

**SPECIAL ISSUE: SYMBIONTS IN INSECT
BIOLOGY AND PEST CONTROL**

MINI REVIEW

The microbiome of pest insects: it is not just bacteria

Kiran Gurung* , Bregje Wertheim[#]  & Joana Falcao Salles[#] 

Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands

Accepted: 28 January 2019

Key words: microbial communities, protozoa, virus, yeast, molds, archaea, symbionts, review, host fitness, behavior

Abstract

Insects are associated with multiple microbes that have been reported to influence various aspects of their biology. Most studies in insects, including pest species, focus on the bacterial communities of the microbiome even though the microbiome consists of members of many more kingdoms, which can also have large influence on the life history of insects. In this review, we present some key examples of how the different members of the microbiome, such as bacteria, fungi, viruses, archaea, and protozoa, affect the fitness and behavior of pest insects. Moreover, we argue that interactions within and among microbial groups are abundant and of great importance, necessitating the use of a community approach to study microbial–host interactions. We propose that the restricted focus on bacteria very likely hampers our understanding of the functioning and impact of the microbiome on the biology of pest insects. We close our review by highlighting a few open questions that can provide an in-depth understanding of how other components of the microbiome, in addition to bacteria, might influence host performance, thus contributing to pest insect ecology.

Introduction

Insects comprise a hugely diverse group of organisms and constitute a key component in the ecosystem. Some insects are harmful to humans, for example, causing damages to agriculture, forestry, stored products, or human health, and are therefore considered pest species. Like all other organisms, insects live in close association with microorganisms, which profoundly influence their ecology and evolution. Microbes, such as bacteria, archaea, fungi, protozoa, viruses, may be associated with their insect host permanently or transiently, and such associations may be beneficial or harmful to the insects' fitness (Kaufman et al., 2000; Feldhaar, 2011; Hammer et al., 2017) (see Figure 1 for beneficial interactions). For instance, endosymbionts (i.e., microbes that live inside host cells or tissues) tend to be dependent on the insect hosts for obtaining nutrients, whereas they can provide fitness advantages in terms of nutritional provisioning, overcoming host defenses, and protection from pathogens, parasites, or

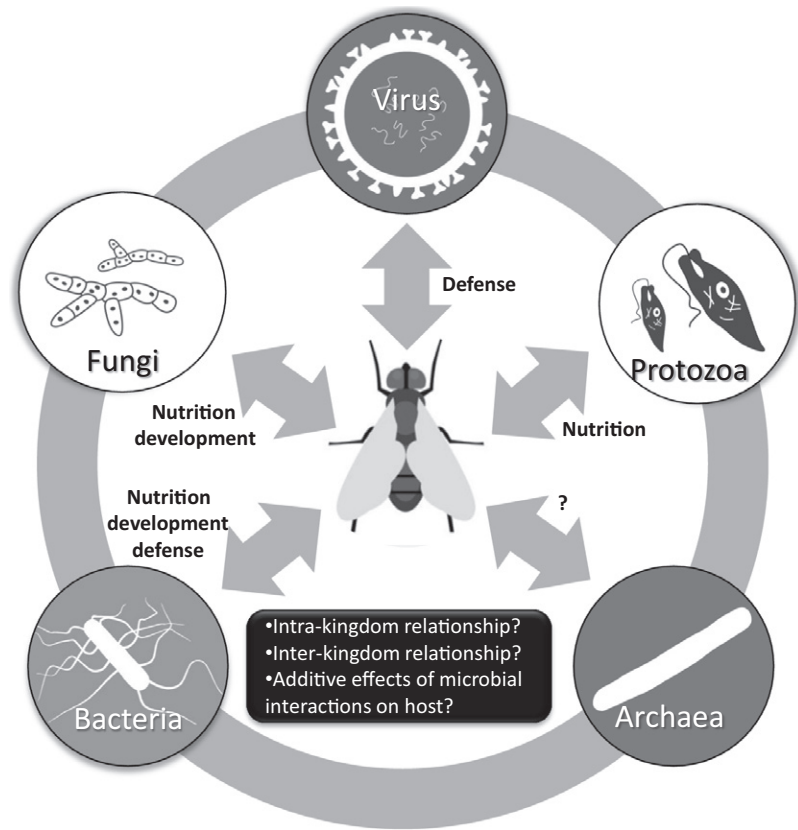
other environmental stressors (Engel & Moran, 2013; Mereghetti et al., 2017). However, microbes might also be pathogenic, reducing viability and causing morbidity. Furthermore, harboring endosymbionts can incur physiological costs (Krams et al., 2017).

Until 2 decades ago, most research on insect–microbe interactions focused on single species of bacteria. One of the best studied models for beneficial host–microbiome interactions in pest insects is aphids and their endosymbiotic bacteria of the genus *Buchnera* (Douglas, 1998), which are obligately associated with each other in terms of nutrition. Another well-studied insect–endosymbiont interaction is between (many different) insect species and parasitic bacteria of the genus *Wolbachia*. These reproductive parasites can induce feminization, male-specific killing, cytoplasmic incompatibility, and parthenogenesis in their hosts to enhance their own transmission (Kageyama et al., 2002; Werren et al., 2008). In addition, *Wolbachia* can also be considered mutualistic for many insects, as it may provide its host with resistance against viruses, insecticides, or plant defenses, and contribute to nutritional provisioning (Berticat et al., 2002; Hedges et al., 2008; Miller, 2013; for an extended discussion on how *Wolbachia* influences host

*Correspondence: E-mail: k.gurung@rug.nl

[#]These authors contributed equally to senior authorship.

Figure 1 The complexity of the microbiome associated with pest insects. The microbiome encompasses various microbial groups that are connected to each other and to their hosts (gray circle and arrows). All microbes, individually or together, can affect the biology of the pest insect, as exemplified by the processes indicated next to the arrows. Given the complexity of potential interactions, pest insects should be seen as mini ecosystems, in which the microbiome and the host interact as a system. To understand these interactions, we highlight key questions for future research (black box).



biology, see Kageyama et al., 2002; Nikoh et al., 2014). Although these examples illustrate the large and varied effects that a single species of microbe may have on its insect host, there has been a growing recognition that all organisms harbor a rich diversity of microbial species, collectively known as the microbiome (Douglas, 2018).

The past decade has witnessed an enormous rise in studies to characterize the microbiome of insects, mostly through next-generation sequencing approaches. These studies have been focusing on describing the composition of the microbiome and its putative functional role in the biology of the insects (Broderick & Lemaitre, 2012; Deutscher et al., 2018), thus expanding our understanding from a single microbial species to the whole community. All components of the microbiome can potentially and collectively affect the behavior and physiological traits of insects through genetic and metabolic interactions (Janson et al., 2008). Nevertheless, maybe due to historical reasons or feasibility issues, a common denominator among these studies is the focus on bacterial communities, with little or no integration of the rest of the microbiome. A search for the terms 'insect' and 'microbiome' in Web of Science provided nearly 1 000 articles since 2004, the vast majority of which is devoted to bacterial communities in insects,

and these often do not include descriptions on the composition or functional roles of other microbes such as the fungi (including yeasts), viruses, and protozoa. However, these other microbial members can also influence their insect hosts' life history and fitness. We argue that it is also the joint action and the interactions among diverse members of the microbial communities that affect the insect host (Figure 1). Therefore, we need to expand our studies of insect pest microbiome (1) to include the various microbial components and (2) to address them from a community perspective, in order to explore their role in insect ecology.

Here, we provide an overview of some well-studied examples of how members of the insect microbiome can influence the host. As it is impossible to be exhaustive in describing all these associations for such a diverse class of organisms as insects, which interact with an even more diverse group of microbes, we discuss a selection of available examples with a focus on pest insects. These examples are selected to illustrate the wide range of effects that microbes may have on their host, and how it is often their joint action that is required for these effects. Based on the overview from these examples, we argue that the additive role played by all the members of the microbiome in insect pests is still in its infancy. We provide some examples on

how interactions among microbes within the microbiome are of relevance in pest insect ecology. We conclude with a plea for adopting a community ecology approach to study the microbiome, where insects are addressed as miniature ecosystems in which several microbial components are addressed simultaneously.

Bacteria

Bacteria represent the most studied microbial kingdom interacting with insect pests. These microbes can endow their hosts with nutritional benefits, but also help them coping with temperature stress and provide protection against natural enemies. Below, we provide some examples of such interactions.

Nutrient provisioning by bacteria

Many insects inhabit nutritionally limited environments that challenge their physiological activities (Skidmore & Hansen, 2017). Under such conditions of nutritionally restricted surroundings, microbes of these hosts can provide nutrients. A well-studied example is the phloem-feeding aphids, which require the essential amino acid tryptophan that is not present in the phloem sap. These are provided by the bacterium *Buchnera aphidicola* Munson et al., a primary symbiont of aphids (Douglas & Prosser, 1992; Birkle et al., 2002).

In the case of the xylem sap-feeder, glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), nutrient provision relies on the mutualistic interaction between two bacterium species, *Baumannia cicadellinicola* Moran et al. and *Sulcia muelleri* Moran et al. Although *B. cicadellinicola* furnishes vitamins and cofactors, *S. muelleri* most likely furnishes the essential amino acids thereby complementing each other's metabolism (Wu et al., 2006). This represents a kind of dual symbioses wherein the bacterial symbionts in addition to being mutualistic to each other, also benefit the host. *Sulcia* spp. have evolved such dual symbioses with a variety of endosymbionts in phloem- and xylem-feeding hemipteran insects, as their reduced genomes make them dependent on other bacteria for the synthesis of all the essential amino acids (Bennett & Moran, 2013; Ankrah et al., 2018). Although other bacteria belonging to the genera *Bacillus*, *Delftia*, *Moraxella*, *Pectobacterium*, *Serratia*, and *Xylella* have been found in the sharpshooters, it remains unclear whether and, if so, how they contribute to the hosts' biology (Hail et al., 2011; Rogers & Backus, 2014).

A further example is the tephritid olive fruit fly, *Bactrocera oleae* (Rossi), that lays eggs in unripe olives that are nutritionally restricted in amino acids and are rich in secondary metabolites like phenolics. This olive fruit fly has

an obligate symbiont, the bacterium *Candidatus Erwinia dacicola* Capuzzo et al., that furnishes essential amino acids to adult flies from nitrogen sources of proteinogenic origin (like bird droppings), increasing their reproductive output (Ben-Yosef et al., 2014). In addition, larvae rely on their microbiome to overcome the host defenses and develop in unripe olive fruits, which contain high levels of the phenolic oleuropein. This dependency was not observed in larvae infecting ripe olive fruits where this compound had been degraded. The caeca of the midgut of the larvae growing on unripe fruits showed high abundances of the symbiont *E. dacicola*. The variation in densities was correlated with abundances of bacterial species belonging to the genera *Pantoea* and *Burkholderia*, indicating potential stable associations and modulated interaction between these bacteria in the gut of olive fruit fly larvae (Ben-Yosef et al., 2015). Whether or not these other bacteria are also required for nutrient provisioning and overcoming host defenses is yet unclear (Ras et al., 2017).

Another common interaction that contributes to insect nutrition is the association between insects and diazotrophic bacteria, which can fix atmospheric nitrogen, an essential element for physiological activities of the host. The ability to fix atmospheric nitrogen is found in several bacterial phyla such as Proteobacteria and Firmicutes. For instance, the family Enterobacteriaceae (Gammaproteobacteria) is one of the dominant bacterial families in the gut microbiota of the bark beetles *Dendroctonus* spp. obtained from pine trees in Mexico and USA (Hernández-García et al., 2017) and in wood-boring beetles collected from Italy (Rizzi et al., 2013), and in these associations, the genus *Enterobacter* contributes to host nutrition by fixing atmospheric nitrogen (Durand et al., 2015; Gauthier et al., 2015). In another tephritid, the medfly *Ceratitis capitata* (Wiedemann), a whole community of nitrogen-fixing bacteria belonging to the family Enterobacteriaceae – genera *Citrobacter*, *Klebsiella*, *Pectobacterium*, and *Pantoea* (Behar et al., 2008) – supports fly development and reproduction. Medflies are also colonized by other Proteobacteria, including the families Burkholderiaceae, Methylobacteraceae, Pseudomonadaceae, and Sphingomonadaceae (Malacrino et al., 2018), all harboring a few nitrogen-fixing species, but little is known about their effects on their host.

Protective function of bacteria

Microbes can also provide a protective function to their hosts, aiding them to cope with abiotic (temperature) and biotic (natural enemies, such as parasitoids, nematodes, and fungal pathogens) stress. These benefits have been observed in aphids (Montllor et al., 2002; Oliver et al., 2014; Frago et al., 2017), where *Buchnera* endosymbionts recurrently carry a mutation that governs thermal

tolerance of their pea aphid hosts, affecting their fitness positively under lower temperatures (Dunbar et al., 2007). The bacterium *Hamiltonella defensa* Moran et al. can confer protection against parasitoids in pea aphids (Oliver et al., 2003), whereas *Regiella insecticola* Moran et al. can largely increase pea aphid survival following a fungal infection (Scarborough et al., 2005) and confer resistance to parasitoids (Jamin & Vorburger, 2019). *Spiroplasma* bacteria have been shown to provide protection in *Drosophila* spp. against parasitoids (Xie et al., 2010) and parasitic nematodes (Jaenike et al., 2010).

Fungi

Similar to the bacterial components of the pest–insect microbiome, fungi that encompass molds and yeasts contribute to the provision of nutrients and regulation of host defenses. As overcoming (insect) host defenses is frequently achieved by inducing plant disease, the relationship between the fungi and the insects is extensively studied through the lens of pathogenic invasions and vector biology (Paine et al., 1997). Furthermore, fungal partnerships have been identified in insects such as ants and mound-building termites that have an agricultural symbiosis with fungi, which they cultivate in large underground chambers in their colonies for the digestion of wood and for food supply (Zoberi & Grace, 1990). These interactions of insects with yeasts as well as molds had been reported since the 1950s (Jankevica, 2004; Vega & Dowd, 2005).

Nutrient provisioning and protective functions by molds

The importance of molds to the life history of pest insects have been documented in bark beetles belonging to the genus *Dendroctonus*, which feed on phloem tissues and rely on fungi for nutrients throughout their life cycle (Ayres et al., 2000; Bentz & Six, 2006; Davis, 2015). Moreover, some bark beetles of the same genus are able to attack healthy trees, which involve defeating host defense mechanisms (resin flow and toxins). In this specific case, *Dendroctonus* overcomes the tree defenses through mass inoculation of fungi (e.g., *Ophiostoma* spp., the causative agents of Dutch Elm disease) that the beetles carry in specialized cuticular structures, that is, mycangia (Paine et al., 1997).

Recent studies based on meta-barcoding using fungal-specific primers have revealed a rich fungal community in both pest and non-pest insects (Chandler & Kopp, 2012; Hu et al., 2015; Miller et al., 2016; Boccazzi et al., 2017; Ramírez-Camejo et al., 2017; Mohammed et al., 2018; Quan & Eisen, 2018). Fungal species that have been associated with pest insects comprise, among others, species of molds belonging to the genera *Aspergillus*, *Beauveria*,

Metarhizium, *Cordyceps*, *Isaria*, and *Pandora* (Shang et al., 2015). The exact functions that these fungi play in pest insects are yet to be discovered for most associations (Vega & Dowd, 2005). Even in the case of the bark beetles that have been extensively studied in the context of the fungal–insect interactions, the exact position of the fungi in the life history of these pest insects is not fully resolved, and the key role for fungi in the tree-killing of primary bark beetles has come under debate as the bark beetles do not necessarily have to harbor the pathogenic fungi in the tree killing pursuit (Six & Wingfield, 2011).

Nutrient provisioning by yeasts

In addition to molds, molecular methods have revealed the presence of yeasts belonging to the genera *Saccharomyces*, *Pichia*, *Kluyveromyces*, *Candida*, *Hanseniaspora*, *Debaryomyces*, *Metschnikowia*, and *Cryptococcus* in several insects (Nguyen et al., 2007; Suh, 2008; Piper et al., 2017; Stefanini, 2018). Yeasts or yeast-like symbionts in the insect digestive tract are involved in metabolic pathways of amino acids and fatty acids and the absence of yeasts can lead to incomplete metamorphosis (Vega & Dowd, 2005; Carvalho et al., 2010).

Some evidence on a functional role of yeasts in nutrient provisioning comes from research on the non-pest insect *Drosophila melanogaster* Meigen, in which the mechanisms for this interaction have also been studied. Specifically, *D. melanogaster* larvae cannot develop on sterile fruit substrates, and strongly prefer fruits containing filamentous fungi or yeast over food without microbial growth, even when the fungi produce insecticidal mycotoxins (Trienens et al., 2017). By actively feeding on fungi, the larvae of *D. melanogaster* acquire sterol, which is required to support their growth and development (Starmer & Fogleman, 1986; Carvalho et al., 2010). This intricate dependency is also shown by the extensive repertoire of defensive chemicals that the larvae possess to cope with the mycotoxins produced by some of the fungi (Trienens et al., 2017). A close relative of *D. melanogaster* and emerging pest in several countries worldwide, *Drosophila suzukii* (Matsumura), lays eggs on fresh rather than fermenting fruits, where the density of molds and yeasts is initially very low. An important question is which strategies it may have evolved to acquire sterol in this niche. As infestation with *D. suzukii* is typically associated with inducing rot and fruit collapse, we hypothesize that this pest may inoculate the fruits with yeasts and fermenting bacteria to provide essential nutrients to their developing offspring.

Similarly, yeast-like symbionts provide nutritional benefits to the phloem feeding plant-hoppers of the genus *Nilaparvata* (Horgan & Ferrater, 2017). Under the event of the restricted nitrogen content, these symbionts recycle the

excretory material uric acid of the plant-hopper into reusable nitrogenous products. Additionally, they enhance the reproductive investment by the adults and shorten the developmental time of the various stages of the progenies (Ferrater et al., 2013). Finally, many bark beetles also host yeasts inside them, which are implicated in vitamin assimilation in these insects, although the exact mechanism of this is yet to be unraveled (Suh et al., 2008; Houseknecht et al., 2011).

Viruses

The insect–virus interaction is particularly well-studied for insects that act as vectors of viral pathogens, which is often the reason for giving these insects their ‘pest’ status. However, insect–virus associations are not limited to this. First, insects are not only vectors for viruses, but a virus can simply be pathogenic to its insect host, and in this context, viruses are also studied as potential biological control agents to kill pest insects (Carter, 1984; Winstanley & Rovesti, 1993; Lacey et al., 2001). Viruses might manipulate the host behavior or physiology, ultimately to increase its own transmission and replication, for example, inducing increased egg laying in their host (Gandon et al., 2009), or causing the infected hosts to migrate to locations that increase the chance of viral dispersal (van Houte et al., 2014). Additionally, non-pathogenic viruses may provide benefits to their host in mutualistic interactions. Finally, viruses may also infect other members of the microbiome, such as bacteriophages – viruses that infect bacteria – which can result in a pathogenic (lytic) or more symbiotic (temperate) association with the bacteria (Leigh et al., 2018).

Viruses associated with pest insects have been described using classic approaches and metagenomic analyses, the latter being a more sensitive method to reveal the ubiquity and diversity of insect viruses (Liu et al., 2011). Viruses belonging to the Baculoviridae, Parvoviridae, Flaviviridae, Ascoviridae, Togaviridae, Bunyavirales, and Rhabdoviridae have most commonly been associated with insects, but many new insect viruses have been discovered with metagenomic approaches (Nouri et al., 2018). There are still many questions that remain unanswered in terms of diversity of the viruses, the predominance of viruses over the life cycle, and their impact on the physiology and life history of pest insects. Here, we present a few examples that illustrate the versatile roles they have in pest insect biology.

Vectoring of viruses

Animal viruses can be transmitted by various blood-feeding arthropod vectors. Ticks from China hosted the animal viruses belonging to Bunyaviridae, Anelloviridae, and Rhabdoviridae, some of which cause blood infections (Xia

et al., 2015). The composition of the viral part of the microbiome, the virome (i.e., the community of viruses in a given host or environment), has also been extensively studied for various mosquito species (e.g., *Aedes* spp., *Culex* spp.), in order to characterize the disease-causing viruses as well as the insect-specific viruses (Atoni et al., 2018; Nouri et al., 2018). Interestingly, there is growing evidence that the vector competence of the insect can be affected by the insect-specific viruses they also carry; that is, several of the insect-specific viruses have been shown to suppress or enhance the transmission of medically important viruses (Hobson-Peters et al., 2013; Hall-Mendelin et al., 2016; Zhang et al., 2017).

Many plant viruses also rely on insect vectors to transmit them to other plants, and these interactions can be rather specific, with members of a particular genus of plant viruses (e.g., *Potyvirus*) being transmitted mostly by a particular insect taxon (e.g., aphids) (Whitfield et al., 2015). The mechanisms for this specificity, and for the transmission of the virus from the insect to the plant, have been studied in detail for many plant–virus–insect interactions (Whitfield et al., 2015; Dietzgen et al., 2016). Some insect species can transmit many different viruses. For example, over 100 viral species can be transmitted by the whitefly *Bemisia tabaci* (Gennadius), mostly belonging to the genus *Begomovirus* (Geminiviridae), but also members of the genera *Crinivirus*, *Closterovirus* (both Closteroviridae), *Ipomovirus* (Potyviridae), and *Carlavirus* (Betaflexiviridae) (Jones, 2003). Also in plants, virus–virus interactions are known to occur. For example, some viruses are dependent on other viruses for producing infectious progeny and their transmission, as was reported for the *Carrot mottle virus* that requires viruses of the Luteoviridae as ‘helper’ virus for transmission by aphids (Waterhouse & Murrant, 1983).

Viral functions in biological control of pest insects

The family of Baculoviridae has been the best studied group for use as biocontrol agents against coleopteran, hemipteran (sawflies), and lepidopteran pests (Carter, 1984; Winstanley & Rovesti, 1993; Lacey et al., 2001; Williams et al., 2017). An advantage of these viral biocontrol agents is their high specificity for a particular pest insect, with limited possibilities for non-target effects (e.g., on beneficial insects or humans), and they can naturally spread through a pest population. In contrast, viruses are also known to hamper pest management strategies. For example, it is known that viruses can influence insecticide resistance (Yoshikawa et al., 2018). Furthermore, when viruses infect larvae of pests, they could potentially prevent parasitoids to parasitize them (Robertson et al., 2013).

Viral effects on host behavior and physiology

Some changes that viruses may induce in the hosts' behavior and physiology may be primarily to benefit the viral replication and transmission, whereas others can be considered more mutualistic for the insect and the virus. The complexity of the interactions between viruses, plants, and insects is exemplified by the plant-pathogenic *Tospovirus* (Tospoviridae) and the western flower thrips, *Frankliniella occidentalis* (Pergande). Studies in thrips showed that the virus had a positive effect on thrips development, with more offspring and shorter developmental time, when the thrips fed on virus-infected compared to uninfected plants (Maris et al., 2004). The virus can also infect the insect host, which can act as a vector to transmit the virus to other plants, but this actually induces an immunological response in the thrips, indicating that the virus may (also) be pathogenic to the insect host (Medeiros et al., 2004). Thus, the interactions between plants, *Tospovirus*, and thrips suggest to be highly complex, and go beyond mere transmission and pathogenicity (Whitfield et al., 2005).

Additionally, parasitoid behavior has been shown to be manipulated by viruses, increasing their rate of superparasitism, which allows the virus to jump from infected to uninfected parasitoids (Varaldi et al., 2003). Baculoviruses are known to induce profound changes in the behavior of *Spodoptera exigua* (Hübner) larvae, triggering phototactic responses and hyperactivity to increase their own spread (Goulson, 1997; van Houte et al., 2012, 2014).

Protective functions of viruses

One prominent example of viruses that confer protective benefits to their host comprises the Polydnviridae, symbiotic viruses harbored by two families of parasitoid wasps, Ichneumonidae and Braconidae. These viruses reside in the reproductive organs of the wasp – first in the ovaries, then in the reproductive tract. The wasp, while ovipositing on its insect hosts, transmits the virus to its host. These viral particles tend to suppress the immune defense in the host thereby allowing the development of the wasp's offspring (Herniou et al., 2013; Strand & Burke, 2015).

In addition to considering viruses that are associated directly with insects, there are also viruses that infect the bacteria in the insects' microbiome, that is, bacteriophages, and may provide their protective function through them. Aphids are a well-studied insect system in terms of bacteria–phages–insect associations. For instance, aphids that harbor *H. defensa* with APSE (*Acyrtosiphon pisum* secondary endosymbiont) phages tend to have a greater defense capacity against the attack of parasitoids than the

ones without APSE (Degnan & Moran, 2008; Degnan et al., 2009). The phage infects its bacterial host; the bacteria then defend the aphid against its natural enemies (Oliver et al., 2009). The bacteriophages of the insects are also represented in the *Arsenophonus*–phage system and *Wolbachia*–phage system, to name but a few (Gavotte et al., 2006; Duron, 2014). We return to these bacteria–phage–insect interactions in the paragraph on community perspectives of the host microbiome.

Archaea

Archaea form another important domain of the microbes that not only inhabit extreme regions, but are also found in strong association with soils and oceans (Alves et al., 2018). Their rich diversity and huge abundances have been demonstrated with the help of metagenomics (Bates et al., 2011; Edwards et al., 2015; Siles et al., 2018), which also revealed that these organisms play important roles in the nitrogen cycling in soils – a function that was discovered not much more than a decade ago (Treich et al., 2005). Archaea might directly affect their plant host (Taffner et al., 2018), which could indirectly affect pest insects. Yet, information on their importance for plants and other hosts, and how they interact with both, is largely missing, mostly due to methodological constraints associated with culturing these organisms in the laboratory. Molecular approaches indicate the presence of archaea in many non-insects and their presence tends to influence the bacterial or viral communities that shift in the presence of archaea.

In insects, methanogenic and non-methanogenic archaea belonging to the phylum Euryarchaeota, have been reported in beetles, cockroaches, termites, and millipedes. The methanogenic archaea are usually present in the hindguts, an environment with limited oxygen availability (Shinzato et al., 1999; Hara et al., 2002; Šustr et al., 2014; Tinker & Ottesen, 2016). Ziganshina et al. (2018) also reported the presence of Crenarchaeota in the larval gut of beetles.

Protozoa

Protozoa are single-celled microbial eukaryotes with or without locomotory organelles. The contribution of protozoa to the microbiome of the insects has been rarely addressed. One prominent example in which protozoa proved to be very important for an insect host is in termites that feed on wood which does not provide a suitable resource for necessary vitamins for growth and development (Poinar, 2009). Protozoan members help termites to sustain on these nutrient-restricted substrates by synthesizing a number of hydrolytic enzymes (Husseneder,

2010). Flagellated protozoa have been reported in termites through in vivo studies (Ohkuma, 2008), whereas their exact influence on the arthropod hosts is not fully understood (Reyes, 1966).

Many species of protozoa are parasitic, and several similarities exist between the protozoa–insect interactions and the interactions between insects and pathogenic viruses. For example, insects can also vector these parasitic protozoa. The best-studied association in this respect is between *Anopheles* spp. mosquitoes and the malaria parasite *Plasmodium falciparum* Welch. Other examples include the protozoan species *Trypanosoma brucei* Plimmer & Bradford that induces sleeping sickness and is vectored by the tsetse flies, and the *Leishmania* protozoa that are spread by sandflies. Protozoa have a narrow host range and are often highly specific (Henry, 1981; Cleveland, 1923; Apuya et al., 1994). Consequently, parasitic protozoa have also been used as effective biocontrol agent of pest insects (Henry, 1990; Boucias & Pendland, 1998; Burges & Jones, 1998). Similar as with the viruses, however, non-parasitic protozoa may provide benefits to their insect hosts in mutualistic interactions, as shown for the termites. At the moment, our knowledge on the protozoan members of the microbiome is very restricted, and there is a paucity of metagenomic studies on this part of the microbiome. It is time that we start examining the diversity of these protozoan members in insects, to characterize both their functional roles and as a putative means to manage pest insects.

Community perspectives of the host microbiome

Given the multitudes of microbial species living in association with insects, it is important to go beyond one-to-one interactions between the microbe and the insect host. Studying a single species of bacterium might be essential to understand the basic biology of the organism and potential mechanisms driving its interaction with the host. At the same time we should realize that microorganisms live in diverse communities and interact with each other (Figure 1). It is therefore important to also consider this community ecology perspective of the microbes in the insect pests, in order to understand the drivers of microbial interactions and how these interactions, which might influence host and microbial gene expression patterns or genomes, shape host ecology (Douglas, 2018).

The role played by all the members of the microbiome in insect pests is still in its infancy. Results from well-studied communities such as soil have demonstrated that microbial interactions result in additive, synergistic, or competitive effects (Trabelsi & Mhamdi,

2013; Mallon et al., 2015a,b; Dini-Andreote et al., 2018). Microbial interactions influence both how communities are formed (Dini-Andreote et al., 2015, 2018; Jia et al., 2018) and how they respond (Jurburg et al., 2018; Mallon et al., 2018). Such patterns have also been observed in microbial communities associated with hosts such as plants (Wang et al., 2018), corals (Leite et al., 2018a,b), and birds (van Veelen et al., 2017), indicating that similar rules drive microbiome assembly in several habitats.

To witness this kind of additive effects in insects, we need to investigate the interplay among the microbial partners, to know how all the various microbes, as well as their intra- and inter-kingdom interactions, affect the development, fitness, physiology, and behavior of insects (Figure 1). Below, we discuss three community-based approaches associated with insect–microbiome research that could help elucidate the complexity of the interactions and their consequence for the host.

Approaches to study microbiome–host interactions

To study the link between the community composition and the function of the microbes in insect pests, it would be very helpful if we could experimentally mix-and-match microbes. This requires, however, that we can culture them outside of their insect hosts. In fact, most microbes are difficult to propagate or cannot be cultured. Thus, we have to rely on culture-independent approaches such as metagenomics, for the taxonomic (amplicon sequencing) and functional (shotgun sequencing, metatranscriptomics) characterization of microbial communities. The former approach allows us to characterize ‘who is there’ (species identification) whereas the latter allows us to characterize the variety of genes they possess or are expressing, which signifies ‘what they are doing’ within the host. Another approach that can be used to experimentally manipulate components of the bacterial microbiome is the use of (cocktails of) antibiotics that target specific bacterial groups, or temperature-shock treatments that affects some microbes more than others. With these approaches, the microbiome changes in composition, and the performance of insects can be compared with vs. without, or among different combinations of treatments (Bordenstein & Bordenstein, 2011; Lin et al., 2015). Although these methods provide an indication of potential links between microbiome and host, these are usually mere correlations and should therefore be interpreted with caution, as they might not infer causation.

Within-kingdom interactions

Despite methodological issues linked to microbiome studies, several studies report interactions occurring within

taxonomic groups at kingdom level. An obvious example is quorum sensing in bacteria–bacteria interactions. Quorum sensing is the regulation of gene expression between cells in response to increased cell population density, by secreting chemical signals (Papenfort & Bassler, 2016). This means that bacterial populations co-ordinate its cooperative behavior in response to the synthesis of chemicals called auto-inducers. This phenomenon of regulating cell density has been reported as beneficial within biofilms, and has also been reported for the gut microbiome in mice and in tsetse flies (Thompson et al., 2015; Enomoto et al., 2017). Additionally, metabolic exchanges are important among various bacterial members associated with the insect hosts (Ankrah et al., 2017; Opatovsky et al., 2018). A nice example is the interaction described in ‘Nutrient provisioning by bacteria’, between the symbionts *B. cicadellinicola* and *S. muelleri*, that jointly provide essential nutrients to the glassy-winged sharpshooter *H. vitripennis* (Wu et al., 2006). Another good example is the virus–virus interactions, where viruses may depend on other viruses for infectivity, and suppress or enhance the vector competence of insects (see ‘Vectoring of viruses’). Although we know that bacterial communities associated with the insect hosts are diverse, little is still known about cooperation and conflict between bacterial taxa. Even less is known about the complexity of interactions within fungal, protozoan, and archaeal communities.

Cross-kingdom interactions

In addition to interactions within taxonomic microbial groups, it seems fair to assume that the members of various microbial kingdoms (bacteria, archaea, and microbial eukaryotes – molds, yeasts, protozoan) and viruses are also interacting with each other in the host (Figure 1). In this context, the production of penicillin by fungi in response to growth inhibition from bacteria is a well-studied and a common example of how microbes from different kingdoms may have evolved properties for their interactions (Kester et al., 2011). Other examples of important bacteria–fungi interactions exist in the human (Nguyen et al., 2015; Sartor & Wu, 2017) and in plant microbiome (Vandenkoornhuys et al., 2015; Toju et al., 2018). Thus, in insects, these interactions are likely relevant too.

One example focusing on the interactions among host, fungi, and bacteria is witnessed in bark beetles of the genus *Dendroctonus* that transport the fungi (*Ophiostoma*) in membranous pockets. Zhou et al. (2016) recently showed how bacteria and fungi in bark beetles interact when it comes to scavenging the carbon sources. In their study with the red turpentine beetle, *Dendroctonus valens*

LeConte, they showed that in the presence of bacterial symbionts, the fungi tend to utilize D-pinitol as their carbon substrate, whereas in the absence of bacteria these fungi would grow on D-glucose, their most preferred substrate. When the larvae grow on D-glucose, the unused D-pinitol antagonizes the survival of the beetle larvae that negatively affects the survival of both the fungi and bacterial symbionts. Thus, the bacteria–fungi form a relationship which is also responsible for the distribution of carbohydrates in the larvae eventually resulting in the survival of all three.

The bacteriophages are another clear example of cross-kingdom interactions. Again, by borrowing examples from other systems and hosts, we do know that bacteriophages can regulate bacterial communities (Obeng et al., 2016) – being important drivers of the composition of bacterial communities in the human gut (Ogilvie & Jones, 2015) – and even a three-way interaction among fungi, bacteria, and phages has been reported in soil (Pratama & van Elsas, 2018). Our knowledge regarding bacteriome–virome interactions in insects is limited to a few examples (see ‘Viruses’ section; Handley, 2016). The complexity of such interactions is exemplified in aphid–bacteria–phage interactions, in which the phage APSE infects its bacterial host *H. defensa*, and the bacterium then defends the aphid against its natural enemies (Oliver et al., 2009). Generally, the absence of phage leads to a rise in the bacterial count inside the aphid host, which leads to reduced fitness of the aphid. The negative impact on aphid fitness is due to *H. defensa* scavenging essential amino acids from its host, which are in fact synthesized by another bacterial symbiont of the aphids, *B. aphidicola*; second, the absence of the phage reduces the protective function of *H. defensa* for the aphid against parasitoids (Weldon & Oliver, 2016).

Another example of cross-kingdom interactions are the dramatic effects that *Wolbachia* bacteria can have on transmission of pathogenic viruses and parasitic protozoa that are vectored by insects (Moreira et al., 2009; Bian et al., 2010; Rainey et al., 2014; Johnson, 2015). These interactions are now also being studied to explore how this may be exploited for vector and disease control (Bourtzis et al., 2014; Caragata et al., 2016; Brinker et al., 2019).

Previous examples on protozoa revealed their relevance in supplying termites with nutrients such as acetates, vitamins, and amino acids (see ‘Protozoa’ section). However, given that bacteria and fungi are also present in termite hindguts, how do these three groups – bacteria, fungi, and protozoa – interact among themselves? Benjamino & Graf (2016) have shown the presence and abundance of protozoa and the protozoa-associated bacteria *Treponema* and *Endomicrobia*, along with the presence of other

bacterial communities, but their specific functions remain unknown.

Outlook

When studying the microbiome of pest insects, the restricted focus on bacteria is very likely to severely hamper our understanding of its functioning and impact on the biology of the pest. It is important to know how all the various microbes, as well as their intra- and inter-kingdom interactions, affect the development, fitness, physiology, and behavior of insects (Figure 1). We have made large advances in the past decade in characterizing and understanding the great importance of the bacterial species for host functioning. We now need to expand this research on the role of the microbiome and more explicitly include the non-bacterial components of the microbiome and their various interactions, as well as their functioning. Given that the field of microbiome is progressing in metagenomics, emphasis on culturomics (Bilen et al., 2018) is the need of the hour to get a better grasp about what is actually going on in this network where all the microbial partners are interconnected. In closing, we highlight three open-standing questions that we consider of primary importance for understanding the ecology of the microbiome associated with pests.

- (1) Culture-independent techniques have captured how microbial community composition in insects varies largely in time and space-in response to diet, geography, season, and developmental stage (Hu et al., 2013; Wilhelm et al., 2014; Chaplinska et al., 2016; Lv et al., 2016; Vacchini et al., 2017; Bascuñán et al., 2018). The relevance of these parameters might vary according to the microbial groups, with environmental factors being more important for bacteria and fungi than in shaping the viral communities (Nouri et al., 2016). How do these variations in microbiome composition affect the interplay among these members of the microbiome, and how does that affect pest insects? And do we see similar results in endemic as well as in invasive insect pests?
- (2) Some insects harbor obligate symbionts, whereas others seem to have more ephemeral, stochastic, and flexible associations with microbes. The causes and consequences of these differences are highly relevant to understand the evolutionary and ecological dynamics between the microbes within the microbiome. Insects evolve strong associations with obligate primary endosymbionts, which may lead to the formation of a core microbiome in which these primary endosymbionts are well integrated. This contrasts with pests that can independently derive the necessary

nutrients from their resource or from naturally occurring environmental microbes on their resources. In these cases, it might be difficult to pinpoint a core microbiome and identify key organisms associated with the pest insect. Does the lack of a core species make it difficult to work on the microbial control of pests? Or shall we look for a core microbial genome (set of specific microbial functions rather than species) required for pest survival to build microbial control strategies?

- (3) How do the additive effects of the total microbiome work? We are yet to witness how the interplay among microbial partners influences insect biology. In order to do so, we argue that we need to use an 'ecosystem' approach, in which the biotic and abiotic interactions of associated microbial components are addressed simultaneously (insect host as a complex ecosystem). Given that the field of microbiome research is progressing in metagenomics, emphasis on both taxonomic and functional characterization of microbial communities should allow us to raise hypotheses about their interactions (amplicon sequencing), their functions, and their impact on hosts (shotgun sequencing and metatranscriptomics), as well as how these patterns vary in space and time (between and within developmental stages). However, given the correlational nature of this type of data, culturable approaches should be performed concomitantly with genomics, and both should be used to design experiments to manipulate the microbiome and draw causal relationships from microbiome interactions and their insect hosts.

Acknowledgements

We thank Jean-Christophe Billeter for his comments on the role of microbes in insect biology. We thank two anonymous reviewers for their insightful comments and suggestions in improving our article. This work was supported by a grant from the Adaptive Life programme of the University of Groningen, The Netherlands. The authors declare no conflict of interest.

References

- Alves RJE, Minh BQ, Urich T, Haeseler A & Schleper C (2018) Unifying the global phylogeny and environmental distribution of ammonia-oxidising archaea based on *amoA* genes. *Nature Communications* 9: 1517.
- Ankrah NY, Luan J & Douglas AE (2017) Cooperative metabolism in a three-partner insect-bacterial symbiosis revealed by metabolic modeling. *Journal of Bacteriology* 199: e00872-16.

- Ankrah NY, Chouaia B & Douglas AE (2018) The cost of metabolic interactions in symbioses between insects and bacteria with reduced genomes. *mBio* 9: e01433-18.
- Apuya LC, Stringham SM, Arends JJ & Brooks WM (1994) Prevalence of protozoan infections in darkling beetles from poultry houses in North Carolina. *Journal of Invertebrate Pathology* 63: 255–259.
- Atoni E, Wang Y, Karungu S, Waruhiu C, Zohaib A et al. (2018) Metagenomic virome analysis of *Culex* mosquitoes from Kenya and China. *Viruses* 10: 30.
- Ayres MP, Wilkens RT, Ruel JJ, Lombardero MJ & Vallery E (2000) Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. *Ecology* 81: 2198–2210.
- Bascuñán P, Niño-García JP, Galeano-Castañeda Y, Serre D & Correa MM (2018) Factors shaping the gut bacterial community assembly in two main Colombian malaria vectors. *Microbiome* 6: 148.
- Bates ST, Berg-Lyons D, Caporaso JG, Walters WA, Knight R & Fierer N (2011) Examining the global distribution of dominant archaeal populations in soil. *The ISME Journal* 5: 908.
- Behar A, Yuval B & Jurkevitch E (2008) Gut bacterial communities in the Mediterranean fruit fly (*Ceratitidis capitata*) and their impact on host longevity. *Journal of Insect Physiology* 54: 1377–1383.
- Benjamino J & Graf J (2016) Characterization of the core and caste-specific microbiota in the termite, *Reticulitermes flavipes*. *Frontiers in Microbiology* 7: 171.
- Bennett GM & Moran NA (2013) Small smaller smallest: the origins and evolution of ancient dual symbioses in a phloem-feeding insect. *Genome Biology and Evolution* 5: 1675–1688.
- Bentz BJ & Six DL (2006) Ergosterol content of fungi associated with *Dendroctonus ponderosae* and *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). *Annals of the Entomological Society of America* 99: 189–194.
- Ben-Yosef M, Pasternak Z, Jurkevitch E & Yuval B (2014) Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *Journal of Evolutionary Biology* 27: 2695–2705.
- Ben-Yosef M, Pasternak Z, Jurkevitch E & Yuval B (2015) Symbiotic bacteria enable olive fly larvae to overcome host defences. *Royal Society Open Science* 2: 150170.
- Berticat C, Rousset F, Raymond M, Berthomieu A & Weill M (2002) High *Wolbachia* density in insecticide-resistant mosquitoes. *Proceedings of the Royal Society of London B: Biological Sciences* 269: 1413–1416.
- Bian G, Xu Y, Lu P, Xie Y & Xi Z (2010) The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathogens* 6: e1000833.
- Bilen M, Dufour JC, Lagier JC, Cadoret F, Daoud Z et al. (2018) The contribution of culturomics to the repertoire of isolated human bacterial and archaeal species. *Microbiome* 6: 94.
- Birkle LM, Minto LB & Douglas AE (2002) Relating genotype and phenotype for tryptophan synthesis in an aphid–bacterial symbiosis. *Physiological Entomology* 27: 302–306.
- Boccazzi IV, Ottoboni M, Martin E, Comandatore F, Vallone L et al. (2017) A survey of the mycobiota associated with larvae of the black soldier fly (*Hermetia illucens*) reared for feed production. *PLoS ONE* 12: e0182533.
- Bordenstein SR & Bordenstein SR (2011) Temperature affects the tripartite interactions between bacteriophage WO, *Wolbachia*, and cytoplasmic incompatibility. *PLoS ONE* 6: e29106.
- Boucias DG & Pendland JC (1998) *Principles of Insect Pathology*. Springer, Boston, MA, USA.
- Bourtzis K, Dobson SL, Xi Z, Rasgon JL, Calvitti M et al. (2014) Harnessing mosquito–*Wolbachia* symbiosis for vector and disease control. *Acta Tropica* 132: S150–S163.
- Brinker P, Fontaine MC, Beukeboom LW & Salles JF (2019) Host, symbionts, and the microbiome: the missing tripartite interaction. *Trends in Microbiology* (in press).
- Broderick NA & Lemaitre B (2012) Gut-associated microbes of *Drosophila melanogaster*. *Gut Microbes* 3: 307–321.
- Burges HD & Jones KA (1998) Formulation of bacteria, viruses and protozoa to control insects. *Formulation of Microbial Biopesticides – Beneficial Microorganisms, Nematodes and Seed Treatments* (ed. by HD Burges), pp. 33–127. Springer, Dordrecht, The Netherlands.
- Caragata EP, Dutra HL & Moreira LA (2016) Exploiting intimate relationships: controlling mosquito-transmitted disease with *Wolbachia*. *Trends in Parasitology* 32: 207–218.
- Carter JB (1984) Viruses as pest-control agents. *Biotechnology and Genetic Engineering Reviews* 1: 375–419.
- Carvalho M, Schwudke D, Sampaio JL, Palm W, Riezman I et al. (2010) Survival strategies of a sterol auxotroph. *Development* 137: 3675–3685.
- Chandler JL & Kopp A (2012) Yeast communities or various *Drosophila* species: comparison of two symbiont groups in the same host. *Applied and Environmental Microbiology* 78: 327–336.
- Chaplinka M, Gerritsma S, Dini-Andreote F, Salles JF & Wertheim B (2016) Bacterial communities differ among *Drosophila melanogaster* populations and affect host resistance against parasitoids. *PLoS ONE* 11: e0167726.
- Cleveland LR (1923) Symbiosis between termites and their intestinal protozoa. *Proceedings of the National Academy of Sciences of the USA* 9: 424–428.
- Davis TS (2015) The ecology of yeasts in the bark beetle holobiont: a century of research revisited. *Microbial Ecology* 69: 723–732.
- Degnan PH & Moran NA (2008) Diverse phage-encoded toxins in a protective insect endosymbiont. *Applied and Environmental Microbiology* 74: 6782–6791.
- Degnan PH, Yu Y, Sisneros N, Wing RA & Moran NA (2009) *Hamiltonella defensa*, genome evolution of protective bacterial endosymbiont from pathogenic ancestors. *Proceedings of the National Academy of Sciences of the USA* 106: 9063–9068.
- Deutscher AT, Burke CM, Darling AE, Riegler M, Reynolds OL & Chapman TA (2018) Near full-length 16S rRNA gene next-generation sequencing revealed *Asaia* as a common midgut bacterium of wild and domesticated Queensland fruit fly larvae. *Microbiome* 6: 85.

- Dietzgen R, Mann K & Johnson K (2016) Plant virus–insect vector interactions: current and potential future research directions. *Viruses* 8: 303.
- Dini-Andreote F, Stegen JC, van Elsas JD & Salles JF (2015) Disentangling mechanisms that mediate the balance between stochastic and deterministic processes in microbial succession. *Proceedings of the National Academy of Sciences of the USA* 112: E1326–E1332.
- Dini-Andreote F, van Elsas JD, Olf H & Salles JF (2018) Dispersal-competition tradeoff in microbiomes in the quest for land colonization. *Scientific Reports* 8: 9451.
- Douglas AE (1998) Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annual Review of Entomology* 43: 17–37.
- Douglas AE (2018) *Fundamentals of Microbiome Science: How Microbes Shape Animal Biology*. Princeton University Press, Princeton, NJ, USA.
- Douglas AE & Prosser WA (1992) Synthesis of the essential amino acid tryptophan in the pea aphid (*Acyrtosiphon pisum*) symbiosis. *Journal of Insect Physiology* 38: 565–568.
- Dunbar HE, Wilson AC, Ferguson NR & Moran NA (2007) Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biology* 5: e96.
- Durand AA, Bergeron A, Constant P, Buffet JP, Déziel E & Guertin C (2015) Surveying the endomicrobiome and ectomicrobiome of bark beetles: the case of *Dendroctonus simplex*. *Scientific Reports* 5: 17190.
- Duron O (2014) *Arsenophonus* insect symbionts are commonly infected with APSE, a bacteriophage involved in protective symbiosis. *FEMS Microbiology Ecology* 90: 184–194.
- Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podshetty NK et al. (2015) Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences of the USA* 112: E911–E920.
- Engel P & Moran NA (2013) The gut microbiota of insects—diversity in structure and function. *FEMS Microbiology Reviews* 37: 699–735.
- Enomoto S, Chari A, Clayton AL & Dale C (2017) Quorum sensing attenuates virulence in *Sodalis praecaptivus*. *Cell Host & Microbe* 21: 629–636.
- Feldhaar H (2011) Bacterial symbionts as mediators of ecologically important traits of insect hosts. *Ecological Entomology* 36: 533–543.
- Ferrater JB, de Jong PW, Dicke M, Chen YH & Horgan FG (2013) Symbiont-mediated adaptation by planthoppers and leafhoppers to resistant rice varieties. *Arthropod-Plant Interactions* 7: 591–605.
- Frago E, Mala M, Weldegergis BT, Yang C, McLean A et al. (2017) Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. *Nature Communications* 8: 1860.
- Gandon S, Varaldi J, Fleury F & Rivero A (2009) Evolution and manipulation of parasitoid egg load. *Evolution* 63: 2974–2984.
- Gauthier JP, Outreman Y, Mieuxet L & Simon JC (2015) Bacterial communities associated with host-adapted populations of pea aphids revealed by deep sequencing of 16S ribosomal DNA. *PLoS ONE* 10: e0120664.
- Gavotte L, Henri H, Stouthamer R, Charif D, Charlat S et al. (2006) A survey of the bacteriophage WO in the endosymbiotic bacteria *Wolbachia*. *Molecular Biology and Evolution* 24: 427–435.
- Goulson D (1997) Wipfelkrankheit: modification of host behaviour during baculoviral infection. *Oecologia* 109: 219–228.
- Hail D, Lauziere I, Dowd SE & Bextine B (2011) Culture independent survey of the microbiota of the glassy-winged sharpshooter (*Homalodisca vitripennis*) using 454 pyrosequencing. *Environmental Entomology* 40: 23–29.
- Hall-Mendelin S, McLean BJ, Bielefeldt-Ohmann H, Hobson-Peters J, Hall RA & van den Hurk AF (2016) The insect-specific Palm Creek virus modulates West Nile virus infection in and transmission by Australian mosquitoes. *Parasites & Vectors* 9: 414.
- Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP & Fierer N (2017) Caterpillars lack a resident gut microbiome. *Proceedings of the National Academy of Sciences of the USA* 114: 9641–9646.
- Handley SA (2016) The virome: a missing component of biological interaction networks in health and disease. *Genome Medicine* 8: 32.
- Hara K, Shinzato N, Seo M, Oshima T & Yamagishi A (2002) Phylogenetic analysis of symbiotic archaea living in the gut of xylophagous cockroaches. *Microbes and Environments* 17: 185–190.
- Hedges LM, Brownlie JC, O’neill SL & Johnson KN (2008) *Wolbachia* and virus protection in insects. *Science* 322: 702.
- Henry JE (1981) Natural and applied control of insects by protozoa. *Annual Review of Entomology* 26: 49–73.
- Henry JE (1990) Control of insects by protozoa. *UCLA Symposia on Molecular and Cellular Biology* 112: 161–176.
- Hernández-García JA, Briones-Roblero CI, Rivera-Orduña FN & Zúñiga G (2017) Revealing the gut bacteriome of *Dendroctonus* bark beetles (Curculionidae: Scolytinae): diversity, core members and co-evolutionary patterns. *Scientific Reports* 7: 13864.
- Herniou EA, Huguet E, Thézé J, Bézier A, Periquet G & Drezen JM (2013) When parasitic wasps hijacked viruses: genomic and functional evolution of polydnviruses. *Philosophical Transactions of the Royal Society B* 368: 20130051.
- Hobson-Peters J, Yam AWY, Lu JWF, Setoh YX, May FJ et al. (2013) A new insect-specific flavivirus from northern Australia suppresses replication of West Nile virus and Murray Valley encephalitis virus in co-infected mosquito cells. *PLoS ONE* 8: e56534.
- Horgan FG & Ferrater JB (2017) Benefits and potential trade-offs associated with yeast-like symbionts during virulence adaptation in a phloem-feeding planthopper. *Entomologia Experimentalis et Applicata* 163: 112–125.
- Houseknecht JL, Hart EL, Suh SO & Zhou JJ (2011) Yeasts in the *Sugiyamaella* clade associated with wood-ingesting beetles and the proposal of *Candida bullrunensis* sp. nov. *International*

- Journal of Systematic and Evolutionary Microbiology 61: 1751–1756.
- van Houte S, Ros VID, Mastenbroek TG, Vendrig NJ, Hoover KM et al. (2012) Protein tyrosine phosphatase-induced hyperactivity is a conserved strategy of a subset of baculoviruses to manipulate lepidopteran host behavior. *PLoS ONE* 7: e46933.
- van Houte S, van Oers MM, Han Y, Vlak JM & Ros VID (2014) Baculovirus infection triggers a positive phototactic response in caterpillars to induce ‘tree-top’ disease. *Biology Letters* 10: 20140680.
- Hu X, Wang C, Chen H & Ma J (2013) Differences in the structure of the gut bacteria communities in development stages of the Chinese white pine beetle (*Dendroctonus armandi*). *International Journal of Molecular Sciences* 14: 21006–21020.
- Hu X, Li M & Chen H (2015) Community structure of gut fungi during different developmental stages of the Chinese white pine beetle (*Dendroctonus armandi*). *Scientific Reports* 5: 8411.
- Husseneder C (2010) Symbiosis in subterranean termites: a review of insights from molecular studies. *Environmental Entomology* 39: 378–388.
- Jaenike J, Unckless R, Cockburn SN, Boelio LM & Perlman SJ (2010) Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science* 329: 212–215.
- Jamin AR & Vorburger C (2019) Estimating costs of aphid resistance to parasitoids conferred by a protective strain of the bacterial endosymbiont *Regiella insecticola*. *Entomologia Experimentalis et Applicata* 167 (doi: 10.1111/eea.12749).
- Jankevica L (2004) Ecological associations between entomopathogenic fungi and pest insects recorded in Latvia. *Latvijas Entomologs* 41: 60–65.
- Janson EM, Stireman JO III, Singer MS & Abbot P (2008) Phytophagous insect–microbe mutualisms and adaptive evolutionary diversification. *Evolution* 62: 997–1012.
- Jia X, Dini-Andreote F & Salles JF (2018) Community assembly processes of the microbial rare biosphere. *Trends in Microbiology* 26: 738–747.
- Johnson K (2015) The impact of *Wolbachia* on virus infection in mosquitoes. *Viruses* 7: 5705–5717.
- Jones DR (2003) Plant viruses transmitted by whiteflies. *European Journal of Plant Pathology* 109: 195–219.
- Jurburg SD, Natal-da-Luz T, Raimundo J, Morais PV, Sousa JP et al. (2018) Bacterial communities in soil become sensitive to drought under intensive grazing. *Science of the Total Environment* 6: 1638–1646.
- Kageyama D, Nishimura G, Hoshizaki S & Ishikawa Y (2002) Feminizing *Wolbachia* in an insect, *Ostrinia furnacalis* (Lepidoptera: Crambidae). *Heredity* 88: 444–449.
- Kaufman MG, Walker ED, Odelson DA & Klug MJ (2000) Microbial community ecology & insect nutrition. *American Entomologist* 46: 173–185.
- Kester M, Vrana KE & Karpa KD (2011) Elsevier’s Integrated Review: Pharmacology, 2nd edn. Elsevier, Philadelphia, PA, USA.
- Krams IA, Kecko S, Jõers P, Trakimas G, Elferts D et al. (2017) Microbiome symbionts and diet diversity incur costs on the immune system of insect larvae. *Journal of Experimental Biology* 220: 4204–4212.
- Lacey LA, Frutos R, Kaya HK & Vail P (2001) Insect pathogens as biological control agents: do they have a future? *Biological Control* 21: 230–248.
- Leigh BA, Bordenstein SR, Brooks AW, Mikaelyan A & Bordenstein SR (2018) Finer-scale phyllosymbiosis: insights from insect viromes. *mSystems* 3: e00131-18.
- Leite DC, Salles JF, Calderon EN, Castro CB, Bianchini A et al. (2018a) Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. *Frontiers in Microbiology* 9: 833.
- Leite DC, Salles JF, Calderon EN, van Elsas JD & Peixoto RS (2018b) Specific plasmid patterns and high rates of bacterial co-occurrence within the coral holobiont. *Ecology and Evolution* 8: 1818–1832.
- Lin XL, Kang ZW, Pan QJ & Liu TX (2015) Evaluation of five antibiotics on larval gut bacterial diversity of *Plutella xylostella* (Lepidoptera: Plutellidae). *Insect Science* 22: 619–628.
- Liu S, Vijayendran D & Bonning BC (2011) Next generation sequencing technologies for insect virus discovery. *Viruses* 3: 1849–1869.
- Lv X, Ma B, Yu J, Chang SX, Xu J et al. (2016) Bacterial community structure and function shift along a successional series of tidal flats in the Yellow River Delta. *Scientific Reports* 6: 36550.
- Malacrinò A, Campolo O, Medina RF & Palmeri V (2018) Instar and host-associated differentiation of bacterial communities in the Mediterranean fruit fly *Ceratitis capitata*. *PLoS ONE* 13: e0194131.
- Mallon CA, Poly F, Le Roux X, Marring I, van Elsas JD & Salles JF (2015a) Resource pulses can alleviate the biodiversity–invasion relationship in soil microbial communities. *Ecology* 96: 915–926.
- Mallon CA, van Elsas JD & Salles JF (2015b) Microbial invasions: the process patterns and mechanisms. *Trends in Microbiology* 23: 719–729.
- Mallon CA, Le Roux X, van Doorn GS, Dini-Andreote F, Poly F & Salles JF (2018) The impact of failure: unsuccessful bacterial invasions steer the soil microbial community away from the invader’s niche. *The ISME Journal* 12: 728–741.
- Maris PC, Joosten NN, Goldbach RW & Peters D (2004) *Tomato spotted wilt virus* infection improves host suitability for its vector *Frankliniella occidentalis*. *Phytopathology* 94: 706–711.
- Medeiros RB, Resende RDO & de Ávila AC (2004) The plant virus *Tomato Spotted Wilt Tospovirus* activates the immune system of its main insect vector, *Frankliniella occidentalis*. *Journal of Virology* 78: 4976–4982.
- Meregghetti V, Chouaia B & Montagna M (2017) New insights into the microbiota of moth pests. *International Journal of Molecular Sciences* 18: 2450.
- Miller WJ (2013) Bugs in transition: the dynamic world of *Wolbachia* in insects. *PLoS Genetics* 9: e1004069.
- Miller KE, Hopkins K, Inward DJ & Vogler AP (2016) Metabarcoding of fungal communities associated with bark beetles. *Ecology and Evolution* 6: 1590–1600.

- Mohammed WS, Ziganshina EE, Shagimardanova EI, Gogoleva NE & Ziganshin AM (2018) Comparison of intestinal bacterial and fungal communities across various xylophagous beetle larvae (Coleoptera: Cerambycidae). *Scientific Reports* 8: 10073.
- Montllor CB, Maxmen A & Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrtosiphon pisum* under heat stress. *Ecological Entomology* 27: 189–195.
- Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT et al. (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue Chikungunya and *Plasmodium*. *Cell* 139: 1268–1278.
- Nguyen NH, Suh SO & Blackwell M (2007) Five novel *Candida* species in insect-associated yeast clades isolated from *Neuroptera* and other insects. *Mycologia* 99: 842–858.
- Nguyen LD, Viscogliosi E & Delhaes L (2015) The lung mycobiome: an emerging field of the human respiratory microbiome. *Frontiers in Microbiology* 6: 89.
- Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M & Fukatsu T (2014) Evolutionary origin of insect–*Wolbachia* nutritional mutualism. *Proceedings of the National Academy of Sciences of the USA* 111: 10257–10262.
- Nouri S, Salem N, Nigg JC & Falk BW (2016) Diverse array of new viral sequences identified in worldwide populations of the Asian citrus psyllid (*Diaphorina citri*) using viral metagenomics. *Journal of Virology* 90: 2434–2445.
- Nouri S, Matsumura EE, Kuo YW & Falk BW (2018) Insect-specific viruses: from discovery to potential translational applications. *Current Opinion in Virology* 33: 33–41.
- Obeng N, Pratama AA & van Elsas JD (2016) The significance of mutualistic phages for bacterial ecology and evolution. *Trends in Microbiology* 24: 440–449.
- Ogilvie LA & Jones BV (2015) The human gut virome: a multifaceted majority. *Frontiers in Microbiology* 6: 918.
- Ohkuma M (2008) Symbioses of flagellates and prokaryotes in the gut of lower termites. *Trends in Microbiology* 16: 345–352.
- Oliver KM, Russell JA, Moran NA & Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the USA* 100: 1803–1807.
- Oliver KM, Degnan PH, Hunter MS & Moran NA (2009) Bacteriophages encode factors required for protection in a symbiotic mutualism. *Science* 325: 992–994.
- Oliver KM, Smith AH & Russell JA (2014) Defensive symbiosis in the real world—advancing ecological studies of heritable, protective bacteria in aphids and beyond. *Functional Ecology* 28: 341–355.
- Opatovsky I, Santos-Garcia D, Ruan Z, Lahav T, Ofaim S et al. (2018) Modeling trophic dependencies and exchanges among insects' bacterial symbionts in a host-simulated environment. *BMC Genomics* 19: 402.
- Paine TD, Raffa KF & Harrington TC (1997) Interactions among scolytid bark beetles, their associated fungi, and live host conifers. *Annual Review of Entomology* 42: 179–206.
- Papenfert K & Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nature Reviews Microbiology* 14: 576.
- Piper AM, Farnier K, Linder T, Speight R & Cunningham JP (2017) Two gut-associated yeasts in a Tephritid fruit fly have contrasting effects on adult attraction and larval survival. *Journal of Chemical Ecology* 43: 891–901.
- Poinar GO (2009) Description of an early Cretaceous termite (Isoptera: Kalotermitidae) and its associated intestinal protozoa, with comments on their co-evolution. *Parasites & Vectors* 2: 12.
- Pratama AA & van Elsas JD (2018) The 'neglected' soil virome—potential role and impact. *Trends in Microbiology* 26: 649–662.
- Quan AS & Eisen MB (2018) The ecology of the *Drosophila*-yeast mutualism in wineries. *PLoS ONE* 13: e0196440.
- Rainey SM, Shah P, Kohl A & Dietrich I (2014) Understanding the *Wolbachia*-mediated inhibition of arboviruses in mosquitoes: progress and challenges. *Journal of General Virology* 95: 517–530.
- Ramírez-Camejo LA, Maldonado-Morales G & Bayman P (2017) Differential microbial diversity in *Drosophila melanogaster*: are fruit flies potential vectors or opportunistic pathogens? *International Journal of Microbiology* 2017: 8526385.
- Ras E, Beukeboom LW, Cáceres C & Bourtzis K (2017) Review of the role of gut microbiota in mass rearing of the olive fruit fly, *Bactrocera oleae*, and its parasitoids. *Entomologia Experimentalis et Applicata* 164: 237–256.
- Reyes RP (1966) Insect protozoa. II. Devescovina and other flagellates from termites. *Proceedings of the First International Congress of Parasitology* 1: 601.
- Rizzi A, Crotti E, Borruso L, Jucker C, Lupi D et al. (2013) Characterization of the bacterial community associated with larvae and adults of *Anoplophora chinensis* collected in Italy by culture and culture-independent methods. *BioMed Research International* 2013: 420287.
- Robertson JL, Tsubouchi A & Tracey WD (2013) Larval defense against attack from parasitoid wasps requires nociceptive neurons. *PLoS ONE* 8: e78704.
- Rogers EE & Backus EA (2014) Anterior foregut microbiota of the glassy-winged sharpshooter explored using deep 16S rRNA gene sequencing from individual insects. *PLoS ONE* 9: e106215.
- Sartor RB & Wu GD (2017) Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. *Gastroenterology* 152: 327–339.
- Scarborough CL, Ferrari J & Godfray HCJ (2005) Aphid protected from pathogen by endosymbiont. *Science* 310: 1781.
- Shang Y, Feng P & Wang C (2015) Fungi that infect insects: altering host behavior and beyond. *PLoS Pathogens* 11: e1005037.
- Shinzato N, Matsumoto T, Yamaoka I, Oshima T & Yamagishi A (1999) Phylogenetic diversity of symbiotic methanogens living in the hindgut of the lower termite *Reticulitermes speratus* analyzed by PCR and in situ hybridization. *Applied and Environmental Microbiology* 65: 837–840.
- Siles JA, Öhlinger B, Cajthaml T, Kistler E & Margesin R (2018) Characterization of soil bacterial, archaeal and fungal communities inhabiting archaeological human-impacted

- layers at Monte Iato settlement (Sicily, Italy). *Scientific Reports* 8: 1903.
- Six DL & Wingfield MJ (2011) The role of phytopathogenicity in bark beetle–fungus symbioses: a challenge to the classic paradigm. *Annual Review of Entomology* 56: 255–272.
- Skidmore IH & Hansen AK (2017) The evolutionary development of plant-feeding insects and their nutritional endosymbionts. *Insect Science* 24: 910–928.
- Starmer WT & Fogleman JC (1986) Coadaptation of *Drosophila* and yeasts in their natural habitat. *Journal of Chemical Ecology* 12: 1037–1055.
- Stefanini I (2018) Yeast–insect associations: it takes guts. *Yeast* 35: 315–330.
- Strand MR & Burke GR (2015) Polydnnaviruses: from discovery to current insights. *Virology* 479: 393–402.
- Suh SO, Nguyen NH & Blackwell M (2008) Yeasts isolated from plant-associated beetles and other insects: seven novel *Candida* species near *Candida albicans*. *FEMS Yeast Research* 8: 88–102.
- Šustr V, Chroňáková A, Semanová S, Tajovský K & Šimek M (2014) Methane production and methanogenic Archaea in the digestive tracts of millipedes (Diplopoda). *PLoS ONE* 9: e102659.
- Taffner J, Erlacher A, Bragina A, Berg C, Moissl-Eichinger C & Berg G (2018) What is the role of Archaea in plants? New insights from the vegetation of Alpine bogs. *mSphere* 3: e00122-18.
- Thompson JA, Oliveira RA, Djukovic A, Ubeda C & Xavier KB (2015) Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. *Cell Reports* 10: 1861–1871.
- Tinker KA & Ottesen EA (2016) The core gut microbiome of the American cockroach, *Periplaneta americana*, is stable and resilient to dietary shifts. *Applied and Environmental Microbiology* 2: AEM-01837.
- Toju H, Peay KG, Yamamichi M, Narisawa K, Hiruma K et al. (2018) Core microbiomes for sustainable agroecosystems. *Nature Plants* 4: 247–257.
- Trabelsi D & Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. *BioMed Research International* 2013: 863240.
- Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk HP & Schleper C (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic Crenarchaeota in nitrogen cycling. *Environmental Microbiology* 7: 1985–1995.
- Trienens M, Kraaijeveld K & Wertheim B (2017) Defensive repertoire of *Drosophila* larvae in response to toxic fungi. *Molecular Ecology* 26: 5043–5057.
- Vacchini V, Gonella E, Crotti E, Prosdociemi EM, Mazzetto F et al. (2017) Bacterial diversity shift determined by different diets in the gut of the spotted wing fly *Drosophila suzukii* is primarily reflected on acetic acid bacteria. *Environmental Microbiology Reports* 9: 91–103.
- Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A & Dufresne A (2015) The importance of the microbiome of the plant holobiont. *New Phytologist* 206: 1196–1206.
- Varaldi J, Fouillet P, Ravallec M, López-Ferber M, Boulétreau M & Fleury F (2003) Infectious behavior in a parasitoid. *Science* 302: 1930–1930.
- van Veelen HPJ, Salles JF & Tieleman BI (2017) Multi-level comparisons of cloacal skin feather and nest-associated microbiota suggest considerable influence of horizontal acquisition on the microbiota assembly of sympatric woodlarks and skylarks. *Microbiome* 5: 156.
- Vega FE & Dowd PF (2005) The role of yeasts as insect endosymbionts. *Insect-Fungal Associations: Ecology and Evolution* (ed. by FE Vega & M Blackwell), pp. 211–243. Oxford University Press, Oxford, UK.
- Wang M, Veldsink JH, Dini-Andreote F & Salles JF (2018) Compositional and abundance changes of nitrogen-cycling genes in plant-root microbiomes along a salt marsh chronosequence. *Antonie van Leeuwenhoek* 111: 2061–2078.
- Waterhouse PM & Murrant AF (1983) Further evidence on the nature of the dependence of carrot mottle virus on carrot red leaf virus for transmission by aphids. *Annals of Applied Biology* 103: 455–464.
- Weldon SR & Oliver KM (2016) Diverse bacteriophage roles in an aphid–bacterial defensive mutualism. *The Mechanistic Benefits of Microbial Symbionts* (ed. by CJ Hurst), pp. 173–206. Springer, Cham, Switzerland.
- Werren JH, Baldo L & Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* 6: 741.
- Whitfield AE, Ullman DE & German TL (2005) Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43: 459–489.
- Whitfield AE, Falk BW & Rotenberg D (2015) Insect vector-mediated transmission of plant viruses. *Virology* 479: 278–289.
- Wilhelm SW, LeCleir GR, Bullerjahn GS, McKay RM, Saxton MA et al. (2014) Seasonal changes in microbial community structure and activity imply winter production is linked to summer hypoxia in a large lake. *FEMS Microbiology Ecology* 87: 475–485.
- Williams T, Virto C, Murillo R & Caballero P (2017) Covert infection of insects by baculoviruses. *Frontiers in Microbiology* 8: 1337.
- Winstanley D & Rovesti L (1993) Insect viruses as biocontrol agents. *Exploitation of Microorganisms* (ed. by DG Jones), pp. 105–136. Springer, Dordrecht, The Netherlands.
- Wu D, Daugherty SC, Van Aken SE, Pai GH, Watkins KL et al. (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biology* 4: e188.
- Xia H, Hu C, Zhang D, Tang S, Zhang Z et al. (2015) Metagenomic profile of the viral communities in *Rhipicephalus* spp. ticks from Yunnan, China. *PLoS ONE* 10: e0121609.
- Xie J, Vilchez I & Mateos M (2010) *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. *PLoS ONE* 5: e12149.
- Yoshikawa K, Matsukawa M & Tanaka T (2018) Viral infection induces different detoxification enzyme activities in insecticide-resistant and-susceptible brown planthopper *Nilaparvata lugens* strains. *Journal of Pesticide Science* 43: 10–17.

- Zhang G, Asad S, Khromykh AA & Asgari S (2017) Cell fusing agent virus and dengue virus mutually interact in *Aedes aegypti* cell lines. *Scientific Reports* 7: 6935.
- Zhou F, Lou Q, Wang B, Xu L, Cheng C et al. (2016) Altered carbohydrates allocation by associated bacteria-fungi interactions in a bark beetle-microbe symbiosis. *Scientific Reports* 6: 20135.
- Ziganshina EE, Mohammed WS, Shagimardanova EI, Vankov PY, Gogoleva NE & Ziganshin AM (2018) Fungal bacterial and archaeal diversity in the digestive tract of several beetle larvae (Coleoptera). *BioMed Research International* 2018: 6765438.
- Zoberi MH & Grace JK (1990) Fungi associated with the subterranean termite *Reticulitermes flavipes* in Ontario. *Mycologia* 82: 289–294.