Symbioses: A Key Driver of Insect Physiological Processes, Ecological Interactions, Evolutionary Diversification, and Impacts on Humans*

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ABSTRACT Symbiosis is receiving increased attention among all aspects of biology because of the unifying themes it helps construct across ecological, evolutionary, developmental, semiochemical, and pest management theory. Insects show a vast array of symbiotic relationships with a wide diversity of microorganisms. These relationships may confer a variety of benefits to the host (macrosymbiont), such as direct or indirect nutrition, ability to counter the defenses of plant or animal hosts, protection from natural enemies, improved development and reproduction, and communication. Benefits to the microsymbiont (including a broad range of fungi, bacteria, mites, nematodes, etc.) often include transport, protection from antagonists, and protection from environmental extremes. Symbiotic relationships may be mutualistic, commensal, competitive, or parasitic. In many cases, individual relationships may include both beneficial and detrimental effects to each partner during various phases of their life histories or as environmental conditions change. The outcomes of insect–microbial interactions are often strongly mediated by other symbionts and by features of the external and internal environment. These outcomes can also have important effects on human well being and environmental quality, by affecting agriculture, human health, natural resources, and the impacts of invasive species. We argue that, for many systems, our understanding of symbiotic relationships will advance most rapidly where context dependency and multipartite membership are integrated into existing conceptual frameworks. Furthermore, the contribution of entomological studies to overall symbiosis theory will be greatest where preoccupation with strict definitions and artificial boundaries is minimized, and integration of emerging molecular and quantitative techniques is maximized. We highlight symbiotic relations involving bark beetles to illustrate examples of the above trends.

KEY WORDS symbiosis, insects, microorganisms, bark beetles, context dependency

Symbiosis: Definitions, History, and Theory

Even as symbiosis is becoming increasingly recognized as a significant and growing field of enquiry, its definition remains unsettled at best. The term arose independently, but nearly simultaneously, from two investigators. Albert Frank first used "symbiosis" in 1877 (as cited in Sapp 1994) to describe "all the cases where two different species live on or in one another . . . based on mere coexistence." Anton DeBary, among the first experimental botanists, defined "symbiose" a year later as "the living together of differently named organisms" (Sapp 1994). DeBary attributed the greatest significance of symbiosis to its apparent ability to lead to morphological variations, a source of evolutionary change (Sapp 1994). Since these early days, the term has been used to describe a wide variety of interactions, from beneficial associations to states of parasitism to complex ecological interactions (H. G. Thornton in Sapp 1994). Such semantic vagaries, and the accompanying debates, have led some to observe that "defining symbiosis has become something of a life science cliché, an act of verbal, and often verbose, masochism" (Zook 1998). We will use (a slight modification of) the definition offered by Zook (1998): "the acquisition and maintenance of one or more organisms by another that may result in novel structures and (or) metabolism." We add the words "may" and "or" to include complex interactions (e.g., such as *Wolbachia,* certain antibiotic-secreting bacteria) in which symbionts directly or indirectly affect the organism with which they are associated, without special structures or metabolism.

The study of symbiosis has now become central to some of the most fundamental and far-reaching themes of biology. These include mechanisms of evolution, coadaptation, and speciation (Carroll 1988, Wilson and Sober 1989, Six and Paine 1998, Shoemaker et al. 1999, Stouthamer et al. 1999, Currie 2001, Zilber-Rosenberg and Rosenberg 2008), plant– herbivore and tritrophic interactions (Moran 1992, Gehring and Whitham 1994, Faeth and Hammon 1997, Beckage 1998, Oliver et al. 2003, Webb et al. 2006, Conn et al.

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2007), animal communication and social organization (Dillon et al. 2002, Richard et al. 2007), population dynamics (Hofstetter et al. 2006a, b), biological invasions (Mota et al. 2006), and pest management (Hosokawa et al. 2007).

Major Themes in Symbiosis: Mutualism, Commensalism, and Cooperation

As pointed out by several authors (Sapp 1994, Paracer and Ahmadjian 2000), the late 19th century Darwinian emphasis on struggle and cooperation was often at odds with, or at least reduced consideration of, the role of cooperation in biological evolution. As the study of symbiosis grew, the organismal emphasis of much of the past research continued, focusing on the perspectives of individuals (Relman 2008). Our intent in this article (and in this new subject area within the journal) is to focus as much on the interactions themselves, trying to foster a holistic approach that features inquiries into "interactions, interplay, codependence, and synergy" (Relman 2008).

We highlight interactions among bark beetles and their microsymbionts to examine specific details of these interactions. Several characteristics of these symbioses make them attractive model systems (Six and Klepzig 2004): Within a relatively small genus, *Dendroctonus,* mycangia have evolved at least three times, allowing comparisons of symbioses involving these structures within and among lineages. In addition to possessing highly specific symbionts that exhibit parallel cladogenesis with their hosts, many beetles are also associated with additional symbionts that seem to be "invaders." These additional fungi may function as complementary symbionts, growing more successfully at different optimal temperatures than the main symbionts (Hofstetter et al. 2006a, 2007; Six and Bentz 2007). These bark beetle–fungal complexes also exhibit considerable variation in life history and host tree range, factors that greatly affect the degree and manner in which they interact with one another and likewise affect the manner and extent to which they impact forest resources (Paine et al. 1997, Six and Klepzig 2004).

Areas of Overlap: The Futility of Drawing Firm Boundaries. Symbiotic interactions may frequently transition from one type to another because of external or internal (generated by the symbionts themselves) influences (Paracer and Ahmadjian 2000, McCreadie et al. 2005). In some cases, microorganisms may have some beneficial effects but primarily function as parasites (entomopathogenesis) (Bourtzis and Miller 2003). In others, mutualistic microbes may exploit an insect's host plant to the extent that the plant becomes diseased (phytopathogensis) (Bourtzis and Miller 2003).

In this review, we focus mostly on relationships that benefit the macrosymbiont (insect) while also benefiting (mutualism) or having no effect on (commensalism) the microsymbiont (protozoa, algae, fungus, bacterium, virus). In the broadest sense, symbiosis also includes parasitism. We agree with Paracer and Ahmadjian (2000), who described parasitism as ". . . a symbiosis in which one of the symbionts benefits at the expense of the other (with) some parasites (being) so pathogenic that they produce disease in the host shortly after the parasitism begins. In other associations the symbionts have coevolved into a controlled parasitism where death of the host cells is highly regulated." However, we feel this subject is well covered within the area of Insect Pathology and thus omit it from our discussion, except where the context can greatly affect the direction of the interaction and so is of special importance to symbiosis theory. For example, relationships with *Wolbachia* can include elements of both parasitism and mutualism (Relman 2008). Likewise, gut symbionts that usually exert no apparent adverse effect on their hosts may exert pathogenic effects after disruption of the midgut by entomopathogens (e.g., *Bacillus thuringiensis;* Broderick et al. 2006, 2009).

Benefits to Members of Symbiotic Associations

Some common patterns are for the microsymbionts to receive nurturing, protection, and transport and the macrosymbionts to receive nutrition, assistance in overcoming host defenses, protection from natural enemies, and enhanced (or at least altered) reproduction. We provide an overview of some of the diversity of examples of symbioses observed with insects.

Nutrition. A common pattern among insect–microbe symbioses is the utilization of nutritionally unbalanced substrates. Insects that feed on plant sap, for instance, are challenged with low levels of nitrogen (Mattson 1980). These insects provide many examples of endosymbiosis, typically obligate symbiosis in which the microsymbiont lives within the insect's (macrosymbiont's) cells (Moran 1992). As one indicator of this abundance and diversity, roughly 10% of insect species (e.g., cockroaches, leaf hoppers, aphids) are known to have well-defined intracellular organs that house symbiotic bacteria (Paracer and Ahmadjian 2000). This is likely a conservative estimate, because many groups of insects have not been thoroughly examined.

Aphids and their endosymbionts are among the most studied of mutualistic systems. All aphids carry bacteria in the genus *Buchnera* in their cell cytoplasm, with estimates of ≈5.6 million cells per insect. In these obligate relationships, aphids die or do not reproduce when treated with antibiotics and the microsymbiont, *B. aphidicola,* has lost genes required for independent growth (Houk and Griffiths 1980). *Buchnera* bacteria provide the amino acid tryptophan (rare in plant sap) to the aphid host, as well as aiding in the production of leucine and vitamins (Paracer and Ahmadjian 2000). Other sap-feeding insects, such as scale insects, leaf lice, and cicadas harbor such bacteria (Koch 1967).

Other groups of insects feeding on wood, and in some cases leaves and seeds, have evolved strategies based around external associates. These ectosymbiotic associations involve digestion of plant tissues by fungi and subsequent ingestion of the fungal tissue (or digested wood) by the insects (Francke-Grosmann 1967, Hartzell 1967).

Fungus-growing termites, roaches, and other wood-feeding insects may benefit from cellulose fermentation mediated by anaerobic bacterial symbionts (Paracer and Ahmadjian 2000, Warnecke et al. 2007). Additionally, these higher termites use fungi, generally species of *Termitomyces,* as a supplemental source of vitamins and cellulolytic enzymes (Martin and Martin 1978). Lower termites rely on protozoa, which are themselves associated with endosymbiotic bacteria, for sources of cellulases and hemicellulases (Ohkuma et al. 2007).

Among ants, *Acromyrmex* and *Atta* species construct elaborate nests within which they culture fungal symbionts (*Attamyces* sp.) on a matrix of leaves harvested from a variety of plants. This form of insect agriculture includes harvesting (cutting of the leaves into smaller pieces), planting (inoculation of the substrate with fungus), fertilization (application of ammonia and amino acid–rich drops of ant exudates to the fungus), weeding (removal of fungal and bacterial invaders from the fungal garden), and chemical treatment (biopesticides) (Paracer and Ahmadjian 2000, Currie 2001). Attine leaf-cutting ants house symbiotic actinomycete bacteria within specialized structures in their exoskeleton (Currie et al. 2006). These bacteria produce antibiotics that specifically inhibit the growth of fungal parasites of the garden (Currie 2001). However, recent work has shown an additional antagonistic symbiont, a black yeast, which may compromise the ability of these bacteria to protect the ant colony food source (Little and Currie 2008).

A similar symbiosis occurs between wood wasps and fungi. The most notable of these host organisms is *Sirex noctilio* F., the European woodwasp (Hymenoptera: Siricidae). In its native range (Eurasia and northern Africa), like the majority of wood wasps, it usually attacks weakened or stressed trees (Madden 1988). However, in non-native habitats, attacking female wasps oviposit in a variety of pines, including healthy individuals (Madden 1988, Carnegie et al. 2006). In doing so, they also inject trees with phytotoxic mucus, the killing agent (Coutts 1969). Additionally, trees are inoculated with a wood decay fungus, *Amylostereum areolatum* (Fr.) Boidin, a known cellulose degrading basidiomycete that provides digestive enzymes to the feeding wasps (Kukor and Martin 1983). This important forest pest has spread extensively within the southern hemisphere (Carnegie et al. 2006) and has recently become established within parts of North America (Dodds et al. 2007).

Associations between fungi and bark and ambrosia beetles are well known, with literature going back >160 yr (Paracer and Ahmadjian 2000, Six 2003). Generally associated with ascomycete fungi (*Ambrosiella, Cephalosporium, Endomycopsis, Fusarium, Ceratocystis,* and *Ophiostoma* species), as well as a few basidiomycetes (e.g., *Entomocorticium* sp.), these beetles excavate galleries and inoculate their fungi within the inner bark and/or wood of their host trees (Harrington 2005). In all ambrosia beetles and a subset of bark beetles, the fungal associates are grown on the gallery walls and consumed by the developing larvae. To varying degrees (more so in ambrosia than bark beetles), this fungal crop garden is maintained free of contaminants. All ambrosia beetles and some bark beetles transport fungal symbionts to new trees through specialized structures called mycangia (alternatively mycetangia), which may be located within the mouthparts, thorax, or elytra. Most of these symbioses are obligately mutualistic. The fungus obtains transport to suitable hosts, inoculation into susceptible tissues, nitrogen from beetle excrement, as well as shelter and nutrition from the beetle mycangium. The beetle receives nutritional benefits from the fungus.

As these fungi grow, they may produces cellulases and other enzymes (Valiev et al. 2009) that break down the otherwise indigestible wood into nutritious compounds for the beetle. These fungi may concentrate nitrogen from surrounding host tissues into the edible fungal biomass in the galleries (Ayres et al. 2000, Klepzig et al. 2001a, Bleiker and Six 2007). They also synthesize steroids that are crucial in beetle development, pheromone production, and cold hardiness (Paracer and Ahmadjian 2000, Bentz and Six 2006).

Overcoming Host Defenses. Microsymbionts may also aid their macrosymbiont hosts in overcoming the defenses of their plant or animal hosts. For example, the wasp (*Cotesia congregata*) is an endoparasite of the tobacco hornworm (*Manduca sexta*) (Beckage 1997). When it oviposits, it transmits a polydnavirus that disables the caterpillar's immune system. This relationship has been observed in other systems as well (Webb et al. 2006).

Bark beetles may provide some analogous examples of this benefit in plant–herbivore interactions. Initial studies into fungal roles in bark beetle–fungus symbioses concentrated on putative pathogenicity of the fungi. In reviewing the evidence, however, Paine et al. (1997) concluded that "recent studies have brought into question the inference that fungi are primarily responsible for mortality of trees colonized by bark beetles," and proposed that some of these fungi are "facilitators or expeditors of beetle colonization success through localized interactions with the beetles in exhausting tree resistance rather than as tree killers."

Recent enquiry, therefore, has largely examined the role of fungi as co-factors, biotic agents that are not pathogenic by themselves but function in compromising host defenses (Beckage 1998) in tree mortality (Lieutier 2002, Kopper et al. 2004, Klepzig et al. 2005). Bark beetle symbionts may function more by aiding in exhausting tree defenses and hence facilitating beetle avoidance of tree toxins than by killing trees by sapwood occlusion as originally proposed. Lieutier (2002) argued that associations with fungi lowered the critical threshold of beetle density needed to overwhelm tree defenses. He developed this idea further into an evolutionary argument, in which he proposed that associations with highly pathogenic fungi would be counteradaptive, because such fungi would rapidly invade the tree's tissues and render the host unsuitable for beetle development. By contributing to the depletion of the host tree's ability to synthesize secondary metabolites, the microsymbionts may improve the probability of successful beetle mass attack (Raffa and Berryman 1983, Lieutier 2002).

The ultimate death of the tree may thus be caused by a combination of bark beetle and fungal effects (Paine et al. 1997, Lieutier 2002, Klepzig et al. 2005). One mechanism may be by the production of toxins (McGraw and Hemingway 1977). For example, DeAngelis et al. (1986) found that rates of water loss in seedlings doubled within 24 h of inoculation with isocoumarin metabolites from fungi. However, the extent to which such trials relate to actual tree colonization is uncertain (Paine et al. 1997, Lieutier 2002, Wullschleger et al. 2004). An alternate, and not exclusive, mechanism by which fungi might reduce host tree resistance against bark beetles is through metabolism of host allelochemicals. Direct metabolism of host compounds to less deleterious forms or concentrations has not been shown, although studies with ophiostomatoid or related fungi reveal a diversity of biochemical pathways by which this might occur (Lanza and Palmer 1977, Mironov et al. 1982, Sprecher and Hanssen 1983, Hanssen 1993, Dorado et al. 2000, Faldt 2000, DiGuistini et al. 2007). The biosynthetic capabilities of *Grosmannia clavigera* in regard to synthesis of terpenes, which are toxic and repellent to bark beetles (Raffa et al. 2005), is particularly relevant in this regard (DiGuistini et al. 2007). This fungus has been reported to also exert some phyopathogenicity (Plattner et al. 2008). Moreover, the bark beetle–vectored fungus *Ophiostoma piliferum* is used as a biopulping agent, primarily because of its ability to degrade diterpene acids (Blanchette et al. 1992), important components of conifer defense (Kopper et al. 2005). In addition, the growth of some bark beetle associated fungi is actually stimulated by the presence of host allelochemicals and/or bark beetle symbiotic bacteria (Hofstetter et al. 2005, Adams et al. in review).

Protection from Natural Enemies: Competitors, Parasites, and Predators. The intracellular life of endosymbionts represents one of the best examples of the acquisition of protection from antagonists through symbiosis. Early in the evolutionary history of the symbiosis, the microsymbiont may use a pathogenic strategy for invading the macrosymbiont, as has been observed with weevils (Lefèvre et al. 2004) and aphids (Dale et al. 2002). As this symbiotic relationship evolves from facultative to obligate, endosymbionts lose the ability for genetic recombination and horizontal gene transfer (Silva et al. 2003), and proceed to lose genetic material, retaining at times only 10–20% of their original genome (Dale and Moran 2006). This reduction requires dependence on the macrosymbiont for nutrient acquisition and for protection from competitors and pathogens (reviewed in Dale and Moran 2006).

Endosymbionts may also protect the macrosymbiont from natural enemies. When infected with one or both of its endosymbionts, the pea aphid is more resistant to attack by parasitoids (Oliver et al. 2006). However, this protection can come at a cost of reduced fecundity. A similar relationship may occur with the red gum lerp psyllid (Hansen et al. 2007). In another example of defensive qualities obtained through symbioses, a *Paederus* beetle uses a chemical, pederin, acquired from its endosymbiont for defense against predators (Piel 2002).

In a relationship with overlapping aspects of parasitism and mutualism, scale insects (*Aspidiotus osborni* Newell and Ckll) are infected by a basidiomycete fungus (*Septobasidium retiforme* (B. and C.) Pat.) (Couch 1931). Infected insects feed on plant sap, nutrients from which are also assimilated by the fungus within the insect. The insect is housed within chambers formed by the fungus that protect the insects from environmental extremes and predation (Paracer and Ahmadjian 2000).

Associations with symbionts can also increase vulnerability to natural enemies. Some parasitoid wasps use odors from fungal associates to aid in locating their bark beetle prey (Sullivan and Berisford 2004, Adams and Six 2008, Boone et al. 2008). Similar relationships have been observed with parasitoids and fungal symbionts of wood wasp larvae (Madden 1968, Martinez et al. 2006). This exploitation of mutualistic fungi by parasitoids provides one of many examples of the mixed benefits and detriments to many if not most symbiotic associations.

Growth, Physiology, Reproduction, and Communication. *Wolbachia* is an intracellular bacterium found within ≈16% of all insects. Inherited through the maternal cytoplasm and not housed in any specialized structures, in many cases this symbiont apparently contributes little to its host. The bacterium can have important reproductive effects, and has been suggested to sometimes promote speciation among insects (Werren 1997, Shoemaker et al. 1999, Stouthamer et al. 1999). These same effects may be exploited for insect control (through the introduction of cytoplasmic incompatibility caused sterility of populations) (Paracer and Ahmadjian 2000). Alphaproteobacteria, *Wolbachia* are extremely widespread symbionts that effectively function as reproductive parasites. These microorganisms enhance the production of infected females (Relman 2008), *Wolbachia* may also exist in relationships with insects that have mutualistic components. For example, infection of *Drosophila* flies may result in increased resistance to RNA viruses and entomopathogenic fungi (Relman 2008).

The effects of microsymbionts on bark beetles remain much less well understood. However, some studies have shown positive impacts of nutritional mutualistic fungi on insect growth and development (Barras 1973, Coppedge et al. 1995, Six and Paine 1998, Ayres et al. 2000). Some fungal and bacterial symbionts may play roles in the communication systems of bark beetles, primarily through roles in oxidation of host compounds into pheromone components (Brand et al. 1975, 1976; Leufvén et al. 1988), although this has been questioned by recent biochemical studies showing *de novo* synthesis by the insect host (Seybold et al. 1995). Other examples of symbiotic bacteria producing components of insect pheromones include locusts (Dillon et al. 2002) and scarab beetles (Hoyt et al. 1971).

Adaptations to Maintenance and Efficiency of Symbiotic Associations

Given the mostly obligate, or at least highly beneficial, nature of many of these associations, it is not surprising that both insects and their symbionts have evolved a diverse array of structures and strategies to maintain them.

Morphological Adaptations. Structural features may have received more study than any other component of insect-symbiont interactions. Numerous reviews catalog the diverse ways in which insects protect, nourish, and transport their microbial partners. Koch (1967) lists fermentation chambers, bucal pockets, blind sacs, crypt guts, diverticula, crypt cells, bacteriocytes, mycetocytes, mycetomes, and such phenomena as the entire lymph being "flooded" with bacterial endosymbionts. Francke-Grosmann (1967), likewise, lists and describes numerous morphological adaptations for fungal ectosymbionts. Mycetangia (a term largely replaced by mycangia) are described as being located in the prothorax, pronotum, coxa, mesonotum, mandibles, pharynx, and elytra.

Looking specifically at bark beetles, Six (2003) developed a two-tiered system (fungus carrying structures with or without glands) to describe glandular and nonglandular pit, sac, and brush mycangia. Recent works (Stone et al. 2005, Pechanova et al. 2008, Scott et al. 2008) have shown further insights into mycangial structure and function.

Biochemical Adaptations. After acquisition of a bacteriocyte and ≈200 million years of coevolution, the endosymbiont genome has been reduced to a smaller size than for any free-living bacteria observed to date (Gil et al. 2002). Even with such a reduction in the genome, the aphid endosymbiont, *Buchnera,* retains many properties of its ancestor (Baumann et al. 1995). However, adaptations to a life history in which a symbiotic relationship is so pivotal likely include an increase in the production of products used by the macrosymbiont.

Ectosymbiotic relationships also result in biochemical adaptations. Insects with structures adapted for housing microsymbionts can be lined or attached to specialized glands that penetrate the cuticle. These glands are thought to provide for the ectosymbionts and thus assist in maintenance of associations with bacteria in the leaf cutter ants (Currie et al. 2006, Caldera et al. 2009) and with fungi in ambrosia and bark beetles (reviewed in Francke-Grosmann 1967, Beaver 1989). The ectosymbiotic microsymbionts also coevolve with their macrosymbionts with the outcome being the production of novel chemicals used for defense against antagonists. Examples include the leaf-cutter ant symbiosis (Caldera et al. 2009) and the southern pine beetle–fungus association (Scott et al. 2008).

Ecological Adaptations: Space, Time, and Environment. In bark beetle–fungal symbioses, ecological and (for the beetles) behavioral adaptations play strong roles in the maintenance and impacts of the symbionts by and for each other. Carefully timed inoculation of the mutualistic mycangial fungi by the beetles provides spatial and temporal separation from antagonistic fungi. Time, in the form of seasonal temperature changes, also influences beetle–fungal interactions (Six and Bentz 2007). Apparent redundancy in symbionts, in at least two systems, seems to be partially caused by competition, or at least cohabitation, among mutualistic fungi with differing optimal growth temperatures (Hofstetter et al. 2006a, Six and Bentz 2007).

Evolution of Complex Associations. Entire books have been devoted to the role of symbiotic interactions in the evolution and diversification of life (Margulis and Fester 1991, Sapp 1994, Margulis 1998). The role of symbiosis in the development of cells, the movement of life to land, and subsequent radiation to diverse hosts and life history strategies has been well established and analyzed. Such an evolutionary pathway has been assumed or speculated on for bark beetles, and the array of specialized structures and behaviors suggests a long co-evolutionary history, but this aspect has not been as thoroughly studied as in other systems.

Context Dependency, Net Effects. Much as initial symbiosis studies were marked by an organismal or individualistic approach, early research on bark beetle–fungal interactions tended to focus on specific stages in time to judge the impacts of one symbiont on the other. A framework of context dependency (Callaway and Walker 1997) seems most applicable to bark beetle–microbial symbioses. A holistic approach should consider these interactions (which seem to range from mutualism to antagonism to commensalism within one symbiont pairing) across the extent of the insect life cycle (time) and tree attack process (environment) (Klepzig and Wilkens 1997, Klepzig et al. 2001a). In some cases, changing points in the beetle life cycle bring changes in the relative prevalence of various symbionts (Adams and Six 2007) and in the symbiont interaction (Klepzig and Wilkens 1997). In other cases, the interaction remains relatively consistent (Eckhardt et al. 2004). Changing environmental conditions such as water potential (Klepzig et al. 2004, Bleiker and Six 2009) can alter competitive interactions among fungal symbionts. Changing host defensive chemistry can likewise have differential effects on symbionts (Hofstetter et al. 2005, Kopper et al. 2005). Our argument is for a holistic, overarching view, concentrating on net effects of association with a given complement of symbionts (Lombardero et al. 2003, Klepzig and Six 2004, Raffa et al. 2005, Hofstetter et al. 2006b).

Multipartite Symbioses. Along with consideration of context-dependent and net effects has come in-

Table 1. Examples of insect pests of forests and forest products whose symbiotic associations intensify their impacts

Insect order	Forest insect pest	Impact	Symbiont	Relationship	Reference
Coleoptera	Bark beetles	Can kill or weaken trees	Ophiostomatales fungi, Entomocorticium fungi	Nutritional supplement	Reviewed in Paine et al. 1997, Six 2003
			Streptomyces sp.	Protection from antagonists	Cardoza et al. 2006, Scott et al. 2008
	Ambrosia beetles	Infests wood products	Fungi	Food source	Reviewed in Farrell et al. 2007
	Wood borers	Can kill or weaken trees	Gut bacteria	Cellulose degradation	Delalibera et al. 2007
Hymenoptera	Leaf-cutting ants	Defoliation	Multiple fungal cultivars	Food source	Chapela et al. 1994, Mueller et al. 1998
	Wood wasps	Can kill or weaken trees	*Amylostereum* sp.	Cellulose degradation	Kukor and Martin 1983
Isoptera	Lower termites	Eats wood products	Gut protozoa	Cellulose degradation	Ohkuma et al. 2007
	Higher termites	Eats wood products	Gut bacteria	Cellulose degradation	Warnecke et al. 2007

creasing realization that the classic model of one macrosymbiont–one microsymbiont was overly simplistic. Closer examination of bark beetle systems, for example, using new approaches and exploring a broader range of questions has shown associations with mites, nematodes, and bacteria. In some cases, these "additional" symbionts may have very important roles as mediators or regulators of beetle–fungal–host interactions.

The spruce beetle, *Dendroctonus rufipennis* (Kirby), system is closely associated with the fungus *Leptographium abietinum* (Peck) Wingfield, but the nature of this interaction is only partially understood. For example, it may be a source of sterols for beetles (Bentz and Six 2006) but may also exert some negative effects on gallery construction (Cardoza et al. 2006a). The frequency of association between this and several other species of fungi, spruce beetles, and their host trees can vary with beetle population density (Aukema et al. 2005). At least eight mite species are associated with spruce beetles, the most prevalent of which feeds preferentially on *L. abietinum* (Cardoza et al. 2008). In addition, adult beetles contain a specialized structure (nematangium), in which *Bursaphelenchus* sp. nematodes and a variety of fungi are transported (Cardoza et al. 2006b). These nematodes also may feed on the symbiotic fungi. When bark beetles overcome live trees by mass attack, they do so by depleting tree chemical defenses, but this also renders the substrate suitable for opportunistic fungi such as *Aspergillus* and *Trichoderma* that greatly reduce brood establishment and survival (Cardoza et al. 2006a). Spruce beetles respond by egesting fluids from their mouthparts and smearing these fluids with their legs along the sides of their galleries. These fluids contain several species of fungicidal bacteria that protect eggs and larvae from these fungi and also from *L. abietinum* that can be somewhat antagonistic during the beetle's development phase (Cardoza et al. 2006a).

Similar complexities occur in the southern pine beetle, *Dendroctonus frontalis* Zimmermann, system, which includes complex multipartite symbiotic interactions among the beetle, at least three fungi, and many mites (Lombardero et al. 2000, Klepzig et al. 2001b). The macrosymbiont benefits from its relationship with a mycangial fungus, *Entomocorticium* sp. A., benefits somewhat less from a second mycangial fungus *Ceratocystiopsis ranaculosus* (Klepzig and Wilkens 1997) and is negatively impacted by its blue-staining fungal symbiont, *Ophiostoma minus.* Mites carried by the beetle benefit from the latter two fungi, which they vector, indirectly impacting the beetle. As in the case with spruce beetles (Cardoza et al. 2006a), southern pine beetles have an actinomycete bacterium with fungicidal activity (Scott et al. 2008). This bacterium produces a previously undescribed antibiotic, mycangimycin, that is selective in its activity, strongly inhibiting the fungal antagonist *O. minus*, but only weakly inhibiting the mutualist, *E. sp.* A. (Scott et al. 2008). Additionally, Vasanthakumar et al. (2006) identified gut bacteria with possible nitrogen-fixing capabilities.

Consequences of Symbiotic Associations to Human Welfare

As the most successful organisms on earth (at least as measured by diversity and abundance), it is not surprising that insects impact many human values. Given their abundance within the insects, symbiotic organisms are likewise predominant within such pestiferous systems. In at least one case, gut symbionts largely determine whether or not a stink bug achieves pest status in argoecosystems (Hosokawa et al. 2007). Some examples of the multiple impacts of symbiotic relations on human values include invasive species and plant and animal pathogens. Selected examples with insects damaging to trees and forest products are shown in Table 1. Some have suggested exploiting or targeting such symbiotic relationships (and vulnerabilities within) for biological control of pests (Klepzig 1998). Vasanthakumar et al. (2008) have argued that 'invasive species' should instead be viewed as invasive species complexes. Symbiotic interactions are prominent features of many invasive aphids, roaches, termites, flies, ants, and beetles that have become established and currently cause significant adverse economic, environmental, and health effects. As with insect pathology, entire journals are focused on the vectoring of plant and animal pathogens. As such, we do not elaborate on these important subsets of insect–microbe interactions in our survey.

Future Directions in Insect–Symbiont Interaction Research

The study of symbiosis is currently receiving increased and broadly reaching attention as a discipline (see Relman 2008, part of a special issue of *Nature Reviews*). As recently noted (McFall-Ngai 2008), "The study of symbiosis is quintessential systems biology. It integrates . . . all levels of biological analysis—from molecular to ecological—(and) the interplay between organisms in the three domains of life." Certainly the availability of sequencing is facilitating the field. Research once focused on species identification of symbiotic partners may now turn to consideration of new species, intraspecific variability and gene for gene interactions. The relatively advanced stage (from a reductionist perspective) of symbiotic studies in other areas of symbiosis studies (e.g., *Rhizobium*, mycorrhizae, marine symbioses) points to the rapid progress—and additional complexities—that await this field. Likewise, proteomics may provide another tool to determine the functional contributions of symbionts to insect life processes (Pechanova et al. 2008).

Improved interplay between molecular and ecological approaches will yield new insights that were previously not possible (e.g., the use of genetic approaches to probe ecosystem functions in which community members are viewed as analogous to genes within an organism) (Little et al. 2008, Relman 2008). However, as McFall-Ngai (2008) points out, these technical advances will also serve to show the scale of the task of understanding symbiotic interactions. For example, there is a need for improved statistical approaches and tools for analyzing community structures, making comparisons among various communities, and estimating how degrees of association between various insects and microorganisms vary in space and time and with plant hosts and population phase. The complexities unearthed by the explosion in technique and interest in this field will only reemphasize the need for interdisciplinary, collaborative, holistic approaches.

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