Research Focus

Biological pest control in beetle agriculture

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Bark beetles are among the most destructive tree pests on the planet. Their symbiosis with fungi has consequently been studied extensively for more than a century. A recent study has identified actinomycete bacteria that are associated with the southern pine beetle and produce specific antibiotics against an antagonist of the beetles' mutualistic fungus. In addition to highlighting the ecological complexity of bark-beetle–microbial symbioses, this work reveals a potential source of novel antibiotics.

The complexity of mutualistic interactions

> *'...I will be an enemy to your enemies and will oppose those who oppose you.' Exodus 23:22*

The traditional view of mutualism is one of a reciprocally beneficial interaction between two species (Box 1). However, it is becoming increasingly evident that the distinction between mutualistic and parasitic interactions is far from clear and, also, that the nature of these relationships can vary for a single interaction. Moreover, although the net result of a mutualistic interaction must by definition be positive for the interacting species, these species usually also pay a price. For example, gut bacteria usually provide benefits to their host and, therefore, must be categorized as mutualistic, but the benefits come at a cost for the host. This explains why swine farmers add antibiotics to livestock feed (i.e. to suppress their gut flora).

The symbiosis between fungus-growing beetles and fungi illustrates clearly that the distinction between mutualistic and parasitic interactions can be context dependent. A large group of wood-inhabiting beetles live in a mutualistic symbiosis with various fungi, which provide nutrition to their larvae. In the well-studied southern pine beetle, the main mutualistic fungus is a species of the genus *Entomocorticium*, but an additional symbiont, *Ophiostoma minus*, has a context-dependent effect [1]: it can assist adult beetles in depleting tree resources during the early part of an attack, which is beneficial for the host at that stage; however, at a later stage, it competes with the more beneficial *Entomocorticium* sp. A, which makes it a parasite of the mutualism [1].

This example illustrates yet another complication of the traditional view of mutualisms: mutualistic interactions are not usually restricted to two species. A recent paper by Scott *et al.* [2] has identified an additional bacterial symbiont in the previously mentioned symbiosis between southern pine beetles and their associated fungi. The bacterium belongs to the actinomycete genus *Streptomyces*, a group well known to humans (and other organisms [3]) because it is an important source of antibiotics. The *Streptomyces* sp. isolated by Scott *et al.* [2] shows antibiosis towards *O. minus*, the fungus which is – at least sometimes – a parasite of the mutualism. This bacterium appears to be another mutualist in this symbiosis because it can protect the mutualistic fungus against the parasite.

Protecting your mutualists

In mutualistic host–symbiont interactions, hosts employ various mechanisms to protect their symbionts against parasites. One form is simply physical protection (for example, by providing a protective growth environment, which can even be the host body or cell itself, in endosymbioses). Another possible form of protection can be found in the domestication of secondary symbionts that are harmful to parasites and thereby protect the primary symbiont against parasites. The first example of such a protective symbiont was discovered by Currie *et al.* [4] in the well-studied mutualism between attine ants and fungi. These ants were found to carry specific bacteria (actinomycete bacteria of the family Pseudonocardiaceae) that produce antibiotics targeted at a specialized parasitic fungus (genus *Escovopsis*) of this fungus-growing insect mutualism [4,5]. It has been suggested that other insect–fungus mutualisms are also likely to have additional symbionts or even consortia of multiple symbionts and that this might lie at the heart of the success that has stabilized such interactions for more than 50 million years [6].

Scott *et al.* [2] have provided intriguing evidence that fungus-growing beetles carry bacterial symbionts to protect their mutualistic fungi against antagonistic fungi. First, they have shown that one of the two isolated bacterial morphotypes specifically inhibits the antagonist *O. minus*, and much less so the mutualistic fungus. Second, the authors have isolated and identified the chemical (which they have named mycangimycin [7]) that causes this specific antibiosis. Third, the bacterium seems to be specialized to this mutualism: it has been isolated from most beetle individuals tested (92 of 110) and from five of ten tested mycangia, which are specialized structures for the vertical transmission of the mutualistic fungus. Fourth, using scanning electron microscopy techniques, abundant filamentous growth of the bacterium could be shown in natural galleries.

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Box 1. The evolution of mutualisms

Mutualisms – reciprocally beneficial interactions between different species, or 'reciprocal exploitations that nonetheless provide net benefits to each partner' – are widespread in nature [19]. They range from loose interactions, such as those between pollinating insects and flowering plants, to tightly coevolved symbioses, such as those between some insects and endosymbionts inside their cells. The evolution of mutualisms is difficult to explain. Theory predicts that in the short term, cheating individuals, which profit but do not pay the cost of reciprocating the help of their partner, will be selected because they have a selective advantage in competition with reciprocating competitors [20]. The identification of factors that stabilize mutualisms forms an important research area [19,21,22].

The most fundamental requirement for cooperation to be stable is that cooperating individuals, on average, receive more help from their partner than non-cooperative individuals do [20]. For a mutualism, this requirement can be fulfilled through various types of feedback mechanisms [21]. The most important of these are partner-fidelity feedback and partner choice or its negative equivalent, partner sanctions [19,21,22]. Within-species relatedness among multiple symbionts associated with a single host is important for these feedbacks: if the returned benefits go to unrelated individuals of the same species, the feedback mechanisms do not work [21].

Partner-fidelity feedback occurs if the benefit given by one partner species to the other results in a direct benefit in return [21]. An extreme form of partner-fidelity feedback occurs in uniparental vertical symbiont transmission, which aligns the reproductive interests of the two partners. Indeed, it has been found that some of the most ancient and stable mutualistic symbioses have uniparental vertical transmission. Examples include various forms of endosymbioses between organelles and eukaryotic cells and between *Buchnera* and aphids [23]. However, vertical transmission is not a universal characteristic of mutualisms, and interactions with vertical transmission are not necessarily mutualistic. This shows that vertical transmission is neither a necessary nor a sufficient condition for the evolution of mutualism [19,24].

Partner choice occurs if one of the players in a mutualism is able to select its partner or sanction non-cooperative partners. Several examples of partner choice have been found. One example comes from some yucca species, which live in an obligate mutualism with pollinating moths. In exchange for the favor of pollinating the flower, the insects use some of the developing seeds as the food source for their developing larvae. Yucca plants 'sanction' insects that overexploit the plant, by aborting fruit that contains too many eggs [25]. Other plants with partner choice are legumes that abort root nodules that have uncooperative (non-nitrogen-fixing) bacteria [26].

A mutualistic ecosystem within the wood: bark beetles, mites, fungi and bacteria

Bark beetles are well known for complex interactions with micro-organisms (Box 2). The most conspicuous interactions are with a group of fungi, collectively called the Ophiostomatoid fungi, that have specialized mechanisms of spore dispersal that facilitate interactions with the beetles [8]. A few beetle species are also associated with basidiomycete species. Associations between fungi and bark beetles can be very complex (many fungal species can be carried by a single beetle, and some fungal species are shared between beetle species), they vary in space and time, and their outcome can change from mutualistic to antagonistic depending on their context [1]. These roles, however, are not always clear. For example, the southern pine beetle frequently carries an additional Ophiostomatoid fungus, *Ceratocystiopsis ranaculosus*, in its mycangia, but its ecological role is not as clear as those of the other fungi in the system that have been mentioned here [2,9].

Mites have an important role in bark-beetle–fungal ecosystems [10]. The mites, specifically the fungivorous *Tarsonemus* spp., feed on some of the associated Ophiostomatoid fungi and collect them in specialized flaps on their exoskeletons. The mites cling to the beetles for dispersal and in the process, promote the spread of their collected fungi. In the southern pine beetle system studied by Scott *et al*. [2], the mites frequently carry *O. minus* (the antagonist of the beetle) and *C. ranaculosus*, on which they feed.

It is well known that yeasts and bacteria occur in the galleries of bark beetles, but their ecological roles are poorly understood. A recent study by Adams *et al*. [11] has provided some evidence of their potential importance; yeasts and bacteria from the galleries of *Dendroctonus ponderosae* (the mountain pine beetle) collectively enhanced the growth of *Ophiostoma montium* but reduced the growth of *Grossmania clavigera*, whereas an apparently endophytic bacterium in the tree inhibited the growth of both fungi. Both fungi are carried in the beetle mycangia (vertical transmission) and are thought to be mutualistic, although to different degrees and in context-dependent ways [12]. The identity and specific roles of the microbes involved, however, are not known.

Scott *et al*. [2] have provided strong evidence to suggest that bacteria are important to the bark beetles, supposedly to suppress antagonistic fungi that they carry and to enhance the success of at least one symbiotic fungus. The bacterium has a clear benefit for the southern pine beetle, by selectively suppressing the antagonist *O. minus* but not the mutualist, *Entomocorticium* sp. A. This is expected to provide an advantage to *Entomocorticium* sp. A in competing with *O. minus* for establishment in the galleries. In this way, the beetle ensures a food source for its larvae. It has been shown that once *Entomocorticium* sp. A is established, it can successfully defend its colonized domain against invading *O. minus* [1,10]. Therefore, the presence of the bacteria is probably most valuable early on in the establishment of the beetle and its mutualistic fungus.

Why do the bacteria produce antibiotics?

A general question with respect to the evolution of mutualisms is why symbionts would provide a (costly) benefit to their host (Box 1). For the association between southern pine beetles and actinomycetes, this question translates into why the bacteria would produce costly antibiotics. One possibility is that the bacteria enjoy a direct growth benefit in resource competition with *O. minus*. Alternatively, if bacterial fitness depends on host fitness, they benefit indirectly by fighting an antagonist of their host. An extreme form of such a host-related benefit would occur if the bacteria are transmitted vertically via dispersing beetles because bacterial dispersal is then coupled to host reproduction. Moreover, strict vertical transmission also reduces the effective population size of the bacteria associated with a single gallery. Regular bottlenecks of the bacteria (keeping their relatedness high) might be important; high symbiont relatedness prevents the evolution of 'free riders' (i.e. bacteria that do not produce the antibiotic themselves but profit from others producing it). If future

Box 2. Bark-beetle–fungus associations

J.B.S. Haldane is quoted as having said that ‘God must have an inordinate fondness for beetles’, referring to the diversity of the Coleoptera. Of the beetle families, the true weevils (Curculionidae) are the most diverse. One equally diverse subfamily of this group is the bark beetles (subfamily Scolytinae). These small (~5 mm) beetles feed on the phloem of trees, causing characteristic tunnels under the bark or in the case of ambrosia beetles, into the xylem and heartwood.

If God has an inordinate fondness for beetles, He must have an extraordinary fondness for fungi. Many bark beetles are known to carry a diverse assemblage of associated fungi many times as diverse as their hosts. Münch [18] first graphically described the Ophiostomatoid fungi, the most common associates of the bark beetles [27]. These fungi have elongated necks or stalks that carry sticky spore droplets at just the right height to expose the spores to passing insects (Figure I). The Ophiostomatoid fungi are not a natural grouping but include fungi in two unrelated orders (namely, the Ophiostomatales and the Microascales) that have independently evolved similar adaptations for insect dispersal. A few bark beetles are also known to carry basidiomycete fungi in the genera *Entomocorticium* and *Phlebiopsis*. Although some of the fungi are carried on the exoskeletons of the insects or by mites associated with them, some bark beetle species have specially adapted mycangia for carrying fungal spores.

Despite the common association between bark beetles and fungi, the nature of the interactions, especially where multiple fungi are involved, is poorly understood. Associations ranging from mutualism to commensalism and antagonism have been shown. These relationships are sometimes also context dependent, changing from one type to another depending on the external factors [1,10]. The fungi gain a clear advantage from the association through transport to suitable host trees and inoculation into the woody substrate. In some – but certainly not all – cases, the fungi help in overcoming the defenses of the tree or contribute to the nutrition of the developing larvae in the wood.

A few bark beetle species attack and kill live trees. These beetles can be among the most destructive biological agents on the planet. For example, an ongoing outbreak of the mountain pine beetle (*Dendroctonus ponderosae*) in British Colombia, Canada covers >130 000 km^2, in most cases killing whole stands (www.for.gov.bc.ca/hre/bcmpb/cumulative/2008.htm). Kurz *et al.* [28] has shown that the extent of the damage of this outbreak will change these forests from a small carbon sink to a large carbon source. This could exacerbate the climate change effect, which is thought to have been part of the cause of the outbreak in the first place.

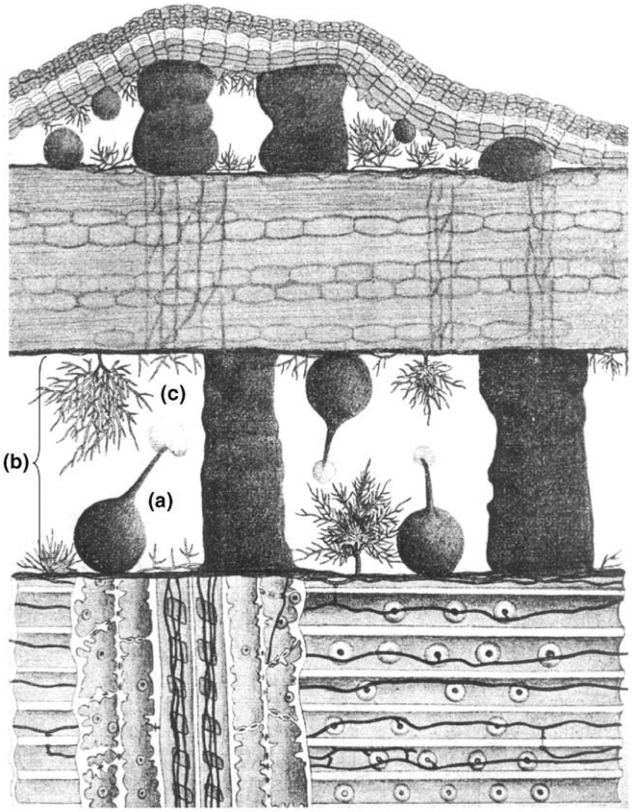

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Figure I. A graphic illustration of *Ophiostoma minus* (=*Certostomella pini*), the fungal antagonist in the system described by Scott *et al.* [2], by Münch [18]. The illustration shows how well the fungus is adapted to the bark beetle niche with **(a)** long necks carrying drops of sticky spores at their apices, **(b)** thus enabling the beetles to collect spores as they crawl through their tunnels. *O. minus* is an antagonist of the mutualist fungus *Entomocorticium* sp. that is carried by *Dendroctonus frontalis* in specialized mycangia, and Scott *et al.* [2] have now shown that the antagonist is suppressed by a bacterial associate in beetle galleries and mycangia. **(c)** Intriguingly, some of the unidentified branched structures in this remarkable illustration might well represent filamentous Actinomycetes, as can be seen in Figure 1b of Ref. [2].

research shows that the bacteria are frequently recruited from the environment (see below), an urgent question is that of how the specific association with antibiotic-producing bacteria arises. Evolutionary theory predicts that some form of feedback must occur to secure the association with antibiotic-producing bacteria (Box 1). An intriguing possibility is that the beetles are somehow able to employ partner choice of antibiotic-producing bacteria or sanctioning of non-producing symbionts (Box 1).

Co-evolved mutualism vs recent acquisitions

In the study by Scott *et al.* [2], bacteria have also been isolated from the mycangia, which are specialized structures for the vertical transmission of the fungal symbiont. It is possible, therefore, that these bacteria, like the fungal symbionts of the beetle, are also vertically transmitted between generations. However, because the bacterium could be isolated from only half of the mycangia tested, the frequency of this vertical transmission remains to be understood.

Recently, it has been questioned whether tight co-evolution between ants and actinomycete bacteria has occurred [13]. Some evidence now suggests that the actinomycete bacteria associated with this mutualism over evolutionary time have been acquired repeatedly from the environment, although they still show some host specificity [13]. For the southern pine beetle, the specificity of the association between the newly discovered actinomycetes and their host remains to be tested, as does whether any coevolution has occurred between them. Actinomycetes such as *Streptomyces* are well known from various environments, including trees (where they might have a role as endophytes), soil and water [14,15]. These bacteria are also known to be associated with other insects, as was shown in recent studies that identified *Streptomyces thermosacchari* (the closest relative of the *Streptomyces* sp. isolated by Scott *et al.*) in the guts of termites in Australia [8] and *Streptomyces* sp. associated with wasps [4], respectively. It is, thus, conceivable that these bacteria represent recently recruited symbionts from the environment.

Conclusions and future studies

The work of Scott *et al*. [2], Adams *et al*. [11] and Cardoza *et al*. [16] suggest that microbes other than those previously known might play an important part in mediating the interactions between the bark beetles, filamentous fungi and mites. In fact, they might be the key to the long-term stability and success of these co-operations, as was predicted by Mueller *et al*. [6]. These studies highlight the need to study bacterial and other microbial symbionts in other bark-beetle–fungal symbioses and in insect–fungal interactions in general.

Future studies should explore basic details of the evolution of the mutualism between bark beetles and antibiotic-producing bacteria. For example, it will be important to estimate the phylogenetic relationships between bacteria from related beetles and free-living bacteria. Furthermore, studies of symbiont transmission mode, partner choice and reciprocal adaptations in the beetle and bacteria are needed to determine the extent to which the interests of the *Streptomyces* sp. and the southern pine beetle are aligned (Box 1).

Much remains to be learned about the ecological role of the bacteria reported by Scott *et al*. [2]. For example, they also isolated a different, closely related, strain of *Streptomyces*, which had a different growth-inhibition spectrum than the strain described above: it showed comparable antagonism towards both the mutualistic and the antagonistic fungi of the southern pine beetle, and even showed slightly more to the mutualistic fungus. It remains to be established, therefore, whether that strain has any role in the symbiosis. The interaction with the other symbiont of the southern pine beetle, *C. ranaculosus*, has also not yet been explained. Whatever the role of these bacteria is for the bark beetle symbiosis, as Berenbaum and Eisner [17] point out, their discovery reveals an important, diverse (Box 2) and as yet unexplored niche of biologically active substances that humans might come to find useful for biological pest control.

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