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The Role of Yeasts as Insect Endosymbionts

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Insect associations with fungi are common and may be casual or highly specific and obligate. For example, more than 40 fungal species are associated with the coffee berry borer (*Hypothenemus hampei*, Coleoptera: Curculionidae; Pérez et al. 2003) and about the same number with the subterranean termite *Reticulitermes flavipes* (Zoberi and Grace 1990; table 9.1). In one system 28 species of yeasts were isolated from the external parts of *Drosophila serido* and 18 species, including some not found on the external surfaces, from their crop (Morais et al. 1994; table 9.1).

In relatively few cases a specific role for the fungus has been identified, as is the case for associations with ants (chapter 7), termites (chapter 8), and bark beetles (Chapter 11; Six 2003). These associations imply that different species are living together, reinforced by specific interactions, a concept popularized as symbiosis by de Bary (1879). Symbiotic associations have been classified as ectosymbiotic when the symbiont occurs outside the body of the host or endosymbiotic when the symbiont occurs internally, either intra- or extracellularly (Steinhaus 1949; Nardon and Nardon 1998; Margulis and Chapman 1998). Several interesting symbiotic associations occur between insects and yeasts. In all cases that are well studied, the benefit that accrues for the insect is better understood than the benefit to the yeasts.

The term "yeast" is used to describe a particular fungal growth form (Steinhaus 1947; Alexopoulos et al. 1996). These predominantly unicellular ascomycetes divide by budding at some point in their life cycle (e.g., *Saccharomyces*). A surprising number of yeasts, however, also produce filamentous hyphae. At present, almost 700 species in 93 genera (Barnett et al. 2000) have been described in the ascomycete class Saccharomycetes, a group known informally as "true yeasts." True yeasts lack specialized sex organs, and sexual spores (ascospores) are produced in

Table 9.1. Yeasts internally isolated from insects.

Insect Species	Order: Family	Yeast Location (Species)[a]	Reference
Stegobium paniceum (= *Sitodrepa panicea*)	Coleoptera: Anobiidae	Mycetomes (Saccharomyces)[b] Cecae (*Torulopsis buchnerii*) Mycetome between foregut and midgut Mycetomes (*Symbiotaphrina buchnerii*) Mycetomes and digestive tube (*Torulopsis buchnerii*) Gut cecae (*Symbiotaphrina buchnerii*)	Escherich 1900 Buchner 1930 Gräbner 1954 Pant and Fraenkel 1954 Kühlwein and Jurzitza 1961 Bismanis 1976 Noda and Kodama 1996
Lasioderma serricorne	Coleoptera: Anobiidae	Mycetome between foregut and midgut (*Symbiotaphrina kochii*)	van der Walt 1961; Jurzitza 1964 Gams and von Arx 1980 Noda and Kodama 1996
Ernobius abietis	Coleoptera: Anobiidae	Mycetomes (*Torulopsis karawaiewii*) (*Candida karawaiewii*)[c]	Jurzitza 1970 Jones et al. 1999
Ernobius mollis	Coleoptera: Anobiidae	Mycetomes (*Torulopsis ernobii*) (*Candida ernobii*)	Jurzitza 1970 Jones et al. 1999
Hemicoelus gibbicollis	Coleoptera: Anobiidae	Larval mycetomes	Suomi and Akre 1993
Xestobium plumbeum	Coleoptera: Anobiidae	Mycetomes (*Torulopsis xestobii*) (*Candida xestobii*)	Jurzitza 1970 Jones et al. 1999
Criocephalus rusticus	Coleoptera: Cerambycidae	Mycetomes	Riba 1977
Phoracantha semipunctata	Coleoptera: Cerambycidae	Alimentary canal (*Candida guilliermondii*, *C. tenuis*) Cecae around midgut (*Candida guilliermondii*)	Chararas and Pignal 1981 Nardon and Grenier 1989
Harpium inquisitor	Coleoptera: Cerambycidae	Mycetomes (*Candida rhagii*)	Jurzitza 1960
Harpium mordax H.sycophanta	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*)	Jurzitza 1960
Gaurotes virginea	Coleoptera: Cerambycidae	Cecae around midgut (*Candida rhagii*)	Jurzitza 1960 Jones et al. 1999
Leptura rubra	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*) Cecae around midgut (*Candida parapsilosis*)	Jurzitza 1960 Jurzitza 1959 Jones et al. 1999

Leptura maculicornis *L. cerambyciformis*	Coleoptera: Cerambycidae	Cecae around midgut (*Candida parapsilosis*)	Jurzitza 1960
Leptura sanguinolenta	Coleoptera: Cerambycidae	Cecae around midgut (*Candida sp.*)	Jurzitza 1960
Rhagium bifasciatum	Coleoptera: Cerambycidae	Cecae around midgut (*Candida tenuis*)	Jurzitza 1960
Rhagium inquisitor	Coleoptera: Cerambycidae	Cecae around midgut (*Candida guilliermondii*)	Jurzitza 1959
Rhagium mordax	Coleoptera: Cerambycidae	Cecae around midgut (*Candida*)	Jurzitza 1959
Carpophilus hemipterus	Coleoptera: Nitidulidae	Intestinal tract (10 yeast species)	Miller and Mrak 1953
Odontotaenius disjunctus	Coleoptera: Passalidae	Hindgut (*Enteroramus dimorphus*)	Lichtwardt et al. 1999
Odontotaenius disjunctus *Verres sternbergianus*	Coleoptera: Passalidae	Gut (*Pichia stipitis, P. segobiensis Candida shehatae, C. ergatensis*)	Suh et al. 2003
Scarabaeus semipunctatus *Chironitis furcifer*	Coleoptera: Scarabaeidae	Digestive tract (10 yeast species)	Malan and Gandini 1966
Unknown species	Coleoptera: Scarabaeidae	Guts (*Trichosporon cutaneum*)	do Carmo-Sousa 1969
Dendroctonus and *Ips* spp.	Coleoptera: Scolytidae	Alimentary canal (13 yeast species)	Shifrine and Phaff 1956
Dendroctonus frontalis	Coleoptera: Scolytidae	Midgut (*Candida* sp.)	Moore 1972
Ips sexdentatus	Coleoptera: Scolytidae	Digestive tract (*Pichia bovis, P. rhodanensis, Hansenula holstii,* (*Candida rhagii*)	Pignal et al. 1987, 1988
		Digestive tract (*Candida pulcherina*)	Le Fay et al. 1969, 1970
Ips typographus	Coleoptera: Scolytidae	Alimentary canal	Grosmann 1930
		Alimentary tracts (*Hansenula capsulata*, *Candida parapsilosis*)	Lu et al. 1957
		Guts and beetle homogenates (*Hansenula holstii*, *H. capsulata, Candida diddensii, C. mohschtana, C. nitratophila, Cryptococcus albidus, C. laurentii*)	Leufvén et al. 1984 Leufvén and Nehls 1986
Trypodendron lineatum	Coleoptera: Scolytidae	Not specified	Kurtzman and Robnett 1998b
Xyloterinus politus	Coleoptera: Scolytidae	Head, thorax, abdomen (*Candida, Pichia, Saccharomycopsis*)	Haanstad and Norris 1985

(*continued*)

Table 9.1. (continued)

Insect Species	Order: Family	Yeast Location (Species)[a]	Reference
Periplaneta americana	Dictyoptera: Blattidae	Hemocoel (*Candida* sp. nov.)	Verrett et al. 1987
Blatta orientalis	Dictyoptera: Blattidae	Intestinal tract (*Kluyveromyces blattae*)	Henninger and Windisch 1976
Blatella germanica	Dictyoptera: Blattellidae	Hemocoel	Archbold et al. 1986
Cryptocercus punctulatus	Dictyoptera: Cryptocercidae	Hindgut (1 yeast species)	Prillinger et al. 1996
Philophylla heraclei	Diptera: Tephritidae	Hemocoel	Keilin and Tate 1943
Aedes (4 species)	Diptera: Culicidae	Internal microflora (9 yeast genera)	Frants and Mertvetsova 1986
Drosophila pseudoobscura	Diptera: Drosophilidae	Alimentary canal (24 yeast species)	Shihata and Mrak 1952
Drosophila (5 spp.)	Diptera: Drosophilidae	Crop (42 yeast species)	Phaff et al. 1956
Drosophila melanogaster	Diptera: Drosophilidae	Crop (8 yeast species)	de Camargo and Phaff 1957
Drosophila (4 spp.)	Diptera: Drosophilidae	Crop (7 yeast species)	Starmer et al. 1976
Drosophila (6 spp.)	Diptera: Drosophilidae	Larval gut (17 yeast species)	Fogleman et al. 1982
Drosophila (20 spp.)	Diptera: Drosophilidae	Crop (20 yeast species)	Lachaise et al. 1979
Drosophila (8 species groups)	Diptera: Drosophilidae	Crop (*Kloeckera, Candida, Kluyveromyces*)	Morais et al. 1992
Drosophila serido	Diptera: Drosophilidae	Crop (18 yeast species)	Morais et al. 1994
Drosophila (6 spp.)	Diptera: Drosophilidae	Intestinal epithelium (*Coccidiascus legeri*)	Lushbaugh et al. 1976 Ebbert et al. 2003
Protaxymia melanoptera	Diptera	Unknown (*Candida*, *Cryptococcus*, *Sporoblomyces*)	Bibikova et al. 1990
Astegopteryx styraci	Homoptera: Aphididae	Hemocoel and fat body	Fukatsu and Ishikawa 1992
Tuberaphis sp. *Hamiltonaphis styraci* *Glyphinaphis bambusae* *Cerataphis* sp.	Homoptera: Aphididae	Tissue sections	Fukatsu et al.1994
Hamiltonaphis styraci	Homoptera: Aphididae	Abdominal hemocoel	Fukatsu and Ishikawa 1996
Cofana unimaculata	Homoptera: Cicadellidae	Fat body	Shankar and Baskaran 1987

Leofa unicolor	Homoptera: Cicadellidae	Fat body	Shankar and Baskaran 1987
Lecaniines, etc.	Homoptera: Coccoidea[d]	Hemolymph, fatty tissue, etc.	Buchner 1965
Lecanium sp.	Homoptera: Coccidae	Hemolymph, adipose tissue	Tremblay 1989
Ceroplastes (4 sp.)	Homoptera: Coccidae	Blood smears	Mahdihassan 1928
Laodelphax striatellus	Homoptera: Delphacidae	Fat body	Noda 1974 Mitsuhashi 1975
		Eggs	Kusumi et al. 1979
		Eggs (*Candida*)	Eya et al. 1989
Nilaparvata lugens	Homoptera: Delphacidae	Fat body	Chen et al. 1981a
		Eggs (2 unidentified yeast species)	Nasu et al. 1981
		Eggs, nymphs (*Candida*)	Shankar and Baskaran 1992
		Eggs (7 unidentified yeast species)	Kagayama et al. 1993
		Eggs (*Candida*)	Eya et al. 1989
Nisia nervosa N*isia grandiceps* *Perkinsiella* spp. *Sardia rostrata* *Sogatella furcifera*	Homoptera: Delphacidae	Fat body	Shankar and Baskaran 1987
Sogatodes orizicola	Homoptera: Delphacidae	Fat body	Lienig 1993
Amrasca devastans	Homoptera: Jassidae	Eggs, mycetomes, hemolymph	Gupta and Pant 1985
Tachardina lobata	Homoptera: Kerriidae	Blood smears (*Torulopsis*)	Mahdihassan 1928
Laccifer (=*Lakshadia*) sp.	Homoptera: Kerriidae	Blood smears (*Torula variabilis*)	Přibram 1925; Mahdihassan 1929 Tremblay 1989
Comperia merceti	Hymenoptera: Encyrtidae	Hemolymph, gut, poison gland	Lebeck 1989
Solenopsis invicta *S. quinquecuspis*	Hymenoptera: Formicidae	Hemolymph (*Myrmecomyces annellisae*)	Jouvenaz and Kimbrough 1991

(*continued*)

Table 9.1. (continued)

Insect Species	Order: Family	Yeast Location (Species)[a]	Reference
Solenopsis invicta	Hymenoptera: Formicidae	Fourth instar larvae (*Candida parapsilosis, Yarrowia lipolytica*)	Ba et al. 1995
		Gut and hemolymph (*Candida parapsilosis, C. lipolytica, C. guillermondii, C. rugosa, Debaryomyces hansenii*)	Ba and Phillips 1996
Apis mellifera	Hymenoptera: Apidae	Digestive tracts (*Torulopsis* sp.)	Cited in Gilliam et al. 1974
		Intestinal tract (*Torulopsis apicola*)	Hajsig 1958
		Digestive tracts (8 yeast species)	Cited in Gilliam et al. 1974
		Intestinal contents (12 yeast species)	Cited in Gilliam et al. 1974
		Intestinal contents (7 yeast species)	Cited in Gilliam et al. 1974
		Intestines (14 yeast species)	Cited in Gilliam et al. 1974
		Intestinal tract (*Pichia melissophila*)	van der Walt and van der Klift 1972
		Intestinal tracts (7 yeast species)	Gilliam et al. 1974
		Alimentary canal (*Hansenula silvicola*)	Gilliam and Prest 197
		Crop and gut (13 yeast species)	Grilione et al. 1981
Apis mellifera	Hymenoptera: Apidae	Midguts (9 yeast genera)	Batra et al. 1973
Anthophora occidentalis	Hymenoptera: Anthophoridae		
Nomia melanderi	Hymenoptera: Halictidae		
Halictus rubicundus	Hymenoptera: Halictidae		
Megachile rotundata	Hymenoptera: Megachilidae		

Bombus sp. *Lasioglossum* sp.	Hymenoptera: Apidae Hymenoptera: Halictidae	Crop (*Hansenula anomala, Saccharomyces spp., Schizoaccharomyces spp., Rhodotorula spp*.)	Batra et al. 1973
Adelura apii	Hymenoptera: Braconidae	Midgut	Keilin and Tate 1943
Comperia merceti	Hymenoptera: Encyrtidae	Hemolymph, gut, fat body, poison gland	Lebeck 1989
Pimpla turionellae	Hymenoptera: Ichneumonidae	Hemolymph, fat body, and most tissues	Middeldorf and Ruthman 1984
Termites (5 species)	Isoptera: Termitidae	Gut (*Candida, Pichia, Sporothrix, Debaryomyces*)	Schäfer et al. 1996
Termites (6 species)	Isoptera: Termitidae	Hindgut (12 yeast species)	Prillinger et al. 1996
Sigelgaita sp.	Lepidoptera: Phycitidae	Larvae (12 yeast species)	Rosa et al. 1992, 1994
Unknown species	Lepidoptera: Cossidae	Larval gut (*Endomycopsis wickerhamii*)	van der Walt 1959
Orgyia pseudotsugata	Lepidoptera: Lymantriidae	Alimentary tract (*Candida zeylanoides*)	Martignoni et al. 1969
Chrysopa (=*Chrysoperla*) *carnea*	Neuroptera: Chrysopidae	Crop (*Torulopsis* sp.)	Hagen et al. 1970
Chrysoperla rufilabris	Neuroptera: Chrysopidae	Foregut, midgut, hindgut (*Metschnikowia pulcherrima*)	Woolfolk and Inglis 2003

[a]The insect organ where the yeast was isolated is given as presented in the original paper. In many cases it is not possible to ascertain the exact location from which the yeast was isolated due to authors using general terms, such as "intestinal tract," "alimentary canal," "intestines," and so on.

[b]Note progression in taxonomical identification: from *Saccharomyces* (Buchner 1930), to *Torulopsis buchnerii* (Gräebner 1954), to *Symbiotaphrina* (Kühlwein and Jurzitza 1961). The latter was disputed by Bismanis (1976), who revived the name *Torulopsis buchnerii*, but Gams and von Arx (1980) validated the species as *Symbiotaphrina buchnerii*. Noda and Kodama (1996) and Jones and Blackwell (1996) present evidence indicating that this classification is inappropriate.

[c]*Candida karawaiewii* is considered as a synonym of *C. ernobii* (Kurtzman and Robnett 1998a).

[d]Dozens of examples of yeast endosymbionts in the superfamily Coccoidea have been reported by Buchner (1965).

asci converted from somatic cells that are not produced in an ascocarp. In the scientific literature, some fungi that have been isolated from insects are referred to as yeastlike fungi or yeastlike symbionts (YLS), and they often are evolutionarily reduced, derived from the subphylum Pezizomycotina, the largest clade of ascomycetes, variously known as filamentous or ascocarpic ascomycetes (chapter 10). Some yeastlike fungi are dimorphic, alternating between a yeast phase and a hyphal phase according to environmental conditions (e.g., body temperature or CO_2). The dimorphic yeasts include human pathogens (e.g., *Coccidiomyces*) but also certain associates of insects (e.g., *Ophiostoma*). Other yeasts that will not be discussed further as insect associates are classified as basidiomycetes and zygomycetes.

Several roles have been determined for yeast and yeastlike fungi associated with insects, the most important being a nutritional role in which yeasts provide enzymes for digestion, improved nutritional quality, essential amino acids, vitamins, and sterols. Yeasts also play an important role in the detoxification of toxic plant metabolites in the host's diet. In this chapter we focus on examples of yeasts that are located both intra- and extracellularly in certain insects, the benefits yeasts provide for the insects and their role in insect ecology, and hypotheses on how these systems evolved. It is important to note that endosymbiosis seems to be much more significant to the insect than to the yeast, and as such it is hard to discern the mutualism (see Douglas and Smith 1989), but benefits to the yeasts are suggested to be dispersal and a protected and favorable environment rich in nutrients (Cooke 1977). Finally, we discuss the evidence for the way in which these systems evolved.

The Endosymbiotic Systems

Yeastlike Symbionts and Rice Planthoppers

One of the best known insect–YLS systems involves three rice planthoppers: the brown planthopper (*Nilaparvata lugens*), the small brown planthopper (*Laodelphax striatellus*), and *Sogatella furcifera* (table 9.1). These insects are of economic importance because they transmit several rice viruses. Vertical transmission of yeasts in planthoppers occurs by a mechanism involving symbiont movement from the fat body to the primary oocyte in the ovariole, where the yeasts form a symbiont ball from which eggs become infected (Nasu 1963; Noda 1974, 1977; Mitsuhashi 1975; Chen et al. 1981a; Cheng and Hou 2001).[1] Reduction of the yeast population via heat treatment in *N. lugens* results in aposymbiotic insects in which the following effects have been observed: (1) death of fifth-instar nymphs after failing to moult or complete ecdysis (Chen et al. 1981b); (2) reduction of egg hatch and increased nymphal duration (Bae et al. 1997; Zhongxian et al. 2001); (3) interference with normal embryonic and postembryonic development due to the absence of specific proteins involved in these processes (Lee and Hou 1987); and (4) reduction in weight, growth rate, and concentration of protein per unit of fresh weight (Wilkinson and Ishikawa 2001).

In heat-treated *L. striatellus*, adult molt is affected, indicating that the yeast is involved in sterol metabolism in the insect (Noda and Saito 1979a,b). Noda et al.

(1979) reported that in *L. striatellus*, the YLS is responsible for the production of 24-methylenecholesterol and that cholesterol concentrations were greatly reduced in heat-treated insects. Similarly, Eya et al. (1989) reported dramatically reduced levels of ergosterol and 24-methylenecholesterol in heat-treated *N. lugens* and *L. striatellus* and found that YLS isolated from eggs of *L. striatellus* produced lanosterol, 24-methylenelanosterol, dihydroergosterol, and ergosterol in culture broth. Wetzel et al. (1992) isolated another sterol, ergosta-5,7,24(28)-trien-3β-ol (trienol 6) from the *N. lugens* and *L. striatellus* YLS.

Eya et al. (1989) conducted the first study in which a planthopper symbiont was identified to genus, in this case, *Candida*, implying that it is a member of the true yeasts (Saccharomycetes). *Candida*, however, is a polyphyletic grouping of asexual yeasts from many clades and is largely without phylogenetic significance today. In addition, the modern phylogenetic placement of planthopper YLS outside of the true yeasts has bearing on hypotheses of the origin of symbioses and is discussed later.

N. lugens yeastlike symbionts also play a role in nitrogen recycling and, more specifically, in uric acid metabolism, whereby nitrogen waste products are converted into compounds that have nutritional value (Sasaki et al. 1996). In this process, uric acid is stored for subsequent transformation and use. In heat-treated (and therefore aposymbiotic) *N. lugens*, there was no uricase (urate oxidase) activity, and the concentrations of uric acid were much higher than in the control insects. Symbionts grown in artificial culture had uricase activity 15 times higher than that detected in control insects.

Yeasts and Drosophila

Associations between true yeasts with *Drosophila* are well known (table 9.1; Begon 1982), and all studies indicate that the fungi are necessary for optimal development due to their nutritional role. Yeasts provide essential nutrients, vitamins, and sterols (Sang 1978), and the associations secondarily may involve detoxification of plant metabolites and pheromone production (Starmer et al. 1986). Shihata and Mrak (1952) isolated 24 yeast species from the alimentary canal of *D. pseudoobscura*. Individual flies contained three or fewer different yeast species, and the taxonomic of makeup of the yeast flora varied depending on the time of year they were collected (table 9.1).

In most yeast–*Drosophila* associations, the yeasts clearly play a nutritional role at an extracellular level. For example, *Candida ingens* metabolized toxic fatty acids in cactus tissues, with positive consequences for *D. mojavensis* (Starmer et al. 1982). Similarly, *Candida sonorensis* and *Cryptococcus cereanus* have been shown to metabolize 2-propanol (toxic to *Drosophila* larvae and adults at moderate to high concentrations) in decaying cactus tissue, resulting in positive effects on three *Drosophila* species (Starmer et al. 1986). Yeasts, in turn, are reported to benefit by being transported by the fly to different habitats (Gilbert 1980; Starmer and Fogleman 1986, Morais et al. 1994) and by being provided with adequate moisture conditions (Gilbert 1980). In all the cases mentioned above, the presence of yeast within the insect appears to reflect what is present on the feeding substrate (Morais

et al. 1992), and thus the yeasts are not intracellular. The only clear example of an intracellular yeast–*Drosophila* association is the yeastlike fungus *Coccidiascus legeri* (Lushbaugh et al. 1976), which accelerates development and improves eclosion rates (Ebbert et al. 2003).

Other YLS Associations with Insects

Other examples of yeasts associated with insects include members of the Coleoptera, Dictyoptera, Diptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, and Neuroptera (table 9.1). Of these families, Coleoptera is the best represented. One of the best studied Coleopteran–yeast associations is that of *Symbiotaphrina* and two anobiids, the cigarette beetle (*Lasioderma serricorne*) and the drugstore beetle (*Stegobium* [= *Sitodrepa*] *paniceum*). In these insects the YLS live intracellularly in enlarged cells associated with grape-bunch–like proliferations of tissues around the midgut (Buchner 1965). Yeasts are transmitted vertically when the female smears them on the eggshell, which is consumed by the hatching larva (Buchner 1965). These YLS are important in providing nutrients for the host insects and also in detoxifying plant toxins (see below).

Benefits of Yeast Associations to Insects

Parasitoids and Yeasts

Various examples of parasitoids vertically transmitting yeasts at the time of oviposition have been reported: (1) *Comperia merceti*, an encyrtid parasitoid of *Supella longipalpa* (Blattodea: Blatellidae) ootheca (Lebeck 1989); (2) *Adelura apii*, a braconid parasitoid of the celery fly (*Philophylla* (*Acidia*) *heraclei;* Diptera: Agromyzidae) (Keilin and Tate 1943); and (3) *Pimpla turionellae*, an ichneumonid parasitoid (Middeldorf and Ruthmann 1984). Keilin and Tate (1943) reported that *A. apii* larvae developing within the host feeds on the yeast-filled hemolymph, suggesting a nutritional role for the yeast, about which little is known. These studies did not determine whether yeasts become established in nonkilled hosts. It is possible that yeast transmission during oviposition might have a role in egg protection (Lebeck 1989), as has been reported for hymenopteran polydnaviruses that interfere with hemocytes involved in egg encapsulation (Schmidt et al. 2001).

Yeasts and Plant Allelochemicals

Responses of endosymbiotic yeasts to plant allelochemicals have not received much attention. In evolutionary terms it would be advantageous for a plant to produce chemicals that inhibit yeast growth in cases when the yeast is providing essential nutrients to the insect pest. This would serve as an indirect pest control method. It is obvious, then, that endosymbiotic yeasts are exposed to the chemistry of the plant on which the insect is feeding, as evidenced by the detoxification mechanisms presented in the next section. But what happens when yeastlike symbionts are not pre-

pared to handle plant chemistry? Milne (1961) showed that nicotine inhibited growth of *Lasioderma serricorne* YLS, and further showed that sorbic acid at 0.25% and higher inhibited growth of the symbiont. This work resulted in a proposal to use sorbic acid as a pest control mechanism due to its effect on the endosymbiont (Milne 1963). Similarly, Vega et al. (2003) found that increased concentrations of caffeine in *in vitro* studies resulted in reduced levels of *Pichia burtonii*, a yeast isolated from the coffee berry borer (*H. hampei*). Although not working with yeastlike symbionts, Jones (1981) examined the effects of 2-furaldehyde, a bald cypress (*Taxodium distichum*) allelochemical, and found a significant reduction in seven bacterial and two fungal (*Mucor*, *Curvularia*) enteric isolates of *Bombyx mori* larvae. This kind of research is of value not only for developing novel insect pest management strategies, but also in formulating evolutionary hypotheses aimed at understanding YLS associations with insects.

Yeasts and Pheromones

Ectosymbiotic yeasts are involved in the production of pheromones in bark beetle systems. Hunt and Borden (1990) showed that *Hansenula capsulata* and *Pichia pinus* associated with *Dendroctonus ponderosae* are responsible for the conversion of *cis*- and *trans*-verbenol to verbenone in the beetle galleries. Verbenone serves as an antiaggregation pheromone. Leufvén et al. (1984) also reported a similar yeast-mediated transformation in *Ips typographus*.

Yeasts and Insect Nutrition

Most insects have an obligate requirement for 10–14 amino acids, including aromatic and sulfur-containing types, sterols, several B vitamins, and specific fatty acids, usually linolenic and/or linoleic (Dadd 1985). Only sterols with certain functional groups are acceptable, depending on the insect (Dadd 1985; Nes et al. 1997). Many nutritional substrates of insects (e.g., plant sap and wood) provide very low levels of these nutrients or, in some cases, none. Because the biochemical constituents of certain yeasts and other fungi contain these essential requirements (Brues 1946; Southwood 1973), simple digestion of them will help provide the nutrients. Essential nutrient provision through digestion of yeast associates may be occurring in cases where yeasts are present in localized areas of the insect gut where absorption of nutrients occurs. For example, the yeasts associated with *Carpophilus* sap beetles were rapidly digested (Miller and Mrak 1953). What was described as an intracellular yeast (but which may be a filamentous fungus based on mycelium production in old cultures) symbiont of the soft scale *Pulvinaria innumerabilis* (Homoptera: Coccidae) produced lipase, diatase (amylase), and protease (Brues and Glaser 1921).

Many studies suggesting that yeast associates provide nutrients have inferred this role based on determination of the growth rate of the insects with and without the microbes. Removal of the microbes to provide aposymbiotic insects was accomplished through rearing under aseptic conditions or by interfering with the transmission process of the yeast from one generation to the next (such as surface

sterilizing the eggs of *L. serricorne* or *S. paniceum*). Although these methods should lead to valid comparisons, studies in which antibiotics have been used to remove associates should be viewed with caution because the antibiotics may also affect processes in the insect itself.

Several studies have shown that insect survival, growth, or reproduction is deterred in the absence of their yeast associates, without identifying the nutritional reason. In several instances the biomass of colonies of *Solenopsis invicta* was significantly greater in colonies containing symbiotic yeasts than those that did not (Ba and Phillips 1996). In another study of unspecified benefits, the intracellular yeastlike fungus of *Drosophila*, *Coccidiascus legeri* (Lushbaugh et al. 1976), accelerated development and improved eclosion rates (Ebbert et al. 2003).

In other studies the nutrients provided by the yeast associates have been identified, mainly through studies in which nutritional supplements were added to the diets of insects in the absence of their yeast associates. For example, nitrogen appears to be provided by yeasts in the scale insect *Pseudococcus citri* (Koch 1954). Vitamins synthesized by cultures of the yeasts appear to have benefitted two woodboring cerambycids (*Leptura* and *Rhagium*; Gräbner 1954; Jurzitza 1959).

Some of the best information on the role of symbionts has been provided in studies involving the intracellularly derived yeasts of the genus *Symbiotaphrina* of two anobiid beetles (*L. serricorne* and *S. paniceum*), in which it is relatively easy to eliminate the symbionts by surface-sterilizing eggs. These yeasts have been demonstrated to provide nitrogen (Pant et al. 1959; Jurzitza 1969a; Bismanis 1976), sterols (Pant and Fraenkel 1954; Pant and Kapoor 1963), and vitamins (Fraenkel and Blewett 1943a,b; Gräbner 1954; Pant and Fraenkel 1954; Jurzitza 1964, 1969a,b,c, 1972, 1976; Bismanis 1976) to their hosts. However, nutritional studies with defined diets indicated the symbionts could not supply all of the B vitamins necessary for optimal growth of *L. serricorne* (Pant and Anand 1985).

Other studies have demonstrated that the yeasts provide essential amino acids. In studies with *S. paniceum* involving defined diets with and without amino acids and with and without symbionts, the symbionts appeared to provide various amino acids, although the restoration of growth to levels noted with the symbiont-containing hosts varied (Pant et al. 1959). Tryptophan was clearly provided by the symbionts, because in its absence survival was close to that for a casein diet; there were no survivors among the symbiont-free insects (Pant et al. 1959). Survival of symbiont-containing insects in the absence of histidine was about 50% that of the casein diet, and for symbiont-free insects the survival was about half again that many (Pant et al. 1959). Overall, at least some survivors were present for all individual amino acid deficiencies in symbiont-containing insects, but there were no survivors in symbiont-free insects when arginine, isoleucine, leucine, lysine, phenylalanine, threonine, tryptophane, or valine were absent (Pant et al. 1959). Similar results were obtained in studies with *L. serricorne* (Jurzitza 1969a,b). Later Jurzitza (1972) also showed that with *L. serricorne*, the symbionts appeared to be recycling uric acid, as its addition to protein-deficient diets helped growth of symbiont-containing larvae, but not symbiont-free ones.

The symbionts also appear to provide *L. serricorne* with vitamin B_1, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and choline (Fraenkel and Blewitt 1943). Similar results in a study using symbiont elimination, followed by nutrient supplementation, indicated that the yeasts did in fact provid thiamine, folic acid, biotin, riboflavin, and nicotinic acid, in addition to pyridoxine, pantothenic acid, and choline (Pant and Fraenkel 1954). These results conflict somewhat with another study of similar design in which a different species of anobiid, *S. paniceum*, was not supplied with thiamine by the yeast (Pant and Fraenkel 1954). Additional studies with *L. serricorne* reared on totally artificial diets indicated insects were able to develop through four generations on a vitamin-free diet, although insects grew more slowly (Jurzitza 1969c). Through addition of vitamins to these diets, it appeared that the symbionts supplied choline, lactoflavin, nicotinic acid, pantothenic acid, pyrodoxine, thiamine, and a sterol (Jurzitza 1969c). Studies with different plant materials and symbiont-free *L. serricorne* indicated tobacco leaves supplied enough vitamins for normal development of the beetle; deciduous wood, however, had lower amounts of vitamins (resulting in slower development), and coniferous wood had almost no vitamins, allowing no development (Jurzitza 1976).

Studies using powders from different plant sources indicated growth of symbiont-free larvae of *L. serricorne* was improved with addition of casein and/or vitamins, except for material from *Angelica archangelica* (Umbelliferae), which contains toxic isopsoralens, suggesting the symbionts were needed to detoxify these toxic compounds (Jurzitza 1969d).

Symbiont exchange experiments indicated *S. paniceum* with *L. serricorne* yeasts could better tolerate thiamine deficiency (Pant and Fraenkel 1954). Presence of the symbionts allowed larvae to develop in the absence of vitamins, although growth was slower than if vitamins had been present (Jurzitza 1969c). Although some differences in nutrient requirements for *S. buchnerii* compared to prior studies were noted, the yeasts were capable of providing amino acids and vitamins to the host as they were able to use inorganic nitrogen and required only biotin as a vitamin (Bismanis 1976). Pantothenic acid, pyridoxine, and choline chloride were essential to *L. serricorne* in artificial diet studies, even in the presence of their symbionts (Blewett and Fraenkel 1944; Pant and Anand 1985).

In studies using different proteins and amino acids, gelatin-based diets supplemented with tryptophan permitted some growth of symbiont-free *L. serricorne*, while histidine and methionine did not (Jurzitza 1969b). Symbiotic yeasts were able to use inorganic sulfur to synthesize methionine (Jurzitza 1969b). Symbionts appeared to be more important in protein metabolism than vitamin provision for foliage feeders: Foliage provides an adequate supply of vitamins but not essential amino acids, whereas wood is both deficient in essential amino acids and vitamins (Jurzitza 1969d).

Sterol was provided to the anobiids by their respective symbionts, as indicated by similar types of nutrient supplement studies where cholesterol restored normal survival and growth of symbiont-free insects (Pant and Fraenkel 1950, 1954). Similar results with cholesterol and *L. serricorne* were reported in similarly designed studies (Pant and Fraenkel 1950; Pant and Kapoor 1963). When cholesterol was

excluded from artificial diets, 89% of larvae of *L. serricorne* matured into adults in the presence of symbionts, but none matured in the absence of symbionts (Pant and Kapoor 1963). When 1% cholesterol was added to the diets, 88% of *L. serricorne* larvae without symbionts matured to adults (Pant and Kapoor 1963).

Yeast-mediated Digestive–Detoxifying Reactions

The next section provides a discussion of symbiotic associations that are generally catabolic in nature, resulting in digestive or detoxifying reactions. In several cases, it appears that the same enzyme or enzyme group can catalyze reactions that are both digestive and detoxifying, sometimes involving the same substrate. For example, removal of glucose molecules from tannic acid to form gallic acid detoxifies the tannic acid (Dowd 1990) and at the same time releases glucose molecules that can be absorbed as nutrients. Other detoxifying reactions involve conjugation and will also be considered.

The kinds of information available to support the symbiont roles in digestion or detoxification are variable. New techniques such as gene cloning or genome sequencing similar to that done with the bacterial symbionts of aphids (Clark et al. 1998; Shigenobu et al. 2000), followed by specific gene knockout or replacement will be necessary to definitively establish which enzymes are involved in the degradative reactions of yeast endosymbionts of insects.

Studies demonstrating digestive/detoxifying capabilities of yeast endosymbionts include those involving symbionts cultured apart from the host and studies involving symbionts as they naturally occur in insect tissues (see below). In some cases standard assimilation studies used to describe new species have used substrates that may be relevant in considering digestive capabilities that may be contributed to the host (e.g., starch, cellulose, cellobiose, inulin) or detoxifying capabilities (e.g., of salicin, a phenolic glycoside from willow).

Studies using cultures of symbionts can be useful in determining what the capabilities of the symbionts are but may not reflect what actually occurs in the natural host state (either under- or overrepresenting what actually occurs). In studying the capabilities of insect symbionts in culture substrate utilization studies, enzyme type or presence (using indicator substrates involving colorimetric detection, for example) or enzyme induction studies have been used. Certainly, using biologically relevant substrates is most appropriate, but indicator substrates are often useful in determining the spectrum of enzyme activity.

In studies involving the presence or absence of symbionts in the host, the same strategies are used that have been used to study enzyme activity of symbionts in culture. Some studies have indicated the symbionts (or perhaps their hosts) produce different enzymes in association with the host insect than apart from the insect. In some cases, it has been possible to obtain aposymbiotic strains of insects to compare performance in the presence of different nutrient sources or toxins. This type of study can be particularly useful if symbiont-free insects can be obtained without use of antibiotics because of their potentially confounding effects. The sections below cover digestive and detoxification reactions separately, organized

on the basis of nutrient resource degraded by the different yeasts. Some associations have been studied more than others; wood ingesting insects have been of paramount interest.

Digestive Reactions

Digestive reactions include (1) protein/peptide degradation (catalyzed by a variety of endo- and exoproteases and peptidases), (2) polysaccharide/starch/sugar degradation (catalyzed by myriad hydrolytic enzymes), and (3) fat/fatty acid degradation (catalyzed by lipases). In the case of starches/sugars, substrate specificity is of particular importance. Protein degradation theoretically would occur with nearly all proteins, but forms resistant to degradation (such as allergens) may be more highly *N*- or *O*-glycosylated and require additional enzymes. Binding of different compounds (e.g., phenolics) to proteins also may inhibit their degradation (Dowd 1990).

Typically, insects do not produce many of the enzymes necessary to degrade polysaccharides or even simpler sugar molecules. They are limited even in their ability to break α-glucose bonds, principally with amylases (Applebaum 1985). Many starches or other plant polysaccharides (including cellulose, hemicellulose, and pectins) and lignins are linked by other types of bonds, such as β forms. Thus, the availability of many of these potential sources of sugars depends on having the appropriate enzymes. Complete cellulose degradation to simple sugars typically requires endo-β-glucanases (Cx-cellulases), exo-β-glucanases (cellobiohydrolases), and a β-glucosidase (cellobiose) (Klemm et al. 2002). A different enzyme, β-xylanase, will break down both cellulose and hemicellulose (Klemm et al. 2002). Binding of lignins to cellulose requires further degradative enzymes, which many fungi possess (Klemm et al. 2002).

Inulin, a β-fructose polymer, is found in high concentrations in some plant species, especially the tubers of plants such as *Dahlia*, *Helianthus*, and *Cichorium* (Franck and De Leenheer 2002). Exo- and endoinulanases produced by yeasts and molds degrade inulin to fructose monomers (Franck and De Leenheer 2002). Fructose can be absorbed and assimilated by insects (Turunen 1985). Pectins are highly complex polysaccharides that require multiple enzymes for effective degradation, including endo- and exo-polygalacturonase, methyl and acetyl esterases, endopectin lyase and endopectate lyase, endoarabinase, arabinofuranosidase, feruloyl esterase, endogalactanase, and rhamnogalacturonan dimer and galacturonohydrolases (Ralet et al. 2002). Again, interconversion of various sugar monomers to glucose, fructose, or other monomers may be necessary for insects to use them.

Even though cellulose degradation in insects is traditionally associated with the presence in the termite gut of flagellates that can break down cellulose (Bignell 2000; but see chapter 8, this volume), yeasts and their fungal relatives can degrade a variety of recalcitrant sugar sources through the activity of such enzymes as β-glucosidases, xylases, cellulases, and so on. (Klemm et al. 2002). Many studies on insect yeast endosymbionts have identified these enzymatic reactions using various substrates in indicator reactions generating a chromophore (e.g., Shen and Dowd 1989, 1991a,b). Supplying a compound of interest as a sole carbon (or

nitrogen) source can also be useful in identifying enzymes capable of degrading various complex sugars, polysaccharides, or proteins.

Although sometimes producing toxic aglycones (Applebaum 1985), glycoside removal from some toxic compounds can also be interpreted as a digestive reaction because the liberated glucose or other sugar can then be absorbed as a nutrient (see "Detoxifucation Reactions" below). Most of the information available on polysaccharide or complex polymer degradation by yeast endosymbionts has come from beetle endosymbionts. For example, in studies with anobiid beetles, *Symbiotaphrina kochii* from the cigarette beetle *L. serricorne* (van der Walt 1961; Jurzitza 1964) and *Symbiotaphrina buchnerii* from the drugstore beetle *Sitodrepa panicea* (Kuhlwein and Jurzitza 1961; Bismanis 1976) assimilated cellobiose. Breakdown of indicator substrates suggested that cultures of *S. kochii* produced lipases, α- and β-glucosidase, phosphatase, and trypsin (Shen and Dowd 1991a). Cellobiose assimilation by yeast species isolated from the wood-boring anobiids *Ernobius mollis* and *E. abietis* (e.g., *Candida ernobii*), and *Xestobium plumbeum* (e.g., *C. xestobii*) was positive, negative, and limited, respectively (Meyer et al. 1998).

The degradative abilities of the *Candida* yeasts of cerambycid beetles have been studied to some extent. Some symbiont species have free-living strains (e.g., *C. guilliermondii* of *Phoracantha semipunctata*), so interpretations are difficult without the use of markers that resolve at infraspecific levels. However, *C. guilliermondii* is closely related to *C. xestobii*, an obligate symbiont of *X. plumbeum* (Jones et al. 1999). Previous studies primarily have investigated the ability of the microbes to assimilate different sugars or to degrade cellulose. Larval stages of most Cerambycidae are wood boring, so the capability to degrade wood polymers would be important. Thus far, studies of the microbial enzyme systems have involved only pure cultures of the yeast. *Candida* spp. isolated from *P. semipunctata* assimilated pectin (Chararas et al. 1972) and exhibited glycosidase (Chararas and Pingal 1981) and β-glucosidase activity (Chararas et al. 1983). Symbionts from *Stromatium barbatum* (Mishra and Singh 1978) and *Homochambyx spinicornis* (Mishra and Sen-Sarma 1985) produced several polysaccharide-hydrolyzing enzymes, including xylanase, which is important in cellulose degradation. *Candida rhagii* (endosymbiont of *Rhagium inquisitor* and *Gaurotes virginea*), and *C. tenuis* (endosymbiont of *Leptura rubra*) were tested in culture for their ability to assimilate cellobiose; they did not, however, utilize starch as a carbon source (Meyer et al. 1998).

Other yeast endosymbionts of beetles also have been studied. Yeasts closely related to *Pichia stipitis* have been isolated from the passalid beetle *Odontotaenius disjunctus* from a wide region in eastern North America (chapter 10). It is of interest that all isolates were identical based on 600 bp of the internal transcribed spacer (ITS) region as well as large subunit rDNA (Suh et al. 2003). Another passalid (*Verres sternbergianus*) is associated with a yeast of very similar genotype. Cultural studies indicated that the yeast degrades xylose, and it may assist in the digestion of the wood into which these insects bore (Suh et al. 2003). Cultures of *Pichia burtonii* and *Candida fermentati* isolated from the coffee berry borer (*H. hampei*) produced trypsinlike protease, α- and β-glucosidase, phosphatase, and lipase activity (Vega and Dowd, unpublished data).

Other species of insects with yeast endosymbionts also have been examined, including several species of yeasts discovered in termites. Cellobiose was assimilated by previously undescribed species of yeast from the termites *Zootermopsis nevadensis*, *Z. angusticollis*, *Reticulitermes santonensis*, *Heterotermes indicola*, *Mastotermes darwiniensis*, *Neotermes jouteli*, and the wood cockroach, *Cryptocercus punctulatus* (Prillinger et al. 1996). Inulin was used by a yeast isolated from the termites *Z. nevadensis* and *M. darwiniensis*, and starch was used by another yeast isolated from *Z. nevadensis*, *Z. angusticolis*, *H. indicola*, and *M. darwiniensis* (Prillinger et al. 1996). Hemicellulose degradation by termite yeasts also has been reported (Schäfer et al. 1996). The strains of *Candida guilliermondii* and *Debaromyces hansenii* isolated from the red imported fire ant *Solenopsis invicta* could use inulin and cellobiose (Ba and Phillips 1996).

Comparative symbiont removal studies demonstrating digestive contributions of yeasts to their hosts (similar to those already described for nutrition) to determine the relative importance of the symbiont to the health of the insect host under conditions of varying recalcitrant nutrient sources have not been performed. However, some interesting possibilities exist. For example, the major polysaccharide in the coffee berry is composed of an unusual complex of sugars: arabinogalactan, mannan, and an unsubstituted glucan (Bradbury 2001). β-glucosidase activity demonstrated using naphthol glucoside may be an indicator that the yeasts associated with the coffee berry borer are capable of breaking down this polysaccharide to a form that can be used by its insect host (Vega and Dowd, unpublished data).

Detoxification Reactions

Detoxification reactions consist primarily of hydrolytic reactions (performed by esterases or proteases), oxidative reactions (performed by monooxygenases), or conjugating reactions (performed by such enzymes as glutathione transferases; Brattsten and Ahmad 1986). The result of these reactions is either to degrade a complex polymer into a relatively smaller molecular weight compound that can be used or excreted (for protein or carbohydrate toxins) or to convert the toxins into more polar forms, which instead of being absorbed into tissues are excreted through the digestive system. Although there are some exceptions, yeast and fungi do not commonly produce monooxygenases or other oxygenases capable of detoxification (laccases are an exception). However, these organisms have potent hydrolytic capabilities. Conjugating reactions are also less common compared with arthropod or vertebrate sources. Thus, as for digestive reactions, it appears that the host insect contributes detoxifying enzymes (e.g., monooxygenases) while symbionts provide enzymes characteristic to yeasts or fungi. This is consistent with evolutionary gain and loss of function reported for genome studies of bacterial symbionts of aphids, whereby the bacterial symbionts have many amplified copies of some amino acid biosynthetic enzymes but have lost the enzymes responsible for biosynthesizing cell-surface lipids and defensive genes, unnecessary defenses due to host provisions (Shigenobu et al. 2000).

Detoxification reactions have been demonstrated for a variety of insect symbionts, including both those that are involved in farming associations (e.g., cultivated fungi

of ants and termites) and those that are intracellular (e.g., bacteria and yeasts). These reactions have degraded compounds such as aromatic hydrocarbons, insecticides, aromatic esters, benzoxazolinones, and phenolic acids (Dowd 1991, 1992). Most of the information on yeast or yeastlike symbionts came from studies done with the YLS *Symbiotaphrina kochii* associated with the cigarette beetle *Lasioderma sericorne*. Because the symbiont is culturable and can be eliminated without including antibiotics in the insect's diet, it has been a particularly useful system for investigating insect–symbiont interactions. A variety of studies have explored the detoxification capabilities of this organism.

Earlier studies indicated salicin could be assimilated by *S. kochii* (van der Walt 1961; Jurzitza 1964) but not by *S. buchnerii* (Kuhlwein and Jurzitza 1961; Bismanis 1976). More recently, studies have shown that cultures of *S. kochii* used a variety of plant flavonoids, plant aromatic acids, and plant meal toxins, as well as aromatic alcohols, mycotoxins, insecticides, and herbicides as sole carbon sources; not all compounds tested, however, could be used (Shen and Dowd 1991a). Enzymes produced in culture were consistent with compounds used, and these included indicator substrates for esterase, α- and β-glucosidase, phosphatase, glutathione transferase, trypsin, and specifically parathion hydrolase; oxidative *O*-demethylation and laccase activity were not detected (Shen and Dowd 1991b). Esterase activity was induced significantly by flavone, griseofulvin (a *Penicillium* mycotoxin), *cis*-β-pinene, and malathion, with flavone causing almost two times higher levels of induction (Shen and Dowd 1989). Isozymes were induced with griseofulvin, malathion, and β-pinene (Shen and Dowd 1989). Thus, in culture the symbionts appeared to produce enzymes capable of detoxifying a variety of compounds, which were in some cases apparently inducible. It is interesting that compounds that were not targeted substrates induced enzymes; these may serve as general triggers for inducing detoxifying enzyme complexes.

Some comparative studies were also performed with intact insect–symbiont systems of *S. kochiii* and *L. serricorne*. Histological assays indicated the mycetomes were a concentrated source of the total gut enzymes capable of hydrolyzing the esterase substrate 1-naphthyl acetate and tannic acid (Dowd 1989). The yeasts appeared to be the source of the enzyme activity when examined using histochemical tests; the symbiont-free mycetomes showed only low enzyme levels (Dowd 1989). Symbiont-free insects took three to four times as long to emerge as adults when flavone or tannic acid was present in the diet, compared to insects that contained symbionts (Dowd and Shen 1990). A major band of esterase activity also was absent from the gut of symbiont-free insects compared to those with symbionts (Dowd and Shen 1990). The yeasts *C. ernobii* (as *C. karawaiewii*) and *C. xestobii*, isolated from the wood-boring anobiids, *Ernobius mollis*, *E. abietis*, and *Xestobium plumbeum*, differed in their ability to assimilate salicin; the reactions were positive, negative, and limited, respectively (Meyer et al. 1998). *Candida guilliermondii* and *Debaromyces hansenii* isolated from the red fire ant *Solenopsis invicta* have been shown to assimilate salicin (Ba and Phillips 1996).

The information available for cerambycid detoxification of certain substrates has been discussed previously in regard to digestive enzymes. Cultures of *Candida rhagii* (endosymbionts of *Rhagium inquisitor* and *Gaurotes virginea*) and *C. tenuis* (endo-

symbionts of *Leptura rubra*) assimilated salicin (Meyer et al. 1998), indicating production of β-glucosidases. These enzymes may be important in degrading hydrolyzable tannins or other phenolic glycosides, although increased toxicity may result when aglycones of certain chemicals are produced, as previously discussed (Dowd 1992).

Pichia burtonii and *Candida fermentati* were isolated from the coffee berry borer, *H. hampei* (Vega et al. 2003). Another *Pichia* species, *P. guilliermondii*, has also been implicated as a symbiont in cerambycids. Yeast-derived esterase isozymes were produced only by these yeast strains while in association with the insect, but wild strains produced them in artificial culture (Vega and Dowd, unpublished data). Enzymes were not induced in the symbiotic isolates even when coffee berry material was added to culture media, whereas Dopa was oxidized by yeast culture homogenates (Vega and Dowd, unpublished data). Caffeine was not detoxified by *P. burtonii*, however (Vega et al. 2003).

Origin of Endosymbiotic Associations

How did endosymbiotic yeast–insect associations come about? Two hypotheses have been proposed. The first states that symbionts were originally pathogenic parasites or nonpathogenic commensals (Steinhaus 1949), while the second presents them as descendants of phytopathogenic or saprophytic fungi (Dowd 1991). A third hypothesis involving feeding habits has been suggested on the basis of very recent work (Vega, unpublished data).

Taming the Insect Pathogen

Phylogenetic analysis of yeastlike fungi associated with planthoppers suggests that the endosymbionts are derived from within a clade of insect pathogens. Fukatsu and Ishikawa (1995, 1996) conducted a phylogenetic analysis of a YLS from the hemolymph of the aphid *Tuberaphis* (*Hamiltonaphis*) *styraci* (Tribe Cerataphidini; Stern et al. 1997). The symbiont was not a true yeast, but it was closely related to three other symbionts present in the planthoppers *N. lugens*, *L. striatellus*, and *S. furcifera* (Noda et al. 1995; Fukatsu and Ishikawa 1996). These YLS were all placed among the filamentous ascomycetes (Ascomycota: Pezizomycotina). Fukatsu and Ishikawa (1996) suggested that because filamentous ascomycetes include the most common entomopathogenic fungi (e.g., *Beauveria*, *Cordyceps*, and *Metarhizium*), it was possible that the ancestor of the present endosymbiont in *T. styraci* might have been an entomopathogen. Fukatsu et al. (1994) also used this hypothesis to explain the presence of yeasts in some aphid genera in the Cerataphidini.

Fukatsu and Ishikawa (1996) presented three possible explanations for the close phylogenetic relation between the aphid and planthopper symbionts: (1) horizontal symbiont transfer; (2) a common ancestor for both aphids and planthoppers having acquired the symbiont; and (3) independent acquisition of the symbiont in both aphids and planthoppers. A phylogenetic analysis of the uricase gene in aphids and planthoppers by Hongoh and Ishikawa (2000) supports the first explanation, (i.e., horizontal transfer from the aphids to the planthoppers).

The taxonomic status of the aphid and planthopper YLS (Fukatsu et al. 1994; Noda and Kodama 1995; Noda et al. 1995; Fukatsu and Ishikawa 1996) was not determined to a taxonomic level specific enough to place it among known insect pathogens. However, a study using the small and large subunit rRNA genes (Suh et al. 2001) concluded that the YLS of *N. lugens*, *L. striatellus*, and *S. furcifera* should be placed within one of several clades of the polyphyletic genus *Cordyceps* (Pezizomycotina: Hypocreales: Clavicipitaceae), all well known as insect pathogens.

Plant Pathogen Progenitor

It has been suggested that endosymbionts might have originated as plant pathogens because the detoxifying ability for the symbiont would have to be similar when acting as a plant pathogen or saprophyte or when detoxifying components of the insect diet that originate in the plant (Dowd 1991). The relationships of *Symbiotaphrina* spp. are not well resolved, but these yeastlike fungi appear to have arisen from filamentous ascomycetes (Pezizomycotina) in a notoriously poorly resolved area including discomycetes and loculoascomycetes (Jones and Blackwell 1996). The plant pathogen progenitor hypothesis implies that insects acquired the YLS from the plant, suggesting a feeding-habit component as a possible mechanism for the origin of yeasts as endosymbionts (discussed below).

Feeding Habits and Endophytes

Another possibility for the origin of yeasts as endosymbionts involves insect feeding habits and the presence of endophytic yeasts. Leafhoppers and planthoppers feed on phloem, xylem, or mesophyll tissue (depending on the species), whereas most aphids feed only on phloem (Backus 1985). Feeding behavior would bring insects in contact with yeasts occurring endophytically or on the phylloplane. Endophytic yeasts have been reported in wheat (*Triticum aestivum*; Larran et al. 2002), *Eucalyptus* (de Sá Peixoto Neto et al. 2002), and pines (Zhao et al. 2002), and yeasts on flowers, fruits, and the phylloplane are quite common (Phaff and Starmer 1987). *Drosophila* is a typical example of an insect that has close associations with yeasts present on food substrates, although these yeasts are not intracellular. Thus, insect feeding behavior presents the opportunity either for acquiring yeasts from the plant vascular tissue and/or the phylloplane and, if already acquired, for inoculating them into the plants in a manner similar to the transmission of plant pathogens. Various piercing-sucking insects can serve as vectors for plant pathogenic yeasts in the genera *Ashbya* and *Nematospora* (Phaff and Starmer 1987).

The possible role of endophytes as eventual YLS remains unexplored. Vega et al. (unpublished data) recently isolated various unidentified endophytic yeasts from coffee plants, as well as two yeast species isolated internally from the coffee berry borer (*P. burtonii*, *C. fermentati*; Vega et al. 2003). Pérez et al. (2003) isolated various yeasts (e.g., *Candida diddensiae*, *C. fermentati*, *P. burtonii*, *Hanseniaspora* sp.) from coffee berry borer's guts, feces, and cuticles, as well as from the galleries bored by the insect inside the coffee berry. Fungal endophytes also have been reported on the feces of grasshoppers, indicating that the insects are not only con-

suming them, but serving as a mechanism for their dispersal (Monk and Samuels 1990).

It is worth noting that one of the possibilities presented by Fukatsu and Ishikawa (1996) and Hongoh and Ishikawa (2000) to explain the close phylogenetic relation between the aphid and planthopper symbionts was horizontal symbiont transfer. Placement of the aphid and planthopper YLS within a clade of insect pathogens implies that a possible mechanism for horizontal transfer was sharing an entomopathogen. Another possible mechanism for horizontal transfer is the plant via the feeding habits of the insect. It would be worthwhile to search for endophytic yeasts in the plants shared by *T. styraci* and the planthoppers *N. lugens*, *L. striatellus*, and *S. furcifera* to determine whether these, if present, are the same as the YLS in the insects. By definition, endophytes are asymptomatic in the plant, and consequently endophytic yeasts have not been a cause of concern for plant pathologists, mycologists, or ecologists. Thus, the presence of yeasts as endophytes is likely to have been undersampled based on the low number of reports in the literature compared to reports of other endophytic fungi.

Future Directions and Practical Concerns

Despite the amount of work that has been done on insects and their yeast endosymbionts, considerable work will be necessary to comprehend the complexities of the endosymbiotic relationship. Certainly, based on the preceding discussion, it appears that symbionts are playing a vital role in the life strategy of insects, in a relatively unsubtle manner. Molecular techniques have indicated the association between *Symbiotaphrina* spp. and their anobiid hosts is ancient compared to that between other anobiids and *Candida* yeasts, which may have been perpetuated due to the detoxifying capabilities of the *Symbiotaphrina* (Jones et al. 1996).

Recent studies with vertebrate gut microbes may give clues about additional roles played by insect endosymbionts. Research on the human gut symbiont *Bacteroides thetaiotamicron* indicates that is capable of providing a wide variety of polysaccharide-degrading enzymes on the cell surface that are not produced in the human digestive system, suggesting that this organism can provide additional sugars for its host (Xu et al. 2003). Vertebrates without their normal complement of gastrointestinal tract microflora require significantly more calories to maintain weight, presumably because gut microflora are responsible for liberating additional sugars or other nutrients from undigested materials (Gilmore and Ferretti 2003). Similar roles almost certainly are played by insect symbionts, but more exacting studies analogous to vertebrate studies are necessary. Considering the numbers of microorganisms insects are exposed to and the numbers they may harbor, we need to determine how these organisms are interacting. Cascade-type metabolism has been reported in the termite and its symbionts (Breznak 2000; Slaytor 2000). Does this also occur in other insects? Can symbionts act as biocompetitors for other organisms that are potentially neutral or pathogenic?

Molecular biology has clarified the relationships between symbionts and their relatives, resolving long-standing questions about nature and identity (Noda and

Kodama 1995, 1996; Noda et al. 1995; Jones and Blackwell 1996; Jones et al. 1999; Suh et al. 2001). As discussed previously, genome studies of the bacterial symbionts of aphids indicated many similarities but some differences among the three species of aphids. Knowledge of phylogenetic relationships is helpful in evaluating hypotheses about the origin of such symbioses. The available information also has helped clarify some relationships of yeasts with digestive and detoxifying capabilities. Histological work with aphid symbionts of *Myzus essigi* indicated the potential ability to detoxify aryl esters and benzoxazolinones, tannic acid, and the insecticide diazinone (Dowd 1991). If the genome of the *M. essigi* symbiont is closely related to the genome of other aphid symbionts (e.g., *Baizongia pistaciae*, *Acyrthosiphon pisum*, *and Schizaphis graminum*), whose genomes are very similar, it might be possible to identify some strong candidate genes for detoxification. A variety of proteases were reported, including serine proteases (e.g., GenBank AAO26942, NP 660570, NP 777837) and aminopeptidases (e.g., GenBank AAO27053, NP 777948, BAB13071). Other than simple protein degradation/housekeeping in the cells, it does not make sense for these symbionts to produce so many protein-degrading enzymes. However, some aminopeptidases have been reported to hydrolyze toxins, including pyrethroid insecticides (Dowd and Sparks 1988), so it is likely that the hydrolytic enzymes involved in detoxification in *M. essigi* are the proteases sequenced in genomic studies of the other aphid species symbionts. Although mutability studies of specific enzymes from these unculturable aphid bacterial symbionts are impractical, once similar genomic information becomes available on intracellular yeast/fungal symbionts that can be cultured apart from the insect, more definitive studies can be undertaken to determine which genes code for degradative enzymes contributing to host welfare by using transformation techniques already available for yeasts or fungi.

The enzymatic capabilities of yeast endosymbionts could be exploited in a number of areas. Imbalances in amino acids, B vitamins, or other nutrients that can be provided by these eukaryotic symbionts could be corrected in crop plants by using their genes, in a manner similar to that reported for carotenoid biosynthesis in golden rice or other such nutrient-fortified plants (e.g., Potrykus 2001). But will these nutrient-fortified plants also have more problems with diseases and insect pests because they provide them with a more balanced diet? As has been suggested (for review, see Dowd 1991), the essential nature of the symbiont makes it a potentially unique target for control of insect pests that contain symbionts.

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Note

1. In contrast to planthoppers, in Anobiidae and Cerambycidae there are distinct structures associated with the reproductive system that result in the yeast being smeared on the egg surface; these are consumed by the emerging larvae upon hatching (Buchner 1965).

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