Review article.
Dendroctonus Bark Beetles as Model Systems for Studies on Symbiosis

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Abstract

Several characteristics of *Dendroctonus*-fungus symbioses make these associations ideal model systems for studying the development and maintenance of ectosymbioses. Within this relatively small genus, structures specialized for dissemination of symbionts (mycangia) have evolved at least three times, allowing comparisons of symbioses within and among mycangial and non-mycangial lineages. In addition to possessing highly specific symbionts that exhibit parallel cladogenesis with their hosts, many beetles are also associated with what appear to be "invaders". *Dendroctonus* species also exhibit considerable variation in life history and host tree range, factors which are likely to greatly affect the degree and manner in which they interact with fungi. In this paper, we develop a framework for developing and testing predictions on *Dendroctonus*-fungus symbioses. To develop the framework we categorized the nineteen species of *Dendroctonus* into five groups based on phylogenetic lineage. For each species, we summarized their life histories and associations with fungi. Using this framework, we then develop predictions on where particular association types are most likely to occur. Finally, we present three examples of *Dendroctonus*-fungus symbioses.

1. Introduction

Distribution

The geographic distribution of the scolytine genus *Dendroctonus* Erichson ranges from arctic North America to Nicaragua (seventeen species) to northern Europe (one species) and Asia (one species) (Wood, 1982). Most *Dendroctonus* colonize the bark of conifers larger than 14 cm in diameter. All species except one, *D. approximatus* Hopkins, can kill trees, although many species are relatively un-aggressive, either typically colonizing living hosts without killing the tree, or colonizing trees severely weakened or killed by other factors including other bark beetles (Wood, 1982). A few are highly aggressive and can develop extensive outbreaks where tens to hundreds of thousands of host trees may be killed over the span of just a few years (Wood, 1982).

Fungal associations

All *Dendroctonus* appear to be associated with fungi, although specificity and consistency of associates may vary (Six, 2003a). Symbioses among *Dendroctonus* beetles and fungi vary greatly in type and include antagonisms, and possibly commensalisms, as well as mutualisms, and can range from obligate to facultative (Six, 2003a).

Most fungal associates of *Dendroctonus*, as well as other bark beetles, are ascomycetous fungi in the teleomorph (sexual) genus *Ophiostoma* and its anamorph (asexual) genus *Leptographium* (Harrington, 1993; Paine et al., 1997; Six, 2003a). This is a group of closely related fungi that possess similar biologies; however, individual species can have greatly varying effects on host beetles. Some act as nutritional mutualists (Barras, 1973; Bridges, 1983; Goldhammer et al., 1990; Coppedge et al., 1995; Six and Paine, 1998; Ayres et al., 2000) or with variable effects including antagonism (Barras, 1970; Six and Paine, 1998), while others appear to have no measurable effect on their hosts. The impact of a given fungus on a host beetle can also vary with the context within which the organisms interact (Klepzig and Six, 2004).

In addition to ascomycetes, some *Dendroctonus* are also associated with basidiomycetous fungi in the genera *Entomocorticium* and *Phlebiopsis* (Hsiau and Harrington, 2003). Unfortunately, our knowledge of this group of fungi is still quite limited.

However, with only a small number of beetles (or wood samples) surveyed to date, already five species of *Entomocorticium* have been found to be associated with *D. ponderosae* Hopkins, and one species each with *D. jeffreyi* Hopkins, *D. frontalis* Zimmermann, and *D. brevicomis* LeConte (Hsiau and Harrington, 2003). At least four species of *Dendroctonus* that possess glandular pronotal sac

mycangia, *D. approximatus* Hopkins, *D. brevicomis* LeConte, *D. frontalis*, and *D. mexicanus* Blandford, carry *Entomocorticium* species in their mycangia (Klepzig et al., 2001a; Hsiau and Harrington, 2003; R. Hofstetter, pers. com.) (Table 2). *D. approximatus* carries *Phelbiopsis gigantea* (Fr.) Julich (Hsiau and Harrington, 2003). With *D. frontalis*, and likely with all pronotal sac mycangium-bearing beetles, the basidiomycete associate provides a source of nutrients, particularly nitrogen, to the host beetles (Ayres et al., 2000).

The two *Dendroctonus* species that possess maxillary sac mycangia, *D. ponderosae* and *D. jeffreyi*, are also found in association with *Entomocorticium* species (Hsiau and Harrington, 2003) and at least one of these fungi may confer nutritional benefits to the host (Whitney et al., 1987). However, these fungi have not yet been isolated from mycangia nor from the exoskeleton of either of these beetles (Whitney and Farris, 1970; Six and Paine, 1997; Six, 2003b; D.L. Six and B.J. Bentz, unpublished data) making vector relationships unclear and close association with the beetles uncertain. Further research is critically needed to characterize the distribution, effects and extent of association of *Entomocorticium* with these beetles.

Mycangia

Several characteristics of *Dendroctonus*-fungus symbioses combine to make these associations ideal model systems for studying the factors that influence the development and maintenance of ectosymbioses. Within this relatively small genus, for example, mycangia have evolved at least three times allowing comparison of the development of symbioses in three separate mycangial lineages. Sac mycangia have evolved independently in two lineages in *Dendroctonus* and are known to be present in at least seven species (Whitney and Farris, 1970; Barras and Perry, 1971b; Paine and Birch, 1983; Six and Paine, 1997; Six and Paine, 1999; R. Hofstetter, pers. comm.). In two other lineages, pit mycangia occur in at least one species and possibly in others (Lewinsohn et al., 1994; Chen and Yuan, 2000; Six, 2003a).

In addition to diversity in type, location, and presence of mycangia, the structures also vary by lineage in degree of sexual dimorphism. Pronotal sac mycangia are only fully developed and functional in females of the species in which they occur (Barras and Perry, 1971b). Maxillary sac mycangia, however, are present and functional in both sexes of the species in which they are found (Whitney and Farris, 1970; Six and Paine, 1997). Pit mycangia in *Dendroctonus* are also functional in both sexes, although in at least one species, they appear to be more efficient at spore acquisition and transport in female beetles (Lewinsohn et al., 1994).

Life histories

The broad range of variation in life histories occurring within *Dendroctonus* may provide insight into the development of symbioses with these, as well as other, bark beetles, and among other organisms in general. Colonization behavior varies considerably among *Dendroctonus* species. Some species are aggressive or moderately aggressive tree killers, while others are relatively non-aggressive, dependant upon other factors to kill the host tree, while still others exploit living hosts without killing them (Wood, 1982).

Further variation is observed after the initial colonization of the host is complete. While all parental *Dendroctonus* excavate galleries in the inner bark, laying eggs in niches in the phloem, once eggs hatch, larval feeding patterns may differ by species. The brood of some species spend their entire developmental period feeding in the phloem layer in almost constant contact with the cambium. Other species begin to feed as early instars in the inner bark but then move to the nutrient deficient outer bark to complete development (Wood, 1982). The larvae of some species are solitary feeders, while the larvae of other species feed gregariously (Wood, 1982).

All *Dendroctonus* are restricted to feeding on trees within the Pinaceace, with most feeding strictly on *Pinus* (Wood, 1982; Kelley and Farrell, 1998). Feeding on *Pinus* is probably the ancestral state for *Dendroctonus*; however, one early lineage shifted to feeding on other genera within the Pinaceae including *Picea*, *Larix*, and *Pseudotsuga*. Within this ancient lineage, only one species feeds on *Pinus*, the apparent result of a later reversal (Kelley and Farrell, 1998). Using the functional definitions of specialist and generalist (based on proportional use of available host species within the geographic range of a beetle) developed by Kelley and Farrell (1998) for this group, most (approx. 70%) *Dendroctonus* can be considered generalists. Kelley and Farrell (1998) developed a phylogeny of *Dendroctonus* using mtDNA sequences and found specialization to be a highly derived state in these beetles, with specialists found only at tips of branches.

Exogenous factors affecting interactions

Degree of aggressiveness, location of larval feeding, and degree of host tree specialization, are likely to greatly affect the quality of the environment experienced by both a beetle and its associated fungi, and potentially the type, degree of benefit, and consequence of association. For example, an aggressive beetle and its associated fungi that colonize relatively vigorous host trees are challenged by stronger host defenses during the initial attack phase than are less aggressive beetles (and their fungal associates) that colonize weakened or recently killed trees. However, host tree secondary chemical compounds decline

rapidly once a tree has died so that after a relatively short period of time, development of brood of both types of beetles occurs in a mostly undefended host. In contrast, beetles that develop in living hosts experience relatively intense host defense responses, not only during the colonization phase, but during their entire developmental period.

Contrasts can also be made of food quality experienced by beetles with different life histories. All bark beetles feed on host tree tissues that are relatively poor in nutrients indicating that most, if not all, depend upon fungal associates for nutritional supplementation. However, the nature of dependence on fungi may vary depending upon the feeding behavior and food quality experienced by the beetle during larval development. Beetles that complete their entire development in galleries in the phloem that are continually extended during larval feeding may rely less on fungi than beetles whose larvae feed only a short time in galleries and then feed in stationary feeding chambers.

Additionally, beetles that kill trees, or colonize recently killed trees, and whose brood experience a continual reduction in food quality as host tree tissues decline after death, may be more dependant on fungi than beetles whose brood develops in living hosts and experience little to no reduction in nutritional content of host tissues. Thus, we can speculate that beetles that feed strictly in the phloem of recently-killed trees may be more dependant upon nutritional symbionts (in the form of associated fungi) than beetles that feed in living hosts.

Oligophily in fungal associations

Oligophilic associations (two to a few consistent symbionts per host) appear to be common in *Dendroctonus* (Six, 2003a). Some fungal associates exhibit parallel cladogenesis with their hosts indicating long shared evolutionary histories, while other fungal associates appear to be relative newcomers ("invaders" of established symbioses) (Six and Paine, 1999; Six, 2003a). Multiple associates of a given bark beetle can have quite disparate effects on their hosts. The fact that many bark beetles have more than one associate, each with a potentially different effect on host beetle fitness, greatly adds to the difficulties of characterizing effects and interactions in these systems. However, such variation does provide an excellent opportunity to investigate the evolution of these associations as well as the mechanisms and consequences of symbiont invasion and replacement.

For example, *D. ponderosae* is associated with two mycangial fungi, *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington and *O. montium* (Rumbold) von Arx. *Ophiostoma clavigerum* exhibits parallel

cladogenesis with its host while *O. montium* does not (Six and Paine, 1999). *Ophiostoma clavigerum* is mutualistic while the invader, *O. montium*, appears to be an antagonist or, at best, a weak mutualist (Six and Paine, 1998). Even though the newcomer may reduce overall reproduction of its host, it is found in most populations of the beetle, and it is often the most abundant associate (D.L. Six and B.J. Bentz, unpubl. data). This begs the question as to how this fungus is maintained at high levels in many populations when it should be strongly selected against.

D. frontalis is also associated with two mycangial fungi, one a weak mutualist ascomycete, *O. ranaculosum* Perry and Bridges) Jacobs (formerly *Ceratocystiopsis ranaculosus*) and the other a strong mutualist basidiomycete, *Entomocorticium* sp. A. The weak mutualist exhibits parallel cladogenesis with the host (Six and Paine, 1999). It is unknown whether the other associate, an *Entomocorticum* species, also exhibits parallel cladogenesis.

However, because most, if not all, beetles possessing mycangia in the lineage containing *D. frontalis* are also associated with *Entomocorticium* species, there is a strong probability that both fungi may have long shared evolutionary histories with their host. Indeed, phylogenies of *Entomocorticium* (Hsiau and Harrington, 2003) and their *Dendroctonus* vectors (Kelly and Farrell, 1998) show strong, though not perfect, congruence. The continued presence of the weak mutualist (discussed below) may be explained both by its ability to enter and compete for the mycangium and by its co-association with phoretic mites (Klepzig et al., 2001b).

2. A Framework for the Study of *Dendroctonus*-Fungus Symbioses

Symbioses that occur across a group of related organisms, yet vary in their presence and type, allow the consideration and comparison of factors that may help explain the forces behind their development. To construct inclusive meaningful hypotheses, a framework is necessary that allows investigators to envision how host phylogeny and life history influence, and are influenced by, associations of fungi with *Dendroctonus*.

To this end, we categorized the nineteen species of *Dendroctonus* into five groups based on phylogenetic lineage, and then, for each species, we summarized several important aspects of their life histories (Table 1) and associations with fungi (Table 2). Each of the five lineages of beetles generally corresponded well to natural groupings based on presence and type of mycangia and other general aspects of their biologies (Table 1).

Separating the effects of ecology from those of phylogeny can be difficult given that life history, host specificity, and fungal interactions all may covary with phylogeny.

Table 1. Framework for *Dendroctonus*-fungus associations. Part I. Summary of phylogenetic diversification, host tree range, degree of specialization, presence and type of mycangium, and life history characteristics of *Dendroctonus* bark beetles.

Phylogenetic lineage[a]	Beetle species	Diversification[b]	Hosts[c]	Host tree range[d]	Mycangium[e]	Life history[f]	Location of development[f]
Group I	*D. ponderosae*		*Pinus*	Polyphagous	Maxillary sac	Kills host/solitary larvae	Phloem
	D. jeffreyi	Derived	*Pinus*	Monophagous	Maxillary sac	Kills host/solitary larvae	Phloem
Group II	*D. vitei*		*Pinus*	Oligophagous	Unknown	Kills host/solitary larvae	Phloem
	D. mexicanus		*Pinus*	Polyphagous	Pronotal sac	Kills host/solitary larvae	Phloem
	D. frontalis		*Pinus*	Polyphagous	Pronotal sac	Kills host/solitary larvae	Phloem
	D. adjunctus		*Pinus*	Oligophagous	Pronotal sac	Kills host/solitary larvae	Phloem
	D. brevicomis		*Pinus*	Oligophagous	Pronotal sac	Kills host/solitary larvae	Phloem
	D. approximatus		*Pinus*	Polyphagous	Pronotal sac	Dead host/solitary larvae	Phloem
Group III	*D. rhizophagus*		*Pinus*	Oligophagous	Unknown	Kills host/gregarious larvae	Phloem
	D. valens		*Pinus*	Polyphagous	Unknown	Live host/gregarious larvae	Phloem
	D. terebrans		*Pinus*	Polyphagous	Unknown	Live host/gregarious larvae	Phloem
	D. parallelocollis		*Pinus*	Oligophagous	Unknown	Kills host/solitary larvae	Phloem
Group IV	*D. micans*		*Picea*	Polyphagous	Unknown	Live host/gregarious larvae	Phloem
	D. punctatus		*Picea*	Oligophagous	Unknown	Live host/gregarious larvae	Phloem
	D. murrayanae		*Picea*	Oligophagous	Unknown	Kills host/gregarious larvae	Phloem
	D. rufipennis		*Pinus*	Polyphagous	Unknown/pit?	Kills host/solitary larvae	Phloem
	D. simplex		*Larix*	Monophagous	Unknown	Kills host/solitary larvae	Phloem
	D. pseudotsugae		*Pseudotsuga*	Oligophagous	Pit	Kills host/solitary larvae	Phloem
Group V	*D. armandi*	Ancestral	*Pinus*	Polyphagous	Pit	Kills host/solitary larvae	Phloem

[a]Kelley and Farrell, 1998. [b]Kelley and Farrell, 1998; Zuniga and Salinas-Moreno, 2002. [c]Wood, 1982; Kelley and Farrell, 1998. [d]Monophagous = one host tree species; Oligophagous = two–three host tree species; Polyphagous = more than three host tree species. Wood, 1963; Wood, 1982; Zuniga and Salinas-Moreno, 2002. [e]Francke-Grosmann, 1967; Whitney and Farris, 1970; Barras and Perry, 1971b; Paine and Birch, 1983; Six and Paine, 1997; Six and Paine, 1999. [f]Thomas and Bright, 1970; Wood, 1982; Langor and Raske, 1987; Zhonghi, 1989; Furniss, 1995; Cibrian Tovar et al., 1995.

Table 2. Framework for *Dendroctonus*-fungus symbioses. Part II. Summary of fungal associates and their effects on *Dendroctonus* bark beetles.

Phylogenetic lineage[a]	Beetle species	Common fungal associates[b] (known mycangial species in bold)	Effect of common fungal associates on host beetle[d]	Fungi with restricted distribution/uncertain relationship with host[e]
Group I	*D. ponderosae*	*O. clavigerum*	Mutualist	*Entomocorticium dendroctoni,*
		O. montium	Antagonist or weak mutualist	*Entomocorticium* sp. D, E, F, G, and H
	D. jeffreyi	*O. clavigerum*	Mutualist	*Entomocorticium* sp. E
Group II	*D. vitei*	Unknown	Unknown	
	D. mexicanus	Unknown	Unknown	
	D. frontalis	*Entomocorticium* sp. A	Mutualist	
		Ophiostoma ranaculosum	Mutualist	
		Ophiostoma sp. (*ranaculosum* –like)	Mutualist	
		Ophiostoma minus	Antagonist	
	D. adjunctus	*Leptographium pyrinum*	Unknown	
	D. brevicomis	*Entomocorticium* sp. B	Mutualist	
		Ophiostoma brevicomi	Mutualist	
	D. approximatus	*Phlebiopsis gigantea*	Unknown	
		Leptographium spp.	Unknown	
Group III	*D. rhizophagus*	Unknown	Unknown	
	D. valens	*O. ips* in western US	Unknown	*Leptographium procerum*
		L. terebrantis in WI		
	D. terebrans	*L. terebrantis*		*L. procerum, O. ips*
	D. parallelocollis	Unknown	Unknown	
Group IV	*D. micans*	*O. canum*	Unknown	
	D. punctatus	Unknown	Unknown	
	D. murrayanae	Unknown	Unknown	
	D. rufipennis	*L. abietinum*	Unknown	
	D. simplex	*O. americanum*	Unknown	*Ceratocystis rufipenni*
	D. pseudotsugae	*O. pseudotsugae*	Unknown	*L. terebrantis, Pesotum* sp.
		L. abietinum	Unknown	
Group V	*D. armandi*	*L. terebrantis*	Unknown	
		O. minus		

[a]Kelley and Farrell, 1998. [b]Whitney and Farris, 1970; Klepzig et al., 1989; Lieutier et al., 1994; Lewinsohn et al., 1994; Six and Paine, 1996; Hsiau and Harrington, 2003; Six and Paine, 1997; Six and Paine, 1999; Klepzig et al., 2001; Hsiau and Harrington, 2003; Six and Bentz, 2003; Six, unpubl. data. [c]Six and Paine, 1999. [d]Barras, 1973; Bridges, 1983; Goldhammer et al., 1990; Coppedge et al., 1995; Six and Paine, 1998; Ayres et al., 2000. [e]Lewinsohn et al., 1994; Solheim, 1995; Jacobs et al., 1997; Hsiau and Harrington, 2003.

However, within *Dendroctonus* exist several species that do not possess life histories similar to other species in their lineage, but rather, more similar to those of species in other lineages. This presents the opportunity to investigate whether fungal associations of the deviant species are more similar to those of closely related beetle species, or more similar to species with similar ecologies regardless of relatedness.

Unfortunately, considerable gaps exist in our knowledge of many beetle species, especially in the identity of fungal associates and their effects on the host (Table 2), leaving this framework far from complete. However, as more information becomes available, the framework, and any hypotheses based upon it, can be further refined. Below, we begin to develop predictions for mutualistic associations among *Dendroctonus* bark beetles and fungi based on this framework.

Group I

Group I contains the sibling species *D. ponderosae* and *D. jeffreyi* (Table 1). These beetles possess nearly identical morphologies (Wood, 1982), and are genetically very similar (Six and Paine, 1999). These beetles share a mutualistic mycangial associate, *O. clavigerum* (Six and Paine, 1997; 1999; Six et al., 2003), while only *D. ponderosae* possesses a second mycangial associate, *O. montium*, which can be considered an antagonist or weak mutualist, depending on context (see section on mountain pine beetle below). These beetles are characterized by the presence of maxillary sac mycangia, tree-killing behavior, and development of solitary larvae in phloem (Wood, 1982). Both species typically require one year to complete a generation.

Because beetles in Group I feed exclusively and extensively in phloem and have relatively long life cycles over which phloem quality declines substantially, we predict that these beetles also require nutritional supplementation by fungi. Nutritional supplementation may come from feeding on fungi in phloem as larvae and/or as new adults during feeding on fungal spore layers in pupal chambers (Six, 2003a). An early study concluded that *D. ponderosae* fed upon fungi as first instar larvae and later as teneral adults in the pupal chamber, but relatively rarely as larvae (Whitney, 1971).

However, more recent work has found that *D. ponderosae* larvae are associated with fungi throughout their development (A.S. Adams and D.L. Six, unpubl. data). Adults of both species of beetle also feed extensively on fungal spores lining the pupal chamber for 1–3 weeks after eclosion and prior to emergence from the natal host tree (Whitney, 1971; Six and Paine, 1998; Six, 2003a).

Group II

Beetles in Group II are characterized by the presence of glandular pronotal sac mycangia, tree-killing behavior, and solitary feeding by larvae that first develop in the phloem, but later move to the outer bark (Table 1). We predict that fungal associates are required to meet the nutritional requirements of Group II beetles. All but one Group II species has multiple generations per year. This indicates that larval development in phloem occurs relatively rapidly and before substantial decline in phloem resources occurs. However, larval feeding behavior indicates that fungi are still important in supplementing beetle nutrition. Group I larvae are solitary feeders, a strategy which may have evolved in response to competition among siblings for a poor food resource. These larvae feed in galleries in the phloem for only a short period of time and then excavate a feeding chamber where they apparently feed primarily on fungi (Klepzig et al., 2001b). Fourth instar larvae move to the nutrient deficient outer bark to complete development (Wood, 1982).

In comparison to beetles that feed only in galleries (Group II), these larvae consume relatively little tree tissue during development. For example, *D. frontalis* larvae consume 21% less phloem than *Ips grandicollis* Eichhoff larvae (also scolytine bark beetles and strict phloem feeders), although *D. frontalis* adults are larger than *I. grandicollis* adults. For this group of beetles, feeding on fungi that concentrate nitrogen may allow the beetles to grow larger while consuming less tree tissue (Ayres et al., 2000).

Dendroctonus approximatus differs from other *Dendroctonus* in this group in that it is not a tree-killing species colonizing trees killed by other more aggressive bark beetles (Wood, 1982). Thereafter, the development of this beetle in the host tree follows the same overall pattern exhibited by other species in this group. Therefore, we predict that mutualistic associations with fungi are likely to be similar among all beetles in this group.

Group III

Group III contains two species whose brood commonly feed in living trees, *D. valens* LeConte and *D. terebrantis* (Olivier), and two species that kill trees, *D. rhizophagus* Thomas & Bright and *D. parallelocollis* Chapuis (Thomas and Bright, 1970; Wood, 1982; Cibrian Tovar et al., 1995) (Table 1). All four species feed opportunistically in fresh stumps and at the bases of recently killed trees. Colonization and feeding by *D. valens* and *D. terebrans* seldom kills the host tree except when numbers of attacks are unusually high. The larvae of *D. valens*, *D. terebrans*, and *D. rhizophagus* feed gregariously in phloem at the base of trees or in roots. *Dendroctonus parallelocollis*, although typically rare, kills the host tree prior to laying eggs and its brood feeds as solitary larvae in

phloem in the roots and at the base of trees (Cibrian Tovar et al., 1995) (Table 1).

Little is known about relationships of fungi with beetles in Group III. Few studies have attempted to identify, let alone quantify the prevalence of the associates of these beetles, and none have looked at effects of the fungi on beetle fitness. While mycangial associates of beetles in Groups I and II are found with most individuals in a population indicating very close associations, the presence and prevalence of particular fungi appears to be more variable with the three non-aggressive beetle species in Group III. The fungi associated with *D. valens* LeConte vary somewhat by geographic region. Klepzig et al. (1991) found that 73% of *D. valens* in a population in Wisconsin carried *L. terebrantis* Barras & Perry, 20% carried *O. ips* (Rumbold) Nannf. and 7% carried *L. procerum* (Kendrick) Wingfield.

In contrast, D.L. Six (unpublished data) found 70–76% of *D. valens* in Montana to carry *O. ips*. In California, *D. valens* is known to carry both *L. terebrantis* and *O. ips* (Fox et al., 1992), but the proportion carried of each is unknown. Rane and Tattar (1987) found 100% of *D. terebrans* (Olivier) (sister species to *D. valens*) in a population in Massachusetts to be associated with *L. terebrantis*, although 20% and 10% of these beetles also carried *L. procerum* and *O. ips*, respectively.

Interestingly, in contrast with other ophiostomatoid associates of *Dendroctonus*, *L. terebrantis* exhibits relatively strong virulence in host pines (Rane and Tattar, 1987). This virulence may be an adaptation to association with beetles that develop in a living host where less virulent, more saprophytic, fungi would not survive. The fungus develops a sporogenous layer in the pupal chamber much like those produced by mycangial fungi of other *Dendroctonus* (Barras and Perry, 1971b). However, it is not known if teneral adults feed upon this layer.

The nutritional content of phloem fed upon by Group III beetles that exploit living trees is unlikely to decline substantially over time, unlike for Group III beetles that feed in phloem of recently dead trees. However, in living hosts, phloem in areas where larvae are feeding often becomes resin-soaked which may impact its quality as a nutritional substrate for beetles or fungi. While the gregarious feeding behavior of the larvae may be indicative of a relatively nutritious food resource, demonstrating that competition among siblings for food resources is not strong, it may also merely be a behavioral adaptation to feeding in a living defensive host.

D. valens and *D. terebrans* also attack newly created stumps, and dying and recently killed trees. When developing in these resources, they may be at a similar disadvantage as bark beetle species that feed in declining phloem (beetles in Group II). In this context, fungal associates may be of increased importance to these beetles.

For the single species in this group that kills trees and whose brood feed as solitary larvae, *D. parallelocollis*, we predict similar requirements for fungi as for beetles in Group I. This beetle is associated with an ophiostomatoid fungus that produces an ambrosial spore layer in the pupal chamber (pictured in Cibrian Tovar et al., 1995). However, neither the identity nor the effects of this fungus on the beetle host are known.

Considerable work remains to be done on the beetles in this group and their fungi. The presence of mycangia has not been investigated for any members of this group, nor have the impacts of their associated fungi.

Group IV

Beetles in Group IV differ in many ways from *Dendroctonus* found in all other groups. All but one species feeds on members of the Pinaceae other than *Pinus* (*Picea, Larix,* and *Pseudotsuga*) (Table 1) (Wood, 1982). Furthermore, this group contains species that exhibit a wide variety of feeding and colonization behaviors. The brood of two Group IV species feed gregariously in live trees. One Group IV species kills its host tree yet its larvae feed gregariously, and three species kill their host and exhibit solitary larval feeding (Table 1). Associations with fungi for most beetles in this group remain poorly or completely uncharacterized. Of the three beetles that develop in living hosts, fungal associates have been investigated for only one species. *Ophiostoma canum* (Munch) H. & P. Syd. is the most common fungus associated with *D. micans* (Kugelann) (Lieutier et al., 1992). However, the highly variable frequency at which this fungus has been isolated (0.5–90%) is consistent with our prediction that beetles that feed in phloem of living trees may not be highly dependant upon fungal associates. However, as with most phloem or wood feeding insects, associations with some sort of microbe (e.g. filamentous fungi, yeasts, bacteria) are likely essential to successful development (Martin, 1987).

The fungal associates of *D. punctatus* (LeConte) and *D. murrayanae* Hopkins have not been investigated. *D. murrayanae* is the only *Dendroctonus* species that feeds gregariously as larvae, yet kills its host tree (Wood, 1982). *D. murrayanae* is also the only beetle in Group IV to feed on *Pinus*; a trait that may be the result of a reversal to the ancestral *Pinus*-feeding state (Kelley and Farrell, 1998).

The remaining three species of beetles in this group kill the host tree during colonization or colonize recently killed trees and feed as solitary larvae (Table 1). *Dendroctonus pseudotsugae* Hopkins is commonly associated with *O. pseudotsugae* (Rumbold) von Arx and *Leptographium abietinum* (Peck) Wingfield which are transported by the beetle in pit mycangia located on the

elytra and pronotum (Lewinsohn et al., 1994; D.L. Six and K. Skov, unpubl. data). These pits contain pores in the exoskeleton indicating that these mycangia may be associated with endodermal glands (Lewinsohn et al., 1994). *Dendroctonus simplex* LeConte carries *O. americanum* Jacobs & Wingfield (Jacobs et al., 1997; D.L. Six, unpubl. data). Unfortunately, no studies have been conducted to investigate impacts of these fungi on their hosts.

Dendroctonus rufipennis (Kirby) is consistently associated with *L. abietinum* (Reynolds, 1992; Six and Bentz, 2003) which is disseminated in what appear to be pit mycangia (Solheim, 1995). For all three of these beetles, fungi are predicted to fill a role similar to that predicted for fungi associated with Group II beetles. However, these beetles experience very different environments during development than beetles in all other groups, (non-*Pinus* hosts with different chemical and physical defenses, and potentially very different nutritional contents).

Pit mycangia are the only type of mycangium described for species in Group IV. Pit mycangia may have evolved to facilitate spore transfer, or may merely consist of structures that developed for other purposes but are fortuitously positioned to trap spores prior to beetle emergence and for providing protection to the spores during transit. Further investigations into the prevalence and structure of pit mycangia within *Dendroctonus* may help answer questions as to their origin and importance in maintaining symbioses with fungi.

Group V

Group V contains but a single Asian species, *D. armandi* Tsai & Li ,that feeds on *Pinus* (Wood, 1982). The basic biology of this beetle (Zhongqi, 1989) is similar in many ways to *Dendroctonus* in Group I. Therefore, we predict that this beetle is likely to have similar requirements for nutritional supplementation by fungi. Pronotal pit mycangia (glandular status unknown) that carry *L. terebrantis* and *O. minus* (Hedgc.) H. and P. Sydow (Chen and Yuan, 2002) have been described for this beetle (Ming and Hui, 1999); however, the effects of these fungi on the host remain uninvestigated. Like beetles in Group II, *D. armandi* has been observed to maturation feed in the pupal chamber prior to dispersal from the natal host (Zhongqi, 1989).

3. Established Associates vs. Invaders in *Dendroctonus*-Fungus Symbioses

Some associations between *Dendroctonus* beetles and fungi have been established for long periods of time, while others appear to be relatively new. Six and Paine (1999) found evidence for cospeciation among several sac

mycangium-bearing *Dendroctonus* and their most common fungal associates, except for a single instance of an apparent host shift. However, several beetles that exhibit parallel cladogenesis with some associates also posses associates that appear to be relatively recent "invaders". The presence of still other associates appears to be, at least in part, determined by the presence of alternate hosts such as phoretic mites (discussed below).

Given the common occurrence of multiple fungal associates with individual bark beetle species, and the fact that some of these fungal partners are relatively new in association with their host, it is important to consider what factors or conditions may allow the invasion of established associations and new associations to form. Invasibility may be an inherent risk in ectosymbioses where contact between associates may lapse during periods of growth and development of each partner. For bark beetles, the period when larvae and fungi are developing in phloem presents just such a situation where contact among the associates can be lost. Some new associations may then potentially arise from cross-contamination with fungi associated with other beetles or insects cohabiting a tree (Six, 2003b). Cross-contamination may provide an avenue of exposure to new fungi. If establishment of a new associate occurs, isolation with the new host potentially may lead to adaptations to the new host, and eventually to speciation. Evidence for host switching has been found for an undescribed mycangial *Leptographium* associate of *D. approximatus* (Six and Paine, 1999).

Certain characteristics may increase the ability of particular fungi to invade already established associations between beetles and fungi. Most invaders, as well as long established associates, are ophiostomatoid fungi that possess many similar characteristics such as insect-dispersed spores (Six and Paine, 1999). The possession of such shared characteristics may pre-adapt some fungi to successful invasion of non-typical hosts. Hosts may not be able to select against these newcomers without also selecting against their established symbionts.

Competitiveness may also play an important role in the development of new associations. Highly competitive fungi may capture and retain host tree resources (and consequently host beetles) ahead of less competitive established associates. Many invader species and more incidental associates are pathogenic to the host tree, while many established associates are not pathogenic, or are only weakly virulent. Potentially, pathogenicity, by increasing competitiveness, may allow some fungi to invade established associations because it allows them to grow in, and capture, still living or recently killed tree tissues ahead of the less pathogenic established associates.

Some associations of fungi with beetles may truly be considered "new", having developed very recently (in ecological time). However, most "invader" fungi can probably only be considered newcomers on an evolutionary time scale.

Many share extensive geographic ranges with their hosts indicating relatively long association, ostensibly long enough for selection to act, allowing some degree of adaptation to the host to occur. If this is the case, using a combination of descriptive, phylogenetic, and experimental approaches, it may be enlightening to look at variation in characteristics among incidental (no adaptation predicted), invader (moderate adaptations to the host), and established (high level of adaptations to the host) associates.

4. Specific Examples: Dendroctonus-Fungus Symbioses

Southern pine beetle

The southern pine beetle (Group I), *D. frontalis*, is an aggressive beetle that attacks and kills weakened or healthy living trees through mass colonization (Raffa et al., 1993; Paine et al., 1997). As the adult female lays eggs within the inner bark, fungi from the mycangia and exoskeleton are inoculated into the phloem tissue (Bramble and Holst, 1940). Three fungi in particular, are known symbionts of this beetle: *O. minus*, *O. ranaculosum*, and *Entomocorticium* sp. A (an undescribed basidiomycete) (Hsiau and Harrington, 2003).

Ophiostoma minus is an ascomycete fungus carried phoretically on the exoskeleton of *D. frontalis* (Rumbold, 1931; Bridges and Moser, 1983) as well as by phoretic mites (Bridges and Moser, 1983). Although this fungus is very frequently carried by attacking beetles, its presence is not necessary for tree death to occur (Hetrick, 1949; Bridges, 1985; Bridges et al., 1985). The benefit of association with the beetle to the fungus is clear: consistent transport to an ephemeral resource, a newly killed tree. The fungus produces sticky spores, within pupal chambers, that easily adhere to emerging beetles. In addition, these spores are actively collected by *Tarsonemus* spp. mites which themselves are phoretic on the beetles. Thus, bark beetles and their mite associates serve as effective means by which this fungus can be transported to new host material (Dowding, 1969). The mites have a vested interest in ensuring transport of *O. minus* to new hosts; feeding on this fungus supports their development and reproduction (Lombardero et al., 2000). This mite-fungus relationship, therefore, is a classic mutualism, albeit superimposed on an insect-fungus relationship that is not so easily categorized.

Benefits, if any, to the beetle are less clear (Hofstetter, 2004). During the early stages of colonization of the tree, the *D. frontalis-O. minus* relationship might be categorized as a mutualism. At this point, the pathogenicity of the fungus may aid the beetles in overcoming tree defenses (Paine et al., 1997). However, later in the colonization process, *O. minus* becomes highly antagonistic to the beetle. As *D. frontalis* eggs hatch within pine phloem and

larvae begin to tunnel, *O. minus* spores deposited in the gallery earlier by parental beetles or mites germinate and the fungus begins to grow and colonize the phloem. When *O. minus* colonizes areas of phloem where larvae are feeding, the larvae fail to complete development. The relationship between fungus and beetle at this point is clear; increased levels of *O. minus* lead to reduced beetle brood survival and an overall reduction in beetle fitness (Barras, 1970; Franklin, 1970; Hofstetter, 2004).

Interactions of *O. minus* with the beetle are mediated by interactions with two other fungal associates of the beetle, *O. ranaculosum* and *Entomocorticium* sp. A. that are carried in a pair of glandular pronotal mycangia on female beetles (Barras and Perry, 1972; Happ et al., 1971). Each mycangium can contain either a pure culture of *O. ranaculosum* or of *Entomocorticium* sp. A, or occasionally, no fungus (Barras and Perry, 1972; Happ et al., 1976; Bridges, 1985; Hsiau, 1996; Hofstetter, 2004).

Once a tree is successfully attacked, female beetles construct egg galleries in the phloem where they oviposit and where spores from her mycangia inoculate the gallery walls. After eggs hatch, the larvae feed first in individual galleries in the phloem, but eventually excavate obovate "feeding chambers" partially within the phloem but extending into the outer bark. Within these chambers develops luxuriant growth of either, but not both, of the two mycangial fungi. Although it has never been explicitly demonstrated, it is generally held that the larvae feed on fungal hyphae and spores while developing within these chambers. Pupation later occurs within these same chambers.

Feeding on the two mycangial fungi positively impact brood development and survival of *D. frontalis* (Barras, 1973; Bridges, 1983; Goldhammer et al., 1990; Ayres et al., 2000). Female *D. frontalis* carrying *Entomocorticium* sp. A, or *O. ranaculosum* and *Entomocorticium* sp. A, produce more brood (Bridges, 1983; Goldhammer et al., 1990). Furthermore, brood developing with *Entomocorticium* sp. A are larger and contain higher lipid contents than those carrying only *O. ranaculosum* (Coppedge et al., 1995). Differences in lipid content can greatly affect survival (Safranyik, 1976; Botterweg, 1982; 1983; Anderbrandt, 1988), dispersal ability (Atkins, 1969; Thompson and Bennett, 1971) and reproductive capacity of female beetles (Reid, 1962; Amman, 1972; Clarke et al., 1979). The high lipid content of *Dendroctonus* eggs (Barras and Hodges, 1974) also indicates that the nutrition of parental females beetles may be important for optimal egg production. Additional nutritional benefit may come from the provisioning of dietary nitrogen by *Entomocorticium* sp. A and *O. ranaculosum* (Ayres et al., 2000). The two fungi are, although to different degrees, clearly nutritional mutualists with their host beetles.

The relationships among *D. frontalis* and its fungal associates, however, are much more complex than implied by the description presented above. *D.*

frontalis is associated with, and may transport, over 57 species of mites (Moser and Roton, 1971; Moser et al., 1971; 1974). The mites associated with southern pine beetle include parasites, predators, fungivores, and omnivores. In particular, phoretic fungivorous *Tarsonemus* mites including *Tarsonemus ips* Lindquist, *T. krantzii* Smiley and Moser, and *T. fusarii* Cooreman, are all common southern pine beetle associates. All three mite species are transported to new host trees with no directly deleterious effects on the beetle (Moser and Roton, 1971; Smiley and Moser, 1974; Moser, 1976; Bridges and Moser, 1983; Moser and Bridges, 1986). However, all three can impact the beetle-fungus-tree interaction.

All three mite species possess sporothecae, which are specialized, flap-like structures of the integument somewhat analogous to the mycangia of the host beetles. These sporothecae frequently contain ascospores of *O. minus* (Bridges and Moser, 1983; Moser, 1985) and *O. ranaculosum* (Moser et al., 1995). The mites readily feed and develop on *O. minus* as well as occasionally on *O. ranaculosum*, but do not feed or develop on cultures of *Entomocorticium* sp. A (Lombardero et al., 2000). The mites, therefore, transport only their mutualist fungi and not the fungus upon which they do not feed.

Competition among the fungi may determine which fungi individual mites and beetles develop with and carry to the next host tree. Therefore, the outcome of competition among these fungi is of key importance to *D. frontalis* and its associated mites (Klepzig and Wilkens, 1997). In experiments conducted on standard artificial media and within pine logs, *O. minus* is clearly the superior competitor by virtue of its rapid growth rate and aggressive resource capture (Klepzig and Wilkens, 1997). However, under these same conditions, *Entomocorticium* sp. A is able to retain substrate it has colonized in the presence of *O. minus*, while *O. ranaculosum* is not (Klepzig and Wilkens, 1997). Such differences in competitive abilities in a host tree may indicate that *O. minus* can develop in areas colonized initially by *O. ranaculosum* but not in areas initially colonized by *Entomocorticium* sp. A. thus making areas colonized by *Entomocorticium* sp. A safe for feeding by *D. frontalis* larvae. The selection pressure maintaining *O. minus* with *D. frontalis*, which at times is deleterious not only to the host beetle but to at least one of the other fungal symbionts, comes from a third partner in the interaction, the mites. Mites as vectors and mutualists with *O. minus*, as much as, or more than, beetles and their mycangial fungi, may drive symbiotic interactions in the southern pine beetle system (Hofstetter, 2004).

Mountain pine beetle

The mountain pine beetle, *Dendroctonus ponderosae* (Group I), like the

southern pine beetle, is an aggressive bark beetle that exhibits large population eruptions at periodic intervals that result in significant mortality of host pines. Unlike the southern pine beetle, *D. ponderosae* larvae form no feeding chambers and complete all development in galleries in the phloem. Mountain pine beetles are typically univoltine and thus experience substantial declines in host tree quality during development. The phloem of colonized trees is brown, dry, and thin by the time of brood emergence the year after the initial attack.

D. ponderosae possesses sac mycangia on the maxillary cardines that are functional in both sexes (Whitney and Farris, 1970). These mycangia are associated with two fungi, *O. clavigerum* and *O. montium* (Whitney and Farris, 1970; Six and Paine, 1997). Most individual beetles carry only one of the fungi in their mycangia, but occasionally an individual will carry both, although in separate mycangia (Six, 2003b).

The mycangial fungi of *D. ponderosae* can also be carried phoretically on the exoskeleton. *Ophiostoma clavigerum* is very commonly found in mycangia, but only rarely on the exoskeleton (Six, 2003b). *Ophiostoma montium*, however, appears to be equally well suited for phoretic as well as mycangial dissemination. The difference in how the two fungi are disseminated is not surprising. *Ophiostoma clavigerum* has a long shared evolutionary history with its host, and thus is likely to be highly adapted for mycangial dissemination. However, *O. montium* appears to be a more recent "invader" (Six and Paine, 1999) that may have begun its association with *D. ponderosae* as an incidental phoretic fungus, but preadaptations typical for *Ophiostoma* (sticky spores, etc) may have allowed it to exploit the mycangium and establish itself as a mycangial associate (Six, 2003b).

Spores and mycelium from the mycangia and from the exoskeleton become inoculated into the phloem by colonizing parental adult beetles as they excavate galleries in newly attacked trees. The fungi then grow in the phloem, where they are likely fed upon by larvae (A.S. Adams and D.L. Six, unpubl. data), and the sapwood until, nearly a year later, they produce a dense sporogenous layer in host pupal chambers. In these pupal chambers, newly enclosed teneral adults maturation feed on the fungi for a period of days to weeks prior to emergence from the tree (Whitney, 1971; Six and Paine, 1997). At this time the mycangia become charged with fungi.

Ophiostoma clavigerum is a moderately virulent pathogen and is able to colonize living sapwood more rapidly than *O. montium* (Reid et al., 1967; Yamaoka et al., 1990). Only *O. clavigerum* is capable of killing mature trees, but only if inoculated at very high rates (Yamaoka et al., 1995). The presence of *O. clavigerum*, however, is not necessary for tree mortality to occur, as populations of beetles carrying near 100% *O. montium* can successfully attack and kill pines (D.L. Six and B.J. Bentz, unpubl. data).

The two fungi associated with *D. ponderosae* mycangia appear to differentially affect the reproductive potential of their host beetle (Six and Paine, 1998). For *D. ponderosae* reared in bolts, the production of progeny adults was significantly higher, and emergence significantly earlier, when brood developed with *O. clavigerum* than when brood developed with *O. montium* (Six and Paine, 1998). Indeed, brood development in the presence of *O. montium* barely exceeded the parental replacement rate. In contrast, when fungus-free parental adults were introduced into bolts, most rapidly re-emerged and did not excavate galleries; none laid eggs. This reemergence behavior and lack of brood production by fungus-free beetles highlights the fact that, while *O. montium* appears to be an inferior associate to *O. clavigerum* in respect to its effects on beetle reproduction, it's presence still imparts a degree of benefit to the host beetle.

Although Six and Paine (1998) observed no brood production by fungus-free beetles reared in bolts, mountain pine beetles can be successfully reared on axenic diets (Whitney, 1971; Safranyik and Whitney, 1985); however, only if yeast is added (H.S. Whitney, pers. comm. in Strongman (1987). Likewise, Strongman (1987) found that *D. ponderosae* development was much slower on autoclaved fungus-free phloem blocks than in phloem blocks inoculated with fungi. These studies indicate that a fungal product, or products, may be required for optimal development and survival of *D. ponderosae* brood.

Given that beetle-associated fungi may have substantial effects on beetle reproductive potential, the relative abundance of fungal associates within a given population may also have substantial effects on beetle population dynamics. Little attention has been given to determining whether and how fungal associates of bark beetles vary across the geographic range of their hosts. However, it is well known that the frequency of the two mycangial associates of *D. frontalis*, as well as of the mite associate, *O. minus*, varies among populations (Bridges, 1983; Six and Paine, 1999). A recent study on *D. ponderosae* also found substantial variation in the proportion of its two mycangial fungi across its geographic range (D.L. Six and B.J. Bentz, unpubl. data). Potentially, through differential effects on host beetle reproduction, variation in the relative abundance of the fungal associates of *D. ponderosae* may differentially influence its ability to respond to changing environmental conditions, and thus, potentially affect its population dynamics and outbreak behavior.

The spruce beetle

The spruce beetle (Group IV), *D. rufipennis*, is a weak to moderately aggressive beetle that colonizes several species of *Picea*. During colonization,

adult beetles transport fungal spores and yeast cells into host trees in what are likely pit mycangia located on the pronotum and elytra (Solheim, 1995). Once in the tree, the spores germinate and the fungi colonize the phloem and sapwood of the tree in a manner similar to the fungi associated with the mountain pine beetle and the southern pine beetle. It is not known when, or even if, this beetle feeds on its associated fungi; however, given the extensive colonization of tree tissues by its associated fungi, such feeding, at least by larvae, is likely to be unavoidable.

Leptographium abietinum appears to be the most common associate of this beetle (Davidson, 1955; Hinds and Buffam, 1971; Reynolds, 1992; Solheim, 1995; Six and Bentz, 2003) with often greater than 90% of beetles in a given population carrying this fungus (Reynolds, 1992; Six and Bentz, 2003). *Ceratocystis rufipenni* Wingfield, Harrington, & Solheim, a virulent tree pathogen, is also associated with some populations of *D. rufipennis*, but apparently absent in others (Reynolds, 1992; Solheim, 1995; Six and Bentz, 2003). *C. rufipenni* has been hypothesized to be a mutualist of *D. rufipennis* because of its virulence to host trees. However, it does not appear to be a common associate and is not required for tree mortality to occur. Until more is known regarding the distribution and consistency of association of *C. rufipenni* with *D. rufipenni*, it will be difficult to assess what role, if any, this fungus may have with the beetle.

The effect of *L. abietinum* on host beetle fitness is unknown. Experiments are currently in progress investigating the effects of this fungus, as well as *C. rufipenni*, on *D. rufipennis* development and reproduction (B.J. Bentz and D.L. Six, unpubl. data) that may help clarify the role of these fungi with this beetle.

Ophiostoma piceaperdum (Rumb.) von Arx (Rumbold, 1936), *O. penicillatum* (Grosm.) Siemaszko (Davidson, 1955), *O. olivaceum* Mathiesen (Hinds and Buffam, 1971), *O. piliferum* (Fries) H. & P. Sydow, *O. piceae* (Munch) H. & P. Sydow (Safranyik et al., 1983) and *O. ips* (Six and Bentz, 2003) have also been found in association with *D. rufipennis* but only incidentally. The occasional presence of these fungi with *D. rufipennis* may result from cross-contamination with fungal associates of other scolytids that co-infest *Picea* (Six and Bentz, 2003). It is unknown what impact occasional cross-contamination may have on these, and other, bark beetle hosts.

5. Summary

In this paper, we have presented a framework for studying symbioses in *Dendroctonus*-fungus systems. We also presented a set of predictions as to where mutualistic fungal associates (primarily nutritional mutualists) may be most

likely to occur within this genus based on beetle phylogeny and biology. Because significant gaps in knowledge exist within the framework, especially for the less aggressive beetle species and their fungal associates, in many cases our predictions remain highly speculative. However, as information on these beetles and their associates increases with additional research, and many of these gaps are filled, these predictions can be further refined.

This genus of beetles and their fungal associates provide an ideal model system for the study of ectosymbioses. The multiple independent origins of mycangia and the presence of both coevolved fungal associates as well as relative newcomers (invaders), along with the variable colonization and feeding behaviors exhibited by host beetles, provide an ideal system to assess the factors that can lead to, and maintain, ectosymbioses among unrelated groups of organisms.

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