Functional Ecology

British Ecological Society

Functional Ecology 2014, **28**, 315–327 doi: 10.1111/1365-2435.12089

DEFENSIVE SYMBIOSIS

Defensive microbial symbionts in Hymenoptera

Martin Kaltenpoth* and Tobias Engl

Max Planck Institute for Chemical Ecology, Insect Symbiosis Research Group, Hans-Knoell-Str. 8, Jena 07745, Germany

Summary

1. In all stages of their life cycle, insects are threatened by a multitude of predators, parasites, parasitoids and pathogens. The lifestyles and feeding ecologies of some hymenopteran taxa render them especially vulnerable to pathogen infestation. Specifically, development in subterranean brood cells, mass provisioning of resources for the offspring and the life of social insects in large communities can enhance the risk of pathogen infestation and/or the spread of disease among conspecifics.

2. To counteract these threats, insects have evolved mechanical, chemical and behavioural defences as well as a complex immune system. In addition to the host's own defences, some Hymenoptera are associated with protective symbionts. Leaf-cutting ants, solitary digger wasps, bees and bumblebees engage in symbiotic interactions with bacteria that protect the adult host, the developing offspring or the food resources against microbial infections. In the well-studied cases of ants and wasps, the protective activity is mediated by the production of antimicrobial secondary metabolites. In other symbiotic interactions, however, competitive exclusion and immune priming may also play an important role in enhancing protection. Phylogenetic studies indicate that the defensive associations in Hymenoptera are generally more dynamic than the intimate nutritional mutualisms, with horizontal transfer or *de novo* uptake of the symbionts from the environment occurring frequently.

3. Mutualistic micro-organisms can also significantly influence the outcome of host-parasitoid interactions. Some insects are protected by symbiont-produced toxins against parasitic wasps. Ichneumonid and braconid parasitoids, on the other hand, are associated with symbiotic viruses that are injected into the caterpillar host during oviposition and suppress its immune system to the advantage of the parasitoid.

4. The increasing affordability of next-generation sequencing technologies will greatly facilitate the analysis of insect-associated microbial communities and undoubtedly uncover a plethora of as yet unknown protective symbioses. However, a detailed understanding of the host's natural history is indispensable for elucidating the fitness benefits of the symbionts and the molecular basis of symbiont-conferred protection.

Key-words: Actinobacteria, beewolf, defensive mutualism, honeybee, immune system, leaf-cutting ants, parasitoid, pathogen defence, polydnavirus, protective symbiosis

Introduction

The Hymenoptera represent one of the four megadiverse holometabolous insect orders, with more than 110 000 described species to date, and an estimated total number of up to 2·5 million species worldwide (Grissell 1999; Whitfield 2003; Heraty *et al.* 2011). The evolutionary origin of the Hymenoptera dates back to the Triassic, and they have since undergone one of the most successful adaptive radiations among arthropods (Wiegmann *et al.* 2009). The order is generally divided into the paraphyletic 'Symphyta' (sawflies) and the monophyletic Apocrita (Peters *et al.* 2011). The latter comprises the ecologically as well as economically important groups of bees, wasps and ants, which play key roles in terrestrial ecosystem functioning, for example, as plant pollinators, predators, scavengers and parasitoids (Wilson 1971).

---

*Correspondence author. E-mail: mkaltenpoth@ice.mpg.de

A plethora of different lifestyles and feeding ecologies occur within the Hymenoptera. Herbivory as the presumed ancestral condition is still found in the larvae of some lineages of sawflies that feed on leaves, shoots or pollen. Other symphytan groups are wood boring, which probably represents the lifestyle from which ectoparasitism has evolved in the ancestor of all extant Orussoidea and Apocrita (Dowton & Austin 2001; Whitfield 2003). While the Orussoidea maintained the ectoparasitic lifestyle and remained relatively species poor, the Apocrita have experienced an immense radiation with multiple transitions from ecto- to endoparasitism, predation, omnivory, mycophagy or secondary reversals to herbivory (Whitfield 2003). The evolution of nest-building and provisioning behaviour in some groups has probably laid the foundation for the subsequent evolution of differential degrees of social behaviour, ultimately culminating in the complex societies of eusocial bees, wasps and ants (Andersson 1984). The nature of the provisions in social insects ranges from dead arthropods (in many wasps and ants) to pollen and nectar (in most bees) and to actively tended fungal crops that are cultivated by leaf-cutting ants in specialized gardens equivalent to human agriculture (Mueller *et al*. 2005). However, although the social species have been studied most intensively, it has to be noted that the vast majority (around 75%) of extant hymenopteran species are parasitoids (Whitfield 2003).

The diversity in lifestyles not only requires physiological adaptations towards the effective utilization of different food sources, but also the evolution of defence mechanisms against other organisms. While pathogens, predators and parasitoids pose a universal threat that insects in general have to cope with, the ecological characteristics of some Hymenoptera likely increased the selective pressures on evolving particular defence mechanisms. Specifically, parasites and parasitoids have to continuously protect themselves against the immune system of the host or evade detection in the first place (Strand & Pech 1995). In many nonparasitic taxa, on the other hand, the completion of larval development in underground nests, especially in combination with the provisioning and storage of nutrient-rich food resources, entails a significant risk of pathogen infestation from the surrounding soil (Janzen 1977; Jurkevitch 2011). This problem is likely exacerbated in social species, due to the storage of particularly large amounts of resources and the facilitation of within-colony transfer of detrimental microbes by contact of nestmates (Currie, Mueller & Malloch 1999a; Cremer, Armitage & Schmid-Hempel 2007). While behavioural or chemical defences in insects have traditionally received considerable attention, we are only beginning to appreciate the significant and diverse roles that symbiotic micro-organisms can play in protecting the host against detrimental organisms (e.g. Brownlie & Johnson 2009; Kaltenpoth 2009).

Here, we review the defensive symbiotic alliances with bacteria and viruses that are currently known in Hymenoptera, and we discuss these alliances in the light of the hosts' ecology to identify ecological characteristics that may predispose certain taxa towards engaging in defensive symbioses. After a brief introduction into alternative defence strategies, we will focus on symbiont-mediated protection of the adult insect or the developing offspring against pathogens, parasites, parasitoids and predators, consider mutualistic bacteria that protect the host's nutritional resources against detrimental fungi and discuss the complex roles of symbiotic bacteria and viruses in host-parasitoid interactions (Fig. 1). Our aim is not only to provide an overview of this emerging field, but also to suggest novel directions for future research to gain a better understanding of the importance and diversity of defensive mutualisms in Hymenoptera.

Defence strategies in insects

To protect themselves against antagonists, insects evolved a range of defensive mechanisms. A simple solution to the problem is to evade or avoid the antagonist temporally or spatially by behavioural adaptations (Strohm, Laurien-Kehnen & Bordon 2001). If this is not possible, mechanical protection, physical contest with a predator or parasitoid, or active removal of pathogens or parasites can constitute efficient defence strategies. Noxious chemicals provide another common means for protection, and they can act in a variety of ways. Defensive compounds can serve as repellents that deter enemies but have no actual harmful effect (Evans & Schmidt 1990). Alternatively, they can distract attackers, by creating a sensory overload or by physically inhibiting mouthparts, legs or wings (Gross 1993). Finally, toxic substances directly interfere with the enemy's metabolism and have reversible or irreversible ill effects on its physiology (e.g. Bot *et al*. 2002). Generally, defensive substances can be produced *de novo* or sequestered from the environment (Cane, Gerdin & Wife 1983).

Once in contact, the next line of defence is the insect's immune system. As several excellent recent reviews are available on this topic (e.g. Schmid-Hempel 2005; Siva-Jothy, Moret & Rolff 2005), we will only provide a brief summary here to enable an understanding of the contributions of symbiosis to insect immunity. The insect immune system consists of several mechanisms that are complementary or act in concert to provide protection against pathogens and parasites (Schmid-Hempel 2005). The first layer of defence is the cuticle, a tough, flexible and waterproof barrier. The outer layer of the cuticle, the epicuticle, primarily consists of lipids and hydrocarbons, which reduce desiccation but provide no real protection against pathogens. The inner (endo)cuticle is composed of chitin and proteins that gain their rigidity by cross-linking, melanization and sclerotization. Thus, the endocuticle provides mechanical protection against pathogens, and only a few specialized entomopathogenic fungi evolved the ability to actively penetrate this layer. However, the cuticle is not only a passive barrier, but also possesses active immune components. Antimicrobial peptides (AMPs) have been

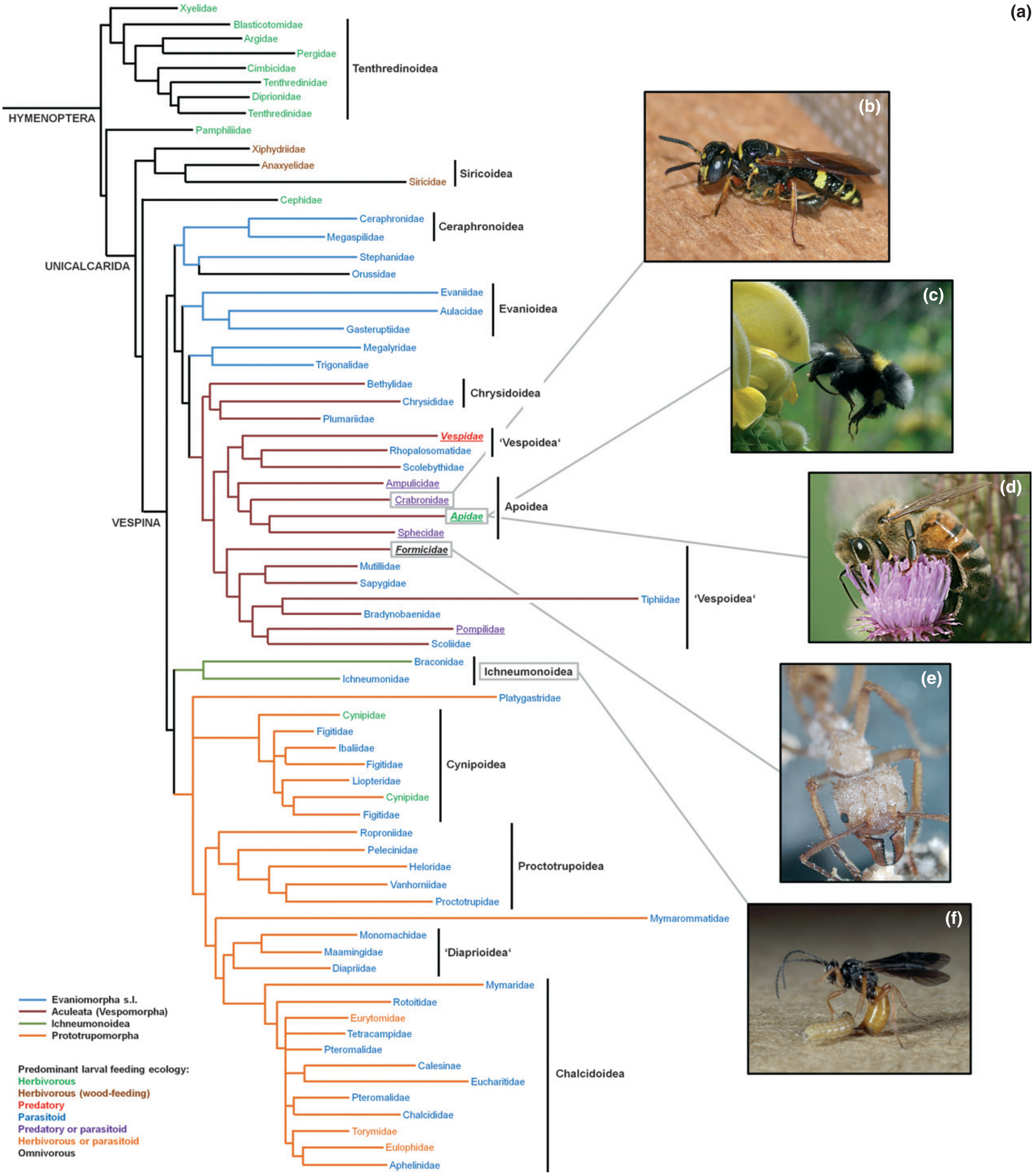

Fig. 1. Defensive microbial symbioses in Hymenoptera. (a) Phylogenetic tree of major hymenopteran families (modified from Heraty *et al.* 2011; with permission). Branches are colour-coded according to supergroups. Colouring of family names indicates the predominant feeding ecology of the larvae (green = herbivorous, brown = wood feeding, red = predatory, blue = parasitoid, purple = predatory or parasitoid, orange = herbivorous or parasitoid, black = omnivorous). Names of families containing social species are highlighted in bold italics, and those containing mass-provisioning species are underlined. (b–f) Hymenopteran families or superfamilies that are known to contain taxa with defensive microbial symbionts. (b) Beewolves (here: *Philanthus gibbosus* with halictid bee as prey) cultivate '*Candidatus* Streptomyces philanthi' in antennal gland reservoirs and on the larval cocoon, where the symbionts produce antibiotics and thereby provide protection for the larva against pathogenic fungi. (c) A betaproteobacterial symbiont in bumblebees (*Bombus terrestris*) has been implicated in the defence against the gut parasite *Crithidia bombi*. (d) The microbial community associated with honeybees (*Apis mellifera*) plays an important role for the protection against fungal and bacterial pathogens (picture from Kaltenpoth 2011). (e) On specific regions of their cuticle, *Acromyrmex octospinosus* and other leaf-cutting ants grow *Pseudonocardia* bacteria for the defence of their fungus gardens (picture kindly provided by Michael Poulsen). (f) *Microplitis demolitor* (Braconidae) and other parasitoid wasps within the families Braconidae and Ichneumonidae are associated with symbiotic viruses that are injected into the host along with the parasitoid egg and protect the developing parasitoid from the host's immune system (picture kindly provided by Michael Strand).

found in both the epicuticle and the endocuticle, and the latter additionally contains phenoloxidase, an enzyme involved in the sclerotization process but also in the defence against invading pathogens (Siva-Jothy, Moret & Rolff 2005).

Once the cuticle is breached, the insect's immune system must recognize the invading pathogen. Pathogen-associated molecular patterns (PAMPs) like lipopolysaccharides, peptidoglycan, beta-1,3-glucans and mannans are recognized by a set of receptor proteins (Schmid-Hempel 2005; Siva-Jothy, Moret & Rolff 2005). These activate the humoral and cellular immune responses, which include opsonization, phagocytosis, melanization, encapsulation, coagulation, the production of reactive oxygen and nitrogen species, AMPs and proteins with lytic activities (Schmid-Hempel 2005). These processes attack or isolate and – if successful – ultimately kill the invading pathogen or parasite.

In addition to the cuticle, there are three main entry routes for pathogens and parasites into the insect's body (Siva-Jothy, Moret & Rolff 2005): the digestive system, the reproductive tract and the tracheae. In all three organs, the cuticle is partially very thin or completely absent, because there is a trade-off between protective function and permeability for nutrients and gases. Although the gut is still protected by a thin cuticular layer, which can also be sloughed to remove attached pathogens, it is especially prone to microbial infestation as it constantly comes into contact with microbes that are ingested with the food. Consequently, the gut epithelium is immunologically very active. Several defensive compounds like defensins, Gram-negative binding proteins, chitinase-like proteins, serine proteases and lectin-like proteins as well as phenoloxidase and small cytotoxic molecules like nitric oxide, radical hydroxide and peroxide are produced for defence against pathogens and parasites (Siva-Jothy, Moret & Rolff 2005). The epithelium of the tracheae seems to be similarly active, and haemocytes are intimately associated with these tissues (Siva-Jothy, Moret & Rolff 2005). Likewise, different mechanisms have been described that protect the genitalia from infections, for example, AMPs in the seminal fluid of fruit flies (*Drosophila melanogaster*; Lung, Kuo & Wolfner 2001) and bed bugs (*Cimex lectularius*; Otti *et al*. 2009).

As social insects live in large colonies that facilitate the transmission of pathogens and thereby increase susceptibility to infections, many social taxa have evolved specialized mechanisms to prevent pathogens from spreading within their colonies, which are collectively referred to as 'social immunity'. Hygienic behaviours, like allogrooming, control of individuals entering the nest, separating groups with different tasks and infection risks, and waste control reduce the risk of pathogen infestation in the colony (Cremer & Sixt 2009). Furthermore, ants, termites, bees and wasps apply antimicrobial substances to colony members (e.g. Baracchi, Francese & Turillazzi 2011) or incorporate materials that contain such compounds into their nests (e.g. Batra 1980; Rosengaus, Guldin & Traniello 1998; Chapuisat *et al*. 2007).

Protection of the adult insect against pathogens and parasites

In addition to the host's own defences, several insect taxa are known to team up with symbiotic micro-organisms for protection. There are three main mechanisms by which symbionts can provide protection: (i) symbiotic micro-organisms can produce chemical compounds that have direct harmful effects on antagonists, (ii) they can colonize vulnerable niches in or on the host and competitively exclude pathogens from successfully establishing an infection or (iii) they can interact with the host immune system and thereby enhance resistance to pathogens or parasites.

The production of antimicrobial compounds is the most common way by which mutualistic microbes participate in the protection of adult insects. As preventive measures, those antimicrobial compounds can act even before the pathogens come in contact with or enter the insect body. Attine ants use not only the secretion from their metapleural glands (Ortius-Lechner *et al*. 2000; Bot *et al*. 2002) but also antibiotics produced by symbiotic Actinobacteria growing on their cuticle for pathogen defence (Oh *et al*. 2009a; Mattoso, Moreira & Samuels 2012). Both the gland secretions and the symbiont-produced antimicrobial compounds protect their fungal gardens from parasitic fungi (see below), but also provide protection for the adult ants themselves against the entomopathogenic fungus *Metarhizium anisopliae* (Mattoso, Moreira & Samuels 2012).

Alternatively, symbionts can modulate the host's immune system in a way that enhances the efficiency of protection against pathogens. The immune system is generally stimulated by contact with low levels of pathogenic bacteria (Evans & Lopez 2004) or fungi (Konrad *et al*. 2012). Previously pathogen-exposed individuals show an activated immune system upon second exposure, which leads to a more efficient defence against the pathogen (Konrad *et al*. 2012). Interestingly, the immune system can not only be primed by pathogens, but also by the presence of symbionts: the encapsulation response of *Camponotus fellah* ants increases significantly with a higher number of intracellular *Blochmannia* bacteria as compared to ants which have been treated with antibiotics to eliminate their symbionts (De Souza *et al*. 2009). Likewise, probiotic *Lactobacillus* bacteria induce the expression of the antibacterial peptide abaecin in honeybee (*Apis mellifera*) larvae (Evans & Lopez 2004). Thus, symbiotic bacteria can activate the immune system of the insect host and thereby increase the efficiency of pathogen defence.

It has long been hypothesized that an important function of the native gut microbiota of insects is to competitively inhibit pathogens from colonizing the gut (Berg 1996; Dillon & Dillon 2004), especially as the gut represents one of the most sensitive entry routes for pathogens (Siva-Jothy, Moret & Rolff 2005). The first empirical support for this hypothesis came from studies on the locust *Schistocerca gregaria* (Dillon & Charnley 1995). Locust faeces contain at least three different quinines, which significantly inhibit the germination of *M. anisopliae* conidiae (Dillon & Charnley 1995). These quinines are absent from germ-free locusts, but present in individuals that are infected exclusively with the dominant gut microbe *Pantoea agglomerans* (Dillon & Charnley 1995), indicating that the protective quinines are produced by this bacterial symbiont.

In Hymenoptera, we are only beginning to understand the composition of microbial gut communities and their possible importance for the host. Due to the recent concerns about the decline of honeybee populations and the concomitant decrease in pollinating services, the microbiota of social bees and its significance for colony health have been subject to especially intense research efforts. Compared to other Hymenoptera and Diptera, honeybees possess a strongly reduced set of immune-related genes (Evans *et al.* 2006), which may be compensated by the presence of protective microbial symbionts, a strategy that has also been suggested for the pea aphid (Moran *et al.* 2005; Gerardo *et al.* 2010). Additionally, the low number of genes encoding lysozyme (Kunieda *et al.* 2006) and the low expression of lysozyme in the gut epithelium (Anderson *et al.* 2011) may support the establishment of a distinct gut microbiota that takes part in immune defence. In both honeybees and bumblebees, a consistent gut microbiota comprising about nine different phylotypes has been found across geographical regions (Gilliam 1997; Cox-Foster *et al.* 2007; Martinson *et al.* 2011).

In bumblebees (*Bombus terrestris*), a betaproteobacterial symbiont in the gut has been implicated in the resistance against the intestinal trypanosomatid parasite *Crithidia bombi*. The bacteria are transferred between nestmates via the faeces, but the mechanisms underlying the protective activity against *C. bombi* are not known yet (Koch & Schmid-Hempel 2011). In honeybees, some evidence for protective activities of the gut microbiota was provided by a series of *in vitro* and *in vivo* studies. Specifically, several lactic acid bacteria (Forsgren *et al.* 2010) as well as *Escherichia coli*, *Providencia* and *Sphingomonas* strains isolated from the honeybee gut (Yoshiyama & Kimura 2009) were found to have inhibitory effects on the brood pathogen *Paenibacillus larvae* and to thereby significantly enhance the survival probability of larvae in infected colonies (Forsgren *et al.* 2010). Other microbial symbionts in honeybee hives, notably strains of the genus *Bacillus*, have been implicated in the defence against fungal pathogens, especially *Ascosphaera apis*, the causative agent of chalkbrood. As *Bacillus* strains have been repeatedly isolated from various bee species including 25 to 40-million-year-old amber-preserved specimens (Cano *et al.* 1994; Cano & Borucki 1995), an intimate association between bees and protective *Bacillus* symbionts has been hypothesized (Gilliam 1997). It has to be noted, however, that the mechanistic basis of the protective activity conferred by both the lactic acid bacteria and the *Bacillus* strains remains largely unknown, although the involvement of organic acids as well as AMPs and fatty acids has been hypothesized (Gilliam 1997; Vasquez *et al.* 2012).

In addition to the gut of a single bee, the entire hive can be a source of symbionts providing resistance or defence (Cremer & Sixt 2009). Concordantly, bacteria with the ability to inhibit bee pathogens have been isolated throughout the bee hive (Gilliam *et al.* 1988; Anderson *et al.* 2011), and Anderson *et al.* (2011) even hypothesized that there might be a sub-caste of bees that is best-suited to nurture the symbiotic bacteria for the aid in food preservation and protection from disease. The bacterial community in bee hives is continuously exchanged between the adult individuals, stored food and larvae, and it is subjected to an incoming flow of bacteria from the environment. In addition, not only bacterial but also fungal symbionts with bioactive potential have been reported: Mucorales, but also Aspergilli and Penicilli, inhibit the highly pathogenic fungus *Nosema apis* (Gilliam *et al.* 1988). Thus, the social bees represent a complex symbiotic system whose individual components are only beginning to be explored. Similar mechanisms of pathogen defence by a beneficial gut microbiota can be expected to occur in the other social hymenopteran communities of wasps and ants, where first studies already provided evidence for stable gut communities associated with certain taxa (in ants: Anderson *et al.* 2012).

Protection of the developing offspring against pathogens

Juvenile stages of insects are often more susceptible to pathogen infection than adults, because some developmental stages are immobile or not able to protect themselves due to the lack of physical defences, an incompletely developed immune system or limited resources that need to be apportioned to both growth and immunity. In addition to defensive chemicals provided by parents, nestmates or the developing insect itself, the offspring of social insects is usually tended by a special sub-caste of workers that is separated from individuals which have more contact to pathogens and therefore pose a higher infection risk (Cremer, Armitage & Schmid-Hempel 2007). Furthermore, the microbial community associated with social insects and their nests can contribute significantly to pathogen defence. As indicated above, the microbiota of bees has been implicated in the protection of honeybee larvae against several specialized bee pathogens (Forsgren *et al.* 2010; Vasquez *et al.* 2012).

However, to our knowledge, the only specific symbiotic protection of hymenopteran offspring has been described for solitary beewolves of the genera *Philanthus, Trachypus* and *Philanthinus* (Crabronidae) (Kaltenpoth *et al.* 2005, 2010b, 2012). Adult females cultivate the Actinobacterium '*Candidatus* Streptomyces philanthi' in specialized antennal gland reservoirs (Goettler *et al.* 2007). In their underground nests, they secrete the bacteria into the larval brood cells (Strohm & Linsenmair 1995; Kaltenpoth *et al.* 2010a). Around a week later, when the larva has fed on the stored provisions and starts to spin its cocoon, it takes the bacteria up from the brood cell and incorporates them into the cocoon silk (Kaltenpoth *et al.* 2010a). Within the first two weeks after cocoon spinning, the *Streptomyces* bacteria produce a cocktail of at least nine different antibiotic substances on the cocoon surface (Kroiss *et al.* 2010). These compounds provide an efficient protection to the beewolf larva against microbial infestation during the long and vulnerable phase of hibernation (Kaltenpoth *et al.* 2005; Kroiss *et al.* 2010; Koehler, Doubský & Kaltenpoth 2013). The symbionts of various beewolf species form a monophyletic group within the genus *Streptomyces*, suggesting an intimate and highly specific symbiotic association (Kaltenpoth *et al.* 2006, 2010b, 2012). However, recent studies also provide evidence for horizontal transmission of symbionts among host species, as phylogenetic analyses of streptomycetes among Philanthini neither showed the expected placement of *Philanthinus* symbionts at the root of the beewolf symbiont clade (Kaltenpoth *et al.* 2012), nor a monophyletic clade of *Trachypus* bacteria (Kaltenpoth *et al.* 2010b).

Many other social and solitary Hymenoptera (as well as a multitude of other arthropods) develop within the soil, but for most taxa, it is currently unknown how the developing offspring is protected against pathogenic micro-organisms. Since eggs, larvae and pupae generally provide a rich source of nutrients for competing and pathogenic bacteria and fungi that are ubiquitous in the soil (Janzen 1977; Keller & Zimmermann 1989), it seems likely that protective alliances with antibiotic-producing micro-organisms are much more common than is currently recognized.

Brood care and the protection of nutritional resources

Many female hymenopterans provide brood care to their offspring. This is not only true for social bees (Apidae, Halictidae), wasps (Vespidae) and ants (Formicidae), but also for many solitary species within the Apoidea, Vespidae, Eumenidae, Masaridae, Sphecidae, Ampulicidae, Crabronidae and Pompilidae (Gauld & Bolton 1988). Provisioning behaviours vary in their complexity across Hymenoptera, with mass provisioning being the most common type among nonsocial species as well as in some eusocial bees (Halictidae, Xylocopinae and Meliponinae), in which a brood cell is supplied with enough resources for the complete larval development and closed after oviposition. Progressive provisioning involves the mother accessing the brood cell and supplying resources to the larva during development, which occurs in a small proportion of several solitary wasp families and in most eusocial taxa (Field 2005). The most complex forms of provisioning can be found in social Hymenoptera, most of which collect the food resources for their offspring and store them in sophisticated nests (Wilson 1971). Notably, leaf-cutting ants are among the few animals that evolved an active form of agriculture, growing fungal cultivars on leaf material and using them as nourishment for the developing offspring as well as for the adult ants (Hölldobler & Wilson 1990).

All insects that store nutritional resources for their offspring have to cope with competing micro-organisms that could not only devour the provisions but also infest the developing offspring. Most social species actively tend the provisions and remove microbial contamination by biting off fungal hyphae and removing contaminated materials (e.g. Currie & Stuart 2001). Additionally, many solitary as well as social Hymenoptera have evolved chemical defences to reduce the risk of microbial infestation. For example, colletid and halictid bees apply a dense lining to their nest, which may inhibit the invasion of detrimental micro-organisms (Batra 1968, 1980), and metapleural gland secretions of leaf-cutting ant workers have antimicrobial activity and thereby probably contribute to the protection of the fungus garden from pathogenic and competing microbes (Ortius-Lechner *et al.* 2000; Bot *et al.* 2002). Similarly, the jewel wasp *Ampulex compressa* sanitizes its cockroach prey with a mixture of antimicrobial compounds (mellein and micromolide) to prevent pathogen infestation (Herzner *et al.* 2013). The solitary European beewolf and related digger wasps apply a secretion mainly consisting of long-chain unsaturated hydrocarbons from a specialized head gland directly to their paralysed prey (Herzner *et al.* 2007; Strohm *et al.* 2008), which prevents water condensation and thereby delays the onset of fungal germination (Strohm & Linsenmair 2001; Herzner & Strohm 2007). However, instead of – or in addition to – producing such protective chemical cocktails themselves, some insects engage in symbioses with micro-organisms that serve to ward off detrimental microbes (Kaltenpoth 2009).

In honeybees, a healthy microbial community is essential for the well-being of a colony and its defence against pathogens. While we already discussed the role of microbial symbionts for the protection of the adult and larval honeybees themselves, mutualistic bacteria are also important for the protection of the bees' food resources from spoilage. It is well established that propolis and honey of honeybees (Simone, Evans & Spivak 2009; Simone-Finstrom & Spivak 2010; Kwakman & Zaat 2012) as well as stingless bees (Temaru *et al.* 2007; Umthong, Puthong & Chanchao 2009) have antimicrobial properties, and some bees line the nest walls with propolis, possibly to reduce microbial infections (Anderson *et al.* 2011). Interestingly, a community of lactic acid bacteria (*Lactobacillus* and *Bifidobacterium*) has been repeatedly isolated from the honey crop as well as the propolis of different honeybee species (Vasquez *et al.* 2012). These bacteria as well as other microbial partners appear to be involved in the fermentation of the bee bread, which plays an important role for the preservation of the food resources (Gilliam 1997;

Vasquez & Olofsson 2009). Apart from honeybees, this lactic acid bacterial community is also present in stingless bees (Meliponini) (Vasquez *et al.* 2012), and in lower abundance also in bumblebees (Olofsson & Vasquez 2009).

Arguably the best-studied symbiosis for the defence of nutritional resources in Hymenoptera is the association between leaf-cutting ants and Actinobacteria. The ants' monocultural fungus gardens are prone to infection by a specialized parasitic fungus of the genus *Escovopsis* (Currie, Mueller & Malloch 1999a; Currie 2001). Infestation by this parasite can be detrimental for whole colonies, so there is a high selective pressure on ants to evolve effective defences (Currie, Mueller & Malloch 1999a). In addition to active removal of pathogens (Currie & Stuart 2001) and the use of metapleural gland secretions for antimicrobial defence (Bot *et al.* 2002), the ants are associated with protective Actinobacteria (Currie *et al.* 1999b). In most attine ant genera, bacteria of the genus *Pseudonocardia* grow on species-specific regions of the cuticle that are probably supplied with nutrients from underlying exocrine glands (Currie *et al.* 2006). *In vitro*, the *Pseudonocardia* symbionts have been demonstrated to produce antimicrobial compounds (Oh *et al.* 2009a; Barke *et al.* 2010; Carr *et al.* 2012) that inhibit the growth of *Escovopsis* (Currie *et al.* 1999b; Poulsen *et al.* 2010; Cafaro *et al.* 2011). Although the *Pseudonocardia*-produced antibiotics have not yet been detected *in vivo*, bioassays provided evidence that the bacteria enhance the fitness of ant colonies by suppressing the growth of the parasitic fungus (Currie, Bot & Boomsma 2003). Interestingly, the symbiont-produced bioactive compounds can also impair the growth of the ants' cultivar fungus *in vitro*, but this does not appear to have any negative effects on fungus garden biomass *in vivo* (Poulsen & Currie 2010).

Despite intensive research on the multipartite leaf-cutting ant symbiosis, the specificity and evolutionary history of the ant-*Pseudonocardia* association remain controversial (Mueller 2012). While Poulsen *et al.* (2005) found little or no genetic variation among *Pseudonocardia* isolates within colonies of *Acromyrmex octospinosus* and *A. echinatior*, another study reported multiple *Pseudonocardia* strains in individual colonies of *Trachymyrmex septentrionalis* (Ishak *et al.* 2011). Broader phylogenetic studies across leaf-cutting ants and their *Pseudonocardia* symbionts revealed some degree of specificity, but also frequent horizontal transmission and *de novo* uptake of symbionts from the environment (Cafaro & Currie 2005; Mueller *et al.* 2008, 2010; Cafaro *et al.* 2011), suggesting that partner choice plays an important role in maintaining the association over evolutionary timescales. Concordantly, bioassays with four *Acromyrmex* species indicated that the ants can differentiate their native microbial symbiont from other *Pseudonocardia* strains (Zhang, Poulsen & Currie 2007). Additionally, there is some evidence that the ants prefer closely related over phylogenetically more distant *Pseudonocardia* strains, regardless of whether the bacteria were previously ant associated or free living (Poulsen *et al.* 2011a). In an attempt to reconcile the contrasting concepts of partner choice and partner fidelity in the ant-*Pseudonocardia* system, Scheuring & Yu (2012) developed a mathematical model demonstrating that nutrient-rich provisioning by the host can selectively favour antibiotic-producing bacterial symbionts by stimulating competition among a community of acquired microbes. Although this model still needs to be tested empirically, it might provide an elegant explanation for the prevalence of both vertical transmission and horizontal exchange of symbionts in the leaf-cutting ant-*Pseudonocardia* association as well as in the beewolf-*Streptomyces* symbiosis.

In addition to *Pseudonocardia*, several other actinobacterial taxa, including members of the genus *Streptomyces* (Kost *et al.* 2007; Haeder *et al.* 2009; Barke *et al.* 2010; Schoenian *et al.* 2011; Zucchi, Guidolin & Consoli 2011) and *Amycolatopsis* (Sen *et al.* 2009), as well as a betaproteobacterial *Burkholderia* sp. (Santos *et al.* 2004) have also been discovered in attine ant nests. Although the importance of this diverse microbial community for ant fitness is not yet clear, the presence of *Streptomyces*-produced antibiotics *in situ* on the cuticle of ant workers suggests that they may also contribute to nest hygiene (Schoenian *et al.* 2011). Interestingly, as in the beewolf-*Streptomyces* symbiosis (Kroiss *et al.* 2010), ant-associated bacteria appear to produce multiple antimicrobial compounds (Barke *et al.* 2010; Schoenian *et al.* 2011; Seipke *et al.* 2011). From *Pseudonocardia* isolates of different ant species, dentigerumycin (Oh *et al.* 2009a), five angucyclines (Carr *et al.* 2012) and a nystatin-like compound (Barke *et al.* 2010) have been isolated, and ant-associated *Streptomyces* species have been found to produce candicidin (Haeder *et al.* 2009; Barke *et al.* 2010) as well as valinomycin, antimycins and actinomycins (Schoenian *et al.* 2011). Thus, both beewolves and leaf-cutting ants may employ a strategy that is comparable to the combination prophylaxis used in human medicine to ward off detrimental micro-organisms (Kroiss *et al.* 2010).

Similar defensive symbioses as in the attine ants have been suggested for two other fungus-growing insect taxa: a recent study isolated antibiotic-producing *Streptomyces* and *Amycolatopsis* species from a nonattine ant genus (*Allomerus*) (Seipke *et al.* 2012a). These ants are associated with a fungal cultivar that is a major component of gallery-like structures that are used to trap prey (Dejean *et al.* 2005; Ruiz-Gonzalez *et al.* 2011). Although specificity, prevalence and function of the association between *Allomerus* ants and Actinobacteria are not known yet, the bacterial symbionts may be involved in the protection of the fungal galleries against pathogenic fungi. Outside of the order Hymenoptera, fungus-growing pine beetles (*Dendroctonus frontalis*) are associated with diverse *Streptomyces* bacteria (Scott *et al.* 2008; Hulcr *et al.* 2011). One isolate was found to produce a compound termed mycangimycin, which inhibits the growth of fungal competitors of the beetles' cultivar (Oh *et al.* 2009b). In termites, the third large group of fungus-growing insects, defensive symbionts have not been discovered yet, despite targeted efforts directed at detecting specific actinobacterial mutualists with protective activities (Visser *et al.* 2011). Likewise, it is currently unknown whether micro-organisms play a role in other wood-dwelling insects that live in close association with nutritional fungi, for example, wood wasps of the genus *Sirex*.

As many Hymenoptera provide stored provisions for their offspring over more or less extensive time periods, it is tempting to speculate that microbes present a widespread solution to the risk of fungal infestation of mass provisions, especially in social insects that live in big colonies and store large amounts of food. However, attine ants currently constitute the only well-documented case of a symbiosis for the protection of the nutritional resources, with the *Allomerus* ants representing a possible second defensive symbiotic system (Seipke *et al.* 2012a). As similar symbionts need not necessarily be associated with the insect itself and could instead be present only in the nest material, their discovery is challenging and requires large-scale metagenomic or culture-based analyses of insects and their nests. A recent study by Poulsen *et al.* (2011b) reported on the isolation of a large number of *Streptomyces* strains (Actinobacteria) from two solitary wasp species (*Sceliphron caementarium* and *Chalybion californicum*, Hymenoptera, Sphecidae). The isolated Actinobacteria produced a range of bioactive compounds, but their functional role *in vivo* remained enigmatic (Oh *et al.* 2011; Poulsen *et al.* 2011b). Other previous efforts to discover defensive symbionts in insects have also focused on Actinobacteria (e.g. Visser *et al.* 2011), as their abundance in the soil, their metabolic versatility and their ability to produce a wide range of antimicrobial secondary metabolites probably predispose them towards engaging into protective symbiotic interactions with soil-living insects (Kaltenpoth 2009; Seipke, Kaltenpoth & Hutchings 2012b). However, after identifying Actinobacteria in nest material or associated with an insect and demonstrating their antimicrobial activity, efforts need to be directed at elucidating their function *in vivo* and the specificity of the association with the host.

Defensive symbiosis in host-parasitoid interactions

Symbiotic bacteria can not only provide protection against pathogenic micro-organisms, but also against eukaryotic parasitoids (Oliver *et al.* 2003). An interesting example is *Hamiltonella defensa* in pea aphids (*Acyrthosiphon pisum*) and black bean aphids (*Aphis fabae*). This gamma-proteobacterial secondary symbiont confers protection against parasitic wasps (Oliver *et al.* 2003), probably by producing a toxin that targets and kills the developing wasp larva in the aphid (Moran *et al.* 2005; Degnan *et al.* 2009). Interestingly, the toxin genes are located on a bacteriophage rather than in the *Hamiltonella* genome itself, and phage-free *Hamiltonella* clones fail to provide protection (Moran *et al.* 2005; Degnan & Moran 2008b; Oliver *et al.* 2009, 2010). Thus, the three-partite association between aphid, bacterium and phage is necessary for successful defence against a parasitoid wasp, and the efficiency of the protection depends on a complex interaction of host, symbiont, phage and parasitoid genome (Degnan & Moran 2008a; Oliver *et al.* 2009; Sandrock, Gouskov & Vorburger 2010; Schmid *et al.* 2012). Recently, several other secondary symbionts of insects have also been implicated in the protection of the host against parasitoid wasps, that is, *Regiella insecticola* in aphids (Vorburger, Gehrer & Rodriguez 2010; Hansen, Vorburger & Moran 2012), *Spiroplasma* in *Drosophila hydei* (Xie, Vilchez & Mateos 2010; Xie *et al.* 2011) and, based on correlational evidence, *Arsenophonus* in a psyllid (Hansen *et al.* 2007).

To our knowledge, microbial anti-parasitoid defence has not yet been described in Hymenoptera, although many of the solitary, social and even of the parasitic taxa are threatened by dipteran or hymenopteran parasitoids. However, an intriguing example of a defensive symbiosis between the potter wasp *Allodynerus delphinalis* and the mite *Ensliniella parasitica* has been reported recently (Okabe & Makino 2008). The wasps house and transport mites in specialized structures called acarinaria (Makino & Okabe 2003). The symbiotic mites attack parasitoids that try to oviposit into the pupal or prepupal potter wasps. By biting and clinging to the parasitoid, they significantly reduce parasitization success and thereby enhance their hosts' – and thus also their own – survival probability (Okabe & Makino 2008). As acarinaria are present in several hymenopteran taxa (i.e. in Eumeninae and Xylocopinae) (Makino & Okabe 2003; Klimov, Vinson & Oconnor 2007), it is possible that symbiotic mites constitute a more widespread defence against parasitoids in solitary Hymenoptera.

Interestingly, in host-parasitoid interactions, not only the host, but also the parasitoid can team up with protective symbionts. Developing endoparasitoids generally have to survive in an extremely hostile environment, as the cellular immune system of an insect host has evolved to recognize, encapsulate and ultimately kill eukaryotic intruders (Strand & Pech 1995). Thus, to survive, the parasitoid needs to evade or suppress the host's immune response. Certain groups of ichneumonid and braconid wasps have independently evolved an especially intriguing mechanism to solve this problem: they are associated with symbiotic polydnaviruses and inject them into the host during oviposition (Edson *et al.* 1981; Fleming 1992; Strand & Pech 1995; Strand 2010; Beckage & Drezen 2012; Strand & Burke 2012). While related nonsymbiotic viruses replicate within the host tissue and cause pathological effects, the mutualistic viruses of Braconidae (bracoviruses) only produce progeny virions in specialized calyx cells in the wasp ovaries (Strand 2010; Strand & Burke 2012). Upon oviposition of the parasitoid egg into the lepidopteran host, the virions deliver genes with immunosuppressive function that enhance parasitoid survival (Edson *et al.* 1981; Fleming 1992; Thoetkiattikul, Beck & Strand 2005). Expression of virus-transferred genes can induce apoptosis or clumping of host haemocytes or inhibit phenoloxidase activity and thereby protect the developing parasitoid against the host's immune system (Beckage 1998, 2012). Additionally, the virus can affect metabolic and developmental processes to the advantage of the parasitoid (Fleming 1992). The association between Braconidae and bracoviruses evolved around 100 million years ago from a nudiviral ancestor (Whitfield 2002; Murphy *et al.* 2008; Bezier *et al.* 2009) and subsequently experienced codiversification of hosts and symbionts (Whitfield & Asgari 2003). The long co-evolutionary history has led to a high degree of integration, and the symbiotic viruses are more reminiscent of cell organelles like mitochondria and chloroplasts rather than representing independent entities (Whitfield & Asgari 2003). In addition to the polydnaviruses of Braconidae and Ichneumonidae, entomopoxviruses, ascoviruses, cypoviruses, as well as the unclassified *Leptopilina boulardi* filamentous virus have been found to enhance the survival of certain parasitoid wasps by suppressing the host immune system (Bigot *et al.* 1997; Lawrence 2005; Renault *et al.* 2005; Martinez *et al.* 2012), indicating that protective symbiotic associations with viruses may be a widespread and common phenomenon in parasitoid wasps.

The examples of aphids and Braconidae/Ichneumonidae demonstrate that symbiotic micro-organisms can affect host-parasitoid interactions in intricate ways, either by defending the host against parasitoid attack or by protecting the parasitoid against the host's immune system. As a large number of hymenopteran taxa are parasitic, defensive symbioses with bacteria, fungi or viruses are likely to play an important ecological and evolutionary role in this insect order by shaping the outcome of the ongoing arms race between hosts and parasitoids.

Protection against predators

To our knowledge, only a single case of bacteria-provided anti-predator defence has thus far been reported in insects. Staphylinid beetles of the genus *Paederus* harbour *Pseudomonas* symbionts and transfer them vertically to their offspring via the egg shell (Kellner 2003). The symbiont genome encodes a mixed polyketide synthase/nonribosomal peptide synthetase gene cluster (Piel 2002) that is responsible for the production of the toxin pederin (Kellner 2001, 2002), which deters wolf spiders as potential predators of the beetle larvae (Kellner & Dettner 1996). Incidentally, the toxin also causes severe cutaneous problems for humans that happen to come into contact with the beetles. As the molecular pathways for the synthesis of noxious chemicals used to deter predators and their eukaryotic origin are unknown in many cases, it is conceivable that other insects including Hymenoptera employ bacterial symbionts for their own protection against predators.

Conclusions and future perspectives: Where and how to look for novel defensive symbioses in Hymenoptera?

Symbiotic micro-organisms can provide protection to insects or their nutritional resources against pathogens, parasites, parasitoids or predators. The Hymenoptera are an especially interesting order in which to investigate such relationships, due to the large diversity of different lifestyles and the ecological and economical relevance of many taxa, specifically the social ants, bees and wasps. To date, only a limited number of defensive alliances involving Hymenoptera have been described, but it seems likely that many associations have so far been overlooked. The leaf-cutting ants and their exosymbiotic antibiotic-producing *Pseudonocardia* bacteria provide a prominent example for a symbiosis that has long awaited discovery, despite the facts that these ants have been studied intensively for decades and the symbionts of several species are easily visible with the unaided eye. The conspicuous white coating on species-specific regions of the cuticle was assumed to be a waxy layer until Currie and colleagues investigated it in detail by scanning electron microscopy and identified it as a dense cover of actinobacterial symbionts (Currie *et al.* 1999b). Similarly, the protective symbionts of beewolves were discovered only recently (Kaltenpoth *et al.* 2005), although the symbiont-containing antennal gland secretion has been described in the 1990s (Strohm & Linsenmair 1995), and the gland reservoirs themselves had been discovered even earlier. Likewise, virtually all other defensive symbionts in insects have been described only during the last one or two decades.

Why are defensive symbioses so much more elusive than nutritional ones, for which numerous examples across most major insect orders have already been known since the seminal work of Paul Buchner (1965)? In our view, the reasons for this are at least threefold. First and foremost, defensive symbionts can be localized in unexpected places within or on the host's body (e.g. Currie *et al.* 2006; Goettler *et al.* 2007). While nutritional symbionts are usually located in close association of the digestive tract or in specialized abdominal bacteriomes, defensive symbionts have been found as endosymbionts in diverse tissues, for example, in the haemolymph (Oliver *et al.* 2003), in the gut (Koch & Schmid-Hempel 2011), in the antennae (Kaltenpoth *et al.* 2005) or as exosymbionts on the cuticle of insects (Currie *et al.* 1999b), which makes them harder to detect by conventional screening efforts. Second, protective symbionts are often facultative, so their infection rate in any given species can vary (e.g. Oliver *et al.* 2008), which complicates their discovery. And third, the fitness benefits conferred by defensive symbionts may be context dependent. Thus, elimination of a defensive symbiont by antibiotic treatment will not necessarily reveal its functional role and the fitness benefits it confers to the host, if the target antagonist is absent, or if the wrong life stage of the host is investigated. The lack of antagonists may be particularly problematic for the detection of defensive symbioses when studying insect populations in the laboratory. Hence, detailed field studies on the natural history of the organism of interest are necessary to identify antagonistic taxa that may be targets of defensive symbionts and thus should be investigated specifically under controlled laboratory conditions.

The first two issues can in part be overcome by the increasing feasibility and affordability of high-throughput next-generation sequencing technologies. Massively parallel amplicon sequencing of microbial 16S rRNA genes by 454 or Illumina technology can be used to comprehensively characterize microbial communities associated with insects, even if some of the micro-organisms are rare or inconsistently present (e.g. Sudakaran *et al.* 2012). Additionally, transcriptome analyses of nonmodel organisms by RNAseq can yield important insights into the molecular interactions of hosts, symbionts and their antagonists. Screening such high-throughput sequencing data sets may reveal the occurrence of putative defensive symbionts (i.e. taxa from bacterial groups that are known as potent producers of antibiotic compounds, e.g. Actinobacteria, Bacilli and Burkholderiales) or the expression of candidate toxin or antibiotic genes (e.g. polyketide synthase or nonribosomal peptide synthase genes) with possible defensive functions. But although high-throughput sequencing and DNA barcoding provide powerful tools that can also contribute significantly towards understanding animal ecology (e.g. Clare *et al.* 2009), none of these techniques can act as a substitute for detailed observations on the natural history of the study organisms. To find novel defensive symbioses, knowledge on the behaviour and ecology of insects is necessary to identify the most vulnerable life stages that need to be protected, as well as potential enemies against which the host needs to be defended. This information will allow designing bioassays to elucidate the function of putative defensive symbionts, which can be complemented by deep sequencing and chemical analyses of symbiont-produced bioactive compounds. Unfortunately, in most defensive symbioses, the identification of such compounds has so far been restricted to *in vitro* analyses or *in silico* predictions. Efforts should be directed towards identifying the presence and activity of candidate compounds *in situ* or even *in vivo* (Kroiss *et al.* 2010; Schoenian *et al.* 2011), and the development of high-resolution mass-spectrometric techniques (e.g. MALDI imaging, nanoSIMS and DESI imaging) provides the powerful tools that are necessary to localize target substances directly within the host tissue.

Acknowledgements

We would like to thank Erhard Strohm and Michael Poulsen for critical comments on the manuscript, and Michael Strand and Michael Poulsen for providing the pictures of *Microplitis demolitor* and *Acromyrmex octospinosus*, respectively. We gratefully acknowledge financial support from the Max Planck Society and the German Science Foundation (MK, DFG KA2846/2-1).

References

Anderson, K.E., Sheehan, T.H., Eckholm, B.J., Mott, B.M. & Degrandi-Hoffman, G. (2011) An emerging paradigm of colony health: microbial balance of the honeybee and hive (*Apis mellifera*). *Insectes Sociaux*, **58**, 431–444.

Anderson, K.E., Russell, J.A., Moreau, C.S., Kautz, S., Sullam, K.E., Hu, Y.I., Basinger, U., Mott, B.M., Buck, N. & Wheeler, D.E. (2012) Highly similar microbial communities are shared among related and trophically similar ant species. *Molecular Ecology*, **21**, 2282–2296.

Andersson, M. (1984) The evolution of eusociality. *Annual Review of Ecology and Systematics*, **15**, 165–189.

Baracchi, D., Francese, S. & Turillazzi, S. (2011) Beyond the antipredatory defence: Honeybee venom function as a component of social immunity. *Toxicon*, **58**, 550–557.

Barke, J., Seipke, R.F., Grüschow, S., Heavens, D., Drou, N., Bibb, M.J., Goss, R.J.M., Yu, D.W. & Hutchings, M.I. (2010) A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. *BMC Biology*, **8**, 109.

Batra, S.W.T. (1968) Behavior of some social and solitary halictine bees within their nests: a comparative study (Hymenoptera: Halicitidae). *Journal of the Kansas Entomological Society*, **41**, 120–133.

Batra, S.W.T. (1980) Ecology, behavior, pheromones, prarasites and management of the sympatric vernal bees *Colletes inaequalis, Colletes thoracicus* and *Colletes validus*. *Journal of the Kansas Entomological Society*, **53**, 509–538.

Beckage, N.E. (1998) Modulation of immune responses to parasitoids by polydnaviruses. *Parasitology*, **116**, S57–S64.

Beckage, N.E. (2012) Polydnaviruses as endocrine regulators. *Parasitoid Viruses: Symbionts and Pathogens* (eds N.E. Beckage & J.-M. Drezen), pp. 163–168. Academic Press, San Diego.

Beckage, N.E. & Drezen, J.-M. (2012) *Parasitoid Viruses: Symbionts and Pathogens*. Academic Press, San Diego.

Berg, R.D. (1996) The indigenous gastrointestinal microflora. *Trends in Microbiology*, **4**, 430–435.

Bezier, A., Annaheim, M., Herbiniere, J., Wetterwald, C., Gyapay, G., Bernard-Samain, S., Wincker, P., Roditi, I., Heller, M., Belghazi, M., Pfister-Wilhem, R., Periquet, G., Dupuy, C., Huguet, E., Volkoff, A.-N., Lanzrein, B. & Drezen, J.-M. (2009) Polydnaviruses of braconid wasps derive from an ancestral nudivirus. *Science*, **323**, 926–930.

Bigot, Y., Rabouille, A., Doury, G., Sizaret, P.Y., Delbost, F., Hamelin, M.H. & Periquet, G. (1997) Biological and molecular features of the relationships between *Diadromus pulchellus* ascovirus, a parasitoid hymenopteran wasp (*Diadromus pulchellus*) and its lepidopteran host, *Acrolepiopsis assectella*. *Journal of General Virology*, **78**, 1149–1163.

Bot, A.N.M., Ortius-Lechner, D., Finster, K., Maile, R. & Boomsma, J.J. (2002) Variable sensitivity of fungi and bacteria to compounds produced by the metapleural glands of leaf-cutting ants. *Insectes Sociaux*, **49**, 363–370.

Brownlie, J.C. & Johnson, K.N. (2009) Symbiont-mediated protection in insect hosts. *Trends in Microbiology*, **17**, 348–354.

Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms*. Interscience Publishers, New York.

Cafaro, M.J. & Currie, C.R. (2005) Phylogenetic analysis of mutualistic filamentous bacteria associated with fungus-growing ants. *Canadian Journal of Microbiology*, **51**, 441–446.

Cafaro, M.J., Poulsen, M., Little, A.E.F., Price, S.L., Gerardo, N.M., Wong, B., Stuart, A.E., Larget, B., Abbot, P. & Currie, C.R. (2011) Specificity in the symbiotic association between fungus-growing ants and protective *Pseudonocardia* bacteria. *Proceedings of the Royal Society B-Biological Sciences*, **278**, 1814–1822.

Cane, J.H., Gerdin, S. & Wife, G. (1983) Mandibular gland secretions of solitary bees (Hymenoptera, Apoidea) – potential for nest cell disinfection. *Journal of the Kansas Entomological Society*, **56**, 199–204.

Cano, R.J. & Borucki, M.K. (1995) Revival and identification of bacterial spores in 25-million-year-old to 40-million-year-old Dominican amber. *Science*, **268**, 1060–1064.

Cano, R.J., Borucki, M.K., Higbyschweitzer, M., Poinar, H.N., Poinar, G.O. & Pollard, K.J. (1994) *Bacillus* DNA in fossil bees – an ancient symbiosis? *Applied and Environmental Microbiology*, **60**, 2164–2167.

Carr, G., Derbyshire, E.R., Caldera, E.J., Currie, C.R. & Clardy, J. (2012) Antibiotic and antimalarial quinones from fungus-growing ant-associated *Pseudonocardia* sp. *Journal of Natural Products*, **75**, 1806–1809.

Chapuisat, M., Oppliger, A., Magliano, P. & Christe, P. (2007) Wood ants use resin to protect themselves against pathogens. *Proceedings of the Royal Society B-Biological Sciences*, **274**, 2013–2017.

Clare, E.L., Fraser, E.E., Braid, H.E., Fenton, M.B. & Hebert, P.D.N. (2009) Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology*, **18**, 2532–2542.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., Vanengelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J.H., Cui, L.W., Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S. & Lipkin, W.I. (2007) A metagenomic survey of microbes in honeybee colony collapse disorder. *Science*, **318**, 283–287.

Cremer, S., Armitage, S.A.O. & Schmid-Hempel, P. (2007) Social immunity. *Current Biology*, **17**, R693–R702.

Cremer, S. & Sixt, M. (2009) Analogies in the evolution of individual and social immunity. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**, 129–142.

Currie, C.R. (2001) Prevalence and impact of a virulent parasite on a tripartite mutualism. *Oecologia*, **128**, 99–106.

Currie, C.R., Bot, A.N.M. & Boomsma, J.J. (2003) Experimental evidence of a tripartite mutualism: bacteria protect ant fungus gardens from specialized parasites. *Oikos*, **101**, 91–102.

Currie, C.R., Mueller, U.G. & Malloch, D. (1999a) The agricultural pathology of ant fungus gardens. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 7998–8002.

Currie, C.R. & Stuart, A.E. (2001) Weeding and grooming of pathogens in agriculture by ants. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 1033–1039.

Currie, C.R., Scott, J.A., Summerbell, R.C. & Malloch, D. (1999b) Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. *Nature*, **398**, 701–704.

Currie, C.R., Poulsen, M., Mendenhall, J., Boomsma, J.J. & Billen, J. (2006) Coevolved crypts and exocrine glands support mutualistic bacteria in fungus-growing ants. *Science*, **311**, 81–83.

De Souza, D., Bezier, A., Depoix, D., Drezen, J.-M. & Lenoir, A. (2009) *Blochmannia* endosymbionts improve colony growth and immune defence in the ant *Camponotus fellah*. *BMC Microbiology*, **9**, 29.

Degnan, P.H. & Moran, N.A. (2008a) Diverse phage-encoded toxins in a protective insect endosymbiont. *Applied and Environmental Microbiology*, **74**, 6782–6791.

Degnan, P.H. & Moran, N.A. (2008b) Evolutionary genetics of a defensive facultative symbiont of insects: exchange of toxin-encoding bacteriophage. *Molecular Ecology*, **17**, 916–929.

Degnan, P.H., Yu, Y., Sisneros, N., Wing, R.A. & Moran, N.A. (2009) *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9063–9068.

Dejean, A., Solano, P.J., Ayroles, J., Corbara, B. & Orivel, J. (2005) Arboreal ants build traps to capture prey. *Nature*, **434**, 973–973.

Dillon, R.J. & Charnley, A.K. (1995) Chemical barriers to gut infection in the desert locust – in-vivo production of antimicrobial phenols associated with the bacterium *Pantoea agglomerans*. *Journal of Invertebrate Pathology*, **66**, 72–75.

Dillon, R.J. & Dillon, V.M. (2004) The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology*, **49**, 71–92.

Dowton, M. & Austin, A.D. (2001) Simultaneous analysis of 16S, 28S, COI and morphology in the Hymenoptera: Apocrita – evolutionary transitions among parasitic wasps. *Biological Journal of the Linnean Society*, **74**, 87–111.

Edson, K.M., Vinson, S.B., Stoltz, D.B. & Summers, M.D. (1981) Virus in a parasitoid wasp – suppression of the cellular immune response in the parasitoid's host. *Science*, **211**, 582–583.

Evans, J.D. & Lopez, D.L. (2004) Bacterial probiotics induce an immune response in the honeybee (Hymenoptera: Apidae). *Journal of Economic Entomology*, **97**, 752–756.

Evans, D.L. & Schmidt, J.O. (1990) *Insect Defenses*. State University of New York Press, Albany, NY.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H., Kanost, M., Thompson, G.J., Zou, Z. & Hultmark, D. (2006) Immune pathways and defence mechanisms in honeybees *Apis mellifera*. *Insect Molecular Biology*, **15**, 645–656.

Field, J. (2005) The evolution of progressive provisioning. *Behavioral Ecology*, **16**, 770–778.

Fleming, J. (1992) Polydnaviruses – mutualists and pathogens. *Annual Review of Entomology*, **37**, 401–425.

Forsgren, E., Olofsson, T.C., Vasquez, A. & Fries, I. (2010) Novel lactic acid bacteria inhibiting *Paenibacillus larvae* in honeybee larvae. *Apidologie*, **41**, 99–108.

Gauld, I. & Bolton, B. (1988) *The Hymenoptera*. Oxford University Press, Oxford, UK.

Gerardo, N.M., Altincicek, B., Anselme, C., Atamian, H., Barribeau, S.M., De Vos, M., Duncan, E.J., Evans, J.D., Gabaldon, T., Ghanim, M., Heddi, A., Kaloshian, I., Latorre, A., Moya, A., Nakabachi, A., Parker, B.J., Perez-Brocal, V., Pignatelli, M., Rahbe, Y., Ramsey, J.S., Spragg, C.J., Tamames, J., Tamarit, D., Tamborindeguy, C., Vincent-Monegat, C. & Vilcinskas, A. (2010) Immunity and other defenses in pea aphids, *Acyrthosiphon pisum*. *Genome Biology*, **11**, R21.

Gilliam, M. (1997) Identification and roles of non-pathogenic microflora associated with honey bees. *FEMS Microbiology Letters*, **155**, 1–10.

Gilliam, M., Taber, S., Lorenz, B.J. & Prest, D.B. (1988) Factors affecting development of chalkbrood disease in colonies of honeybees, *Apis mellifera*, fed pollen contaminated with *Ascosphaera apis*. *Journal of Invertebrate Pathology*, **52**, 314–325.

Goettler, W., Kaltenpoth, M., Herzner, G. & Strohm, E. (2007) Morphology and ultrastructure of a bacteria cultivation organ: the antennal glands of female European beewolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). *Arthropod Structure & Development*, **36**, 1–9.

Grissell, E.E. (1999) Hymenopteran biodiversity: some alien notions. *American Entomologist*, **45**, 235–244.

Gross, P. (1993) Insect behavioral and morphological defenses against parasitoids. *Annual Review of Entomology*, **38**, 251–273.

Haeder, S., Wirth, R., Herz, H. & Spiteller, D. (2009) Candicidin-producing *Streptomyces* support leaf-cutting ants to protect their fungus garden against the pathogenic fungus *Escovopsis*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 4742–4746.

Hansen, A.K., Vorburger, C. & Moran, N.A. (2012) Genomic basis of endosymbiont-conferred protection against an insect parasitoid. *Genome Research*, **22**, 106–114.

Hansen, A.K., Jeong, G., Paine, T.D. & Stouthamer, R. (2007) Frequency of secondary symbiont infection in an invasive psyllid relates to parasitism pressure on a geographic scale in California. *Applied and Environmental Microbiology*, **73**, 7531–7535.

Heraty, J., Ronquist, F., Carpenter, J.M., Hawks, D., Schulmeister, S., Dowling, A.P., Murray, D., Munro, J., Wheeler, W.C., Schiff, N. & Sharkey, M. (2011) Evolution of the hymenopteran megaradiation. *Molecular Phylogenetics and Evolution*, **60**, 73–88.

Herzner, G. & Strohm, E. (2007) Fighting fungi with physics: food wrapping by a solitary wasp prevents water condensation. *Current Biology*, **17**, R46–R47.

Herzner, G., Schmitt, T., Peschke, K., Hilpert, A. & Strohm, E. (2007) Food wrapping with the postpharyngeal gland secretion by females of the European beewolf *Philanthus triangulum*. *Journal of Chemical Ecology*, **33**, 849–859.

Herzner, G., Schlecht, A., Dollhofer, V., Parzefall, C., Harrar, K., Kreuzer, A., Pilsl, L. & Ruther, J. (2013) Larvae of the parasitoid wasp *Ampulex compressa* sanitize their host, the American cockroach, with a blend of antimicrobials. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 1369–1374.

Hölldobler, B. & Wilson, E. (1990) *The Ants*. Springer, Heidelberg.

Hulcr, J., Adams, A.S., Raffa, K., Hofstetter, R.W., Klepzig, K.D. & Currie, C.R. (2011) Presence and diversity of *Streptomyces* in *Dendroctonus* and sympatric bark beetle galleries across North America. *Microbial Ecology*, **61**, 759–768.

Ishak, H.D., Plowes, R., Sen, R., Kellner, K., Meyer, E., Estrada, D.A., Dowd, S.E. & Mueller, U.G. (2011) Bacterial diversity in Solenopsis invicta and Solenopsis geminata ant colonies characterized by 16S amplicon 454 pyrosequencing. *Microbial Ecology*, **61**, 821–831.

Janzen, D.H. (1977) Why fruits rot, seeds mold, and meat spoils. *American Naturalist*, **111**, 691–713.

Jurkevitch, E. (2011) Riding the Trojan horse: combating pest insects with their own symbionts. *Microbial Biotechnology*, **4**, 620–627.

Kaltenpoth, M. (2009) Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology*, **17**, 529–535.

Kaltenpoth, M. (2011) Honeybees and bumblebees share similar bacterial symbionts. *Molecular Ecology*, **20**, 439–440.

Kaltenpoth, M., Gottler, W., Herzner, G. & Strohm, E. (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. *Current Biology*, **15**, 475–479.

Kaltenpoth, M., Goettler, W., Dale, C., Stubblefield, J.W., Herzner, G., Roeser-Mueller, K. & Strohm, E. (2006) *'Candidatus* Streptomyces philanthi', an endosymbiotic streptomycete in the antennae of *Philanthus* digger wasps. *International Journal of Systematic and Evolutionary Microbiology*, **56**, 1403–1411.

Kaltenpoth, M., Goettler, W., Koehler, S. & Strohm, E. (2010a) Life cycle and population dynamics of a protective insect symbiont reveal severe bottlenecks during vertical transmission. *Evolutionary Ecology*, **24**, 463–477.

Kaltenpoth, M., Schmitt, T., Polidori, C., Koedam, D. & Strohm, E. (2010b) Symbiotic streptomycetes in antennal glands of the South American digger wasp genus *Trachypus* (Hymenoptera, Crabronidae). *Physiological Entomology*, **35**, 196–200.

Kaltenpoth, M., Yildirim, E., Gürbüz, M.F., Herzner, G. & Strohm, E. (2012) Refining the roots of the beewolf-*Streptomyces* symbiosis: antennal symbionts in the rare genus *Philanthinus* (Hymenoptera, Crabronidae). *Applied and Environmental Microbiology*, **78**, 822–827.

Keller, S. & Zimmermann, G. (1989) Mycopathogens in soil insects. *Insect-Fungus Interactions* (eds N. Wilding, N. Collins, P. Hammond & J. Webber), pp. 240–270. Academic Press, New York.

Kellner, R.L.L. (2001) Suppression of pederin biosynthesis through antibiotic elimination of endosymbionts in *Paederus sabaeus*. *Journal of Insect Physiology*, **47**, 475–483.

Kellner, R.L.L. (2002) Molecular identification of an endosymbiotic bacterium associated with pederin biosynthesis in *Paederus sabaeus* (Coleoptera: Staphylinidae). *Insect Biochemistry and Molecular Biology*, **32**, 389–395.

Kellner, R.L.L. (2003) Stadium-specific transmission of endosymbionts needed for pederin biosynthesis in three species of *Paederus* rove beetles. *Entomologia Experimentalis Et Applicata*, **107**, 115–124.

Kellner, R.L.L. & Dettner, K. (1996) Differential efficacy of toxic pederin in deterring potential arthropod predators of *Paederus* (Coleoptera: Staphylinidae) offspring. *Oecologia*, **107**, 293–300.

Klimov, P.B., Vinson, S.B. & OConnor, B.M. (2007) Acarinaria in associations of apid bees (Hymenoptera) and chaetodactylid mites (Acari). *Invertebrate Systematics*, **21**, 109–136.

Koch, H. & Schmid-Hempel, P. (2011) Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19288–19292.

Koehler, S., Doubský, J. & Kaltenpoth, M. (2013) Dynamics of symbiont-mediated antibiotic production reveal efficient long-term protection for beewolf offspring. *Frontiers in Zoology*, **10**, doi: 10.1186/1742-9994-10-3.

Konrad, M., Vyleta, M.L., Theis, F.J., Stock, M., Tragust, S., Klatt, M., Drescher, V., Marr, C., Ugelvig, L.V. & Cremer, S. (2012) Social transfer of pathogenic fungus promotes active immunisation in ant colonies. *PLoS Biology*, **10**, e1001300.

Kost, C., Lakatos, T., Bottcher, I., Arendholz, W.R., Redenbach, M. & Wirth, R. (2007) Non-specific association between filamentous bacteria and fungus-growing ants. *Naturwissenschaften*, **94**, 821–828.

Kroiss, J., Kaltenpoth, M., Schneider, B., Schwinger, M.-G., Hertweck, C., Maddula, R.K., Strohm, E. & Svatoš, A. (2010) Symbiotic streptomycetes provide antibiotic combination prophylaxis for wasp offspring. *Nature Chemical Biology*, **6**, 261–263.

Kunieda, T., Fujiyuki, T., Kucharski, R., Foret, S., Ament, S.A., Toth, A.L., Ohashi, K., Takeuchi, H., Kamikouchi, A., Kage, E., Morioka, M., Beye, M., Kubo, T., Robinson, G.E. & Maleszka, R. (2006) Carbohydrate metabolism genes and pathways in insects: insights from the honeybee genome. *Insect Molecular Biology*, **15**, 563–576.

Kwakman, P.H.S. & Zaat, S.A.J. (2012) Antibacterial components of honey. *IUBMB Life*, **64**, 48–55.

Lawrence, P.O. (2005) Morphogenesis and cytopathic effects of the *Diachasmimorpha longicaudata* entomopoxvirus in host haemocytes. *Journal of Insect Physiology*, **51**, 221–233.

Lung, O., Kuo, L. & Wolfner, M.F. (2001) *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. *Journal of Insect Physiology*, **47**, 617–622.

Makino, S. & Okabe, K. (2003) Structure of acarinaria in the wasp *Allodynerus delphinalis* (Hymenoptera: Eumenidae) and distribution of deutonymphs of the associated mite *Ensliniella parasitica* (Acari: Winterschmidtiidae) on the host. *International Journal of Acarology*, **29**, 251–258.

Martinez, J., Duplouy, A., Woolfit, M., Vavre, F., O'Neill, S.L. & Varaldi, J. (2012) Influence of the virus LbFV and of *Wolbachia* in a host-parasitoid interaction. *PLoS One*, **7**, e35081.

Martinson, V.G., Danforth, B.N., Minckley, R.L., Rueppell, O., Tingek, S. & Moran, N.A. (2011) A simple and distinctive microbiota associated with honeybees and bumblebees. *Molecular Ecology*, **20**, 619–628.

Mattoso, T.C., Moreira, D.D.O. & Samuels, R.I. (2012) Symbiotic bacteria on the cuticle of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* protect workers from attack by entomopathogenic fungi. *Biology Letters*, **8**, 461–464

Moran, N.A., Degnan, P.H., Santos, S.R., Dunbar, H.E. & Ochman, H. (2005) The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 16919–16926.

Mueller, U.G. (2012) Symbiont recruitment versus ant-symbiont co-evolution in the attine ant-microbe symbiosis. *Current Opinion in Microbiology*, **15**, 269–277.

Mueller, U.G., Gerardo, N.M., Aanen, D.K., Six, D.L. & Schultz, T.R. (2005) The evolution of agriculture in insects. *Annual Review of Ecology Evolution and Systematics*, **36**, 563–595.

Mueller, U.G., Dash, D., Rabeling, C. & Rodrigues, A. (2008) Coevolution between attine ants and actinomycete bacteria: a reevaluation. *Evolution*, **62**, 2894–2912.

Mueller, U.G., Ishak, H., Lee, J.C., Sen, R. & Gutell, R.R. (2010) Placement of attine ant-associated *Pseudonocardia* in a global *Pseudonocardia* phylogeny (Pseudonocardiaceae, Actinomycetales): a test of two symbiont-association models. *Antonie Van Leeuwenhoek*, **98**, 195–212.

Murphy, N., Banks, J.C., Whitfield, J.B. & Austin, A.D. (2008) Phylogeny of the parasitic microgastroid subfamilies (Hymenoptera: Braconidae) based on sequence data from seven genes, with an improved time estimate of the origin of the lineage. *Molecular Phylogenetics and Evolution*, **47**, 378–395.

Oh, D.C., Poulsen, M., Currie, C.R. & Clardy, J. (2009a) Dentigerumycin: a bacterial mediator of an ant-fungus symbiosis. *Nature Chemical Biology*, **5**, 391–393.

Oh, D.C., Scott, J.J., Currie, C.R. & Clardy, J. (2009b) Mycangimycin, a polyene peroxide from a mutualist *Streptomyces* sp. *Organic Letters*, **11**, 633–636.

Oh, D.C., Poulsen, M., Currie, C.R. & Clardy, J. (2011) Sceliphrolactam, a polyene macrocyclic lactam from a wasp-associated *Streptomyces* sp. *Organic Letters*, **13**, 752–755.

Okabe, K. & Makino, S.I. (2008) Parasitic mites as part-time bodyguards of a host wasp. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2293–2297.

Oliver, K.M., Russell, J.A., Moran, N.A. & Hunter, M.S. (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 1803–1807.

Oliver, K.M., Campos, J., Moran, N.A. & Hunter, M.S. (2008) Population dynamics of defensive symbionts in aphids. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 293–299.

Oliver, K.M., Degnan, P.H., Hunter, M.S. & Moran, N.A. (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science*, **325**, 992–994.

Oliver, K.M., Degnan, P.H., Burke, G.R. & Moran, N.A. (2010) Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Annual Review of Entomology*, **55**, 247–266.

Olofsson, T.C. & Vasquez, A. (2009) Phylogenetic comparison of bacteria isolated from the honey stomachs of honeybees *Apis mellifera* and bumblebees *Bombus spp*. *Journal of Apicultural Research*, **48**, 233–237.

Ortius-Lechner, D., Maile, R., Morgan, E.D. & Boomsma, J.J. (2000) Metaplural gland secretion of the leaf-cutter ant *Acromyrmex octospinosus*: new compounds and their functional significance. *Journal of Chemical Ecology*, **26**, 1667–1683.

Otti, O., Naylor, R.A., Siva-Jothy, M.T. & Reinhardt, K. (2009) Bacteriolytic activity in the ejaculate of an insect. *American Naturalist*, **174**, 292–295.

Peters, R.S., Meyer, B., Krogmann, L., Borner, J., Meusemann, K., Schuette, K., Niehuis, O. & Misof, B. (2011) The taming of an impossible child: a standardized all-in approach to the phylogeny of Hymenoptera using public database sequences. *BMC Biology*, **9**, 55.

Piel, J. (2002) A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 14002–14007.

Poulsen, M. & Currie, C.R. (2010) Symbiont interactions in a tripartite mutualism: exploring the presence and impact of antagonism between two fungus-growing ant mutualists. *PLoS One*, **5**, e8748.

Poulsen, M., Cafaro, M., Boomsma, J.J. & Currie, C.R. (2005) Specificity of the mutualistic association between actinomycete bacteria and two sympatric species of *Acromyrmex* leaf-cutting ants. *Molecular Ecology*, **14**, 3597–3604.

Poulsen, M., Cafaro, M.J., Erhardt, D.P., Little, A.E.F., Gerardo, N.M., Tebbets, B., Klein, B.S. & Currie, C.R. (2010) Variation in *Pseudonocardia* antibiotic defence helps govern parasite-induced morbidity in *Acromyrmex* leaf-cutting ants. *Environmental Microbiology Reports*, **2**, 534–540.

Poulsen, M., Maynard, J., Roland, D.L. & Currie, C.R. (2011a) The role of symbiont genetic distance and potential adaptability in host preference towards *Pseudonocardia* symbionts in *Acromyrmex* leaf-cutting ants. *Journal of Insect Science (Tucson)*, **11**, 1–12.

Poulsen, M., Oh, D.C., Clardy, J. & Currie, C.R. (2011b) Chemical analyses of wasp-associated *Streptomyces* bacteria reveal a prolific potential for natural products discovery. *PLoS One*, **6**, e16763.

Renault, S., Stasiak, K., Federici, B. & Bigot, Y. (2005) Commensal and mutualistic relationships of reoviruses with their parasitoid wasp hosts. *Journal of Insect Physiology*, **51**, 137–148.

Rosengaus, R.B., Guldin, M.R. & Traniello, J.F.A. (1998) Inhibitory effect of termite fecal pellets on fungal spore germination. *Journal of Chemical Ecology*, **24**, 1697–1706.

Ruiz-Gonzalez, M.X., Male, P.-J.G., Leroy, C., Dejean, A., Gryta, H., Jargeat, P., Quilichini, A. & Orivel, J. (2011) Specific, non-nutritional association between an ascomycete fungus and *Allomerus* plant-ants. *Biology Letters*, **7**, 475–479.

Sandrock, C., Gouskov, A. & Vorburger, C. (2010) Ample genetic variation but no evidence for genotype specificity in an all-parthenogenetic host-parasitoid interaction. *Journal of Evolutionary Biology*, **23**, 578–585.

Santos, A.V., Dillon, R.J., Dillon, V.M., Reynolds, S.E. & Samuels, R.I. (2004) Ocurrence of the antibiotic producing bacterium *Burkholderia sp.* in colonies of the leaf-cutting ant *Atta sexdens rubropilosa*. *FEMS Microbiology Letters*, **239**, 319–323.

Scheuring, I. & Yu, D.W. (2012) How to assemble a beneficial microbiome in three easy steps. *Ecology Letters*, **15**, 1300–1307.

Schmid, M., Sieber, R., Zimmermann, Y.-S. & Vorburger, C. (2012) Development, specificity and sublethal effects of symbiont-conferred resistance to parasitoids in aphids. *Functional Ecology*, **26**, 207–215.

Schmid-Hempel, P. (2005) Evolutionary ecology of insect immune defenses. *Annual Review of Entomology*, **50**, 529–551.

Schoenian, I., Spiteller, M., Ghaste, M., Wirth, R., Herz, H. & Spiteller, D. (2011) Chemical basis of the synergism and antagonism in microbial communities in the nests of leaf-cutting ants. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 1955–1960.

Scott, J.J., Oh, D.C., Yuceer, M.C., Klepzig, K.D., Clardy, J. & Currie, C.R. (2008) Bacterial protection of beetle-fungus mutualism. *Science*, **322**, 63–63.

Seipke, R.F., Kaltenpoth, M. & Hutchings, M.I. (2012b) *Streptomyces* as symbionts; an emerging and widespread theme? *FEMS Microbiology Reviews*, **36**, 862–876.

Seipke, R.F., Barke, J., Brearley, C., Hill, L., Yu, D.W., Goss, R.J.M. & Hutchings, M.I. (2011) A single *Streptomyces* symbiont makes multiple antifungals to support the fungus farming ant *Acromyrmex octospinosus*. *PLoS One*, **6**, e22028.

Seipke, R.F., Barke, J., Ruiz-Gonzalez, M.X., Orivel, J., Yu, D.W. & Hutchings, M.I. (2012a) Fungus-growing *Allomerus* ants are associated with antibiotic-producing actinobacteria. *Antonie Van Leeuwenhoek*, **101**, 443–447.

Sen, R., Ishak, H.D., Estrada, D., Dowd, S.E., Hong, E. & Mueller, U.G. (2009) Generalized antifungal activity and 454-screening of *Pseudonocardia* and *Amycolatopsis* bacteria in nests of fungus-growing ants. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 17805–17810.

Simone, M., Evans, J.D. & Spivak, M. (2009) Resin collection and social immunity in honey bees. *Evolution*, **63**, 3016–3022.

Simone-Finstrom, M. & Spivak, M. (2010) Propolis and bee health: the natural history and significance of resin use by honey bees. *Apidologie*, **41**, 295–311.

Siva-Jothy, M.T., Moret, Y. & Rolff, J. (2005) Insect immunity: an evolutionary ecology perspective. *Advances in Insect Physiology*, **32**, 1–48.

Strand, M.R. (2010) Polydnaviruses. *Insect Virology* (eds S. Asgari & K.N. Johnson), pp. 171–197. Caister Academic Press, Norfolk, UK.

Strand, M.R. & Burke, G.R. (2012) Polydnaviruses as symbionts and gene delivery systems. *PLoS Pathogens*, **8**, e1002757.

Strand, M.R. & Pech, L.L. (1995) Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology*, **40**, 31–56.

Strohm, E., Laurien-Kehnen, C. & Bordon, S. (2001) Escape from parasitism: spatial and temporal strategies of a sphecid wasp against a specialised cuckoo wasp. *Oecologia*, **129**, 50–57.

Strohm, E. & Linsenmair, K.E. (1995) Leaving the cradle – how beewolves (*Philanthus triangulum* F.) obtain the necessary spatial information for emergence. *Zoology*, **98**, 137–146.

Strohm, E. & Linsenmair, K.E. (2001) Females of the European beewolf preserve their honeybee prey against competing fungi. *Ecological Entomology*, **26**, 198–203.

Strohm, E., Herzner, G., Kaltenpoth, M., Boland, W., Schreier, P., Geiselhardt, S., Peschke, K. & Schmitt, T. (2008) The chemistry of the postpharyngeal gland of female European beewolves. *Journal of Chemical Ecology*, **34**, 575–583.

Sudakaran, S., Salem, H., Kost, C. & Kaltenpoth, M. (2012) Geographic and ecological stability of the symbiotic mid-gut microbiota in European firebugs, *Pyrrhocoris apterus* (Hemiptera; Pyrrhocoridae). *Molecular Ecology*, **21**, 6134–6151.

Temaru, E., Shimura, S., Amano, K. & Karasawa, T. (2007) Antibacterial activity of honey from stingless honeybees (Hymenoptera; Apidae; Meliponinae). *Polish Journal of Microbiology*, **56**, 281–285.

Thoetkiattikul, H., Beck, M.H. & Strand, M.R. (2005) Inhibitor kappa B-like proteins from a polydnavirus inhibit NF-kappa B activation and suppress the insect immune response. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11426–11431.

Umthong, S., Puthong, S. & Chanchao, C. (2009) *Trigona laeviceps* propolis from Thailand: antimicrobial, antiproliferative and cytotoxic activities. *American Journal of Chinese Medicine*, **37**, 855–865.

Vasquez, A. & Olofsson, T.C. (2009) The lactic acid bacteria involved in the production of bee pollen and bee bread. *Journal of Apicultural Research*, **48**, 189–195.

Vasquez, A., Forsgren, E., Fries, I., Paxton, R.J., Flaberg, E., Szekely, L. & Olofsson, T.C. (2012) Symbionts as major modulators of insect health: lactic acid bacteria and honeybees. *PLoS One*, **7**, e33188.

Visser, A.A., Nobre, T., Currie, C.R., Aanen, D.K. & Poulsen, M. (2011) Exploring the potential for Actinobacteria as defensive symbionts in fungus-growing termites. *Microbial Ecology*, **63**, 975–985.

Vorburger, C., Gehrer, L. & Rodriguez, P. (2010) A strain of the bacterial symbiont *Regiella insecticola* protects aphids against parasitoids. *Biology Letters*, **6**, 109–111.

Whitfield, J.B. (2002) Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7508–7513.

Whitfield, J.B. (2003) Phylogenetic insights into the evolution of parasitism in Hymenoptera. *Advances in Parasitology*, **54**, 69–100.

Whitfield, J.B. & Asgari, S. (2003) Virus or not? Phylogenetics of polydnaviruses and their wasp carriers. *Journal of Insect Physiology*, **49**, 397–405.

Wiegmann, B.M., Trautwein, M.D., Kim, J.-W., Cassel, B.K., Bertone, M.A., Winterton, S.L. & Yeates, D.K. (2009) Single-copy nuclear genes resolve the phylogeny of the holometabolous insects. *BMC Biology*, **7**, 34.

Wilson, E.O. (1971) *The Insect Societies*. Belknap Press, Cambridge, MA.

Xie, J.L., Vilchez, I. & Mateos, M. (2010) *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. *PLoS One*, **5**, e12149.

Xie, J., Tiner, B., Vilchez, I. & Mateos, M. (2011) Effect of the *Drosophila* endosymbiont *Spiroplasma* on parasitoid wasp development and on the reproductive fitness of wasp-attacked fly survivors. *Evolutionary Ecology*, **25**, 1065–1079.

Yoshiyama, M. & Kimura, K. (2009) Bacteria in the gut of Japanese honeybee, *Apis cerana japonica*, and their antagonistic effect against *Paenibacillus larvae*, the causal agent of American foulbrood. *Journal of Invertebrate Pathology*, **102**, 91–96.

Zhang, M.M., Poulsen, M. & Currie, C.R. (2007) Symbiont recognition of mutualistic bacteria by *Acromyrmex* leaf-cutting ants. *ISME Journal*, **1**, 313–320.

Zucchi, T.D., Guidolin, A.S. & Consoli, F.L. (2011) Isolation and characterization of actinobacteria ectosymbionts from *Acromyrmex subterraneus brunneus* (Hymenoptera, Formicidae). *Microbiological Research*, **166**, 68–76.

Received 5 October 2012; accepted 25 February 2013
Handling Editor: Keith Clay