Chapter 13
Yeasts in Insects and Other Invertebrates

Meredith Blackwell

Abstract Minute organisms, including yeasts with diverse physiological capabilities, make possible the existence of arthropods, especially insects, the most speciose group of organisms on Earth. The yeast growth form occurs throughout most of the fungal kingdom and is often associated with insects. Fungi and insects evolved together in the same habitats where casual associations certainly occurred early in their shared geological history and yeast attractants for insects developed over their lives together. Examples of their interactions range from accidental dispersal and the use of yeasts as food to obligate mutualisms described in this chapter. These include discussions of yeast-like symbionts, the use of the wasp gut for yeast outcrossing, extension of endophyte life cycles to include dispersal by insects, the advantage of yeasts in the diet of many insects such as blood-sucking dipterans, and the yeast-like germination of phoretic fungi in ephemeral habitats. Future studies of yeast–insect associations will continue to include species discovery but also approach theoretical questions of sexual and asexual reproduction, host specificity, host switching, advantages of horizontal and vertical dispersal, and studies that include entire interactive communities.

Keywords Erotylidae • Transitions to mutualism • Hemiptera • Ophiocordycipitaceae • Tenebrionidae

Contents

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13.1 Introduction

Haeckel (1899) made an early attempt to popularize small organisms for the general public, but recognition of microbes as important to Earth's environments is only now being widely recognized (Hug et al. 2016). Price (1988), who called for more interest in microbes, chided "Noah's Ark Ecology." He emphasized evidence that the greatest energy transfer in food webs was not by large, familiar animals and plants but by organisms smaller than "bird food." Price's consideration of food webs in a phylogenetic context led him to express an essential principle, "ecology recapitulates phylogeny." Pimentel et al. (1992) estimated that 90% of the animal and plant species present in certain regions are arthropods and microbes, a vast majority of the species on Earth. More recently estimates based on a universal dominance scaling law proposed the existence of as many as 1 trillion (10^{12}) species of microbes, including bacteria, archaea, and microscopic fungi (Locey and Lennon 2016).

Fungal biologists, however, have been interested in fungal associations with other organisms for many years, but recently general attention to microbes has increased due to the human microbiome project (Gill et al. 2006; Turnbaugh et al. 2007; Rodrigues Hoffmann et al. 2016). It is common to see eye-catching headlines in popular literature touting large numbers of microbes on different parts of the human body and T-shirts declaring we are "10% human, 90% bacteria," and although new estimates downplay the number of microbial cells in a person, the numbers are still impressive (Sender et al. 2016). Anthropocentric attention has brought interest to the study of all microbiomes, and the development of high-throughput sequencing, metagenomic analysis, and other methods has benefitted the study of fungi (Hoffmann et al. 2013). Questions, including those focused on all yeast–insect associations and interactions discussed here, should be invigorated by the improved tools of high-throughput sequencing and other methods to provide answers to who, what, when, where, why, and how yeasts and their host organisms became associated; eventually the view will be broadened to consider entire microbial communities (Ganter 2006; Douglas and Werren 2016).

The period from the late nineteenth century until the present has been a time of discovery of associations among microbes and insects (Buchner 1953). The translation of Buchner's (1965) classic work has made the information widely available to English speakers. Kurtzman and Robnett (1998) developed methods not only to identify yeasts rapidly but also to begin to arrange them phylogenetically. Ganter (2006) remedied his perceptive concern for the lack of current discussion of fungi, especially yeasts associated with insects, among biologists by his thorough presentation of the subject. The following list of events marks progress in the study of fungal–invertebrate animal associations, the focus of this chapter, and it provides perspective for the discussion. Items marked by asterisk indicate reviews on the topic:

- 1835—Agostino Bassi di Lodi studied first animal (insect) disease, caused by a fungal agent later named for him (*Beauveria bassiana*).
- 1857—Pasteur determined microbial agents of fermentation.
- 1870—Pasteur saved the French silk industry when he discovered a microsporidian fungus (*Nosema bombycis*), the agent of pébrine disease of silkworms.
- 1952—*The Yeasts: A Taxonomic Study*, first edition (Lodder and Kreger-van Rij 1952).
- 1953*—Publication of *Endosymbiose der Tiere mit Pflanzlichen Mikroorganismen*, a detailed overview of the nineteenth- and twentieth-century literature (Buchner 1953).
- 1965*—Publication of the English translation of Buchner's work on endosymbiotic microbes of animals (Buchner 1965).
- 1970—*The Yeasts: A Taxonomic Study*, second edition (Lodder 1970).
- 1972—Phaff and Heed begin to publish on cactophilic yeast communities.
- 1976—Heed, Fogleman, Starmer, Lachance, and Rosa et al. begin to publish on yeasts associated with insects (see Chap. 8 of this book).
- 1984—*The Yeasts: A Taxonomic Study*, third edition (Kreger van Rij 1984).
- 1988—Lachance and Starmer et al. begin to publish on yeasts associated with ephemeral flowers, bees, and *Drosophila*.
- 1996—Publication of the first eukaryote complete genomic sequence, *Saccharomyces cerevisiae* (Goffeau et al. 1996).
- 1998—*The Yeasts: A Taxonomic Study*, fourth edition (Kurtzman and Fell 1998).
- 1998—Kurtzman and Robnett publish on large subunit ribosomal LSU rDNA (large subunit ribosomal DNA) as a marker and develop a database of yeast sequences (Kurtzman and Robnett 1998).
- 2004—Suh, Nguyen, Urbina, and Blackwell et al. begin to publish on insect gut yeasts.
- 2005*—Vega and Dowd publish often cited chapter on the role of yeasts as insect endosymbionts (Vega and Dowd 2005).
- 2005—Boekhout reports that only 6% of the CBS-KNAW Fungal Biodiversity Centre collection yeasts are from insect sources (Boekhout 2005).
- 2006—Rosa, Cadete, and Lachance begin to publish on yeasts in decaying wood (see Chap. 9 of this book).

- 2006*—Publication of *Biodiversity and Ecophysiology of Yeasts*, Springer, Berlin [includes Ganter (2006) review of yeasts and invertebrate associations] (Rosa and Péter 2006).
- 2011*—*The Yeasts. A Taxonomic Study*, fifth edition (Kurtzman et al. 2011); includes Chap. 6, Yeast Ecology (Starmer and Lachance 2011).
- 2016—First yeast genome-wide study includes some insect-associated yeasts (Riley et al. 2016).
- 2016—Backbone phylogeny of Saccharomycotina published using genome-scale data provided more stable family-level classification (Shen et al. 2016).
- 2016*—Eleven years of increase in insect-associated yeasts accessioned in the CBS—KNAW Fungal Biodiversity Centre collection rises to 7.25% of total with more to be cataloged (see Chap. 15 of this book).

Ganter (2006) distinguished between associations, recognized by established co-occurrence, and interactions, in which a functional relationship has been "established or strongly supported by observational data"; in this chapter association will be used in a general sense, and interaction when a known benefit to a participant is referred to in an interaction. Most of the interactions between yeasts and insects discussed in this chapter are mutualistic, and they probably benefit, not harm, the participants in the association. In many cases, however, we do not understand the basis for the close associations. Diffuse mutualisms are not species specific (Johnstone and Bshary 2002; Starmer and Lachance 2011). Douglas (2009) considered these associations to be some of the more interesting ecologically, because they introduce variables in populations. Some of the associations are casual, while in others organisms interact in a fixed and sometimes obligate manner that affects the fitness of one or both participants; the partners may not benefit to an equal extent in asymmetric interactions. The presence of several classes of microbes (e.g., yeasts and bacteria) in the gut of individual insects is common, perhaps with collaborative roles that are not always considered in the study of the associations (Chandler et al. 2012; Ceja-Navaro et al. 2014, 2015; Douglas and Werren 2016). Certain organisms involved in a mutualism (e.g., multipartite mutualisms), such as a third participant, may be unknown. In a few cases, insects (e.g., planthoppers, roaches, termites) may rely on bacterial mutualists in closely related species but then switch to a fungus under new pressures (Engel and Moran 2013). For example, major switches in symbionts have occurred over evolutionary time coinciding with changes in diet as in roach and termite lineages (Brune and Dietrich 2015).

The work reviewed in this chapter is heavily biased toward terrestrial insects, especially Coleoptera (beetles), Diptera (flies), and a few Hemiptera (planthoppers, leafhoppers, aphids), and their ascomycete yeast associates, because these organisms have been the subject of the most research to date. Perhaps this focus is justified because estimates suggest that one of four animals on Earth is a beetle (Grimaldi and Engel 2005). Ganter (2006) covered the topic in great detail, and that material is not repeated in this chapter except when background information is needed. Another source of a list of specific associations is available on line

(Urubschurov and Janczyk 2011). Other chapters in this book on specific habitats in which yeasts and insects are closely associated include Chap. 8 ("Yeasts in Cactus and Tropical Fruit Habitats"), Chap. 9 ("Yeasts Associated with Decomposing Plant Material and Rotting Wood"), and Chap. 15 ("Census of Yeasts Isolated from Natural Ecosystems and Conserved in Worldwide Collections"). Besides, the book *Yeasts in Natural Ecosystems: Ecology* (Buzzini et al. 2017) includes a few chapters focused on mutualism in yeasts (Mittelbach and Vannette 2017), parasitism in yeasts (Begerow et al. 2017), and antagonistic interactions and killer yeasts (Klassen et al. 2017).

13.1.1 A Paradox of Success

Insects and other arthropods are unable to synthesize many of the essential nutrients and other resources that they require to maintain life (e.g., amino acids, sterols). A paradox exists: the world's most speciose group of organisms on Earth diverged to fill many habitats, often by relying on resources derived from bacteria and fungi. Douglas (2009) pointed out that distinguishing between microbes as food (e.g., ambrosia of beetles, fungal gardens of attine ants) versus symbionts performing other functions is not always clear, but what is certain is that "foodstuffs such as plant sap, vertebrate blood and sound wood, would be unavailable to insects without nutritional input from microorganisms." For example, vitamins for physiological functions and enzymes for degradation of plant cell walls and detoxification of plant secondary metabolites often are acquired from microbes, including yeasts and yeast-like fungi (Vega and Dowd 2005; Ganter 2006; Douglas 2009; Starmer and Lachance 2011). Bacteria may be more effective than fungi as mutualists in providing enzymes for degradation of plant cell walls and fixing nitrogen. Fungi, however, can also supply vitamins, recycle nitrogen in certain cases, and clearly have an advantage in providing sterols that are not synthesized by bacteria.

13.1.2 How Long Has This Been Going On? Dating the Associations

How long have invertebrate animals and microbes been associated? The question is important to consider because we want to understand the evidence for diversification of speciose groups. Based on available evidence, well-established groups of fungi, insects, and plants (including woody plants) could have been living closely in the same habitats by the end of the Carboniferous [~300 million years ago (Ma)] or even earlier (Taylor et al. 2005). Estimates of the ages of fungal taxa and insect orders have been based on DNA, morphology, the fossil record, and environmental data (Lücking et al. 2009; Misof et al. 2014; McKenna et al. 2015; Chang et al.

2015; Toussaint et al. 2017). Misof et al. (2014) dated the origin of insects at Early Ordovician [~479 Ma) and insect flight, Early Devonian (~406 Ma). Based on the illustration (Fig. 1 reported by Misof et al. 2014), estimates suggest that by 350–200 Ma insect orders associated with yeasts today were already established: Isoptera (termites) and Blattodea (Legendre et al. 2015), Hemiptera (bugs, but not necessarily including all members in Fulgoromorpha), Hymenoptera (ants and others), Neuroptera (lacewings), Lepidoptera (moths and butterflies), and the earliest of several divergences of Diptera (true flies) (Wiegmann et al. 2011). Toussaint et al. (2017) provided dates for many groups of beetles based on new fossil calibrations that are generally earlier. Their estimates for crown groups included *holometabolous* insects (those with egg, larva, pupa, and adult stages) before the Carboniferous about ~385 Ma, Coleoptera in Mid-Carboniferous (~325 Ma), and the four extant beetle suborders, including Polyphaga in which most of the fungus-feeding beetles are classified, by Late Carboniferous to Early Permian (~300 Ma). They estimated that many clades (series and superfamily level) survived the mass extinction at the end of the Permian (~250 Ma). The crown groups of beetles important to this discussion (plant-associated families Curculionidae, Cerambycidae, and Chrysomelidae, certain cucujoid families, e.g., Nitidulidae, Erotylidae with fungus-feeding lineages, and Tenebrionidae) were present by the end of the Jurassic (200–150 Ma). These dates are earlier than those given previously for beetles (Hunt et al. 2007; McKenna et al. 2015), but they predate the earliest known fossils available for the different groups and are in better agreement with what is known of radiations of other major groups of organisms.

Dates suggested for fungal evolution vary widely, in part because of the lack of reliable fossils and calibration points. Fossil yeasts have not been reported, even when fossils of insect groups known to harbor them are found, but ascomycetous yeasts certainly are older than the 35- to 45-million-year-old “fossil” yeasts advertised on the Internet for recent brewing ventures. Lücking et al. (2009) relied on previous publications and data from Schoch et al. (2009) to estimate the age of fungal divergences. Based on their most conservative, recalibrated phylogenetic tree ages, they suggested that the divergence of Ascomycota and Basidiomycota coincided with the appearance of nonvascular plants by 500 Ma; divergence of the Saccharomycotina and other Ascomycota, about 400 Ma; the base of Pezizomycotina, about 400–520 Ma; and the Pezizomycotina diversification, 320–400 Ma, corresponding with vascular plant diversification (see Table III and Fig. 2 reported by Lücking et al. 2009). These estimates indicate that yeast–insect associations, including those involving the use of yeasts as food, could be much older than 300 million years. Although yeast feeding has not been recognized in fossil insects, a staphylinid from Burmese amber (~99 Ma) has a spore brush on part of the maxilla, a known spore-feeding mouthpart specialization, as well as possible spores in the gut; this fossil is the earliest known beetle with evidence of a spore-feeding habit (Cai et al. 2016). Analysis of insect mouthparts and gut contents provides evidence of ecological associations with extant insects as well as fossils (Robertson personal communication 2014). Information is available on symbiotic-farming associations of beetles, ants, and termites. The symbioses apparently appear rather late compared to what is surmised about the lineages. Jordal and Cognato (2012) used calibrated molecular divergence rates based on updated fossil records to suggest that the fungus-farming habit in bark and ambrosia beetles arose independently at least ten times with origins about 21 Ma–50 Ma, at least 50 million years after the proposed origin of the subfamily Scolytinae (100–120 Ma). Furthermore, they proposed the origin of farming occurred during two periods of global warming in the Cenozoic. The 50 Ma date was similar to the proposed origins of attine ant associations with Leucocoprineae basidiomycetes (Schultz and Brady 2008) and a somewhat younger origin for African termites and their associated *Termitomyces* spp. of 31 Ma (19–49 Ma). There has been some host switching among termites within *Termitomyces* spp. (Nobre et al. 2011), but one attine lineage developed an association with a completely different group of basidiomycetes, coral fungi in Pterulaceae, about 10–20 Ma (Schultz and Brady 2008).

Yeasts may have been included in the diet of insects much earlier but with little specificity and without vertical transmission of clones as happens in the farming associations (e.g., ants). Elaborate behavioral traits would not have been required for transmission, although morphological adaptations such as gastric caeca and mycangia have developed in some insects. Certain yeast clades (e.g., *Suhomyces*, *Teunomyces*) may have diversified with beetles that favor mushroom diets, but there is only specificity at the level of genus or above. Exceptions are noted in discussions below.

13.1.3 Diversity of Yeasts and Yeast-Like Fungi in Animal Associations

As mentioned before (Blackwell 1984), the observations of Otto von Münchhausen, the eighteenth-century correspondent of Linnaeus, revealed that fungi were the dwellings of animals (Ramsbottom 1953; Findlay 1982). Since the time of Münchhausen, we have learned that some fungi are indeed the houses of animals, the insects within which yeast and yeast-like fungi dwell. It took several hundred years and the development of new molecular methods to determine the general nature of fungi (Alexopoulos et al. 1996; Hibbett et al. 2007), and although more is to be learned about the earliest-diverging lineages, we now have a good understanding of fungal limits (Grossart et al. 2016). Although yeast species are difficult to recognize solely on the basis of morphology, their recognition as fungi was followed rather quickly by classification in different taxonomic groups based largely on ecology, physiology, cell wall composition, guanine–cytosine (G-C) content of nuclear DNA, and in the case of some basidiomycetes by morphology (Suh et al. 2006b; Kurtzman and Robnett 1998; Barnett 2004). Many yeasts are associated with other organisms, and the essential work of determining the species continues (Buchner 1965; Ganter 2006; Starmer and Lachance 2011; Blackwell

2017). There is a high probability that many additional associations will be discovered with the use of high-throughput sequencing.

Yeasts are taxonomically diverse, and the terms “yeast” and “yeast-like” refer to a fungal growth form that includes budding in asexual reproduction, although other types of asexual reproduction also may occur. A number of fungi are dimorphic, and in the same life cycle, they may grow as yeasts in association with insects (e.g., in the mycangia of many ambrosia beetles) and as hyphal filaments when free of the insects (e.g., in a wood substrate). Fungal groups with yeast-like growth forms include Ascomycota, Basidiomycota, and some zygospore-producing Mucoromycotina, although clear evidence of invertebrate animal association is not known for species of Mucoromycotina. Genome studies provided evidence that the irregular occurrence of the yeast growth form throughout the fungal kingdom is due to evolutionary convergence regulated by Zn-cluster transcription factors that regulate yeast–filamentous growth switches over time (Nagy et al. 2014). The evolutionary event that allowed for switches in growth form occurred early in the divergence of the fungal lineage, accounting for the sporadic appearance of the yeast growth form throughout the fungal tree, often in association with insects and other animals.

The yeasts (e.g., *Saccharomyces* spp.) and yeast-like fungi (e.g., *Symbiotaphrina* spp.) discussed below are mostly ascomycetes, but some basidiomycetes (e.g., *Trichosporon* spp. and new genera of Tremellomycetes previously placed in *Cryptococcus*—see below; Liu et al. 2016) also are associated with insects and other invertebrates. Ascomycetous and basidiomycetous yeasts are ecologically very different from each other, and the differences help to explain the more common occurrence of ascomycetous yeasts with animals. Ascomycetes usually have specialized niches rich in organic carbon (copiotrophic) where they are fermentative, often fragrant, and associated with other organisms, including invertebrate animals that disperse them. Basidiomycetous yeasts differ by their use of a wider variety of carbon compounds at low concentrations (oligotrophic), and they are less often fermentative, fragrant, or animal associated. Carotenoid pigments, forcibly discharged basidiospores, and clamp connections, when present in some taxa, allow them to be distinguished easily as basidiomycetes (Lachance and Starmer 1998; Suh et al. 2006b).

Ascomycetes are divided into three subphyla based on morphology and DNA analyses: Taphrinomycotina, Saccharomycotina, and Pezizomycotina. None of the Taphrinomycotina is known to be intimately associated with invertebrate animals, although occasional dispersal of yeast cells may occur. Members of Saccharomycotina (e.g., *Suhomyces*, *Teunomyces*) have extensive associations with animals. Several yeast forms evolved within the filamentous ascomycetes, Pezizomycotina (e.g., *Symbiotaphrina*), and while these taxa are not diverse in numbers, they are essential associates of certain insects, and studies of their genomes have given us data to provide new clues to the way in which strict symbioses evolved. Some arthropod-associated fungi with yeast states also occur among the three major subphyla of basidiomycetes. Pucciniomycotina is a group that most mycologists think of as plant parasites, but it is much more diverse and contains some biotrophic parasites of insects (e.g., *Septobasidium* spp.). Other yeasts are classified in one clade of Agaricomycotina (Tremellomycetes: new genera previously placed in *Cryptococcus*—see below; Liu et al. 2016) and in Ustilaginomycotina (e.g., *Sporisorium*) (Alexopoulos et al. 1996; Hibbett et al. 2007; Kurtzman et al. 2011; Begerow et al. 2014). Ganter (2006) discussed yeast–insect reports (1966–2004) characterized by habitat as well as taxonomic group. His records included a variety of productive plant-associated habitats, including decaying wood, basidiocarps, and the insects associated with these habitats (e.g., Platypodinae, Scolytinae, Biphyllidae, Bostrichidae, Buprestidae, Cerambycidae, Languriidae, Scarabaeidae), and his work should be consulted for detail.

13.1.4 Finding a Partner: Yeast and Animal Associations

Animals may be attracted by taste, feel, or sight. For example, certain insects are attracted to bright orange and yellow colors, and flies have been reported to be attracted to the fungus *Neurospora* growing in a bright mass, or they may react to the sweetness of ergot (Hawker 1957; Witzgall et al. 2012). Insects may use color only (Ernst et al. 2016) or odor at far distances and color in close quarters (Streinzer et al. 2009); many insects rely heavily on odor alone. Yeasts and other fungi produce volatile organic compounds (VOCs), which act as semiochemicals to signal other organisms, especially insects (Davis et al. 2013). The volatiles may reveal the presence of "nutrient resources, competitors, predators, potential mates, and habitat suitability" (Price et al. 2011), and the strength of the signal varies in some cases targeting only one to few organisms or in other cases attracting more broadly to include parasites. The effect of yeast volatile compounds was clearly demonstrated to me the day I inhaled the odor of bananas near the lab transfer chamber, unusual for our odorless (to me) mushroom-feeding beetle yeasts, including a number from Nitidulidae. A check of accession numbers revealed that these yeasts were in fact not from mushroom-feeding beetles but from nitidulid beetles that had been baited with fruit.

Associations between yeasts and invertebrates vary from very casual with the fungi having the advantage of being dispersed to fresh substrates by an animal accidently contacting the cells. Scanning electron micrographs show the variety of fungal diaspores attached to setae and resting in the depressions of arthropod exoskeletons (Blackwell 1984), and casual associations are not regarded as symbioses. Some yeasts are ingested, and after ingestion they are carried internally in the gut of insects; a few cells often survive gut passage and dispersal to new habitats. Douglas (2009) pointed out the benefits of microbial resources to nutrition, including the provision of sterols, amino acids, vitamins, and enzymes for digestion and detoxification of plant materials. She cautioned that some benefits ascribed to microbes have not been tested. For example, some animals, including insects, produce intrinsic cellulases, which have been hard to detect. Also, it is not clear that B-complex vitamins are always supplied to animals unless experimental work and genome annotation support the conclusion.

Studies focusing on yeasts associated with insect pollinators for dispersal have shown that a variety of ascomycetous yeasts are present in nectar, an osmophilic habitat relatively low in competition. Nectar is a well-known habitat for these yeasts, many of which are dispersed by the pollinators (Lachance and Starmer 1998; Andreadis et al. 2015). In a study that compared nectar composition, volume, and pollinators, Mittelbach et al. (2015) found a surprising number of basidiomycetous yeasts present in flowers with nectars high in monosaccharides rather than sucrose and visited by birds, indicating that nectar composition and type of vector are factors that lead to patterns of diversity (Mittelbach et al. 2015). Andréadis et al. (2015) were successful in using yeast cultures (four species of *Metschnikowia* and *Vishniacozyma tephrensis* (former *Cryptococcus tephrensis*)) to bait insects in an organic apple orchard. A diverse group of 93 arthropods from 15 orders, mostly insects, especially flies, were attracted to specific yeasts. Differences in taxa and their abundance were clear in almost all cases. The results suggested that attraction by yeasts and their volatile compounds could be the basis of effective detection and control of pest species.

Specificity has been assumed from observations of repeated associations and experimental studies (Andréadis et al. 2015). In some insects, yeasts are retained for long periods in structures such as mycangia and gut caeca. Often the associations do not show a high degree of species specificity except at the level of a clade as mentioned above or even unrelated groups of species with common ecological traits. The assumption is that evolution proceeds from casual associations with close contact in nature and progresses to a point of no return when a fungus and animal interact to a point that they can no longer live separately in nature, and sometimes evidence exists for such a progression in the examples that follow.

13.2 Examples of Associations

13.2.1 Virulent Pathogen to Obligate Mutualist

Obligate interactions of insects and microbial symbionts are common, but most of the microbes are bacteria and less often fungi. Some lineages, however, are exceptions. The usual symbionts that occur throughout a number of clades of Hemiptera are bacteria (Moran et al. 2008), but in a few species belonging to several clades (aphids, planthoppers, leafhoppers), fungi have replaced bacteria. Planthoppers have sucking mouthparts designed for acquiring phloem sap from plants, a diet that may be poor in nutrients without microbial supplements. The yeast-like symbionts (YLSs) of hemipterans have never been found free living in nature, and the host and microbial organisms cannot be grown separately, characteristics of obligate interactions. The most studied YLS is that of the brown planthopper, *Nilaparvata lugens*. The YLS has gone nameless until recently when the species was called *Entomomyces delphacidicola* (Fan et al. 2015), but apparently the taxon has not been described formally according to the rules of nomenclature. The naming may better await broader taxon sampling, however, because of possible polyphyly as noted below.

The YLSs are intracellular (endosymbiotic), located within specialized cells (mycetocytes) in the fat body, a differentiated cluster of cells connected to the gut, of planthoppers, aphids, and leafhoppers. The YLSs are present in all stages of the insect life history from egg to adult, and vertical transmission from one generation to another is ensured by transmission from the female parent to the egg by transovarial infection. As mentioned earlier, the brown planthopper, *N. lugens*, has been most studied, so generalizations may not always be accurate. During the process yeast cells are released from the mycetocytes to the hemocoel, then to cells surrounding the oocytes, and from that point to the egg surface where the hatching larvae acquire the yeasts as they chew out of the eggs (Buchner 1965; Cheng and Hou 2001). The relationship between *N. lugens* and its symbionts has been an object of investigation for more than 150 years because the insect is a destructive pest of cultivated rice. Although the yeasts cannot be grown in vitro, a method of harvesting cells by density gradient centrifugation has provided material for study (Noda and Omura 1992).

Various researchers helped to develop the planthopper–YLS interaction as a model system in which the YLS provides essential functions for the insect, allowing for survival on a low nutrient diet (Sasaki et al. 1996; Hongoh and Ishikawa 1997, 2000; Noda and Koizumi 2003). Studies of complete genomes of the planthopper, the YLS, a newly discovered bacterial associate, and rice provide insight, not only into the interactions of the organisms, but also into the evolutionary steps leading to obligate symbiosis. The brown planthopper genome size and G-C content are similar to that of the symbiont-associated palm aphid (*Cerataphis brasiliensis*) YLS, but the genome is somewhat reduced and has a higher G-C content when compared to many other ascomycetes (Xue et al. 2014; Fan et al. 2015). The YLS contributes enzymes for synthesis of essential amino acids, nitrogen storage and recycling, and sterols to supplement the poor nutritional quality of the highly specialized rice phloem sap diet of the YLS. Relatively large gene clusters for carbohydrate and amino acid metabolism probably reflect carbohydrate utilization and amino acid biosynthesis functions performed by the YLS (Xue et al. 2014; Fan et al. 2015; Wan et al. 2016). The bacterial associate synthesizes B-complex vitamins for the multipartite association (Xue et al. 2014). The loss of the YLS MAT loci involved in mating results in loss of sexual reproduction (Fan et al. 2015, also see below for apparent asexual reproduction in another yeast group associated with insects).

Not only did the YLS not have a formal name, but until the advent of DNA sequencing, it could not be placed among its close relatives. Early studies using phylogenetic analysis of rDNA placed both planthopper and aphid YLSs among filamentous ascomycetes in Pezizomycotina (Noda et al. 1995; Fukatsu and Ishikawa 1996); later the YLSs were placed near species of necrotrophic insect pathogens (Suh et al. 2001). A major revision of most taxa previously classified in Clavicipitales now places the YLSs in Ophiocordycipitaceae (Hypocreales), a group that includes *Ophiocordyceps sinensis*, renowned in herbal medicine, and *Elaphocordyceps* spp., parasites of hypogeous mycorrhizal ascomycetes, *Elaphomyces* spp. (Sung et al. 2007; Spatafora et al. 2007).

Dramatic symbiont changes have been observed in several hosts, including the roach–termite lineage. *Blattabacterium* sp. resides in mycetocytes of the common ancestor of roaches and termites, but soon after the divergence of termites, the bacterium was lost in all but the earliest-diverging termites, likely due to a change in diet to wood (Sabree and Moran 2014). A similar switch from prokaryotic to eukaryotic symbionts appears to have occurred independently in Hemiptera on several occasions (Nishino et al. 2016), perhaps driven by different dietary needs. The YLSs evolved from within *Ophiocordyceps* on at least two independent occasions, one from within *Ophiocordyceps* to a subclade that contained parasites of beetle grubs, ants, and YLS of planthoppers (*N. lugens* and *Sogatella furcifera*) plus an aphid [*Tuberaphis* (= *Hamiltonaphis*) styraci]; the second subclade contained *Ophiocordyceps* parasites of caterpillars (including *Oph. sinensis*) and YLS of eared leafhoppers (Cicadellidae, Ledrinae) (Nishino et al. 2016). Based on analyses from extant organisms, the phylogenetic evidence suggests stepwise evolutionary transitions with an ancestor containing two bacterial symbionts, then one of the bacteria retained and the other replaced by a fungus, and eventual loss of the remaining bacterium to a fungus symbiont (the YLS) only in the evolutionary history of leafhoppers (Nishino et al. 2016). Although the aphid and planthopper YLS clade and the leafhopper YLS clade are both derived from within *Ophiocordyceps* fungal parasites, they appear to have diverged independently. The switch to the fungus offers an advantage over bacterial symbionts, because, although both types of microbes have uricase genes used in nitrogen recycling, bacteria do not synthesize sterols, essential requirements for insect metabolism (Gibson and Hunter 2010; Fan et al. 2015).

The concept of host switching (as "host jumping") has been used in fungal biology to explain the interkingdom host shifts observed in the *Ophiocordyceps* lineage in evolution from an insect to an *Elaphomyces* host in a common hypogeous environment with connections to plant roots (Nikoh and Fukatsu 2000), and other examples of dramatic host switches also occur in the Hypocreales (Kepler et al. 2012). In a discussion of host–parasite evolution, Araujo et al. (2015) gave evidence that host switching is far more common than co-speciation. In fact, there are few if any examples of co-speciation among fungi. The *Ophiocordyceps* lineage example satisfied the first requirement stipulated by Araujo et al. (2015), opportunity, by temporal and spatial coexistence amid plant roots. Exactly what has been the evolutionary pathway to go another step to overcome changes from virulent filamentous pathogen to obligate YLSs of insects such as planthoppers? Does loss of virulence involve a more efficient immune system of the new host? Does the host switch occur rapidly enough for the newly infected host to survive pathogenicity? Accelerated evolution of a parasite occurs when it changes to a mutualistic lifestyle (Lutzoni and Pagel 1997), and changes in bacterial effects have sometimes been observed in relatively time short periods, perhaps generational time. Weeks et al. (2007) found evidence that cytoplasmic incompatibility in *Drosophila* occurred in matings between males carrying a certain *Wolbachia* infection and females that were either uninfected or infected with a different *Wolbachia*. The fecundity rate (actual reproductive rate), believed to be the only effect on the newly infected population, was measured after 20 years. Evolutionary changes in the parasite *Wolbachia* caused a reduction of an original fecundity disadvantage of 20%, and in less than 20 years, the disadvantage had become an advantage of 10%. The evidence also supported a more encompassing hypothesis that evolution of a mutualism occurs through maternal vertical transmission rather than by horizontal transmission (Weeks et al. 2007).

But could changes in even a relatively short generational time frame allow for coexistence long enough for the virulent parasite to develop a mutualistic life with the planthopper? Perhaps the first steps in the rapid shift from virulent pathogen to obligate mutualist could occur almost instantaneously as has happened with some virus-infected filamentous fungi, for example, hypovirulence of the agent of chestnut blight, *Cryphonectria parasitica*. Immediate change from tree-killing parasite to hypovirulent parasite comes by infection with a virus (Alexopoulos et al. 1996; Anagnostakis 2001). The process occurs naturally when fungal strains are vegetatively compatible, and hypovirulent (virus +) strains fuse with the virulent (virus –) strains, and the virus migrates throughout the cytoplasm converting the virulent strain to hypovirulent, resulting in an infection that does not kill the tree as a result of the now hypovirulent infection. The phylogenetic evidence of Nishino et al. (2016) suggesting amelioration by a bacterium could have aided in the transition of the last step in the development of the mutualism, suggests a way in which the virulent pathogen to YLS could have evolved quickly. Such a progression has also been described as a “stepping-stone” process (Araujo et al. 2015).

13.2.2 Fungi as a Unique Habitat for Insects and Yeasts

Fungus feeding is important to many insects and may be a primitive feeding habit. Lawrence (1989) estimated that half of all beetle families are primarily mycophagous or dependent upon plant material that has been altered by fungal action, a statement reiterated by others (Hammond and Lawrence 1989; Leschen and Buckley 2007; Cai et al. 2016). However, more strictly speaking, about thirty families of beetles are associated with basidiocarps and other fungal fruiting bodies, upon which they feed and breed exclusively. More than 250 beetle species were collected from 2660 basidiocarps of a polypore species in an extensive study in eastern Canada (Gilbertson, 1984). In another study, 136 taxa from 30 different beetle families were reported from *Pleurotus* spp. in North America (Cline and Leschen 2005). Some beetles specialize only on specific parts of a basidiocarp, such as spores or context, and differentiate certain types of context tissue characterized by “hardness” and texture. For example, spore-feeding beetles in three different families of superfamily Staphylinoidea had convergent morphological features linked to the spore-feeding trait (Betz et al. 2003). A group of polypores including species of *Phellinus*, *Inonotus*, and *Phaeolus* judged to have the same "hardness" were inhabited by the same context-eating ciid (Ciidae) beetle species (Lawrence 1973, 1989). Mouthparts have been selected for feeding, and the changes in morphology can be used to track evolutionary changes across insect lineages corresponding to changes in diet in the fossil record (Leschen and Buckley 2007; Cai et al. 2016).

As a distinct major lineage of organisms, fungi differ in a number of ways from other organisms including plants. Many beetles associated with plant nutritional sources have mouthparts that allow them to penetrate living cells, degrade recalcitrant plant cell walls, or more often use microbes to degrade the cell walls. As mentioned above, the ability to determine feeding substrates based on mouthpart morphology underscores the possibility of being able to interpret spore-feeding behavior of insects from fossils in order to search for associated yeasts. Reliance on a single food source such as mushrooms and polypores throughout their lives draws attention to the possibility of an inadequate diet over the long term. It is not clear that insects could subsist on basidiocarp tissues alone, but microbes that inhabit the gut, sometimes in midgut caeca (McHugh et al. 1997), could provide additional nutrients. Suggestions that yeasts supply vitamin supplements and a variety of enzymes have been supported by findings of a variety of B-complex vitamins and assimilation of a wide variety of carbon substrates, including trehalose. Evolution of caeca in the gut of these beetles points to a benefit for the insect, but the exact basis of the association remains uncertain. The dispersal benefit for the microbes is extremely important for their survival, because fungi (e.g., wood decayers) and insects (e.g., fungus feeders) modify or completely destroy their substrates in a relatively short time and need a new, similar habitat.

A large percentage of the yeasts isolated from the gut of fungus-feeding beetles were previously unknown taxa, some more than 40 base pairs of LSU rDNA different from the closest known species in GenBank. The principal clades in the fungus-feeding beetle guts included *Suhomyces* species (previously known as the *Candida tanzawaensis* clade), *Teunomyces* species (previously, *Candida kruisii* clade), *Meyerozyma guilliermondii* clade (previously *Pichia guilliermondii*), and the *Candida mesenterica* and Trichomonascaceae clades. The insects showed no preference in a variety of wood-decaying basidiomycetous fruiting bodies indicating that as in the case of yeasts from ephemeral flowers and desert cacti, the gut yeasts are more closely tied to the host insect than to the specific food substrate used by the insect (Blackwell 2017). This fact indicates that the association likely benefits both insects and fungi, and that, as mentioned just above, dispersal of yeasts to a new habitat is of great importance. Often yeasts can be isolated from frass, and germination of cells on agar indicates survival after gut passage. This fact points to dispersal success inside an insect, but identification of the insect associate may not be possible (Imanishi et al. 2008).

Yeasts are more commonly associated with beetles having certain ecological and morphological similarities. Fungus-feeding beetles may possess gastric caeca located at the anterior part of the midgut, and these have been well characterized in beetles such as the fungus-feeding *Megalodacne heros* (Erotylidae; McHugh et al. 1997). Caeca and mycangia also are found in Anobiidae, Buprestidae, Cerambycidae, Cucujoidea, and Tenebrionidae, many of which are fungus feeders. The deep pockets of gastric caeca may help to retain gut yeasts even during molts in larval beetles (Engel and Moran 2013). Moreover, some beetles exhibit parental care, providing an opportunity for possible vertical transmission to offspring, including fungus-feeding Erotylidae (e.g., *Pselaphacus* spp.) and Tenebrionidae. Other families with parental care known to have fungus and yeast associations include Curculionidae (Scolytinae, Platypodinae), Passalidae, and Scarabaeidae. Evidence of consistent yeast-beetle associations has come from repeated isolation of the same yeast species from the same beetle species over a 5-year period at five different localities in southeastern Louisiana, the association of the same yeast and beetle species along the eastern and gulf coasts of the United States from Vermont to Georgia and Louisiana, and isolation of a yeast from both larvae and adults of a beetle species (Blackwell and Suh, unpublished data). Although species-specific associations do occur on occasion and vertical transmission may assure such an association over the short term, specificity is usually more common at the level of yeast clade.

Fungus substrates were chosen for study in my own work, because yeasts previously had been reported from mushrooms (Prillinger 1987), and the substrate offered an easy target to resample certain insects to determine repeated associations (Suh and Blackwell 2005; Suh et al. 2005). Several clades of yeasts were dominant among the taxa isolated from fungus-feeding beetles. The yeast strain, CBS 7422, had a long and interesting history since it was first isolated from the moss *Polytrichum commune* in Kanagawa Prefecture, Mount Tanzawa, Japan, by Nakase in 1966. Nakase refrained from describing the single strain for 22 years, while he waited unsuccessfully to isolate the yeast a second time before describing it as *C. tanzawaensis* (Nakase et al. 1988). Thirteen more years passed before it or any close relative was found. Kurtzman (2001) described six new related species from a variety of substrates including insect frass, rotted wood, and mushrooms, mostly from the central United States but also one from South Africa. *Suhomyces kilbournensis* was described most recently from maize kernels collected in Illinois, USA, and soil in Mexico (Kurtzman et al. 2016). In a study of yeasts from insect guts, sixteen more species in what is now known as the *Suhomyces tanzawaensis* clade were described from Panama and the southeastern United States in association with fungus-feeding beetles (Suh et al. 2004a).

Suhomyces spp. are common in the gut of a variety of fungus-feeding beetles. Out of about 650 strains from the gut of fungus-feeding beetles, 30% were members of this clade, and 85% of these were isolated from only two beetle families, Tenebrionidae and Erotylidae. While the clade is widespread in association with fungus-feeding beetles, the species may have more restricted distributions. Among the 16 novel species described, *Suhomyces panamericana*, *Suhomyces bribrorum*, and *Suhomyces anneliseae* (Table 13.1) have been found to have broad ranges

Table 13.1 Localities where species of *Suhomyces* and *Teunomyces* were isolated from the gut of fungus-feeding beetles (Blackwell, Luangsa-ard, Nguyen, Suh and Urbina, unpublished data)

Panama	SE United States	Thailand
Suhomyces anneliseae	*Suhomyces anneliseae*	*Suhomyces anneliseae* (0[a])
Suhomyces panamericana	*Suhomyces panamericana*	*Suhomyces* cfr. *panamericana* (13–15)
Suhomyces bribrorum	*Suhomyces bribrorum*	–
Suhomyces ambrosiae	*Suhomyces ambrosiae*	–
Suhomyces guaymorum	–	–
Suhomyces bokatorum	–	–
Suhomyces kunorum	–	–
Suhomyces terraborum	–	*Suhomyces* cfr. *terraborum* (7)
Suhomyces emberorum	–	–
Suhomyces wounanorum	–	–
Suhomyces maxi	–	–
Suhomyces taliae	–	–
–	*Suhomyces yuchorum*	–
–	*Suhomyces chickasaworum*	*Suhomyces* cfr. *chickasaworum* (0–11)
–	*Suhomyces choctaworum*	–
–	*Suhomyces bolitotheri*	–
–	*Suhomyces atakaporum*	*Suhomyces* cfr. *atakaporum* (16–30)
–	–	*Suhomyces tanzawaensis* (0–2)
–	–	*Suhomyces* sp. ST-431 (7)
–	–	*Suhomyces* sp. ST-370 (17–19)
Teunomyces panamensis	–	–
Teunomyces barrocoloradensis	–	–
Teunomyces stri	–	–
Teunomyces aglyptinia	–	–
Teunomyces gatunensis		–
	Teunomyces pallodes	–
	Teunomyces tritomae	–
	Teunomyces lycoperdinae	–
	Teunomyces atbi	–

[a] (n) indicates number of base pairs difference in LSU rDNA from type strain

evidenced by their isolation from a variety of beetles in Panama, the United States (Suh et al. 2004a), and Thailand (Blackwell et al. unpublished data).

Su. anneliseae has been isolated from beetles in seven families of fungus-feeding beetles. In addition *Suhomyces ambrosiae* reported by Kurtzman (2001) from a number of localities in the central and northeastern United States also was isolated in Panama and the southeastern United States (Table 13.1). The basis of the specific relationship between fungus-feeding beetles and *Suhomyces* clade yeasts is not clear, but as mentioned above, the results of metabolic tests indicate that yeasts produce several B vitamins and a wide variety of carbohydrate-degrading enzymes that may be of use to the beetle hosts. It is of interest that the five species that were not isolated from the gut of beetles are in a subclade distinct from the 19 beetle-associated species.

Crosses of related strains of *Suhomyces* have not produced ascospores in culture (Suh et al. 2004a; Kurtzman et al. 2016). Little information from genomes has bearing on the topic, except that the genome of the type of *Su. tanzawaensis* does have a heterothallic *MAT* locus organization with only one *MAT* idiomorph present in the single strain examined (Riley et al. 2016). It is possible that the other mating type will be found at some point. An interesting finding was that many gut isolations recovered only a single species of yeast. The isolation of single species could be an artifact of the methods of isolation, including choice of medium or purification, so that rare yeasts were overgrown or the result of inhospitable insect gut conditions, although an effort was made to avoid these possibilities. In an effort to explain this finding, we looked for inhibition of growth of *Suhomyces* strains, and it is possible that some of the yeasts may inhibit the growth of related strains (Blackwell and Lu, unpublished data).

Species from a second previously rarely isolated yeast clade have been placed in the genus *Teunomyces*, although some strains have been found in habitats other than fungus-feeding beetles. The clade members are known widely from the northern and southern United States, Greece and Crete, Panama, and China (Table 13.1) (Kurtzman et al. 2016). *Teunomyces kruisii* and CBS 9453 (now *Teunomyces cretensis*) were the only members of the clade known until 2006. Of 650 fungus-feeding beetles dissected, about 100 isolates, the majority of which were from beetles in the Nitidulidae, especially *Pallodes*, were isolated, and nine new species were described (Suh et al. 2006a). There are no records of clade members from Thailand, but few nitidulids were collected, perhaps due to drought conditions (Blackwell et al. unpublished data). As in the case of *Suhomyces*, strains of *Teunomyces* have never produced ascospores in culture, despite numerous attempts at crossing closely related strains under different conditions (Suh et al. 2006a; Kurtzman et al. 2016).

A life away from fungus-feeding beetles has been a possibility for species belonging to the genera *Suhomyces* and *Teunomyces*, because strains have been isolated from frass and, more recently, free living associated with trees (Sylvester et al. 2015); isolation from the beetle gut, however, always has had a higher probability of success. A study using 454 sequencing of the LSU rDNA marker detected species of *Suhomyces* and *Teuomyces* in the gut of *Drosophila* spp. visiting *Russula* spp. at Stony Brook, NY, USA (Chandler et al. 2012). These species were distinct from all other yeasts analyzed from 13 other non-fungal substrates (mostly from California). Furthermore, yeasts common to plant-associated habitats were absent in the fungus-associated flies. Bacterial populations from the fungi also were distinctive, and bacteria were more diverse than yeasts in all substrates sampled including those from *Russula*.

The fact that communities centered around a fungal resource are distinct from plant-based communities is of interest but not surprising, because the organisms that specialize on fungal substrates would require distinctive physiology for efficient use of a fungal resource. Most fungi, including mushrooms, differ dramatically from plants in cell wall composition (e.g., chitin and glucan, not cellulose, hemicellulose, and lignin), energy resource (storage as glycogen and lipids, not starch), and sterols as precursors of cell membranes (ergosterol, not 24-alkyl-Δ(5)–sterols) (Alexopoulos et al. 1996; Weete et al. 2010). Starmer (1981) noted another difference: yeasts from relatively low-quality mushrooms had a higher catabolic activity than those from other yeast communities. Contributions by different yeasts to the fitness of *Drosophila* spp. have been determined (e.g., Anagnostou et al. 2010), and the differences in yeast communities from different habitats probably have been significant in *Drosophila* radiations (Broderick and Lemaitre 2012). The discovery of both *Suhomyces* spp. and *Teunomyces* spp. associated with drosophilids adds a new dimension to the life cycle of yeasts that should be integrated with the life histories of the flies. Are the yeasts simply acquired from the surface of the fungus where the drosophilids feed along with beetles, or does some other interaction with beetles occur?

13.2.3 Yeasts and Wood-Ingesting Insects

Several families of beetles (Passalidae, Cerambycidae, Buprestidae, and a Tenebrionidae species *(Phrenapates bennetti*) ingest wood as they tunnel through several year-old white-rotted logs. The most common yeasts associated with wood-ingesting beetles are members of the *Scheffersomyces* and *Spathaspora* clades. Many of the yeasts are marked by a rare attribute, xylose fermentation, among other properties; xylose-fermenting yeasts are consistently present in termites, wood roaches, and other animals that feed in dead wood (Prillinger et al. 1996; Suh et al. 2003; Urbina et al. 2013b). In addition several other clades of xylose-assimilating yeasts have also been present, including species of *Lodderomyces* and basidiomycetous species of *Trichosporon* and Tremellomycetes genera previously included within *Cryptococcus* (Urbina et al. 2013b; see Liu et al. 2016). Comparisons of gut yeasts from fungus-feeding beetles with those of wood-ingesting beetles show that very different communities are associated with each group of yeasts, in part due to physiological adaptations for use of nutrients and survival of the gut physical conditions.

The passalid gut is distinctly compartmentalized, similar to that of the termite gut but larger (Nardi et al. 2006). In addition to the foregut and midgut, the hindgut is divided into several different compartments that are morphologically and physiologically distinct. Parabasalid flagellates, related to termite hindgut organisms, occupy part of the anterior hindgut. The enlarged anterior hindgut has the lowest O_2 availability in the gut, and bacteria are attached to the convoluted gut walls. Although the bacteria in the anterior hindgut are not diverse in species numbers, the taxa present and gut physical conditions indicate that it is the site of decomposition of lignocellulosic materials, N_2 fixation, pH_2 regulation, and CH_4 production (Ceja-Navarro et al. 2014). The elongated posterior hindgut contains other bacteria, fungi, and gut organisms previously placed in a group known as trichomycetes (Lichtwardt et al. 1999). Yeasts are attached to the posterior end of the hindgut, and xylose-fermenting yeasts are always present in both larvae and adults. Adult beetles provide some level of parental care by provisioning the gallery walls with a mixture of chewed wood and frass. The frass contains yeasts, so that the hindgut microbial community can be renewed constantly after molting. Any advantage of these yeasts to the beetle is not clear. Although fermentation can increase the nutrient value of a poor diet and enhance the anaerobic conditions in the gut favoring the bacterial communities, the far posterior position of the yeasts may not promote an advantage because it is not clear how much absorption occurs in that part of the hindgut in insects.

Odontotaenius disjunctus (Passalidae) harbors the xylose-fermenting species, *Scheffersomyces stipitis*, in the hindgut. The yeast has a holdfast by which it attaches to the hindgut wall (Suh et al. 2004b; Nardi et al. 2006). A holdfast, rarely observed as a yeast adaptation, may indicate that selection has been in favor of the yeast that benefits from an advantageous position to acquire nutrients in a dynamic gut region (Nardi et al. 2006). All attempts to cure the beetles of yeasts failed so that experimental studies could not be pursued (Gross 2010). The species *Scheffersomyces cryptocercus* has been isolated several times in association with a wood roach, *Cryptocercus* sp. (Urbina et al. 2013a), and apparently free living from the bark of forest trees (Sylvester et al. 2015). A different xylose-fermenting yeast, *Spathaspora passalidarum*, also has been isolated from the gut of *O. disjunctus* (Nguyen et al. 2006b). *Scheff. stipitis* and *Spath. passalidarum* both form ascospores in culture, although *Spath. passalidarum* has more specific requirements, notably lower temperature (Nguyen et al. 2006b). Sequencing of a single isolate each of *Scheff. stipitis* and *Spath. passalidarum* showed that *Scheff. stipitis* has a homothallic *MAT* locus organization with both *MAT*a and *MAT*α present, but *Spath. passalidarum* has a heterothallic organization with a single idiomorph present (Riley et al. 2016). Related asexual species of both yeasts have been found in rotting wood, and some of them associated with wood-ingesting beetles also have been isolated directly from wood (Cadete et al. 2013; Morais et al. 2013). In tropical localities (Brazil, Guatemala, Panama, and Thailand), *Sc. stipitis* and related yeasts appear to be more species diverse. The list of xylose-fermenting yeasts from rotted wood, beetles, and termites grows longer and now includes species of *Sugiyamaella* and other plant cell wall-degrading yeasts of biotechnological potential (Jeffries et al. 2007; Urbina and Blackwell 2012; Morais et al. 2013; Suh et al. 2013; Urbina et al. 2013b; Cadete et al. 2016; Handel et al. 2016; Riley et al. 2016).

13.2.4 Basidiomycetes Also Inhabit the Gut of Insects

As mentioned earlier the work of Prillinger (1987) and Prillinger et al. (1993, 1996) was the stimulus to look at insects associated with mushrooms and other fungi in order to determine if insects might be active dispersers of yeasts. The possibility was that because the yeasts he isolated were from a mushroom parasitic on other mushrooms, an entire community of organisms might be dispersed between mushroom islands by insects. The basidiomycetous yeasts appeared infrequently (Prillinger et al. 1993), and we also isolated basidiomycetes much less often than ascomycete yeasts by our dissection and streaking methods. Among the Tremellomycetes (Agaricomycotina), species in the genera *Trichosporon*, *Bullera*, and others previously placed in *Cryptococcus* (Liu et al. 2016), including *Vanrija*, *Rhynchogastrema*, *Cystofilobasidium*, *Naganishia*, *Papiliorama*, *Piskurozyma*, *Filobasidium*, *Vanrija*, and *Vishniacozyma*, were isolated in Panama in association with fungus-feeding beetles in a variety of families (Curculionidae, Endomychidae, Nitidulidae, Passalidae, Scaphididae, and Scaraebidae). Ballistosporic yeasts (e.g., *Vanrija humicola*, former *Cryptococcus humicolus*) and relatives of those first reported by Prillinger et al. (1993) from species of *Asterophora* were occasionally found in the same beetles. Other basidiomycetous yeasts only rarely isolated from the gut of beetles included ballistosporic yeasts classified in Pucciniomycotina (e.g., previously classified in the genera *Sporobolomyces*, *Rhodosporidium*, *Rhodotorula*) and members of Ustilaginomycotina (*Pseudozyma*, *Sporisorium*).

Several basidiomycetous yeasts isolated from insect guts are known to interact with other fungi. One new species, *Trichosporon insectorum*, was isolated from the guts of a scarab and a passalid beetle from Gamboa and Barro Colorado Island, Panama, respectively, but also from artisanal cheese where it may have been insect or mite dispersed. Interestingly, all three strains possess an identical killer factor that is active against certain strains of *Cryptococcus neoformans* and *Cryptococcus gattii*. Another basidiomycete yeast from a termite hindgut, *Apiotrichum* (*Trichosporon*) *mycotoxinivorans*, has been reported to detoxify the mycotoxins, ochratoxin A, and zearalenone (Molnar et al. 2004).

13.2.5 Sex in the Gut of Wasps

To date, a great deal of time has been invested in looking for potential symbiotic associations in nature. There is, however, relatively little known about the activities of fungal associates within a host in nature. *S. cerevisiae* is one of the best-known fungi in the world because of its value to scientific research and its industrial importance, but despite the sophisticated knowledge of genomics and molecular biology of the yeast, relatively little was known of the complete life cycle in nature. Cavalieri and colleagues (Stefanini et al. 2012, 2016) determined that the gut of adult female social wasps (*Vespa crabro* and *Polistes* spp.) was a specialized niche for overwintering by *S. cerevisiae*, one in which conditions for outbreeding existed. Their experimental work indicated that the wasp gut also provided an environment conducive to outcrossing not just for *S. cerevisiae* but also for interspecific hybridization with its close relative, *Saccharomyces paradoxus*, both contributing to high genetic diversity. Their experiments showed that pure *S. paradoxus* ascospores were unable to survive gut passage, but hybrid spores remained viable.

Stefanini et al. (2016) have filled a gap in our knowledge of the life history of *S. cerevisiae* not only by determining that the yeasts overwinter in the wasp gut but also by determining that the spores are dispersed to the wasp progeny and then to the fruits in the wild or in vineyards. The wasp-yeast interaction provides a new role for an insect in providing a means of overwintering, outcrossing to increase diversity, and eventually dispersing the yeast. If gut passage of *S. cerevisiae* is compared to the yeasts of fungus-feeding beetles in *Suhomyces* and *Teunomyces*, the life histories are very different, primarily in the dramatic increase in genetic diversity of *S. cerevisiae* in the wasp gut and apparent lack of or only rare sex in the fungus-feeding beetles (see above), two very different life-history strategies.

13.2.6 YLSs of Anobiid Beetles

Symbiotaphrina buchneri and *Symbiotaphrina kochii* have long been known (Escherich 1900; van der Walt 1961) as YLSs associated with *Stegobium paniceum*, the drugstore beetle, and *Lasioderma serricorne*, the cigarette beetle (Anobiidae), respectively. However, unlike the YLS of planthoppers, *Symbiotaphrina* spp. grow readily in vitro, and they have been discovered growing independently in nature (Martin et al. 2015). The yeasts occupy caeca at the anterior end of the insect midgut and do not undergo sexual reproduction (Gams and von Arx 1980). The beetles eat processed plant material that often contains toxic secondary metabolites, and they have been found living in sacks of flour, cigarette tobacco shreds in packs, and other harsh, arid environments. The females pass yeast cells to their offspring by smearing them on the surface of the eggs. As the larvae chew out of the egg, they ingest some of the cells from the shell that will eventually populate the midgut caeca (Jurzitza 1979). Some of the yeast cells reproduce in the larval gut, but most of them never move from the gut into the caeca, so that part of the population is not passed on to the offspring vertically, an important observation that is mentioned again below (Frank 1996). Uninfected beetles are reported to die prematurely, while symbiont-infected beetle hosts survive. The beetles obtain amino acids and B-complex vitamins from the YLS (Nardon and Grenier 1989). The beetles also obtain ergosterol and 5-dihydroergosterol from the YLSs that they convert to 7-dehydrocholesterol (Pant and Fraenkel 1950; Nasir and Noda 2003). Experimental evidence from cultural studies indicates that *Symb. kochii* synthesizes broad-spectrum detoxifying enzymes (e.g., ester hydrolase, glucosidase, phosphatase, and glutathione transferase) to detoxify a variety of harmful natural and human-made products, including mycotoxins, insecticides, and herbicides, including parathion. Some of the compounds tested were used as a sole carbon source by the yeasts (Shen and Dowd 1991; Dowd 1992).

The phylogenetic placement of *Symbiotaphrina* spp. was impossible to determine until molecular methods became available. As the name suggests, the genus first was assumed to be a relative of early diverging ascomycetes (Taphrinomycotina) and then Saccharomycotina and, finally, based on small subunit rDNA, among Pezizomycotina in an uncertain position because it was not close to any sequences in GenBank (Noda et al. 1995; Jones and Blackwell 1996; Noda and Kodama 1996). More recently, Gazis et al. (2012) discovered a new lineage of the Pezizomycota radiation, the Leotiomyceta that contains *Xylona heveae* (Xylonomycetales), an endophyte isolated from *Hevea* spp. (rubber trees) in Amazonian Brazil. In a second study, Gazis et al. (2016) used a concatenated alignment of ITS (internal transcribed spacer) and LSU rDNA with well-chosen taxa for comparison to place the Xylonomycetales as a sister to Symbiotaphrinales, finally placing the YLS among close relatives. In addition, when the genome of *Xyl. heveae* was compared with those of other endophytes and plant-associated ascomycetes, there were fewer carbohydrate-active enzymes (CAZymes) for degradation of plant cell walls encoded, and the genome had more in common with animal-associated fungi that those associated with plants (compare with planthopper enzymes above). Thus, the ability of the endophytic *Xyl. heveae* to enter plants by direct penetration is limited, and Gazis et al. (2016) suggested that the species could be associated with an insect vector. Another unexpected finding from the study was the discovery of two strains of *Symb. kochii* in GenBank originally isolated as endophytes. The possibility that usually vertically transmitted symbionts have some cells that are not transmitted directly to the host offspring may benefit the symbionts. Frank (1996) included the life history of the drug-store beetle in his discussion of alternative horizontal dispersal as an evolutionary strategy favoring symbionts in order to avoid competition with similar genotypes.

13.2.7 Extending Endophyte Life Histories to Include Insects

Many fungi regularly produce budding cells in their life cycles. As Nagy et al. (2014) indicated, a genetic tool kit for directing changes from yeast to filamentous growth may have originated soon after the divergence of the fungal lineage. “Latent homology” was proposed to explain the switches in growth form in which yeasts appear throughout the fungi (Nagy et al. 2014). Some of the yeast-like fungi may have life cycles as endophytes (Vega 2008, Sasan and Bidochka 2012), including “multifunctional lifestyles” as insect pathogens (Barelli et al. 2016). Certain arthropod-associated yeasts have been reported as endophytes, including a mite-associated species of *Meira* (Sánchez Márquez et al. 2008). In addition to the YLS of anobiid beetles discussed above, other fungi with well-known arthropod associations also may live as endophytes (Martin et al. 2015).

The life history of *Septobasidium* species is interesting for their mutualistic associations with scale insects (Coccoidea). The genus is a member of the large, diverse clade of rust fungi (Pucciniomycotina), and it has a worldwide tropical to temperate distribution. The life histories of many species in culture include basidiospores that are forcibly discharged and germinate by undergoing several rounds of budding. Eventually hyphae grow out, and in nature a hyphal mat with a velvety or felt-like surface develops (Henk 2005; Henk and Vilgalys 2007; Henk personal communication). The interior of the colony has a series of chambers and tunnels, and a few to entire colonies of the insects are parasitized by fungal haustoria, typical of biotrophic parasites. The parasitized scale insects support the growth of the fungus, and in return the other scale insects are protected from parasitoid wasps and desiccation. The life cycle associated with scale insects was known for species of *Septobasidium* for more than a century (Couch 1938). Now, however, two strains of *Septobasidium* sp. have been discovered in wild trees of *Hevea* spp. and more than 30 environmental ITS sequences in GenBank may be endophytes (Martin et al. 2015). It is not clear how the endophytes fit into the scale insect-associated life history, but this finding deserves investigation.

13.2.8 Supplements for Blood-Feeding Insects

Some insects (e.g., tsetse flies, bat flies, kissing bugs, lice) are blood feeders for their entire lives, and they often harbor microbial symbionts, mostly bacteria including W*igglesworthia* spp. The bacteria are important because blood diets are deficient in essential B vitamins and some amino acids, and the bacteria help to overcome the dietary deficiencies (Douglas 2009; Gusmão et al. 2010). More recently, both laboratory-reared and wild mosquito populations have been reported to harbor a variety of yeast species in different stages of the insect life cycle (Ignatova et al. 1996; Gusmão et al. 2010). Attention to gut organisms in mosquitoes has increased with the spread of arthropod-borne diseases including malaria and a number of viruses (e.g., dengue, West Nile, Chikungunya, and Zika). The finding that has attracted new attention is that *Wickerhamomyces anomalus*, a mosquito gut yeast, produces an inhibitor of the β-1,3-glucans in the spore walls of several protistan parasites, including an agent of malaria, *Plasmodium berghei*. Inhibition of the protistan spores occurs in vitro but also in the mosquito gut where inhibition is up to 90% greater than in the controls (Ricci et al. 2011; Cappelli et al. 2014; Valzano et al. 2016; van Tol and Dimopoulos 2016).

13.2.9 Beetles that Farm

Beetles are one of the three groups of farming insects (the other two being termites and ants; Mueller and Gerardo 2002), but the beetles that acquired this habit are not a monophyletic group. Instead farming evolved independently in various weevils (Curculionidae) in the subfamilies Platypodinae and Scolytinae, with most ambrosia beetles found throughout Platypodinae and in two derived tribes of Scolytinae (Corthylini and Xyleborini). Host beetles may have simple pits in their exoskeletons or the specialized glandular structures (e.g., mycangia of many ambrosia beetles) in which dimorphic fungi grow as yeasts and are nourished as they are transported to new substrates (Harrington 2005; Six 2012; Mayers et al. 2015; Lu et al. 2016). Harrington (2005) hypothesized that certain beetles might have escaped to the xylem (wood) to avoid competition in bark habitats. Perhaps this was the perfect strategy and the reason for estimates of more than 3400 ambrosia beetles (Batra 1967; Harrington 2005); the fungal associates of very few of the known species of ambrosia fungi (1–5%) have been characterized (Harrington 2005; Hulcr and Stelinski 2017).

The beetles cultivate fungi that have been a cause of taxonomic uncertainty because convergent evolution of the taxonomic characters likely was convergent for insect dispersal (Spatafora and Blackwell 1993). In the past, sexually reproducing species were placed in a single genus, *Ceratocystis*, and sometimes even combined with taxa in Saccharomycotina based primarily on spore shape (Kurtzman 2011; Starmer and Lachance 2011). Currently, these fungi are classified with their asexual relatives (of which there are many) based on DNA analysis in Pezizomycotina as members of the Ophiostomatales, *Ophiostoma* clade (e.g., *Raffaelea* spp., de Beer et al. 2014), and Microascales, *Ceratocystis* clade (*Ambrosiella* and *Meredithiella*, Harrington et al. 2014; Mayers et al. 2015). Although these fungi are not usually counted among the "yeasts," they provide an appropriate example of the way in which many fungi associated with animals have yeast stages that appear over and over, even for short periods during the life cycle (see above, Nagy et al. 2014). Yeast stages are found in most ambrosia fungi, and the fungi have yeast-like budding of ascospores; species in Ceratocystidaceae, however, produce arthrospore-like cells rather than budding cells (Mayers et al. 2015).

Less is known about associations between these beetles and yeasts (Saccharomycotina). A number of yeast species have been reported in associations with the filamentous ambrosial fungi and ambrosia beetles, although some are from frass and some of the beetles were not identified. These genera include species of *Ambrosiozyma*, *Cyberlindnera*, *Dipodascus*, *Lindnera*, *Lipomyces*, *Ogataea*, *Pichia*, *Saccharomycopsis*, and other yeasts (Kurtzman et al. 2011; Davis 2015). Some of these yeasts have hat-shaped ascospores, apparently a convergent character common among some filamentous ascomycetes in the same habitats (Kurtzman et al. 2011). Although many yeasts probably do not form specific associations with certain beetles, a few such as *Kuraishia capsulata* and *Ogataea pini* occur repeatedly with many beetles, and the yeasts are probably important in their communities for production of pheromones (Davis 2015).

13.2.10 Phoresy, Yeasts Ready to Roll

Coprophilous fungi (i.e., fungi which grow on animal feces) are generally thought to exhibit "gut passage syndrome" in their life cycles, with spores surviving or even requiring heat and enzyme treatments as they travel through the gut of a mammal. After the spores are deposited in dung, they germinate and grow and their reproductive structures are oriented toward light for forcible discharge. To overgeneralize about the intriguing gut passage syndrome, however, is to neglect another type of life cycle in dung and other quickly deteriorating habitats, such as decaying plant material and beached wrack (Blackwell and Malloch 1989). Many of these dung fungi are mycoparasites and dependent on a fungal host and beetle and mite dispersers, and the timing of spore maturity and disperser migration is precisely timed for a move to a new dung pile. At maturity species of *Pyxidiophora* (Laboulbeniomycetes) produce ascospores that are passively discharged, oozing to the tip of a long-necked perithecium where they adhere to phoretic mites that then attach to beetles for transport to a fresh habitat, an example of hyperphoresy (Blackwell and Malloch 1989). Yeast cells are produced from the ascospores, which multiply the products of meiosis, and can be spread rapidly over the surface of the new substrate (Blackwell et al. 1986). The increase in number of cells by the time of arrival at the new substrate probably also increases the possibility of contact with the host fungus and speeds up the life cycle that must be completed before desiccation of the substrate (Blackwell and Malloch 1989). Species of *Kathistes* (Ophiostomatales) are poorly known, but in Ontario, Canada, they are dispersed by phoretic mites of parasitic flies of moose, another case of hyperphoresy. These species also have yeast cells that develop from the ascospores, again providing for multiplication of the meiotic products (Malloch and Blackwell 1990).

The holdfast of *Scheff. stipitis* that attaches to the hindgut of beetles, a part of the gut from which the cells could otherwise easily be eliminated, was discussed above. Only a few other yeasts are known to produce holdfasts by which they attach for dispersal (Kerrigan and Rogers 2003, 2013). The pseudohyphal filaments of several species of *Botryozyma* attach to nematodes by bifurcate basal holdfast cells distinct from those of *Scheff. stipitis*, and the filaments often are present in large numbers on nematodes phoretic on long-horned beetles, *Saperda calcarata* (Cerambycidae) in trembling aspen, another example of hyperphoresy. Because of the way we isolate yeasts in culture, they rarely are observed in situ, and it may be possible that other species could have holdfasts if we observed them in nature.

13.3 Concluding Remarks

More yeast–insect associations remain to be discovered. There already are many additional reports scattered throughout the literature of yeasts associated with a variety of insects. Examples include reports of lacewings and caddis flies (Nguyen et al. 2006a), dung beetles (Górz and Boroń 2016), codling moths (Witzgall et al. 2012), as well as many others that need more focused study. Social insects (termites, ants, bees, and wasps) are known for their reliance on microbes that play vital roles in their life cycles. Subsocial insects include a number of beetles that exhibit parental care [Erotylidae (e.g., *Pselaphacus* spp.), Silphidae, Chrysomelidae (Cassidinae), Carabidae, Lampyridae, Passalidae, Scarabaeidae, Staphylinidae, Curculionidae (Scolytinae, Platypodinae), and Tenebrionidae (*Phrenapates* spp.)] that also are potential hosts. Although these insects lack the specialized castes of social insects, they often live in large groups with overlapping generations and care for the young. Younger adult colony members may help perform duties connected with rearing young. Mycangia and gastric caeca (e.g., anobiids, cerambycids, buprestids, cucujoids, tenebrionoids) signal the presence of microbial symbionts.

High-throughput methods will help to discover new associations, but cultures in hand will still be important to determine biological traits. Genomes and other data will help to understand the kind of interactions that occur. Some insects change diets as they mature, and these organisms offer an opportunity to track microbial changes with changes in diet. Interest in diet changes over lineages and examination of the extant hosts, and microbes throughout these lineages may help to understand evolution. Fossils can be targeted for more records (Leschen and Buckley 2007; Cai et al. 2016). The basis of many of the interactions between yeasts and insects has been hypothesized, but seldom tested (but see Nasir and Noda 2003).

A number of theoretical considerations remain to be studied, and emphasis on yeast and animal associations could help to provide illumination on the following points:

- Interactions are rarely restricted to two participants, and unknown participants or multipartite interactions may have important consequences for the associations. A broader view to consider entire microbial communities should be pursued (Douglas and Werren 2016).
- The discovery of new microbial symbionts may be important to the way in which microbes function to benefit the host (Xue et al. 2014). Host switching and host-habitat hypotheses may be relevant in some cases (Nikoh and Fukatsu 2000; Araujo et al. 2015).
- Life-history studies may expand the involvement of insects in questions of horizontal and vertical transmission of microbes; is vertical transmission rare among most microbial associations as has been suggested from restricted examples (Boomsma and Aanen 2009)? Do both commonly occur in the same association? Can specialists be generalists (Agosta et al. 2010)?
- Is lack of sexual reproduction better for some symbionts as theory might suggest (Xue et al. 2014; Fan et al. 2015)? How does asexual reproduction affect associations over the long term (Moran 1996)?
- Is there confirmation that symbiosis is a “key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans,” as Klepzig et al. (2009) suggest?

- Yeast–insect associations are excellent systems for studying applications for agriculture (Witzgall et al. 2012), medicine (Valzano et al. 2016), and evolutionary research.
- Studies resulting from the large amounts of funding available for human genomes can provide newer methods and background information for the study of microbe and insect associations (Putignani et al. 2014).

In the summer of 1970, I worked at Houston NASA during the Apollo 13 mission. The aborted moon launch left us without the anticipated moon rock samples to use in assays to ensure that pathogens had not come to Earth from the moon. To occupy ourselves, my colleagues and I attempted to cultivate symbiotic bacteria from roaches using several kinds of media including at the time sophisticated insect culture media. We never made up for the insect component of the interaction, and at last I understand why our attempts were unsuccessful (Engel and Moran 2013).

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