-Fungi-Bio/>).

When it concerns microbe-insect mutualism, much importance is directed towards exploring associations within the bacterial society, more or less, limited importance being given to the fungal associations that are equally common and important. In contrast to bacterial mutualism, fungal associations with insects are mostly facultative and horizontal, and it is reported that the microbial cells live extracellularly in hemolymph, fat bodies, or other specialized structures of the associated insects (Klepzig et al. 2009). A well-characterized and general example of such a relationship is that between leaf-cutter ants and fungi, in which the ants cultivate the fungi to gain access to their source of nourishment. A similar kind of mutualistic interaction is seen between certain species of bark beetles and fungi too. Other examples of insect–fungi mutualism which may not be too well known include that of wood wasp *Sirex noctilio* and wood decay fungus *Amylostereum areolatum*. The fungus contains wood degrading enzymes which enable the wasps to degrade and colonize tree barks (Nielsen et al. 2009; Kukor and Martin 1983; Talbot 1977) Another example, out of the several species that can be quoted in this context, is that of *Epichloe* species of fungi and *Botanophila* flies. The flies consume the fungal spores and cause deposition of their fecal matter onto unfertilized stroma rendering cross-pollination (Bultman et al. 1998; Bultman and Leuchtman 2008). Fungal mutualists are now also reported to play a pivotal role as producers of chemical indicators for insect communication. The fungi *Pichia pinus* and *Hansenula capsulate* produce are capable of converting cis and trans verbenol to

verbenone which acts as an anti-aggregation pheromone for bark beetles (Hunt and Borden 1990; Leufvén and Nehls 1986).

This chapter also focuses the attention on the evolutionary history as well as on the features that include the mutual relationship between fungi and insects such as leaf-cutting ants, ambrosia beetles, termites, and arthropods.

### 17.3 The Evolutionary Antiquity of Fungus-Farming Insects

Extensive work and research in this area have proposed that the evolution of an obligate mutualism proceeds primarily through five stages: (a) consistent and extended contact (b) avoidance of lethal harm during contact (c) coadaptation leading to reduced virulence and increased tolerance (d) further co-adaptation leading to dependence or interdependence (e) still further coadaptation leading to permanence and stability in the association. These stages give an overview of the progress of any agonistic or antagonistic interaction to stable mutualism (Taylor and FJR 1983).

Since time immemorial, even when the idea of cultivating plants for food to sustain oneself dawned in man's mind, the lineages of three insects namely, termites, ants, and beetles, rose to ecological importance by evolving into fungus cultivating farmers. Since they were completely dependent on the cultivated produce as the sole source of their nutrition several tasks partitioned societies all playing their own individual role in raising of food for their kind and thus in the process, became important players of the ecosystem (Wilson 1971). These insects, mostly being pests and detested by the human population, were put into the task of being exterminated but only recently it had come to the knowledge of the evolutionary glory that runs through their veins.

The transit of termite, ants, and beetles to the arena of fungi culture follows different evolutionary pathways. In termites, fungi probably had been an important source of nutrition derivation before they turned to cultivation. Many non-farming termites are known to feed on fungus infested woods and the termite fungi culture is now believed to be an expansion of such feeding habits of the ancestors (Batra 1979; Rouland-Lefèvre et al. 2006). Studies reveal that out of the 2600 species of identified termites, about 330 species survive in obligate association with a fungus basidiomycete genus, *Termitomyces*. *Termitomyces* are grown in subterranean combs within the heart of termite mound nest (Batra 1979; Abe et al. 2000a). Consumption of the fungal spores and deposition of fecal pellets consisting of the consumed fungal spores and plant forage within the comb serves as the seed for the growth of new fungi cultivar (Mueller and Gerardo 2002). Such a fungi culture practice also serves as a boon for obtaining the genetic material of the fungus from termites, sidestepping the tedious task of nest excavation. To reassemble the evolutionary history, researchers first compared the DNA of *Termitomyces* with that of

non-domesticated fungi, and secondly, comparison was drawn between the DNA of termite farmers and non-farming relatives (Aanen et al. 2002). Studies conducted revealed that the fungus cultivation began in termites with the *Termitomyces* species of cultivars which eventually differentiated into several other cultivar species each almost exclusively associated with a particular termite group. Moreover, each of these cultivar groups has been found to be exchanged between the termite lineages within these group of termites. This led to the adaptation of termite groups to specific fungus cultivars at the same time fungus cultivars have evolved to adapt and survive only in association with certain farming groups (Mueller and Gerardo 2002).

A striking evolutionary parallel can be drawn when comparing the emergence of termite farmers with that of beetle and ant cultivators. In contrast to the ancestral feeding habits of termites that evolved into termite fungi culture, an ancestral vectoring system is responsible for the evolution of beetles into fungus cultivators. Whereas in ants it is still not clear whether fungus cultivation emerged from ancestral mycophagy or ancestral vectoring (Seifert et al. 1993; Mueller et al. 2001). Whatever may be the reason, it is now known that ants started growing fungus (mostly basidiomycetes) in their backyard about 50–60 million years ago (Mueller et al. 2001) and since then till date, roughly about 200 species of fungus-growing ants have emerged (Schultz and Meier 1995). Ants raise their fungus cultivation in subterranean chambers providing manure to their growth in the form of vegetable debris, or in the case of leaf-cutter ants, leaf fragments from live plants. The leaf-cutter ant's sustenance is completely dependent on the fungi they grow and hence, form an obligatory association with their fungal partners (Mueller et al. 2001). As in the case of ants, certain species of beetles commonly known as ambrosia beetles are found in obligatory association with fungi as they grow them as their primary source of food and to derive important nutrition in order to complete their life cycle (Farrell et al. 2001; Batra 1966). The fungus provides nutrition to most of the beetle developmental stages while the insects carry the fungal spores along with them, infecting new trees (Harrington 2005; Paine et al. 1997). The fungi also produce that degrade the indigestible wood into nutritious matter for the insects (Valiev et al. 2009). In stark contrast to termite and ant fungi culture which arose just once in each group and later led to diversification, studies suggest multiple origin of fungus cultivating habit in ambrosia beetles which arose at least seven times giving rise to sheer diversity of beetle species with respect to their feeding habits (Farrell 1998). No records of an evolutionary reversal to a non-fungus-cultivating pattern of life in any of these nine known, independently evolved farmer lineages have been found, suggesting a similar trend to that of humans where transition to way of living depending on agriculture has contributed to a radical and irreversible change that probably has bridled subsequent evolution (Diamond and Renfrew 1997).

## 17.4 Mutualism Between Leaf-Cutter Ants and Fungi

Leaf-cutter ants are considered major players of the ecosystem owing to the significant effects they elicit on local flora (Wirth et al. 2003), seedling recruitment (Costa et al. 2008), distribution of soil nutrients (Sternberg et al. 2007) and human agriculture (Cherrett and Peregrine 1976). These species of ants have known to be in association with fungi species since as long as 40–50 million years which it is believed, originated on the Amazon basins (Schultz and Brady 2008). Fungus-growing ants (*Myrmicinae:Attini*) cultivated fungus for their young ones using organic debris such as dead insect parts and feces as manure for their garden (Weber 1972; Mueller and Wcislo 1998; Mueller et al. 2005). A significant behavioral change in the ancestors *Acromyrmex* and *Atta* in replacing leaf fragments and other plant parts as compost for their fungus gardens instead of the usual organic debris contributed majorly to the deviation in the evolutionary pattern of the species (Schultz and Brady 2008; Hölldobler and Wilson 2010). Such a change in the pattern of cultivation contributed to an astounding increase in colony size, social structure, and ecological footprint. Leaf-cutter ants have now emerged to be the most dominant and diverse in Neotropical ecosystems, harvesting about 2–17% of the foliar biomass of annual leaf production of the forest and savanna woody plants (Wirth et al. 2003; Costa et al. 2008; Weber 1972; Hölldobler and Wilson 2010; Wheeler 1907).

Although the most common associate of attine ants remains to be basidiomycetes fungus, a larger population of the species is involved in farming lepiotaceous fungus of the genus *Leucoagaricus* while a smaller population are farmers of a distantly related pterulaceous group of fungi (Herz et al. 2007; Chapela et al. 1994). The leaf-cutter ants are precise and definite about the plant species, the individual plant, and the leaves within the plant that they cut. Factors that steer the assumption that leaf-cutter ants prefer relatively easy to cut, less defended leaves with high nutritional values include selection of younger leaves than older ones, woody rather than herbaceous and light demanding rather than shade-tolerant species (Villesen et al. 2004; Blanton and Ewel 1985; Coley and Barone 1996; Farji-Brener 2001). The worker ants are involved in cutting the leaves, carrying the fragments to the nest, cleaning, and processing them to form suitable for the fungus to grow and thrive in underground chambers inside the nest (Schultz and Brady 2008). In return, the fungus cultivar partially degrades the leaf material which serves as a source of nutrition to the ant colonies and their developing larvae. Thus the mutualistic interaction endures on the basis of services offered by ants ranging from weeding and grooming to the disposal of various antimicrobial compounds while reaping the benefits of a healthy fungus cultivar (Wirth et al. 2003; Schultz and Brady 2008; Barke et al. 2010; Currie and Stuart 2001, Fernández-Marín et al. 2006, 2009).

Fungus gardens are principally composed of only one fungal mutualist (Aylward et al. 2012) and factors that contribute to the low diversity of fungi in the fungus gardens include cautious cleaning by ants to maintain the hygiene of the fungal cultivars. Three main hygienic practices have been observed in fungus-growing ants which include: (a) weeding removal of dead fungal debris (b) fungus grooming-

removal of spores of foreign fungi (Wirth et al. 2003) (c) application of fecal droplets to the fungal matrix. The fecal droplets of some species of attine ant is reported to contain fungal chitinases and lignocellulases which contributes to both plant biomass degradation as well as eradication of fungal pathogens (Rønhede et al. 2004, 2008; Martin et al. 1973).

Apart from the predominant fungus mutualist, microbes such as *Actinobacterium* of the genus *Pseudonocardia*, that produces antibiotic against fungus attacking parasites (e.g., *Escovopsis*), have been found in association with fungus gardens (Currie 2001; Currie et al. 1999a, b). Many of the bacterial species isolated from these ecosystems have been established to play a major role in not just antibiotic mediated extermination of pathogens but also in nutrient biosynthesis. One particular study carried out on *Klebsiella* and *Pantoea* species of nitrogen-fixing bacteria isolated from leaf-cutter ant nests proves the significance of these bacteria in fixing nitrogen and thus being important nutritional players in the ant–fungi ecosystem (Pinto-Tomás et al. 2009; Hölldobler and Wilson 2009).

The central role of fungus gardens cultivated by ants is the conversion of plant biomass into useful compounds important for the nutrition of the ants. The integrated biomass of fungus gardens includes a rich source of cellulose, hemicellulose, lignin, protein, simple sugars, and other compounds. In gardens of higher attine ants, these compounds are converted to hyphal swellings known as gongylidia, rich in lipids, carbohydrates, and other nutrients (Mueller et al. 2001; Martin et al. 1969). Gongylidia serves as an important food source for the entire colony and is an exclusive nutrient source for the developing larvae and brood (Hölldobler and Wilson 2009; Weber 1966; Nygaard et al. 2011; Suen et al. 2011).

## 17.5 Adaptation of the Ant Genome

The sequenced genomes of the leaf-cutter ants *Atta cephalotes* and *Achromyrmex echinator* have opened up a sea of knowledge about the symbiotic association of these species. Obligate dependence of ants on their fungi associate have led to reductions at the genomic level (Suen et al. 2011; International Aphid Genomics Consortium 2010). On examination, genomes of attine ants were found to be deficient in the levels of amino acid arginine in comparison to other non-farming ant genomes, pointing to the fact that the fungal cultivars provide the required arginine thereby reducing the need for the particular pathway. This hypothesis is supported by the evidence obtained from previous studies of the documented compounds found in *Atta colombica* cultivar which showed presence of arginine (Abe et al. 2000b; Johnson et al. 1981). The *A. cephalotes* genome was also found devoid of the hexamerin gene responsible for amino acid sequestration during larval development. Serine protease was another compound found in significantly low amount, and as with arginine and hexamerin, is hypothesized to be provided by the fungal cultivar (Abe et al. 2000b). These data indicate that over the years, having

established a mutualistic relationship with fungi, leaf-cutter ants have lost their ability and capacity to acquire nutrients on their own.

## 17.6 Fungus Cultivating Termite Species

Symbiotic relationship with a variety of organisms such as protists, methanogenic *Archaea*, and bacteria have always been a major player in the evolution of termites (Martin 1992). However, as on date only a single *Termitidae* sub-family, *Macrotermitinae* is known to have evolved into a mutualistic association with fungi of the genus *Termitomyces* (Abe et al. 2000c) and are a predominant component of the termite species in the African and Indomalayan region (Kambhampati and Eggleton 2000). The fungus is grown on a specialized structure known as fungus comb in the termite nest, maintained by the termites by the continual addition of new predigested plant debris while concurrent degradation of the old material is carried out (Kirk et al. 2001). The *Macrotermitinae* is divided into 11 genera with approximately 330 species and roughly about 40 species involved in *Termitomyces* symbiosis have been identified (Kambhampati and Eggleton 2000; Kirk et al. 2001). They play a significant role in litter removal by rummaging through dead wood, dead grass, and dung of herbivorous mammals. The workers collect substrate, chew them into very small fragments, maintain constant conditions in the nest for growth of the fungus cultivar, prevent the growth of potential competitors and thus in this way not just disperses the fungal spores, but also ensures their healthy growth inside the nest (Ausat et al. 1960).

The mature combs of *Macrotermitinae* which nests the cultivar is a firm intricate structure housing sparse growth of mycelium and populous small white spores called “mycotetes” that are accumulations of conidiophores and conidia, the asexual reproductive fruiting bodies of fungi. The combs are constructed of plant material that has been comminuted by chewing and passing through the workers gut. The cell wall polysaccharides undergoes insignificant breakdown and transformation during its transit through the digestive tract, as indicated by the presence of intact cells and cell walls, high cellulose content of the comb, and negligible difference in the cellulose-to-lignin ratio of the comb to the rummaged plant debris (Sands 1956).

The termite’s diet consists of both the fungus combs and the mycotetes, both performing an integral role in providing a source of excellent nourishment to the termites (Nygaard et al. 2011; Ausat et al. 1960) due to the presence of elevated nitrogen content constituting a range of 5.7–7.9%. Additionally, the fungal enzymes released from the mycotetes after ingestion combines with the enzymes present in the insect gut rendering high catalytic activity that contributes to cellulose and hemicellulose digestion of the plant material in the *Macrotermes* species (Sands 1956; Abo-Khatwa 1978; Martin and Martin 1978; Rouland et al. 1988). Due to the availability of a better and richer source of nutrition, the termites no longer depend on woodchips or filter paper to derive nutrition, unless in cases when they are



destitute of all food obtained through the fungal symbiont (Martin 1987; Wood and Thomas 1989).

It has been observed that the fruiting bodies of *Termitomyces* are always found in association with the termite nests indicating that within the basidiomycetes (Rouland-Lefèvre 2000; Rouland-Lefèvre and Bignell 2001) mutualistic symbiosis traces back to a single evolutionary origin and no record of reverting back to the nonsymbiotic way of living has been reported (Rouland-Lefèvre et al. 2002; Moncalvo et al. 2000, 2002). The patterns of cospeciation and specificity have been found to be steady and undeviating with transmission of fungal symbionts from host to host outside the vertical host lineage (Frank 1996). Horizontal symbiont transmission is the pattern observed in many species of *Macrotermitinae* associated with formation of fruiting bodies in its fungal symbiont. However, for *Microtermes* and species of *M. bellicosus*, clonal uniparental transmission has been observed. The female in *Microtermes* and the male in *M. bellicosus* takes up the task of transmitting the fungus in the absence of sexual fruiting bodies (Darlington 1994).

## 17.7 Mutualistic Association of Beetle With Fungi

The bark and ambrosia beetles are either considered as two families *Scolytidae* and *Platypodidae* within the weevils (*Curculionoidea*) or as sub-families of *Curculionoidea* (Hsiau and Harrington 2003; Vega 2014). Ambrosia beetles are derived from bark beetles that colonize and consume phloem, which is considered to be more nutritious than wood and are mostly found in association with its fungal symbiont Ascomycotan fungi, the level of association ranging from facultative to obligative. Bark beetles, similar to ambrosia beetles, have been found mostly in association with Ascomycotan and hardly ever in association with basidiomycotan fungi. The variability of their association is reported to range from being facultative to obligate mutualists (Harrington 2005; Vega 2014; Li et al. 2016).

*Ambrosiodmus* is a genus consisting of over 80 species within the largest group of ambrosia beetles, *Xyleborini* (Wood 1982, 1992; Hopkins 1915; Batra 1985). Ambrosia beetles are known to have evolved into symbiotic fungi culture after at least 11 subsequent evolutionary patterns (Wood 1982). About 3200 species of ambrosia beetles are known to be fungus farmers of the genus *Ambrosia*. The most prominent ambrosia feeding genera include *Xyleborus*, *Trypodendron*, *Gnathotrichus*, and *Anisandrous*, belonging to family *Scolytidae* and are widely distributed across the temperate and tropical regions (Beaver et al. 1989). The fungus cultivar, responsible for providing nutrition to the beetles during their period of dormancy and inactiveness as well as during the stages of active growth and development, is grown and sheltered by beetles in specialized storage organs known as *mycangia*. Mycangia, also known as mycetangia are ectodermal glandular pockets of beetles where ambrosia fungi are stored and where they grow and multiply (Hulcr and Cognato 2010). Mycangia are known to have evolved in two ways, as pocket like dilation of cuticle or as newly developed hollow glands from

glands that previously produced defensive compounds and oils in order to prevent excessive flow of sap (Vega and Blackwell 2005). Unlike other species of ambrosia beetles that mostly colonize dead but relatively fresh wood tissues, ambrosia beetles are capable of infesting wood throughout the period of its decay, including the final stages of decay when the xylem is inhabited and colonized by other competitive wood-rotting fungi (Beaver et al. 1989). In contrast to the fungus-farming behavior of the *Ambrosiodmus*, its phylogenetically related sister *Ambrosiophilus* is known to rely on mycoclept (fungus theft) to obtain nourishment, having lost the ability to culture their own fungal farms (Vega and Blackwell 2005).

The fungi inoculum carried inside mycangia gets discharged into the beetle tunnels on the bark while burrowing or during oviposition. The nitrogenous waste eliminated by the beetles serve as fertile manure providing nutrient-rich medium for the fungus to grow and thrive. The beetle larvae in turn are dependent on the fungus to derive nourishment. Prior to several days before the eggs are hatched, the fungus weakens the wood elements aiding the larvae in excavation as well as broadening of tunnels. Such a mutualistic interaction has its own importance in the ecosystem as many nonspecific wood-decaying fungi in association with insects such as beetles have been found to augment and accelerate the process of wood decaying, degradation, and nutrient recycling in the forest ecosystem (Beaver et al. 1989).

The mycangial secretions of ambrosia beetles are responsible for morphological characteristics and biology of the ambrosia fungi. Conidia and other reproductive spores of these fungi develop into the ambrosia stage within the mycangia of the beetles. Mycangial secretions found to contain a rich store of oils and proteins, serve as a source of nourishment and preservation of the fungal inoculum. Mycangial contents of other species of ambrosia beetles have also reported to contain compounds such as amino acids, fat, and proteins, confirmed through thin layer chromatographic techniques (Hulcr and Cognato 2010).

## 17.8 Fungi and Insect Mutualistic Association

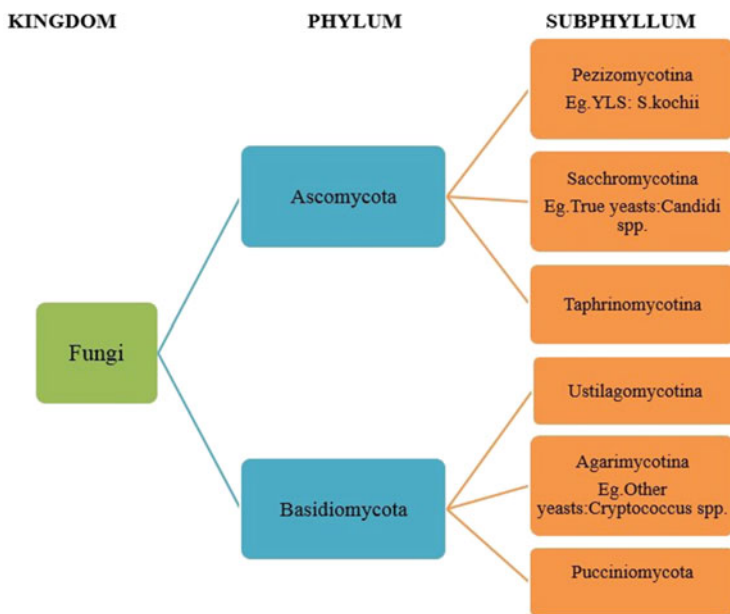
“Yeast” is used to refer to a fungal growth form consisting of only a single cell and lacking specialized sex organs, thereby reproducing through the process of budding via sexual spores from somatic cells, which are not enclosed within fruiting bodies (Vega and Blackwell 2005; Vega et al. 2008; Kurtzman et al. 2011). This group is considered to be omnipresent and found to occupy a variety of ecological niches in both terrestrial and aquatic ecosystems (Kurtzman and Fell 2006). About 1500 species of yeasts have been identified, mostly which belong to the phyla Ascomycota and Basidiomycota (Urubschurov and Janczyk 2011). Almost 700 species from about 93 genera under the class Saccharomycetes of Ascomycota are referred to as “true yeasts” (Batra 1979). Genera belonging to this class include *Candida*, *Kluyveromyces*, *Metschnikowia*, *Pichia*, and *Saccharomyces*. *Fellomyces*, *Tremella*, *Ustilago*, and *Cystofilobasidium* are some examples of fungi that belong to the class

Basidiomycota (Urubschurov and Janczyk 2011; Landell et al. 2009; Suh et al. 2004; Fell et al. 1999; Gibson and Hunter 2005).

Most of the species of yeast discovered to be in mutualistic relationship with insects is found to dwell within the gastrointestinal tract of the host and retrieved from faeces, ovipositors, or other specialized organs of the insects, thus directing a hypothesis of their facultative relationship with the host (Boekhout 2005).

A vast range of variability among the hosts and their habitats have led to the rise of unknown number of new species of yeasts, that almost correspond to the total number of organisms they are found to be in association with since each host is assumed to carry their own particular and specific yeast partner. An example of this is the wide range of yeast species inhabiting the guts of *Erotylidae* and *Tenebrionidae* families of beetles, the number of yeast species being equal to the number of beetle species under each family (Sung-Oui et al. 2005).

Although most often “true yeasts” are involved in symbiosis with insects, studies report that a separate group of fungal endosymbionts known as “yeast like symbionts” (YLS) also exist in association with insects as shown in Fig. 17.2 (Suh et al. 2001, 2004). This group is supposedly believed to have evolved from ascocarpic ascomycetes, especially from the subphylum *Pezizomycotina* (Gibson and Hunter 2010). Notwithstanding their ability to form beneficial association with insects, they are known to be phylogenetically more related to Hypocreales than to Saccharomycetales (true yeasts). This relation directs the attention to an interesting area of evolutionary mutualism, since *Hypocreales* belong to the family of



**Fig. 17.2** Taxonomic Classification of Yeast and Yeast-like Symbionts [adapted from Suh et al. (2004) and (Suh et al. (2001)]

*Clavicipitaceae* which consists of entomopathogenic fungi making it quite evident that YLS is comparatively more related to entomopathogenic fungi than to yeasts (Fukatsu and Ishikawa 1996). This places an evidence of two separate pathways of fungi evolution-mutualistic YLS having their source of origin in insect pathogenic microbes whereas other related yeasts apparently having evolved from commensal interactions (Noda and Kodama 1996). However, the variability in interactions between YLS and insects are sparser than yeast-insect interactions. As per studies and researches to date, examples of mutualistic insect-YLS interactions have been found mostly in anobiid beetles and also in some planthopper and aphid species (Sasaki et al. 1996; Douglas and Smith 1989; Engel and Moran 2013).

## 17.9 Services Offered and Benefits Gained

Though much speculation still exists on the benefits involved in insect–fungi mutualism, studies till date have reported that the major benefits that fungi reap out such a relationship include protection and dispersal of their spores as well as provisions for outbreeding (Kurtzman et al. 2011; Coluccio et al. 2008).

Although insect digestive tracts are considered as one of the highly vulnerable areas for microbial colonization, few microbes have developed resistance to withstand the harsh gut environment and are successfully passed on to host congeners. Although design of the digestive tract varies among different insects and insect orders, the basic anatomy consists of three main regions: foregut, midgut, and hindgut, each possessing their own specialized role in digestion. This variation in turn facilitates distinct abilities in establishing symbiosis with microorganisms. It is generally understood that symbionts are first achieved by ingestion or interaction with the congeners and the environment followed by colonization of the guts and subsequently released from insect molts and faeces for further dispersion of microbial cells. Though the survival and transfer mechanism of yeasts are not well known, it is expected to have similar trends as that of the extensively explored mechanisms of bacterial symbionts (Cory and Ericsson 2009; Carlile et al. 2001; Gonzalez 2014). In contrast to the filamentous structures produced by certain fungi to aid the process of dispersal of conidiospores and ascospores, yeasts are known to produce reproductive spores that are capable of withstanding even extreme conditions of stress such as temperature, salt concentrations, and pH (Carlile et al. 2001; Reuter et al. 2007). The spore wall provides resistance from not just extremes of environmental conditions but also against the stresses encountered in the digestive system, as inferred from the study of survival of spores in the gut of *Drosophila melanogaster* (Carlile et al. 2001). Thus the successful transfer of yeast spores is facilitated through insect feeding and oviposition (Pulvirenti et al. 2002).

Outbreeding is another benefit gained yeast as an extension to the transit and survival of sexual spores through the unwelcoming, harsh environment in the insect gut. Outbreeding is an important concept in order to maintain genetic variation among the descendants to facilitate adaptation and thus evolution. Unfavorable,

harsh conditions mostly contribute to the reproduction in yeasts through the formation of diploid vegetative cells that can undergo meiosis to give rise to a tetrad of haploid spores that can germinate, mate, and restore the diploid state. Most often, it has also come to notice that the four spores contained within the same capsule may undergo selfing or inbreeding. This process is prevented by the gut enzymes that break open the capsule to release the spores, thus inhibiting inbreeding and assisting the phenomena of outbreeding (Guzmán et al. 2013; Lachance and Bowles 2002). A study conducted by making use of genetically marked strains of *S.cerevisiae* divided into two groups, one in contact with fruit flies and another in no contact with insects, demonstrated a significant increase in the number of heterozygotes with insect association than in yeast that was exposed to non-insect mediated mating (Guzmán et al. 2013). An example of yeast receiving benefits in all three forms—protection, facilitation outbreeding, and spore dispersal—is *Metschnikowia* species in association with pollinating insects of the orders *Diptera*, *Coleoptera*, and *Hymenoptera*. The yeast is found particularly in the nectar of flowers thus explaining their association with insect pollinators (Lachance et al. 2001, 2003; Janson et al. 2008; Bismanis 1976).

The benefits achieved by insects as a result of this interaction include nutrition source, detoxication from harmful substances, protection from biotic stress as well as an aid for chemical communication (Noda and Kodama 1996; Jurzitza 1970).

The role played by yeast mutualists in providing nourishment to insects is interpreted from the fact that insect performance and development decreases in the absence of their yeast associate (Kurtzman et al. 2011; Kurtzman and Fell 2006). Yeast cells form an excellent source of nitrogen, containing about 7.5–8.5% of nitrogen by weight, apart from other essential nutrients such as vitamin B3 and B5, proteins, trace metals and amino acids which are broken down and absorbed by simple digestion in the insect gut (96–97,108). Insects such as *Pseudococcus citri*, and wood-boring cerambycids, *Leptura*, and *Rhagium* are reported to depend on yeasts for their dietary source of nitrogen (Starmer and Aberdeen 1990; Noda and Koizumi 2003). The rich source of nitrogen, lipids, and vitamins provide major nutritional support to *Drosophila* flies especially during the stages of egg maturation and larval development (Shen and Dowd 1991a). Similar to the nutritional role played by yeasts, YLS are also reported to play a pivotal role in being nutritional hub to their host. *Symbiotaphrina* and anobiid beetles are supplied by nutrients like nitrogen, sterols, vitamins, and essential amino acids by YLS (Starmer and Aberdeen 1990; Noda and Koizumi 2003). Similarly important intermediate precursors for ergosterol biosynthesis in rice planthoppers are also provided by the yeast-like symbionts (Shen and Dowd 1991b).

The wide range of enzymes produced by insect associated yeasts includes exoproteases and peptidases (involved in protein degradation), lipases (for digestion of fatty acids), and hydrolytic enzymes involved in sugar degradation (Chararas et al. 1983). These enzymes play a role in conversion of complex molecules and polysaccharides to simple compounds like glucose or sugars that are easily and directly absorbed by the insects. This role is however mainly attributed to the enzymes produced by YLS, which release digestive enzymes into their surroundings for

colonization and spreading to new areas, in contrast to true yeasts, which are more sessile and are not known to release digestive enzymes unless they are trapped in their own erosion zones (Noda and Kodama 1996). An exception to this nature is demonstrated by true yeasts belonging to the genus *Candida* which possess the ability to degrade wood components such as cellulose, pectin, and glucosides (Listemann 1988).

The process of detoxification by yeasts have proved that mutualism between insects and fungi is a detail of much significance in herbivory. Detoxification in this context relates to making certain nutrients available by neutralizing or decomposing the toxins, thus aiding the process of digestion. In some cases, detoxification converts these nutrients to more polar forms that can be easily removed from the host's digestive system (Kurtzman et al. 2011; Kurtzman and Fell 2006; Cory and Ericsson 2009). An example of detoxification by yeasts is clearly elaborated in the YLS, *S.kochii* which is found in association with the beetle *L.serricornis* (Kurtzman et al. 2011; Kurtzman and Fell 2006). *S.kochii* is reported to have the potential to detoxify a variety of plant allelochemicals, metal toxins, insecticides, and herbicides in addition to producing detoxifying enzymes such as aromatic ester hydrolase, glucosidase, phosphatase, and glutathione transferase that converts toxic chemicals to important carbon sources (Chararas et al. 1983).

Biotic hazards faced by herbivores include competitors, parasites, predators and plant chemical defenses. Plant diseases could have a negative impact on insects therefore insect–yeast mutualism aides the availability of safe food sources as yeasts have demonstrated various protection mechanism of plant tissues from infesting pathogens (Pulvirenti et al. 2002; Listemann 1988). Yeasts could play a role in limiting the presence of other fungi or microbes inside plant tissues thus favoring the growth and development of associated insects. *Metschnikowia* species decrease the prevalence of molds inside apples which indirectly correspond to lower mortality and larval development time for *Cydia pomonella* (Witzgall et al. 2012). These results are a clear illustration that yeasts not only performs the role as nutrition provider to insects, but also ensures that opportunistic pathogens and microbes that might hinder the development of both participants of symbiosis are kept at bay (Pulvirenti et al. 2002).

## 17.10 Conclusion

Researches and studies conducted so far have pointed to the fact that fungus farmers had not always been involved in mutualistic relations with fungi, but it is a phenomenon that has evolved over a period of nearly 50 million years due to variations and adaptations amongst various species of organisms. It has manifested its effects even at the genome level as in case of attine ants whose genome is deficient of genes responsible for the production of several important enzymes since these are now easily available in ready form from the fungi in association. Yeast and yeast-like organisms have developed resistant mechanisms to survive in the harsh dynamic

environment of insect gut which in turn facilitates spore disposal through feces and insect oviposition. The mutualistic evolution has come a long way in developing mechanisms for the survival and development of both insects and fungi alike. It is a demonstration of the potential of organisms to develop adaptations in the face of unfavorable surroundings and environment giving rise to variations and modifications within different species.

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