REVIEW ARTICLE

The gut microbiota of insects – diversity in structure and function

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Received 16 February 2013; revised 6 May 2013; accepted 13 May 2013. Final version published online 17 June 2013.

DOI: 10.1111/1574-6976.12025

Editor: Sebastian Suerbaum

Keywords
bacterial diversity; digestive system; gut communities; immune system; symbiosis; mutualism.

Abstract

Insect guts present distinctive environments for microbial colonization, and bacteria in the gut potentially provide many beneficial services to their hosts. Insects display a wide range in degree of dependence on gut bacteria for basic functions. Most insect guts contain relatively few microbial species as compared to mammalian guts, but some insects harbor large gut communities of specialized bacteria. Others are colonized only opportunistically and sparsely by bacteria common in other environments. Insect digestive tracts vary extensively in morphology and physicochemical properties, factors that greatly influence microbial community structure. One obstacle to the evolution of intimate associations with gut microorganisms is the lack of dependable transmission routes between host individuals. Here, social insects, such as termites, ants, and bees, are exceptions: social interactions provide opportunities for transfer of gut bacteria, and some of the most distinctive and consistent gut communities, with specialized beneficial functions in nutrition and protection, have been found in social insect species. Still, gut bacteria of other insects have also been shown to contribute to nutrition, protection from parasites and pathogens, modulation of immune responses, and communication. The extent of these roles is still unclear and awaits further studies.

Introduction

Insects are by far the most diverse and abundant animal clade, in numbers of species globally, in ecological habits, and in biomass (Basset *et al.*, 2012). The diversification and evolutionary success of insects have depended in part on their myriad relationships with beneficial microorganisms, which are known to upgrade nutrient-poor diets; aid digestion of recalcitrant food components; protect from predators, parasites, and pathogens; contribute to inter- and intraspecific communication; affect efficiency as disease vectors; and govern mating and reproductive systems. As for essentially all animals, microbial communities are particularly prominent in the digestive tract, where they may be key mediators of the varied lifestyles of insect hosts.

The contribution of microorganisms, particularly gut microorganisms, to insect function is highly relevant from several perspectives, linking to medicine, agriculture, and ecology. Some insect species provide useful laboratory models for experimental work on microbial communities and their interactions with hosts, particularly for the understanding of immunity and metabolic interactions (Lemaitre & Hoffmann, 2007). For insect vectors of disease, symbiotic microorganisms can influence vectoring efficiency (McMeniman *et al.*, 2009; Ricci *et al.*, 2012) or developmental time (Chouaia *et al.*, 2012) and thus provide targets for potential disease control. Insects are responsible for massive agricultural losses and also for pollination of many food crops, and microorganisms associated with both herbivores and pollinators can affect their impact on crop plants. Natural and human-impacted ecosystems depend critically on insects and their gut microbial communities as mediators of biogeochemical cycling; for example, insect–microorganism mediation can be critical in the decomposition of plant biomass and carbon cycle (Bignell *et al.*, 1997; Fierer *et al.*, 2009) and in rates of nitrogen fixation and the nitrogen cycle (Fox-Dobbs *et al.*, 2010).

Despite good reasons for knowing more about insect gut communities and despite the recent massive increase in studies of microorganisms living in insect guts, broad rules about how these communities are organized are just beginning to emerge. The last decade has seen the

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publication of many relevant studies ranging from community diversity surveys to molecular studies on how gut bacteria interact with host immune systems. In this review, we attempt to synthesize current knowledge on this topic. Our emphasis will be on work since 2004; earlier work is summarized in the study by Dillon & Dillon (2004).

The insect gut as a habitat for microorganisms

Structure and properties of insect guts

The basic structure of the digestive tract is similar across insects although they possess a diversity of modifications associated with adaptation to different feeding modes (Fig. 1). The gut has three primary regions: foregut, midgut (or ventriculus), and hindgut (Chapman *et al.*, 2013). The foregut and hindgut originate from embryonic ectoderm and are lined with exoskeleton made up of chitin and cuticular glycoproteins. This exoskeleton separates the gut lumen from the epidermal cells and is shed at each ecdysis. Foregut or hindgut may be subdivided into functionally distinct subsections, with the foregut often having a separate crop or diverticula for temporary food storage and the hindgut encompassing discrete sections such as fermentation chambers and a separate rectum for holding feces before defecation. The midgut is the primary site of digestion and absorption in many insects; it lacks the exoskeletal lining and has a different developmental origin, arising from endodermal cells. In many insects, the midgut epithelial cells secrete an envelope called the peritrophic matrix (or peritrophic membrane), which is typically continuously replaced as it is shed. The peritrophic matrix divides the midgut into the endo- and ectoperitrophic space, and microorganisms are usually confined to the former, preventing their direct contact with midgut epithelium. There are two different types of peritrophic matrix, type I and type II. While type I lines the entire midgut and sometimes is actively produced

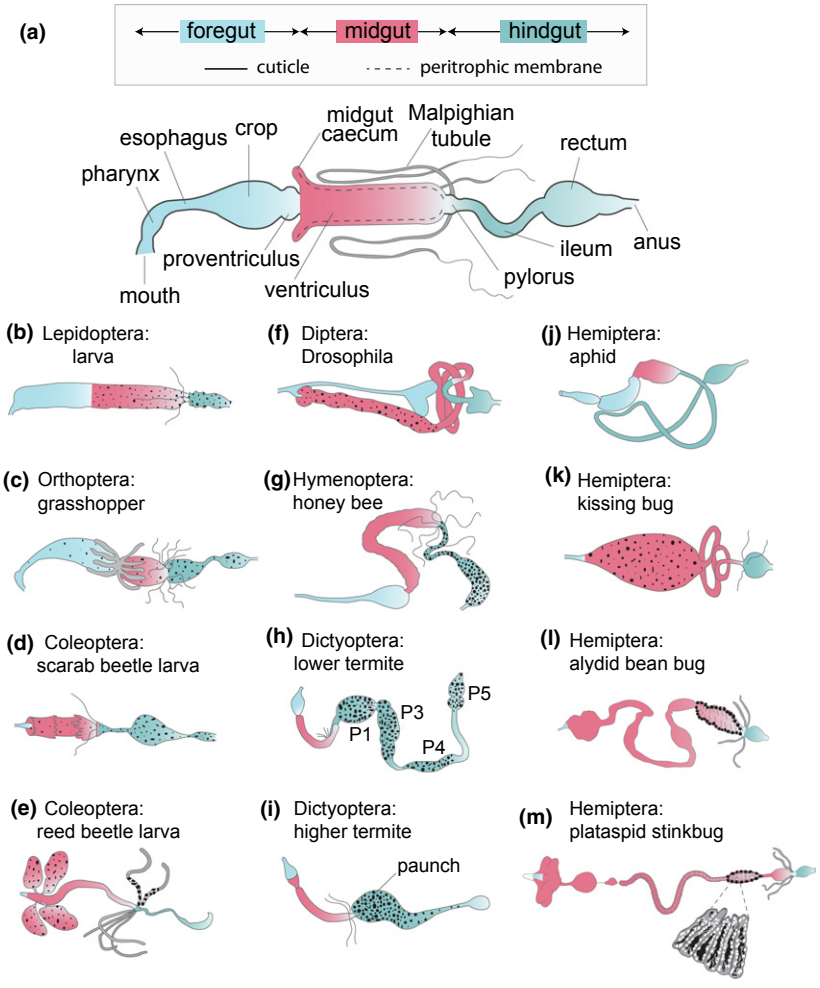

Fig. 1. (a) Generalized gut structure of insects. The foregut and hindgut are lined by a cuticle layer (thick black line), and the midgut secretes a peritrophic matrix (dashed line). (b–m) Gut structures of insects from different orders, focusing on examples from experimental systems considered in this review. If not specifically indicated, the gut of an adult insect is shown. Stipules depict predominant localization of gut bacteria, which have been studied. For plataspid stinkbug, the magnification shows bacteria localized in the midgut crypts, adapted from Hosokawa *et al.* (2012). Midgut crypts are also present in alydid bean bugs (not shown). Blue indicates foregut and hindgut, and red indicates midgut. Gut structures have been adapted from Buchner (1965), Fukatsu & Hosokawa (2002), Brune (2006), and Chapman *et al.* (2013).

when certain food is ingested, the type II is only found in a specific region of the anterior midgut (Lehane, 1997). The peritrophic matrix serves a variety of functions including providing a barrier that protects the epithelium from mechanical damage by food particles, from exposure to large toxin molecules present in food, and from microbial invasion, and also concentrating food and digestive enzymes (Shao *et al.*, 2001). In some cases, the peritrophic matrix packages the undigested food bolus as it moves through the digestive tract. The peritrophic matrix is punctuated by small pores that bar most microorganisms while allowing passage of enzymes and small molecules from digested food (Peters & Wiese, 1986; Spence & Kawata, 1993; Ferreira *et al.*, 1994; Barbehenn & Martin, 1997; Edwards & Jacobs-Lorena, 2000). Some insect species do not produce a peritrophic matrix (Lehane, 1997); among these groups are most sap-feeding Homoptera (order *Hemiptera*), many species of beetles (order *Coleoptera*; Nardi & Bee, 2012), and ants (order *Hymenoptera*) that specialize on nectar or honey dew (liquid feces of sap-feeding insects; Cook & Davidson, 2006).

Insect excretory organs are the Malpighian tubules, which are extensions of the anterior hindgut that extend into the body cavity and absorb wastes, such as uric acid, which are delivered to the anterior hindgut (Fig. 1). Thus, the hindgut contains a combination of nitrogenous waste and food waste, probably creating a different nutritive environment for insect gut bacteria than for gut bacteria of animals, in which these two waste products are separated. While water resorption is a function that is well documented for the hindgut (Chapman *et al.*, 2013), the hindgut can also be a site of nutrient absorption, as demonstrated for numerous insect groups, including crickets (Kaufman *et al.*, 1989), termites (Potrikus & Breznak, 1981), cockroaches (Zurek & Keddie, 1996), and heteropterans (Kashima *et al.*, 2006). For example, the hindgut wall of some cockroaches contains intercellular channels that allow movement of nutrients from hindgut lumen to the hemolymph, including fatty acids and amino acids produced by bacteria within the colon (Cruden & Markovetz, 1987; Zurek & Keddie, 1996).

The basic design of insect guts displays many modifications reflecting adaptations to specialized niches and feeding habits, and many of these specializations have evolved for housing gut microorganisms in specific gut compartments. Examples in Fig. 1 illustrate a few modifications that are relevant to studies discussed in this review.

Stability of the insect gut as a microbial habitat

From the perspective of microbial colonization, insect guts often present unstable habitats (Fig. 2). Insects molt numerous times during larval development, shedding the exoskeletal lining of the foregut and hindgut each time and thus severely disrupting or eliminating any attached bacterial populations. The midgut produces and repeatedly sheds the peritrophic matrix and along with it associated microorganisms, most of which do not cross into the space adjacent to midgut epithelial cells. In holometabolous insects with distinct larval, pupal, and adult stages, there is a radical remodeling of the gut and other organs at metamorphosis, with the elimination of the entire larval gut and contents as a meconium that is enveloped in the peritrophic matrix of the pupal stage. An investigation into gut microorganism persistence through development in several mosquito species found that metamorphosis resulted in complete or near-complete elimination of gut bacteria, with newly emerged adults containing no bacteria in their guts (Moll *et al.*, 2001). However, many insect guts display specialized crypts or paunches that promote microbial persistence.

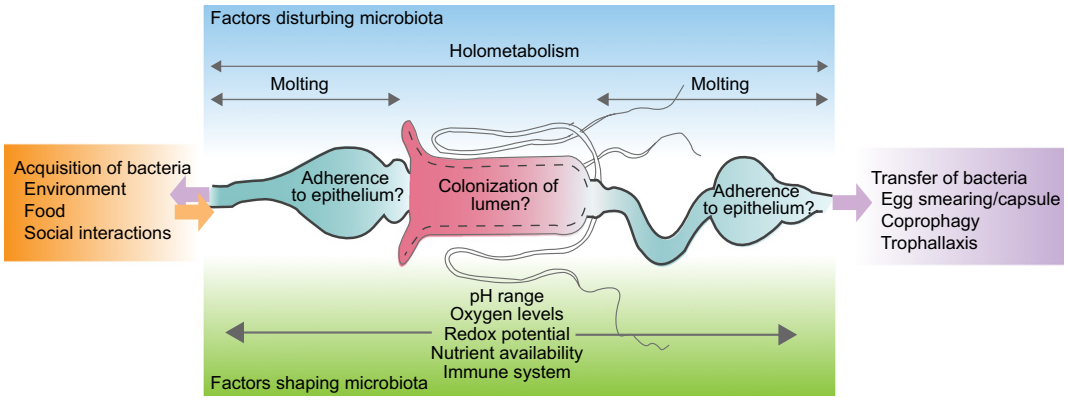

Fig. 2. Factors influencing composition of the gut microbiota of insects include insect development, physiochemical conditions in different gut compartments, available sources for bacteria acquisition, and capability to transfer bacteria to progeny.

And insects do not molt once they reach the adult stage, so following the final molt, the foregut or hindgut wall provides a stable surface for colonization.

The basic insect life cycle also presents potential challenges for transmission of microorganisms between generations. In most insects, females abandon eggs after depositing them, and the only social behavior involves mating of adults. As a result, opportunities for direct transfer of gut symbionts between conspecifics are more limited in most insects as compared to mammals and birds, which have extended parent–offspring contact. However, some insect species, including cockroaches, termites, ants, and some wasps and bees, show gregarious or social behavior, including oral trophallaxis or coprophagy, which can enable direct or indirect social transmission, thus promoting the evolution of specialized host-dependent symbionts (Hongoh *et al.*, 2005, 2006; Martinson *et al.*, 2012). In addition, females sometimes display sophisticated mechanisms for inoculating eggs or progeny with microbial symbionts, thus enabling long-term associations (Hosokawa *et al.*, 2007; Kuechler *et al.*, 2012). These specialized adaptations for transmission to progeny or colony members point to the evolutionary advantages of maintaining a consistent microbiota.

Physical conditions in insect guts

Microbial colonization depends on the physicochemical conditions in the lumen of different gut compartments, and these can display extreme variation in both pH and oxygen availability. The pH of the lumen is actively regulated and often diverges from that of the hemolymph, which is usually near 7. Midguts of lepidopteran larvae show extreme alkalinity, with pH as high as 11–12, and digestive enzymes are adapted to the alkaline conditions (Appel & Martin, 1990; Harrison, 2001). The pH of lepidopteran guts is correlated with feeding on tannin-rich leaves and has been interpreted as an adaptation that lowers the binding of dietary protein with ingested tannins, improving nutrient availability (Berenbaum, 1980), but it also has major consequences for microbial communities as it excludes most bacteria.

In insect guts with large microbial communities, microbial metabolism actively shapes conditions within the lumen of different gut compartments. For example, in detritus-feeding larvae of the scarab beetle *Pachnoda ephippiata*, microbial fermentation products including acetate, formate, and lactate are abundant in both midgut and hindgut, although profiles differ between the two compartments (Lemke *et al.*, 2003). A study of the pH along the gut axis in *P. ephippiata* showed regular, pronounced variation, with values near 8 in the anterior midgut, rising to > 10 in the center of the midgut, and dropping to 7 in the hindgut (Lemke *et al.*, 2003) where microbial densities are highest (Cazemier *et al.*, 1997). In mosquito larvae, highly alkaline conditions in the anterior midgut are dependent on V-ATPase pumps that maintain strong gradients in hydrogen ion concentrations (Boudko *et al.*, 2001). In contrast, the gut lumens of some nonholometabolous insects often show less extreme pH gradients (Appel & Martin, 1990). Termites are an exception, with pH ranging from 5 to > 12 in the compartmentalized guts of some soil-feeding species (Brune & Ohkuma, 2010; Köhler *et al.*, 2012). The extreme alkalinity in some compartments of termite guts does not entirely prevent microbial colonization but instead supports the growth of specialized alkaline-tolerant symbiotic bacteria from *Firmicutes*, *Clostridium*, and *Planctomycetes* (Köhler *et al.*, 2008; Bignell, 2010). Oxygenation of insect guts varies from fully aerobic to anaerobic, with anaerobic conditions more common in larger insects and insects that have enlarged gut compartments and robust gut communities (Appel & Martin, 1990; Johnson & Barbehenn, 2000).

Guts of termites have been characterized most extensively. Termites evolved from cockroach ancestors and have the most elaborate known gut communities of any insects. Their guts display numerous specializations, including several hindgut compartments or paunches (Fig. 1h and i) housing dense, characteristic microbial communities that differ sharply among compartments (Köhler *et al.*, 2012). Gut compartments have volumes of about 1 μL and function as microbial bioreactors with high rates of turnover of hydrogen pools (Pester & Brune, 2007). Microsensors have been used in several studies to precisely delineate conditions within termite guts and to relate these conditions to microbial activities (reviews in Brune & Friedrich, 2000; Brune & Ohkuma, 2010; Köhler *et al.*, 2012).

Structure and evolution of gut microbial communities in insects

Taxonomic compositions

The microorganisms in insect guts can include protists, fungi, archaea, and bacteria. Protists are best studied in the lower termites and wood roaches, in which their maintenance depends on social transmission (Hongoh, 2010; see section on termite nutritional symbiosis). Fungi are frequent in guts of insects that feed on wood or detritus, and they likely play a part in digestion. Methanogenic archaea are mostly known from insects such as beetles and termites that feed on wood or detritus (Egert *et al.*, 2003; Lemke *et al.*, 2003; Brune 2010). Bacterial species comprise all or most organisms in the guts of most insect species. However, most studies have depended on bacterial 16S rRNA gene primers, possibly biasing views of the composition of insect gut communities.

Table 1. Transmission modes, composition, and proposed functions of representative insect gut communities

Insect host species	Transmission route	No. of major spp.	Exemplar taxa	Consistency among hosts	Host food	Proposed roles in hosts	References
Plataspid bug: *Megacopta punctatissima*	Maternal (egg capsule)	1	*Ishikawaella capsulatus* (Proteobacterium)	Uniform	Plant sap	Nutrient provisioning (amino acids)	Fukatsu & Hosokawa (2002), Hosokawa *et al.* (2006)
Alydid bug: *Riptortus clavatus*	Environment	1	*Burkholderia* sp. (Proteobacterium)	Uniform	Plant sap	Nutritional?, degradation of toxin	Kikuchi *et al.* (2007, 2012)
Reed beetle: *Macroplea* sp.	Maternal egg -smearing	1	*Macropleicola* spp. (Proteobacterium)	Uniform	Plant cells	Production of cocoon material	Kölsch *et al.* (2009), Kölsch & Pedersen (2010)
Rhodnius prolixus	Maternal egg -smearing	1?	*Rhodococcus rhodnii* (Actinobacterium)	Uniform	Blood	Nutrient provisioning	Beard *et al.* (2002), Eichler & Schaub (2002)
Honey and bumble bees: *Apis* ssp. *Bombus* spp.	Social transmission	6–9	*Snodgrassella alvi, Gilliamella apicola, Lactobacillus* ssp.	Uniform	Pollen and nectar	Digestion, protection against parasites, other?	Koch & Schmid-Hempel (2011b), Engel *et al.* (2012), Martinson *et al.* (2011)
Lower termite: *Reticulitermes speratus*	Social transmission	> 300	*Flagellates*, *Bacteroidetes*, *Spirochetes*, *Proteobacteria*, *Firmicutes*	Uniform	Dry wood	Nutrient provisioning, N recycling, fixation, lignocellulose digestion, fermentation	Nakajima *et al.* (2005), Hongoh *et al.* (2005, 2008b), Desai & Brune (2012)
Higher termite: *Nasutitermes* species	Social transmission	> 300	*Spirochetes*, *Fibrobacteres*, *Bacteroidetes*, *Firmicutes*, *Acidobacteria*, *Proteobacteria*, TG3	Uniform	Detritus	Nutrient provisioning, N recycling, fixation, cellulose digestion, fermentation	Warnecke *et al.* (2007), Köhler *et al.* (2012)
Grasshopper: *Schistocerca gregaria*	Acquisition from food	< 12	*Enterococcus*, *Serratia*, *Klebsiella*, *Acinetobacter*	Variable	Plant leaves	Produce components of aggregation pheromone	Dillon *et al.* (2008, 2010)
Fruit fly: *Drosophila melanogaster*	Acquisition from food	< 8	*Lactobacillus* spp., *Acetobacteraceae*, *Orbaceae*	Variable	Decaying fruit	Prime immune system, affect metabolism and mating preferences	Reviewed in Broderick & Lemaitre (2012)
Gypsy moth caterpillar: *Lymantria dispar*	Acquisition from food	< 8	*Pseudomonas*, *Enterobacter*, *Pantoea*, *Serratia, Staphylococcus*, *Bacillus*	Variable	Plant leaves	Unknown, may increase susceptibility to toxin by affecting midgut epithelial permeability	Broderick *et al.* (2004, 2009), Mason *et al.* (2011)
Pea aphid: *Acyrthosiphon pisum*	Environment	Few in healthy aphids	*Staphylococcus, Pseudomonas, Acinetobacter, Pantoea*	Variable	Phloem sap	Mostly pathogenic, produce signaling compounds that attract aphid predators	Harada *et al.* (1997), Stavrinides *et al.* (2009, 2010), Leroy *et al.* (2011)

These bacterial communities vary immensely in total size, in composition, and in locations and functions within the gut (Table 1). For example, a honey bee adult contains *c.* 10^9 bacterial cells (Martinson *et al.*, 2012), a similar number is found in adult *Rhodnius* (kissing bugs; Eichler & Schaub, 2002) and in adult *Acheta domestica* (house cricket; Santo Domingo *et al.*, 1998), whereas an adult grasshopper (*Melanoplus sanguinipes*) contains about 10^6 bacteria (Mead *et al.*, 1988), and an adult *Drosophila melanogaster* has about 10^5 bacteria (Ren *et al.*, 2007; Ryu *et al.*, 2010). Most plant sap-feeding insects contain few (Cheung & Purcell, 1993) or no (Douglas, 1988) detectable gut bacteria but instead contain intracellular symbionts (Baumann, 2005). The highest ratios of total gut microbial biomass to host mass are found in some detritivores and wood-feeders, including termites, crickets, cockroaches, and wood-boring and detritivorous beetles (Cazemier *et al.*, 1997). These larger gut communities are usually associated with more compartmentalized guts (Dillon & Dillon, 2004) or with expanded hindguts with an elaborated intima (Cazemier *et al.*, 1997). Insects such as *Drosophila*, mosquitoes, and aphids, with relatively small communities in relation to host body mass, typically possess long narrow guts, sometimes several times the body length (Fig. 1f and j), and may display adaptations for improving nutrient absorption or for limiting water uptake.

Other fundamental distinctions among gut communities of insects involve whether the microorganisms are specifically adapted to living in insects and whether they are transmitted directly between hosts or acquired each generation from the outside environment. These features are often correlated: bacteria with reliable transmission between hosts will evolve specialization to the host gut niche. But environmentally acquired bacteria, whether pathogenic or symbiotic, may have specific regulatory responses to the host-associated niche (Ruby *et al.*, 2004; Wier *et al.*, 2010). For example, in the *Vibrio fischeri*–bobtail squid symbiosis, *V. fischeri* replicates both in the water column and in its host's symbiotic organ where it reorganizes gene expression upon colonization. The entry of symbionts and exclusion of nonsymbionts are mediated by the host innate immune system (Nyholm & Graf, 2012). Similar symbiont policing by the innate immune system has been documented for leeches and their gut symbionts and for *Hydra* and their epithelial symbionts (Silver *et al.*, 2007; Fraune *et al.*, 2010). Such mechanisms may underlie the selective uptake of insect gut bacteria that are acquired environmentally each generation.

A variety of bacterial phyla are commonly present in insect guts, including *Gammaproteobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*, *Firmicutes* including *Lactobacillus* and *Bacillus* species, *Clostridia*, *Actinomycetes*, *Spirochetes*, *Verrucomicrobia*, *Actinobacteria,* and others (see overview in Colman *et al.*, 2012). Community diversity is higher in guts of insects, such as some beetles and termites, which feed on wood or detritus (Colman *et al.*, 2012). Otherwise, there appear to be few broad taxonomic patterns at the level of insect order (Colman *et al.*, 2012), although certain groups have specific associations with particular bacterial species, as in termites (Hongoh, 2010) and honey bees and bumble bees (Martinson *et al.*, 2011; Koch *et al.*, 2013).

Lessons from heritable endosymbionts

The centrality of microorganisms to insect ecology and evolution is evident from studies of intracellular, maternally transmitted symbiotic bacteria, which are widespread in insects and nearly universal within some higher taxa. Many of these are associated with gut tissues or are involved in nutrition. We discuss them briefly here for comparison with typical gut symbionts.

Heritable symbionts can be divided into two intergrading categories: obligate and facultative endosymbionts. Obligate endosymbionts live in the cytosol of specialized host cells (bacteriocytes) and provision limiting nutrients needed by hosts (Baumann, 2005). They are evolutionarily ancient and involve specialization on the part of both host and symbiont. The most intensively studied example is the symbiosis of aphids and the bacterial symbiont, *Buchnera aphidicola*, originating about 200 million years ago (Baumann, 2005). As is typical in obligate symbioses, *B. aphidicola* has a highly reduced genome, but retains genes that enable it to provide its host with nutrients, in this case essential amino acids and vitamins that are rare in the phloem sap diet of the aphid host (Shigenobu *et al.*, 2000; Hansen & Moran, 2011; Poliakov *et al.*, 2011). In other sap-feeding insects, multiple obligate endosymbionts coexist in distinct bacteriocytes within the same host, and their even more reduced genomes retain complementary gene sets encoding nutrient-provisioning biosynthetic pathways needed by the host (McCutcheon & Moran, 2012).

The interaction of these obligate symbioses with host immune systems is mostly not understood. In aphids, the Immune Deficiency (IMD) pathway and most antimicrobial peptides (AMPs) are absent, and dependence on beneficial symbiotic bacteria has been suggested as a selective force leading to this reduction in immune capabilities (Laughton *et al.*, 2011). But some other insects with obligate symbionts may harness immune mechanisms to control symbionts, as in the use of an AMP in grain weevils to prevent a bacteriocyte-associated symbiont from invading other host tissues (Ash, 2011; Login & Heddi, 2012).

These ancient, obligate symbioses can be contrasted with heritable symbionts not required by the host, that is, facultative endosymbionts. These are exemplified by *Wolbachia pipientis*, *Spiroplasma* species, and *Hamiltonella defensa*, which are largely maternally transmitted but undergo occasional horizontal transmission, causing host and symbiont evolution to be decoupled. In these cases, the symbionts retain larger and more dynamic genomes (Werren *et al.*, 2008; Degnan *et al.*, 2009) and possess mechanisms for actively invading host tissues and for affecting host biology in a way that promotes the increased frequency of infected hosts in the host population (Werren *et al.*, 2008; Oliver *et al.*, 2010). These effects are commonly beneficial to the host, as in the case of *H. defensa,* which uses a bacteriophage-encoded mechanism to protect its host against parasitoid wasp larvae, thereby enabling its own survival and transmission (Oliver *et al.*, 2010). Alternatively, symbionts may promote their own spread without benefiting hosts, as in *W. pipientis*, which can cause sex ratio biases or reproductive incompatibilities that lower host fitness (Werren *et al.*, 2008). But even these 'selfish' symbionts may simultaneously confer benefits to hosts; for example, in *Drosophila* species, *W. pipientis* has been found to protect against infections by some RNA viruses (Hedges *et al.*, 2008; Teixeira *et al.*, 2008), and *Spiroplasma* has been shown to protect hosts against nematode parasites (Jaenike *et al.*, 2010). Such benefits might be widespread and unrecognized, because they will only be detected if the right environmental variables are identified for incorporation into experiments.

Thus, heritable symbionts present a continuum with respect to the interdependence of the evolutionary fates of hosts and symbionts and consequently the extent to which symbionts improve host fitness. At one extreme, some obligate symbionts resemble organelles in being degenerate and entirely dependent on host mechanisms for their transmission and maintenance. At the other, they resemble pathogenic bacteria in their ability to invade tissues and cells of completely novel host individuals and species (Dale & Moran, 2006). Some facultative symbiont groups contain lineages, which have become obligate within particular insect hosts (e.g. Dale *et al.*, 2002; Hosokawa *et al.*, 2010a), a shift that is correlated with the loss of mechanisms for cell invasion, genome reduction in the symbiont, and the establishment of specialized host cells that house symbionts.

Findings for heritable symbioses yield several insights relevant to gut microbial communities in insects. First, bacteria can provide a huge variety of services that can affect insects in their various ecological niches; these span the general categories of improving nutrition, protecting against parasites and pathogens, and increasing tolerance to abiotic challenges such as heat stress. Second, different symbionts within the same host may collaborate in contributing complementary services, as in the examples of coresident symbionts supplying different nutrients (McCutcheon & Moran, 2010). Third, transmission fidelity may have a major impact on the extent of specialization of symbiont and host, as coadaptation is most pronounced when there are reliable mechanisms for the direct transmission of symbionts between hosts.

Examples of highly specialized gut bacteria

The wide range in intimacy and continuity of associations of insects with gut microorganisms is illustrated within the Heteroptera (order *Hemiptera*), which includes diverse insects with sucking mouthparts that feed on plant or animal fluids (Kuechler *et al.*, 2012). Many plant-feeding heteropteran species have midguts with caecae or crypts that house populations of symbiotic bacteria (Fig. 1l and m). At one extreme, these gut symbionts can be strictly heritable and approach intracellular symbionts or organelles in their level of specialization. The best-studied example is *Ishikawaella capsulata*, which lives in specialized crypts in guts of the stinkbug species *Megacopta punctatissima* (family: *Plataspidae*; Fukatsu & Hosokawa, 2002). *Ishikawaella capsulata* has all of the hallmarks of an obligate bacteriocyte-associated nutritional symbiont, including strict vertical transmission and coevolution with hosts, reduced genome size, and retention of genes that enable nutrient provisioning to the host, which lives on a restricted diet of plant sap (Hosokawa *et al.*, 2006; Nikoh *et al.*, 2011). While *I. capsulata* resides in the gut lumen and is thus not intracellular or transmitted within eggs, it achieves highly efficient vertical transmission: ovipositioning females defecate to produce a specialized symbiotic capsule on the outside of the egg case, and juveniles immediately ingest the capsule following hatching (Hosokawa *et al.*, 2006). The genome of *I. capsulata* lacks many genes including many involved in cell wall synthesis and lipid metabolism, indicating specialization to conditions provided by the host. Many other heteropterans also possess bacterial symbionts, often in specialized midgut caecae. Some are transmitted vertically through smearing of eggs by the mother as in *I. capsulata*, and in such cases, the symbionts may have the usual genomic features of coevolved obligate symbionts (Prado *et al.*, 2006; Kikuchi *et al.*, 2009, 2010; Hosokawa *et al.*, 2010b; Kaiwa *et al.*, 2010). However, some heteropterans rely on environmental acquisition of a specific symbiont strain every generation, implying that the host gut selects the appropriate bacterial strains from a range of ingested organisms. For example, the bean bug, *Riptortus pedestris* (Heteroptera: *Alydidae*), acquires a specific

Burkholderia symbiont orally every generation, and the symbiont forms dense colonies in midgut crypts (Kikuchi *et al.*, 2005). Hosts that fail to acquire the symbiont during a specific developmental window, coinciding with the appearance of the crypts, display stunted growth (Kikuchi *et al.*, 2007, 2011). Remarkably, *R. pedestris* is efficiently colonized by as few as 80 cells of its *Burkholderia* symbiont (Kikuchi & Yumoto, 2013), paralleling findings for the bobtail squid system (Nyholm & McFall-Ngai, 2004) and suggesting specific recognition capabilities on the part of both host and symbiont. Studies of the gut microbiota of the firebug *Pyrrhocoris apterus* revealed that oxic regions of the midgut contain a variety of bacteria that are likely transient, but that the anoxic M4 section of the midgut contains a highly characteristic community that is maternally transmitted (Sudakaran *et al.*, 2012). These typical taxa include anaerobes from the bacterial phylum *Actinobacteria* and from the *Firmicutes*, and some of the former appear to supplement nutrition and are required for normal growth (Salem *et al.*, 2012).

A representative of another group of plant-feeding Heteroptera, *Nezara viridula* (Heteroptera: *Pentatomidae*), was also found to house a specific symbiont in gut crypts and to acquire the symbiont environmentally each generation (Prado *et al.*, 2006), suggesting that environmental transmission is not always incompatible with high specificity of a symbiotic relationship.

Close and specific associations with particular bacterial symbiont species are also found among blood-feeding heteropterans (Heteroptera: *Reduviidae*: Triatominae). For example, *Rhodnius prolixus*, the kissing bug vector of trypanosome parasites, has a specific association with the actinomycete *Rhodococcus rhodnii*, which forms large populations of up to 10^9 cells in the lumen of the anterior midgut (Beard *et al.*, 2002; Eichler & Schaub, 2002). Acquisition occurs through coprophagy.

In the tsetse fly (*Diptera*: *Glossinidae*), the symbiont *Sodalis glossinidius* lives both intracellularly in different tissues and also in the gut lumen; transmission is achieved as a consequence of the specialized reproductive biology of this insect, in which larvae develop within the maternal uterus and ingest milk secretions containing *S. glossinidius* (Attardo *et al.*, 2008).

Other cases of specific maternally transmitted symbionts associated with the gut are found in some beetle groups. Grain weevils (genus *Sitophilus*) contain true endosymbionts that are transmitted through eggs and that live in cytosol of foregut cells of larvae and migrate to midgut epithelial cells in adults, apparently using bacterial type III secretion systems for cellular invasion (Dale *et al.*, 2002). The symbionts of reed beetles (*Chrysomelidae*: Donaciinae) are also vertically transmitted but reside in the gut lumen; these associations resemble those of the plataspid–*I. capsulata* association described above (Kölsch *et al.*, 2009). The symbionts dominate the gut community and are concentrated in large midgut caecae in larvae and in specialized regions of Malpighian tubules in adults. As in the plataspid–*I. capsulata* association, they undergo efficient maternal transmission, achieved by egg-smearing by mothers followed by ingestion by hatched larvae. Phylogenetic analyses indicate that this has resulted in long-term cospeciation of hosts and symbionts (Kölsch & Pedersen, 2010).

Social transmission of specialized gut bacteria

Other clear cases of specialized gut symbionts that are maintained through vertical transmission are found in social or gregarious insects, including social bees and termites. In honey bees (*Apis mellifera*), bacterial symbionts confined to the hindguts of adults are acquired in the first few days following emergence of adults from the pupal stage, through social interactions with other adult worker bees in the colony (Martinson *et al.*, 2012). Honey bee gut inhabitants belong to a small number of distinctive lineages found only in honey bees and also in other *Apis* species and in *Bombus* species (bumble bees), which are also social and which are closely related to honey bees (Koch & Schmid-Hempel, 2011a; Martinson *et al.*, 2011; Li *et al.*, 2012; Koch *et al.*, 2013). Thus, vertical transmission through sociality may facilitate host–symbiont coevolution and emergence of a distinctive gut community.

Ant species, all of which are social, also show a number of specialized gut bacteria and associated morphological modifications of the gut (Roche & Wheeler, 1999; Cook & Davidson, 2006; Bution & Caetano, 2008; Caetano *et al.*, 2009; Russell *et al.*, 2009a). A broad survey of ants and their bacterial associates revealed relatively simple communities often including characteristic gut symbionts that appear to be linked both to feeding mode and to phylogenetic groupings, suggesting coevolution of particular ant lineages with specialized symbiotic bacteria (Anderson *et al.*, 2012). Specific associations are especially evident in some herbivorous ant groups such as the Cephalotini (turtle ants; Anderson *et al.*, 2012).

Termite gut communities are more complex, usually containing hundreds of species or phylotypes based on 16S rRNA gene surveys; furthermore, most sequences retrieved from termite guts are novel, suggesting that most of these organisms are termite gut specialists and not environmental bacteria ingested with food (Hongoh *et al.*, 2006; Ohkuma & Brune, 2010). Transmission appears to occur primarily through coprophagy or proctodeal trophallaxis within colonies. Different hindgut compartments house different bacterial communities (Köhler, 2011).

The extent of direct transfer of gut bacteria between conspecific hosts in nonsocial insects is unclear. Gregarious insects such as cockroaches and crickets, although lacking parental care and sociality, can transmit bacteria by defecating and feeding in a common area. The firebrat, *Thermobia domestica*, aggregates in groups in response to specific microorganisms present in the feces of conspecifics. This was shown to result in the horizontal transfer of these bacteria between insects (Woodbury & Gries, 2013; Woodbury *et al.*, 2013). In a study of gut microbiota of two termites, a social wood roach, and a solitary cockroach (*Periplaneta americana*), the three social species had guts dominated by specialized communities of symbionts, including bacteria and protozoans, whereas gut communities of the nonsocial *P. americana* were dominated by bacterial species common in the environment (Sabree *et al.*, 2012b). If this pattern were upheld in future studies, it would imply a dominant role of sociality in the evolution of characteristic gut microbiota in insects. On the other hand, even in solitary insects with nonoverlapping generations, females could potentially transmit bacteria to progeny simply by defecating in the vicinity of eggs and having their gut bacteria ingested by their progeny. For this transmission route to be effective, larvae and adults would both need to host the same bacterial types, as in some heteropteran stinkbugs, as described above, and bacteria would need to persist for some time in the environment.

Environmental bacteria as insect gut symbionts

In many insects, most or all gut microorganisms are not transmitted host-to-host, and gut communities are dominated by widely distributed bacteria that appear to colonize hosts opportunistically. In one of the earliest non-culture-based studies of insect gut communities, guts of gypsy moth caterpillars were found to possess bacterial communities that are highly dependent on the diet and that are composed of taxa widespread in other environments, such as *Pseudomonas* and *Bacillus* species (Broderick *et al.*, 2004). A similar study of gut communities of caterpillars of the cabbage white butterfly likewise found that widespread environmental taxa dominated in the gut community (Robinson *et al.*, 2010). Deep sequencing of 16S rRNA gene amplicons from mosquitoes of several species and localities in Kenya revealed that most individual hosts had gut communities dominated by a single bacterial species, but that these varied among hosts of the same species and could be the same for hosts of different species (Osei-Poku *et al.*, 2012). At a minimum, the physicochemical conditions of gut compartments, such as pH, redox potential, and availability of particular substrates, will be selective for particular species (Fig. 2). Thus, even when acquired independently each generation, gut communities are not expected to be random assemblages of bacteria from the food or local environment, and most studies do show differences between profiles of microorganisms in guts vs. those ingested with food.

In many insects, gut bacterial communities vary among individuals within a species and appear to consist largely of bacteria not specifically adapted to living in guts of their host species. In laboratory-reared caterpillars of the lepidopteran pest species *Spodoptera littoralis* and *Helicoverpa armigera*, experiments on gut community composition supported selectivity of the gut environment with some influence of diet, but documented a relatively static community overall (Tang *et al.*, 2012). In contrast, field-collected *H. armigera* from different locations and host plants contained highly variable gut communities with some influence of host plant (Priya *et al.*, 2012).

Several research groups have investigated the *Drosophila* gut microbiota, which exemplify gut communities of low diversity and high variability among hosts. Numerous non-culture-based surveys show that dominant taxa vary among laboratories and are influenced by diet, but that certain taxa recur (reviewed in Broderick & Lemaitre, 2012). In particular, members of *Acetobacteraceae* are often among the few dominant taxa, and *Lactobacillus* species are sometimes also abundant (Roh *et al.*, 2008; Wong *et al.*, 2011). However, dominant taxa differ among studies; for example, a different study of laboratory-reared *D. melanogaster* reported communities with large proportions of *Enterobacter* (*Gammaproteobacteria*) or of *Enterococcus* (*Firmicutes*) along with *Acetobacteraceae*, but few *Lactobacillus* (Cox & Gilmore, 2007); yet another study found that *D. melanogaster* guts contained high numbers of *Enterobacteriaceae* and *Acetobacteraceae*, but few *Lactobacillus* and almost no *Enterococcus* (Chandler *et al.*, 2011). The latter study also showed an influence of diet and of the source laboratory, even though the different source laboratories used the same fly food prepared in the same facility. In comparisons of gut communities of different *Drosophila* species grown on different diets, diet overrode host species, which had no detectable effect (Chandler *et al.*, 2011). Together, these observations indicate that the *Drosophila* gut is colonized by environmental bacteria, and although colonization can be selective, the composition of bacteria in the food is a major determinant of the community profile. Wild-collected flies show even more variation and have communities distinct from those reared in the laboratory with abundance of bacteria in the family *Orbaceae* (Chandler *et al.*, 2011), which also includes gut bacteria from bees (Kwong & Moran, 2012; Engel *et al.*, 2013). Another study of wild *D. melanogaster* recovered many *Acetobacteraceae* and *Lactobacillus* as well as *Enterobacteriaceae* and *Bacteroidetes* and documented differences among

geographic locations (Corby-Harris *et al.*, 2007). Surveys of gut communities in the house fly, *Musca domestica*, and the Mediterranean fruit fly, *Ceratitis capitata*, also revealed a preponderance of *Enterobacteriaceae*, with many retrieved 16S rRNA gene sequences identical to those of known aerobic species (Gupta *et al.*, 2011; Aharon *et al.*, 2013).

Characteristic taxa in dynamic insect gut communities

Even when gut communities associated with an insect species are strongly influenced by diet or location, one or a few bacterial taxa may be shared across most or all hosts (Broderick *et al.*, 2004; Chandler *et al.*, 2011; Schauer *et al.*, 2012; Aharon *et al.*, 2013). The tendency for certain taxa to recur in gut communities of the same and different species potentially reflects direct transmission among host individuals, selective uptake on the part of hosts, or specific adaptation for colonizing insect guts on the part of the microorganisms, or some combination. All three of these processes appear to underlie the finding that species of *Acetobacteraceae* are widespread in guts of diverse insects, particularly those that use plant-derived sugar resources for all or part of their life cycle, including mosquitoes, *Drosophila*, honey bees, leafhoppers, caterpillars, and scale insects (Crotti *et al.*, 2010; Robinson *et al.*, 2010). In *D. melanogaster*, it has been suggested that by choosing food containing byproducts of desirable bacteria, mobile host individuals can shape their own gut microbiota (Broderick & Lemaitre, 2012). Bacterial adaptation to insect guts also appears to be important, even when these microorganisms are also adapted to noninsect niches. Whereas species of *Acetobacteraceae* replicate in plant nectar, some, such as those in the genus *Asaia*, also have specific capabilities for colonizing insect guts and tissues and for maintaining persistent associations with insect hosts, including capabilities for maternal and paternal transmission (Favia *et al.*, 2007; Crotti *et al.*, 2009). Strains of *Asaia* are able to multiply in guts of individual mosquito hosts and to invade divergent mosquito species (Chouaia *et al.*, 2010), and an individual strain can colonize hosts as divergent as leafhoppers and mosquitoes (Crotti *et al.*, 2009). These bacteria sometimes appear to maintain vertically transmitted infections in hosts, resembling facultative endosymbionts such as *H. defensa* in aphids (Damiani *et al.*, 2008).

Analysis of insect gut microbiota – a perspective

There has been a recent proliferation of microbial community studies based on sequenced amplicons from universal 16S rRNA gene primers, and many investigators have reported on insect gut communities (Colman *et al.*, 2012). While these are starting to yield a picture of the community diversity and membership in various insect guts, direct comparisons of diversity measures between studies should be interpreted with caution, because different studies use different starting tissues, different methods for DNA release from bacterial cells, different primers, different sequencing methods, and different cutoffs for distinguishing species (or OTUs). Further, the absolute number of bacteria in the gut can dramatically differ between and within insect species and is only rarely investigated. These factors can have major effects on community profiles detected in samples (Engelbrektson *et al.*, 2010). Nevertheless, multiple studies sometimes do identify the same dominant bacterial taxa in a particular host species or group, through sequencing and comparisons with databases (Ohkuma & Brune, 2010; Köhler *et al.*, 2012; Moran *et al.*, 2012; Sabree *et al.*, 2012a; Sudakaran *et al.*, 2012). A compilation of 16S rRNA gene surveys addressed whether gut community diversity correlates with insect taxonomic group or with diet, but found only a few trends (Colman *et al.*, 2012). However, more patterns might be evident as these surveys progress. Another caution regarding these surveys is that, alone, they enable few functional conclusions. The 16S rRNA gene is a highly conserved molecule, which gives little information about metabolic capabilities of a bacterium; bacterial genomes with nearly identical 16S rRNA gene sequences can have large differences in gene content and metabolic capacities. Combined community functions may often be more critical to hosts than the identities of the particular species, and metagenomic studies of functional capabilities could reveal conservation of metabolism even when species composition varies.

Bacteria–host interaction in the insect gut

Tolerance and resistance

The digestive system of insects is equipped with a multilayered defense system, which likely is a major determinant for shaping microbial communities in the gut. Different mechanisms of this defense system contribute to tolerance and resistance properties of the host toward bacteria in the gut. While tolerance is the ability to reduce negative impacts of a given bacterial load on the host's health, resistance is the ability to reduce the bacterial load, so that it cannot impact the host's health (Schneider & Ayres, 2008). Most studies in immunology have focused on resistance mechanisms, and little is known about mechanisms mediating tolerance. However, these might be of particular relevance to microorganism–host interactions in the gut, because these interactions are

often mutualistic or commensalistic, and the host needs to minimize any negative impacts of the resident microbiota. Insects with large bacterial communities probably exhibit higher levels of tolerance and lower levels of resistance toward bacteria in their guts, in comparison with insects with sparsely populated digestive systems. Consequently, mechanisms underlying gut immunity in different insects might be adapted to the specific needs of the host. In the following, we will summarize immune mechanisms discovered in the gut of insects and discuss their role in microbiota–host interaction.

As described above, most insect midguts secrete a peritrophic matrix that consists of a network of chitin microfibrils embedded in a protein–carbohydrate matrix (Terra, 1990). The peritrophic matrix is semi-permeable and allows the passage of nutrients, digestive enzymes, and defensive molecules, but protects the epithelial cell layer against direct exposure to microorganisms or toxins. In the fore- and hindgut, the cuticle layer lining the epithelial cell layer might provide similar protective functions. These physical barriers between epithelium and lumen are good examples for tolerance mechanisms, because they do not reduce the bacterial load in the gut, but they reduce the impact of the bacteria on the host.

Certain regions of the insect gut can be low or high in pH or produce enzymes, such as lysozymes or peptidoglycan (PGN) hydrolases, which attack bacterial cell wall components (Daffre *et al.*, 1994; Hultmark, 1996; Dubreuil *et al.*, 2001). These mechanisms potentially confer resistance, because they can reduce the number of bacteria in certain gut compartments, but they are also potentially important in digestion of bacterial cells for improved nutrition.

The innate immune system in insect guts

Another line of defense is the innate immune system of insects, which consists of multiple immune reactions, some of which are homologous to immune mechanisms found in mammals (Müller *et al.*, 2008). General principles of innate immunity in insects have been summarized by other reviews (Lemaitre & Hoffmann, 2007; Charroux & Royet, 2010; Ganesan *et al.*, 2011; Chambers & Schneider, 2012), and we cover only aspects relevant to interactions with gut bacteria. Based primarily on studies with *D. melanogaster*, there are two major inducible responses enabling local immunity at the intestinal epithelial cell layer: production of AMPs and synthesis of reactive oxygen species (ROS; Fig. 3). While both of these induced responses might be seen as classical resistance mechanisms, they both include negative feedback loops and modulatory components, which can confer host tolerance toward the commensal gut microbiota.

In the systemic immune response of *D. melanogaster*, Toll and IMD are the two major signaling pathways inducing AMP production (Lemaitre *et al.*, 1995, 1996; De Gregorio *et al.*, 2002). The response in the gut differs, in that only the IMD pathway is active, triggering a local AMP response upon pathogen exposure (Liehl *et al.*, 2006; Nehme *et al.*, 2007; Buchon *et al.*, 2009b). Activation occurs by binding of different variants of bacterial PGN to extra- or intracellular epithelial receptors belonging to the peptidoglycan recognition protein (PGRP) family (Leulier *et al.*, 2003; Kaneko & Silverman, 2005). Downstream signaling via the IMD pathway results in activation of the transcription factor Relish, which in turn induces expression of several AMPs and other immunity-related genes (Fig. 3).

In the gut of *D. melanogaster*, exposure to pathogens also triggers the generation of ROS via the membrane-associated dual oxidase (DUOX) system (Ha *et al.*, 2005a, b; Fig. 3). This happens via PGN-dependent and PGN-independent signaling pathways (Ha *et al.*, 2009; Fig. 3). Production of ROS causes oxidative stress not only on the bacteria but also on the host's epithelial cells. Therefore, *D. melanogaster* eliminates excessive ROS by activating immune responsive catalases (Ha *et al.*, 2005b). This catalase production results in increased tolerance, because it decreases the self-harm caused by the bacteria-induced immune response (Schneider & Ayres, 2008). How these catalases protect host cells without compromising the ROS activity against the pathogens remains to be investigated. One possible explanation could be that the catalase activity is locally restricted, for example, to the proximity of the epithelial surface.

Immune system–gut microbiota interaction

Insects experience constant exposure to bacteria from their commensal microbiota. This poses the question of how the immune system in the gut can distinguish between commensal and pathogenic bacteria and avoid constitutive production of immune effectors, such as AMPs and ROS. *Drosophila melanogaster* has been shown to achieve immune tolerance to the commensal gut microbial community by modulating the IMD pathway and DUOX system activity at various levels (Fig. 3). In the IMD pathway, the homeobox transcription factor Caudal specifically represses AMP gene transcription in the gut by binding to promoter regions. In Caudal-deficient flies, a constitutive AMP production occurs and causes shifts in the gut microbiota and the disintegration of the epithelial cell layer (Ryu *et al.*, 2008). This suggests that Caudal prevents overstimulation of the immune system by commensal gut bacteria. Another immune modulatory mechanism of *D. melanogaster* is governed by

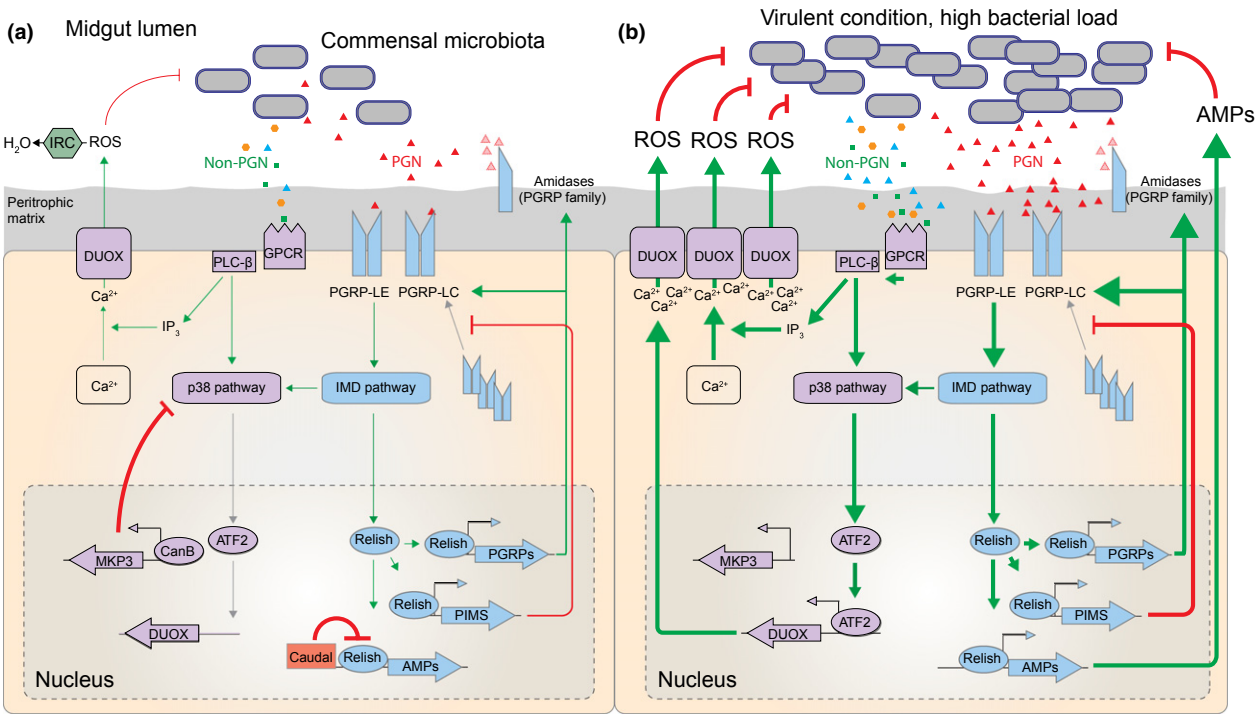

Fig. 3. Signaling network of the insect immune system controlling AMP and ROS production in the midgut of *Drosophila melanogaster*. (a) In the presence of the commensal microbiota, low amounts of PGN bind to PGRPs, which results in a basal activity of IMD pathway. In turn, Relish is translocated into the nucleus and induces expression of negative regulators of the IMD pathway (PIMS and PGRPs). AMP transcription is repressed by Caudal. (b) When bacterial burden is high, for example, in the presence of pathogens, increased levels of PGN result in a strong activation of the IMD pathway. Large amounts of Relish are translocated into the nucleus. Relish overcomes Caudal repression and induces AMP transcription. ROS activity is regulated by three different mechanisms. In the presence of the commensal microbiota (a), immune-regulated catalases eliminate excessive ROS, which can be harmful to the host. Production of ROS via the DUOX system is modulated by intracellular calcium mobilized from the endoplasmic reticulum upon receiving signals from microorganism-associated molecular patterns other than PGN (non-PGNs). Additional expression of DUOX results from p38 activation, which is under the control of both PGN- and non-PGN-mediated signaling. Under normal conditions, p38 activation is inhibited by the dual phosphatase MKP3, which in turn is induced by calcineurin B (CanB). Green and red arrows indicate active pathways. Thicker lines indicate dominant pathways. GPCR, G-protein-coupled receptor; PLC-β, phospholipase C-β; IP_3, phosphatidylinositol-4,5-bisphosphate. For details on specific functions see text. Scheme has been adopted from Leulier & Royet (2009) and Charroux & Royet (2010).

amidases, which are secreted by epithelial cells in the midgut and cleave pro-inflammatory PGN into nonactive forms (Bischoff *et al.*, 2006; Zaidman-Rémy *et al.*, 2006). This is thought to sustain a low basal level of PGN originating from residential microorganisms. Further, the protein Pirk sequesters specific PGN-binding receptors (PGRP-LC) in the cytoplasm, thereby reducing the number of these receptors localizing to the cell surface and retarding IMD pathway signaling (Kleino *et al.*, 2008; Lhocine *et al.*, 2008). Another PGN-binding receptor, PGRP-LE, was recently shown to play a key role in immune modulatory processes in the gut of *D. melanogaster*. On one side, PGRP-LE induces a Relish-dependent immune response to pathogenic bacteria. On the other side, it also ensures immune tolerance to the commensal microbiota via the up-regulation of amidases and Pirk (Bosco-Drayon *et al.*, 2012). PGRP-LE-dependent production of amidases was also implicated in the repression of a systematic immune response by preventing the transfer of PGN originating from the gut microbiota into the hemolymph (Bosco-Drayon *et al.*, 2012). This raises the question of how IMD signaling via a single receptor can enable discrimination of friend and foe. Bosco-Drayon *et al.* (2012) proposed that the bacterial load and the duration of IMD activation could influence the nature of the response. PGN is mostly released when bacteria divide and is therefore a signature of bacterial cell division rather than simply bacterial presence. Consequently, transient colonization with a high load of pathogenic bacteria may induce a strong IMD response characterized by elevated AMP production, while chronic, low-dose exposure to commensal bacteria instead may stimulate IMD-dependent expression of negative regulators of AMP production (Zaidman-Rémy *et al.*, 2006; Bosco-Drayon *et al.*, 2012).

For the DUOX system of *D. melanogaster*, similar modulatory mechanisms have been discovered (Fig. 3; Ha *et al.*, 2009). In the presence of the commensal microbiota, activation of DUOX gene expression is inhibited by MKP3 (Fig. 3). However, a basal ROS production still occurs, because non-PGN ligand binding to the epithelial cell surface can induce DUOX enzymatic activity by mobilizing intracellular calcium (Fig. 3). This basal response is critical, because flies deficient in incorporating such signals from the microbiota display fitness defects attributed to the uncontrolled growth of commensal bacteria (Ha *et al.*, 2009). When bacterial burden increases, for example due to colonization by a pathogen, the basal activity of the DUOX system via calcium signaling is not sufficient to combat the infection. In such a condition, DUOX expression is enhanced via p38 signaling originating from strong PGN-dependent and non-PGN-dependent signals. In addition, DUOX activity is increased by further mobilization of calcium (Ha *et al.*, 2009).

In conclusion, the 'fine-tuned' regulation of two synergistically acting immune responses, consisting of AMP production and ROS synthesis, seems to contribute to homeostasis in the midgut of *D. melanogaster* by tolerating the commensal microbiota and combating deleterious pathogens (Fig. 3). However, differences in strength and duration of signals received from common microorganism-associated patterns, such as PGN, might not be sufficient to explain the distinct immune responses raised against pathogens and commensals in insect guts. For example, the gut of an adult honey bee is colonized by *c.* 10^9 commensal bacteria within a few days of pupal eclosion (Martinson *et al.*, 2012). Whether the host will be able to detect a pathogen under such conditions solely based on PGN release is doubtful and suggests that other factors contribute to the plasticity of the immune system in the gut of insects.

Pathogens typically cause pathology in the host via specific virulence determinants, such as toxins or host cell translocated effector proteins (Casadevall & Pirofski, 2003; Galán, 2009; Ashida *et al.*, 2012). This pathology may induce specific immune responses. In plants, such responses have been studied for years and are generally referred to as effector-triggered immunity (ETI; Flor, 1942; Chisholm *et al.*, 2006; Jones & Dangl, 2006). Recently, evidence has accumulated that similar ETI mechanisms are also present in animals (Brandt *et al.*, 2004; Fontana *et al.*, 2011, 2012; Dunbar *et al.*, 2012; McEwan *et al.*, 2012). For example, in one study, it was shown that *D. melanogaster* can raise an immune response against pathogenic *Escherichia coli* based on the modification of the small GTPase RAC1, as mediated via a specific bacterial effector protein (Boyer *et al.*, 2011). ETI is thought to be of particular relevance in nonimmune cells, like epithelial cells, because these cells may not express the whole set of primary immune system features (Stuart *et al.*, 2013). Hence, ETI could play an important role in distinguishing pathogens from commensals in the gut of insects.

In addition, it is becoming clear that insects have means to generate immune memory and diversity, even though they are lacking B and T cells. The improved response of an insect toward a pathogen upon a second exposure is known as immune priming (Schmid-Hempel, 2005). This can also have important implication for microbiota–host interactions in the gut. Colonization of the gut by commensal bacteria could induce immune priming events resulting in the activation or alteration of the immune response not only toward recurrent colonization of commensal bacteria, but also against pathogens. This was demonstrated in a study on immune priming to *Plasmodium* in mosquitoes: Rodrigues *et al.* (2010) showed that the gut microbiota is essential not only for priming the immune system of the host to *Plasmodium*, but also for eliciting the priming response upon rechallenging the insects with *Plasmodium*. The bacteria-dependent priming response was characterized by the differentiation of prohemocytes into granulocytes and the presence of increased numbers of circulating granulocytes with changed morphology and binding properties. Similarly, in the tsetse fly, bacterial symbionts, including the gut-inhabiting Gammaproteobacterium *S. glossinidius*, were shown to be essential during larval development in order that the adult flies could present a trypanosome-refractory phenotype. In this case, the bacteria seem to influence the formation and integrity of the adult peritrophic matrix, thereby indirectly regulating the fly's ability to detect and respond to the presence of trypanosomes (Weiss *et al.*, 2013). The molecular mechanisms underlying these priming events are still not well understood. However, these studies on the immune priming demonstrated that immune memory in insects does not necessarily have to be elicited by the pathogen, but can also be induced by commensal bacteria in the gut (Rodrigues *et al.*, 2010).

Different properties of the immune system in insect guts

The diversity of insects and their gut structures makes it clear that immunological processes involved in gut microbiota–host interactions cannot be generalized across all insects. Even within the midgut of *D. melanogaster*, regionalization of the immune response exists, with different receptors being expressed in different locations (Tzou *et al.*, 2000; Buchon *et al.*, 2009b; Bosco-Drayon *et al.*, 2012; Neyen *et al.*, 2012). Anterior parts encounter

ingested bacteria first and might need to invoke a different response than more distal parts. Furthermore, almost nothing is known about the immune response in the hindgut of insects. This region, in particular, can be heavily colonized by bacteria in certain insects (Hongoh, 2010; Martinson *et al.*, 2012), and it is expected that the host is equipped with appropriate regulatory systems to sustain gut integrity and tolerate high microbial loads. Genomic sequencing has revealed that certain insects lack some of the well-described immune system components of *D. melanogaster* (Honeybee Genome Sequencing Consortium, 2006; International Aphid Genomics Consortium, 2010); these genomic findings further suggest that mechanisms implicated in gut defense and homeostasis can be divergent across different insect species. Hence, immune mechanisms other than those described for *D. melanogaster* are likely to exist in other insect species.

For example, the mosquito, *Anopheles gambiae*, was shown to transiently reduce the permeability of its extracellular matrix in the midgut by the formation of a dityrosine network in the mucus, a layer underlying the peritrophic matrix (Kumar *et al.*, 2010). This occurs in response to the ingestion of a blood meal and prevents the activation of epithelial immunity by ingested bacteria, which in turn can proliferate in the lumen. As mosquitos are batch feeders, each blood meal exposes the host to a high bacterial load for a short period of time. This can trigger a strong AMP response, and the formation of the dityrosine network hinders such immune activation. Together with the DUOX system, the peroxidase IMPer was shown to be responsible for cross-linking tyrosines into this network in the mucus layer (Kumar *et al.*, 2010). Whether this mechanism is specific to *A. gambiae* or is possibly more widely distributed among insects is unknown at the moment.

Functions of insect gut bacteria

The gut microbiota of mammals governs an immense range of functions contributing to the host's development, pathogen resistance, nutrition, and physiology. Thus, the gut microbiota may be considered as a bacterial organ, which is integrated into the biological system of the host (Bäckhed *et al.*, 2005). A central role of gut microorganisms, as documented for mammals, is likely true for many insects, based on the few species studied to date. However, our current knowledge about microbial functions in the gut of insects is very limited when considered in the context of the immense diversity of insect species that exist on our planet. In the following, we summarize findings, which highlight functional roles of gut microorganisms of specific insects. Figure 4 gives an overview of some of these functions.

Nutritional symbioses

Insects have adapted to an immense range of ecological niches where they often thrive on nutrient-poor or refractory diets. Therefore, nutritional symbioses with microorganisms that amend dietary nutrients are widespread. As discussed above, many insects house endosymbionts in specialized cells or organs for direct provisioning of amino acids and cofactors. However, gut bacteria also can contribute to nutrition of insects. Bacteria passing through the gut can simply be digested and used *per se* as nutrients (nutritional bacteria). In fact, lysozymes expressed in the gut of *Drosophila* were suggested to play a role in nutrition rather than in immunity (Daffre *et al.*, 1994). *Drosophila* expresses more than 10 different lysozymes in the midgut and harbors a transporter exhibiting high affinity for D-amino acids, which are found in PGN (Miller *et al.*, 2008). This indicates that bacteria passing through the gut, ingested with fermented food, might be an important nutrient source. However, as in mammals, commensal gut bacteria can carry out more specific symbiotic functions, including the breakdown of refractory dietary compounds and the production of specific nutrients (Kaufman & Klug, 1991; Kikuchi *et al.*, 2005; Andert *et al.*, 2008; Köhler *et al.*, 2008; Russell *et al.*, 2009a; Gaio Ade *et al.*, 2011; Hongoh, 2011; Engel *et al.*, 2012; Hosokawa *et al.*, 2012; Schauer *et al.*, 2012).

Digestion of recalcitrant plant polymers

Insects feeding on plant matter, especially wood (xylophagy), can harbor gut microbial communities involved in cellulose degradation (Kaufman & Klug, 1991; Slaytor, 1992; Anand *et al.*, 2010). Cellulose is a rich carbon source, but it exists as crystalline or amorphous microfibrils in plant cell walls and thus is not readily accessible to the host (Watanabe & Tokuda, 2010). In the gut, the cellulose fibers first need to be broken down into simpler sugar residues, a process which bacteria are typically involved in (Warnecke *et al.*, 2007; Russell *et al.*, 2009b; Pope *et al.*, 2010; Hess *et al.*, 2011). In contrast to ruminants, which rely solely on gut microorganisms for cellulose digestion, some insects encode cellulases in their own genomes (Watanabe *et al.*, 1998). In termites, these intrinsic enzymes have been shown to be expressed and active in the gut (Watanabe & Tokuda, 2010); however, their relative contribution to the overall cellulose degradation in these insects is not yet clear. Probably, the relative importance of microbial and host-derived enzymes varies depending on the insect species, the presence of a stable gut community, and the composition of the diet. In woody material, cellulolytic components of the plant cell wall are protected by lignin, representing a barrier for

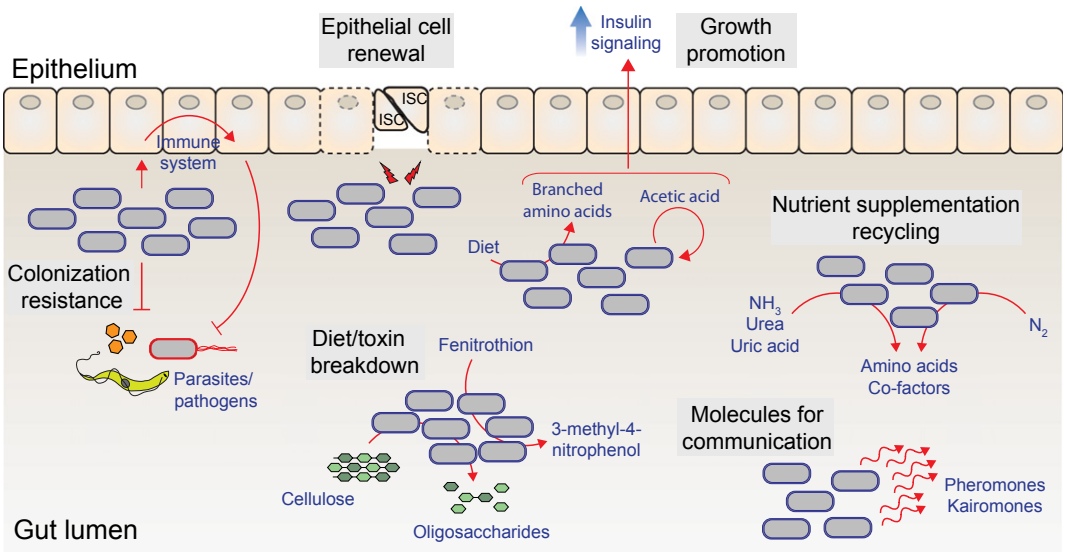

Fig. 4. Known functions of bacteria in insect guts. Colonization resistance against pathogens or parasites has been described for the bumble bee, *Bombus terrestris*, the desert locust, *Schistocerca gregaria*, and various mosquito species (Pumpuni *et al.*, 1993; Gonzalez-Ceron *et al.*, 2003; Dillon *et al.*, 2005; Cirimotich *et al.*, 2011a; Koch & Schmid-Hempel, 2011b). In *Drosophila melanogaster*, the commensal gut microbiota has been shown to be involved in intestinal cell renewal and promotion of systemic growth (Buchon *et al.*, 2009a, b; Shin *et al.*, 2011; Storelli *et al.*, 2011). A prime example for diet breakdown is the degradation of cellulose by the characteristic gut microbiota in the hindgut of termites (Warnecke *et al.*, 2007). Gut bacteria have also been shown to degrade toxins ingested with the diet (Ping *et al.*, 2007; Kikuchi *et al.*, 2012). The insecticide fenitrothion is hydrolyzed into 3-methyl-4-nitrophenol by the *Burkholderia* gut symbiont of the stinkbug *Riptortus pedestris*. Nutrient supplementation, such as the synthesis of vitamins and essential amino acids or the fixation of nitrogen, has been shown for gut symbionts of blood-feeding kissing bugs, stinkbugs, and termites, respectively (Eichler & Schaub, 2002; Hongoh *et al.*, 2008b; Nikoh *et al.*, 2011). Certain gut bacteria of termites can also recycle nitrogenous waste products excreted by the host by converting them into high-value nutrients (Hongoh *et al.*, 2008b). Similar functions might also be carried out by gut bacteria of ants and cockroaches (Russell *et al.*, 2009a; Sabree *et al.*, 2009). In a number of insects, gut bacteria produce molecules involved in intraspecific and interspecific communication, such as pheromones and kairomones (Dillon *et al.*, 2002; Sharon *et al.*, 2010; Leroy *et al.*, 2011).

carbohydrate degradation in xylophagous insects. Many species are thus thought to feed on predegraded wood. However, the Asian longhorned beetle, *Anoplophora glabripennis*, and the Pacific dampwood termite, *Zootermopsis angusticollis*, both degrade lignin during the passage through the gut (Geib *et al.*, 2008). Although fungal microorganisms might be the main players for lignin degradation in these insects, certain metabolic pathways of gut bacteria have also been implicated in contributing to these processes (Schloss *et al.*, 2006; Le Roes-Hill *et al.*, 2011).

A number of plant-feeding insects have specialized on pollen as the main nutrient source. Pollen grains are protected from the environment by a refractory outer shell, which insects must overcome to gain access to the nutrient-rich interior. Besides a typical primary plant cell wall, a so-called exine layer surrounds pollen grains (Ariizumi & Toriyama, 2011). This layer is resistant to degradation, and its digestion has only been reported for certain springtails (subclass *Collembola*) that seem to harbor an enzyme of unknown origin called exinase (Scott & Stojanovich, 1963). In other insects, swelling and pseudogermination of pollen grains were reported to result in the outgrowth of short pollen tubes that promote the exposure of the primary plant cell wall to the gut environment (Haslett, 1983; Peng *et al.*, 1985; Dobson & Peng, 1997). Host- or bacterial-derived enzymes could then act on the exposed primary cell wall resulting in the exudation of the nutrient-rich pollen content. In the honey bee, genes involved in pectin degradation were identified in the gammaproteobacterial gut symbiont *Gilliamella apicola*, and *in vitro* culturing tests confirmed pectin degradation activity of isolates of this bacterial species (Engel *et al.*, 2012). Pectin is part of the primary plant cell wall and is particularly abundant in outgrowing pollen tubes during pollen germination (Taylor & Hepler, 1997). Bacterial degradation of pectin may therefore facilitate perforation and loosening of the primary cell wall of pollen. This stands in agreement with the observations that pectic acids and hemicelluloses of pollen underwent partial digestion during the passage through the gut of honey bees, while exine structure and cellulose remained intact (Klungness & Peng, 1984). Whether bacterial pectin degradation supports pollen

digestion, and thus has a mutualistic function with benefits for both the host and the bacteria, still needs to be experimentally validated.

Nutrient provisioning

As mentioned above, direct supplementation of essential nutrients deficient in the diet of insects is often carried out by intracellular endosymbionts. However, there are a few examples of gut microorganisms that seem to contribute to nutrient provisioning. These include the gut symbionts *R. rhodnii,* which provisions B vitamins to its blood-feeding host *R. prolixus* (Eichler & Schaub, 2002), and *Ishikawaella capsulatus*, which provisions essential amino acids to plant-feeding plataspid stinkbugs (Nikoh *et al.*, 2011).

In the diet of herbivorous animals, nitrogen is often the limiting factor, and many insects probably rely on mutualistic bacteria with a dedicated nitrogen metabolism to compensate these deficits. Gut bacteria in termites, for example, can either utilize nitrogenous waste products excreted by the host and recycle them into high-value nutrients, or directly fix nitrogen from the atmosphere (Hongoh *et al.*, 2008a; Thong-On *et al.*, 2012). Herbivorous ants were also hypothesized to rely on specific gut symbionts for nitrogen provision (Cook & Davidson, 2006). Within different ant genera, herbivory is strongly correlated with the prevalence of a specific clade of *Rhizobiales* gut symbionts (Russell *et al.*, 2009a). Plant-associated *Rhizobiales* are well known for their ability to fix nitrogen (Masson-Boivin *et al.*, 2009), suggesting this possibility for related bacteria in ants feeding on nitrogen-poor plant tissues. However, experimental approaches have thus far failed to confirm nitrogen fixation in ant guts, although the relevant genes were amplified from the gut contents of some species (Russell *et al.*, 2009a). Interestingly, cockroaches, which represent close relatives of termites, and some herbivorous ants (Carpenter ants, Camponotini) harbor endosymbionts for ammonia recycling and biosynthesis of essential amino acids (Feldhaar *et al.*, 2007; Sabree *et al.*, 2012b). Cockroaches store nitrogenous waste as uric acid and utilize this during periods of dietary nitrogen limitation with the aid of their obligate endosymbiont (*Blattabacterium*). However, the *Blattabacterium* genome does not encode a known uricase, which is needed for the initial step in this process, and the source of this activity has been proposed to be either the gut microbiota or the host itself (Sabree *et al.*, 2009).

Transovariole transmission and intracellular housing of endosymbionts might be a more successful strategy for symbiotic provisioning of essential nutrients, because gut microbial associations can be unstable and thus less reliable, for nonsocial insects. Nevertheless, as mentioned above, some solitary insects have found strategies to ensure transmission of gut symbionts to their offspring, such as the transfer of *I. capsulata* colonizing specific midgut regions in the stinkbug species *M. punctatissima*. This symbiont has been shown to be essential for normal host growth and reproduction (Fukatsu & Hosokawa, 2002). Despite having a highly reduced genome, *I. capsulata* has retained biosynthetic pathways for many essential and nonessential amino acids and cofactors (Hosokawa *et al.*, 2006; Nikoh *et al.*, 2011). These genomic features are reminiscent of maternally transmitted intracellular endosymbionts (McCutcheon & Moran, 2012) and suggest that this gut bacterium has an important role in nutritional provisioning. In the alydid stinkbug, *R. pedestris,* the *Burkholderia* symbiont, which is acquired each generation from the environment and colonizes the midgut crypts (Kikuchi *et al.*, 2005, 2011), has similarly important roles in the development of its host (Kikuchi *et al.*, 2007). In contrast, in the pentatomid stinkbug, *N. viridula*, elimination of a similar symbiont has no effect on development or reproduction even after two generations, under laboratory conditions (Prado *et al.*, 2006).

Nutritional roles of gut communities in termites

The most elaborate and best-studied nutritional gut mutualisms are those found in the hindguts of termites. Although these systems might be exceptional in terms of their complexity, they provide unprecedented insights into different types of nutritional symbioses, which can evolve in the digestive systems of insects.

Termites feed on dead plant matter and contribute substantially to the global carbon cycle. The lower termite species, considered to represent more ancestral lifestyles, almost exclusively comprise wood-feeders, while the higher termite species include wood-, litter-, grass-, soil-, and lichen-feeders (Inward *et al.*, 2007; Lo *et al.*, 2007). Each termite species harbors a highly specific microbial gut community consisting of several hundreds of microorganisms including bacteria, archaea, and protists (reviewed in Hongoh, 2010). These microorganisms play a dual mutualistic role for their host. First, they contribute to lignocellulose digestion and produce high levels of acetate, which represents the main carbon source for their host (Hungate, 1943; Yamin, 1981; Odelson & Breznak, 1983; Breznak & Switzer, 1986; Warnecke *et al.*, 2007). Second, they provide their host with nitrogen, which is typically deficient in decomposing plant materials (Benemann, 1973; Breznak *et al.*, 1973). The mutualistic interplay between the gut community and its termite host is extremely complex and consists of multiple symbiotic layers.

After ingestion, termites grind up woody material into small particles using their mandibles and gizzards. Endogenous endoglucanases and β-glucosidases secreted by the salivary gland and/or the midgut of termites are then used to digest lignocellulose. This enzymatic breakdown presents only incomplete digestion of the wood particles, resulting in the production of glucose, which is then transported into the midgut via the peritrophic matrix of the host (Fujita *et al.*, 2010). The main part of lignocellulose digestion is carried out by the specialized gut community present in the hindgut of termites (Inoue *et al.*, 1997; Nakashima *et al.*, 2002; Tokuda *et al.*, 2005; Tokuda & Watanabe, 2007). Interestingly, this degradative process differs between higher and lower termites.

In lower termites, lignocellulose digestion is mostly accomplished by protists (Cleveland, 1923, 1924). These organisms belong to the phyla *Parabasalia* and *Preaxostyla* and are restricted to termites and certain wood-feeding cockroaches related to termites (Hongoh, 2010). Although the precise mechanism of degradation is unclear to date, it has been shown that protists package wood particles into vacuoles (Inoue *et al.*, 1997; Nakashima *et al.*, 2002) and ferment cellulose to acetate, hydrogen, and carbon dioxide (Yamin, 1981). A few bacterial strains with cellulase activity have been isolated from lower termites, but there is no clear evidence that these bacteria substantially contribute to lignocellulose hydrolysis (Wenzel *et al.*, 2002). In lower termites, bacteria predominating in the gut seem to have other essential functions. Fermentation of cellulose results in the production of hydrogen, which is rapidly removed from the hindguts of termites. This occurs via reductive acetogenesis and methanogenesis conducted by various gut bacteria and archaea, respectively (Breznak & Switzer, 1986; Brune, 2010). Bacterial acetogenesis makes up approximately a quarter of the entire acetate production in the termite gut and thus significantly contributes to the host's nutrition. Species of the genus *Treponema*, dominating the hindgut of both lower and higher termite species, seem to be responsible for most of the acetogenic activity. Remarkably, these bacteria are able to grow in axenic cultures on hydrogen and carbon dioxide as the sole carbon source (Leadbetter *et al.*, 1999; Salmassi & Leadbetter, 2003; Graber & Breznak, 2004; Graber *et al.*, 2004; Pester & Brune, 2006). Treponemas are typically present in the gut lumen, the compartment with the highest hydrogen concentration (Ebert & Brune, 1997), or they are found directly attached to the surface of the hydrogen-producing protists. Such ectosymbiotic associations with bacteria are very typical for gut protists of termites. Besides being involved in hydrogen transfer, putative functions of these ectosymbionts include nitrogen metabolism, detoxification of penetrating oxygen, or simply serving as a nutrient source for the protist when phagocytosed. Two specific ectosymbiotic bacteria have been shown to mediate motility of protists by synchronized movements of bacterial cells on the flagellate's surface (Cleveland & Grimstone, 1964; Tamm, 1982; Hongoh *et al.*, 2007). Furthermore, termite gut protists also harbor intracellular symbionts, and the recently sequenced genomes of two of these endosymbionts have provided insights into their functional roles. These are the endosymbiont RS-D17, belonging to the candidate class *Endomicrobia*, and the endosymbiont CfPT1-2, belonging to the order *Bacteroidales*. Despite their distant phylogenetic positions, the genomes of both bacteria are greatly reduced and share a similar functional gene content streamlined for the production of amino acids and cofactors (Hongoh *et al.*, 2008a, b). In addition, the genome of CfPt1-2 encodes genes for nitrogen fixation, ammonium transport, urease, and urea transport. This suggests that CfPt1-2 not only fixes atmospheric nitrogen but also recycles the putative nitrogen waste products of the host protist. The direct coupling of nitrogen fixation and recycling of a bacterial endosymbiont to the cellulolytic activity of a symbiotic protist is a striking mutualistic innovation that provides lower termites with ample nutrients for the bacteria, the protist, and the host, despite a nutrient-poor and refractory diet.

Higher termites typically lack protists in their guts. For a long time, it was assumed that wood-feeding species solely rely on the digestive activity of the endogenous endoglucanase secreted in the midgut (Slaytor, 1992). However, more recent evidence suggests that the cellulolytic activity of bacteria within specific gut segments contributes critically to lignocellulose degradation in the hindgut (Warnecke *et al.*, 2007; Köhler *et al.*, 2012). Ingested food particles pass from the midgut through the so-called mixed segment and different proctodeal segments of the hindgut referred to as P1–P5 (Fig. 1). pH and oxygen levels dramatically shift in these different sections. In the P1 region, the pH reaches values of 10–12, which likely facilitates the solubilization of woody material and other recalcitrant food components of higher termites (Brune *et al.*, 1995; Abe *et al.*, 2000). Cellulolytic activity was found in the posterior proctodeal segments, which are densely populated by bacteria (Tokuda *et al.*, 2005; Tokuda & Watanabe, 2007). Metagenomic and proteomic analysis of these regions revealed a high abundance of bacterial genes and proteins involved in cellulose degradation, acetogenesis, and nitrogen fixation (Warnecke *et al.*, 2007; Burnum *et al.*, 2010). Thus, the cellulolytic activity of bacteria in the higher termites might partially substitute the functions provided by protists in the lower termites.

Other roles of gut communities in nutrition

Closely related to nutritional roles of gut microorganisms are potential roles in detoxification of food. Certain sources of nutrients are available only if toxins can be neutralized, and hydrolysis of some molecules, such as some plant cell wall components, can both detoxify them and make them available as sources of nutrition. Many insects specialize on toxic plants or overcome chemicals used for insect control, and gut bacteria might serve as a portal for acquiring capabilities to digest and detoxify local food sources. The plausibility of this is illustrated in the human gut bacterium, *Bacteroides plebeius*, which acquired the capability, through lateral gene transfer, to degrade porphyrans, polysaccharides of marine red algae, an event that occurred specifically in Japanese populations that consume red algae, in sushi and other foods (Hehemann *et al.*, 2010). In other words, microorganisms associated with the food itself provide a source of genes enabling degradation of food components. A study of *Tenebrio molitor* beetle larvae (mealworms) found that axenic individuals were able to digest diet high in cellulosic compounds with similar efficiency to individuals with normal gut microbiota, but that the specific profile of digestive enzymes differed, with some appearing to be of microbial origin when the gut microbiota is present (Genta *et al.*, 2006). As discussed by the authors, these enzymes have potential not only to make nutrients available but also to hydrolyze toxic plant glucosides, and this could be a source of adaptation of insect populations to local food types. Many insects must cope with plant tannins that reduce availability of proteins in food, and many microorganisms produce tannases (Aguilar *et al.*, 2007a), raising the possibility that insect gut microorganisms may sometimes serve this function. A symbiotic yeast in guts of *Lasioderma serricorne* (cigarette beetle) was able to break down dietary toxins and improve resistance of its host (Dowd & Shen, 1990). In populations of the stinkbug *R. pedestris* exposed to the insecticide fenitrothion, the environmentally acquired *Burkholderia* gut symbiont has gained ability to hydrolyze the compound, thus protecting its host (Kikuchi *et al.*, 2012).

Herbivorous insects typically produce oral secretions that interact with food plants, either stimulating or suppressing plant defense responses. Microorganisms in the gut lumen potentially can produce compounds or enzymes that mediate these responses. For example, feeding by caterpillars of *Spodoptera exigua* (beet armyworm) results in the release of *N*-acyl-amino acids that potentially function in emulsifying food for digestion but that also trigger plants to produce volatiles that in turn attract natural enemies (Alborn *et al.*, 1997). *Microbacterium arborescens* isolated from beet armyworm foreguts was found to produce an *N*-acyl amino acid hydrolase that breaks down these defense elicitors potentially affecting nutrient availability by releasing amino acids and also potentially impacting plant defense responses (Ping *et al.*, 2007). The relative roles of bacterial-produced vs. host-produced *N*-acyl amino acid hydrolases are not clear (Felton & Tumlinson, 2008), but this example illustrates the potential complexity of mechanisms whereby gut microorganisms might impact host nutrition.

Generality of nutritional roles of insect gut bacteria

In contrast to the many examples of nutritional endosymbiosis between intracellular bacteria and insects, we still have only a limited view of the distribution and relevance of gut bacterial symbiosis of insects with roles in nutrient provisioning, breakdown of recalcitrant dietary compounds, or detoxification of molecules in food. Termites provide clear cases of nutritional roles of gut microbiota, but some insects probably do not rely on gut bacteria at all for processing food and gaining nutrients. In some studies, experimental removal of the gut microbiota results in no effect, or even positive effect, on insect fitness; these include pentatomid stinkbugs (Prado *et al.*, 2006) and velvetbean caterpillars (Visôtto *et al.*, 2009). Although *D. melanogaster* shows good survivorship when grown axenically (e.g. Cox & Gilmore, 2007), most *Drosophila* studies support a growth advantage due to microbial colonization of the gut and/or the growth media (overview in Broderick & Lemaitre, 2012).

Effects on insect development and physiology

Gut symbionts with critical roles in nutrient provisioning or digestion facilitate the assimilation of nutrients and thus positively influence development and fitness of the host (Cleveland, 1923, 1924; Fukatsu & Hosokawa, 2002; Hosokawa *et al.*, 2007; Kikuchi *et al.*, 2007). However, gut bacteria can also affect host developmental processes by direct interactions with the host. Perception of bacterial signals via the epithelium contributes to immune and cellular homeostasis and is essential for the host's adaptation to changing conditions in the gut environment. The mammalian gut microbiota provides signals for maturation of the mucosal immune system, influences epithelial cell differentiation, and promotes angiogenesis (Stappenbeck *et al.*, 2002; Xu & Gordon, 2003; Mazmanian *et al.*, 2005; O'Hara & Shanahan, 2006). These processes are mediated by contact-dependent interactions of bacteria with the gut epithelium or via diffusible molecules and metabolites, as for example short-chain fatty acids

(Maslowski *et al.*, 2009; Fukuda *et al.*, 2011; Ashida *et al.*, 2012; Nicholson *et al.*, 2012).

Insights about such processes in insects primarily come from studies on *D. melanogaster*. The existence of well-established genetic tools and the low diversity of its commensal microbiota (Chandler *et al.*, 2011; Wong *et al.*, 2011) make this host species a suitable model to look at interspecies interactions in the gut environment and to study the underlying genetic basis.

Effects of microbiota on midgut development

Two independent studies showed that the host response of *D. melanogaster* to nonlethal or lethal pathogens in the midgut does not only consist of immune system activation, but also involves various aspects of gut cell physiology including stem cell proliferation and epithelial cell renewal (Buchon *et al.*, 2009b; Cronin *et al.*, 2009). The epithelium in the midgut of *D. melanogaster* possesses a self-renewal program in which enterocytes are continuously replaced by underlying intestinal stem cells (Amcheslavsky *et al.*, 2009; Casali & Batlle, 2009). Bacteria in the midgut modulate this stem cell activity, probably by inducing epithelial cell damage and apoptosis, which results in the activation the JAK-STAT signaling pathway (Buchon *et al.*, 2009b; Cronin *et al.*, 2009; Jiang *et al.*, 2009). The extent of the epithelial cell renewal is proportional to the virulence and concentration of the bacteria in the midgut (Buchon *et al.*, 2009a). This raises the question of the extent to which commensal or mutualistic gut microorganisms of insects affect epithelial cell homeostasis. When comparing germ-free vs. conventional flies, Buchon *et al.* (2009a) found elevated epithelial cell turnover in conventional flies harboring commensal bacteria, although the rate of turnover was slower than in the presence of pathogenic bacteria. However, when using mutant flies unable to control the number of commensal bacteria in the gut, a hyperproliferative intestinal stem cell response and abnormal gut morphology were observed; this was not the case when the mutant flies were raised germ-free. Together, these findings suggest that the indigenous microbiota of *D. melanogaster* influences gut cell homeostasis and that the host response depends on the bacterial load and the bacterial community composition in the gut.

Systemic effects of microbiota

Besides local effects on intestinal development and immune homeostasis, gut bacteria of *D. melanogaster* have been implicated in a number of systemic effects. One study suggested that the presence of bacteria at different adult life stages could either increase or decrease the host's life span (Brummel *et al.*, 2004). This finding was challenged by a later study in which no difference in life span was found between conventional and germ-free adult flies (Ren *et al.*, 2007). Despite these contradictory results, there is now compelling evidence that gut bacteria can promote systematic growth and development of *D. melanogaster* by modulating their host's hormone signaling (Shin *et al.*, 2011; Storelli *et al.*, 2011). Germ-free larvae of *D. melanogaster* exhibited reduced growth and developed more slowly than conventionally reared individuals. This effect was shown to be strongly dependent on the nutritional value of the diet. When raised on rich medium, no differences or only slight differences in growth were observed (Shin *et al.*, 2011; Storelli *et al.*, 2011; Ridley *et al.*, 2012). However, when reducing the percentage of yeast extract in the diet, differences between germ-free and conventional larvae became much more pronounced. Feeding a diet containing $< 0.1\%$ yeast extract or substituting yeast extract with casamino acids was lethal for germ-free animals, while conventional larva could develop into puparia (Shin *et al.*, 2011). These beneficial effects were attributed to different members of the gut microbiota of *Drosophila*. In one study, colonization of germ-free flies with *Acetobacter pomorum* (a commensal bacterium frequently isolated from the *D. melanogaster* digestive tract) was sufficient to restore larval development and growth to levels comparable to conventionally reared flies. A transposon mutant screen of *A. pomorum* identified the PQQ-ADH (periplasmic pyrroloquinoline quinone–dependent alcohol dehydrogenase)-dependent oxidative respiratory chain to be essential for restoring development and growth in monoassociated larvae. In addition to slower larval development, adults of larvae monoassociated with PQQ-ADH mutant bacteria had smaller body, wing, and intestine sizes and revealed a reduced intestinal stem cell pool and slower epithelial cell turnover (Shin *et al.*, 2011). The PQQ-ADH system of *A. pomorum* was shown to produce acetic acid *in vitro* and *in vivo,* and strikingly, acetic acid supplementation in the diet could effectively reverse all developmental defects in flies monoassociated with *A. pomorum* PQQ-ADH mutants (Shin *et al.*, 2011).

In a second study, the promotion of systemic growth of *D. melanogaster* was attributed to another commensal gut bacterium, *Lactobacillus plantarum*. Again, significant differences in growth and development between germ-free flies and flies monoassociated with *L. plantarum* were only observed when flies were kept under nutrient-poor conditions (Storelli *et al.*, 2011). Intriguingly, both studies showed that these two microorganisms exert their beneficial functions on the host by promoting insulin signaling. However, the underlying mechanisms seem to be distinct. In the case of *A. pomorum*, acetic acid supplementation

could only rescue the developmental defects in the presence of the PQQ-ADH mutant suggesting that not only bacterial production but also metabolism of acetic acid is required to promote insulin signaling (Shin *et al.*, 2011). In the case of *L. plantarum*, the presence of bacteria seems to enhance protein assimilation from the diet, resulting in increased levels of branched-chain amino acids in the hemolymph and the activation of insulin signaling via TOR kinase activity (Storelli *et al.*, 2011). Key steps in further understanding the implication of the gut microbiota in development and metabolic homeostasis of *D. melanogaster* will be to illuminate the mechanism of acetic acid-mediated growth promotion and to identify the genetic basis of *L. plantarum*'s ability to facilitate protein assimilation. Because acetic acid-producing bacteria are common intestinal inhabitants of sugar-feeding insects (Crotti *et al.*, 2010), it will be interesting to investigate whether these bacteria play a general role in modulating insect host development in response to dietary intake.

Protective functions

Colonization of the gut with commensal or mutualistic microbial communities can increase the resistance of the host against parasite invasion. Underlying mechanisms include nutrient competition, niche occupation, or immune priming (Bartlett, 1979; Ivanov *et al.*, 2009; Endt *et al.*, 2010; Stecher & Hardt, 2011) and are generally referred to as colonization resistance (Vollaard & Clasener, 1994). In mammals, inflammation can reduce colonization resistance by causing shifts in bacterial communities. This disturbance of gut homeostasis makes the host more susceptible (Blumberg & Powrie, 2012) and enables pathogens to colonize liberated niches and cause disease (Lupp *et al.*, 2007; Stecher *et al.*, 2007; Stecher & Hardt, 2008; Keeney & Finlay, 2011; Ashida *et al.*, 2012). Furthermore, pathogens often use the epithelial cell layer in the gut as an entry site for systematic infections (Reis & Horn, 2010), and resident bacteria, which densely populate the epithelial cell surface, impede the access to this entry site for invasion (Lupp *et al.*, 2007; Haag *et al.*, 2012). In insects, these dynamics might be slightly different than in mammals. First, not all insects consistently harbor a residential gut community with cell densities as high as in mammals, and bacteria are frequently picked up from the environment (Boissière *et al.*, 2012). Thus, specific bacterium–epithelium interactions with protective functions might be exceptional in insects. Second, the extracellular peritrophic matrix components secreted in the midgut protect the underlying epithelial cell layer from contact with the gut content and modulate the strength of the immune response (Terra, 1990; Kumar *et al.*, 2010; Kuraishi *et al.*, 2011). In other parts of the insect gut, a cuticle layer separates the epithelium from the gut lumen (Maddrell & Gardiner, 1980). Third, insects lack B and T cells (Schmid-Hempel, 2005), and mechanisms underlying immune priming might be fundamentally different from those in mammals (Sadd & Schmid-Hempel, 2006; Pham *et al.*, 2007; Rodrigues *et al.*, 2010). Another important point is that our knowledge on opportunistic gut pathogens of insects is limited, hindering the study of specific mechanisms of host protection by residential gut bacteria.

Nevertheless, a few studies report on the existence of antagonistic interactions between different gut microorganisms in insects. These include protective functions against host pathogens. For example, the residential gut microbiota of European bumble bees protects against the common trypanosomatid pathogen *Crithidia bombi* (Koch & Schmid-Hempel, 2011b). In this study, bumble bee pupae were raised in the absence of their residential microbial community, and newly emerged adults were fed with either feces from their nest mates, isolates of the resident gut bacterium *G. apicola*, or sterile sugar water. Then, the bees were exposed to a high dosage of the pathogen *C. bombi*. Analysis of parasite load and microbial community composition showed that bees fed feces from their nest mates developed a gut microbial profile indistinguishable from the one of workers from inside the colony and also revealed much lower numbers of *C. bombi* cells in their feces than in the other conditions. Consistent with these results, antibiotic exposure diminished the microbial gut community of these bees, resulting in high infection loads with the pathogen (Koch & Schmid-Hempel, 2011b). The mechanism behind this protective function is not yet clear.

In experiments conducted with the desert locust *Schistocerca gregaria*, an inverse relationship between gut community diversity and the colonization success of the pathogen *Serratia marcescens* was observed (Dillon *et al.*, 2005). This supports the theory that species-rich gut communities are more resistant to invasion, possibly by imposing a higher competitive burden on the pathogen for niche colonization and nutrition or by increasing the immune competence of the host. The latter could be mediated via immune priming mechanisms or via important nutritive roles of gut bacteria. A malnourished insect will not be able to elicit a proper immune response, as indicated by a number of studies focusing on the impact of diet on immunity (Ayres & Schneider, 2009; Srygley *et al.*, 2009; Cotter *et al.*, 2010; Chambers *et al.*, 2012).

Antagonistic interactions among gut inhabitants have also been reported for mosquitoes. Independent studies provided evidence for a protective role of the gut microbiota of *Anopheles* spp. against *Plasmodium falciparum* colonization, the causative agent of malaria (Pumpuni *et al.*, 1993; Gonzalez-Ceron *et al.*, 2003;

Cirimotich *et al.*, 2011a). In *A. gambiae,* clearance of the gut microbiota with antibiotics resulted in enhanced *Plasmodium* infections (Beier *et al.*, 1994; Dong *et al.*, 2009; Meister *et al.*, 2009), and in *Anopheles stephensis*, cocolonization with Gram-negative bacteria inhibited *P. falciparum* development (Pumpuni *et al.*, 1993). Based on results from transcriptome analyses, it was further hypothesized that the antagonistic effect of the gut microbiota could be the result of a bacterially induced immune response including up-regulation of several antiplasmodial factors (Dong *et al.*, 2006, 2009). Thus, colonization with commensal gut bacteria might indirectly control the proliferation of the parasite by stimulation of basal levels of immune effector production (Cirimotich *et al.*, 2011b). In another study, mosquitos collected in natural breeding sites were experimentally challenged with *P. falciparum* and their microbial midgut community assessed with pyrosequencing of 16S rRNA gene amplicons (Boissière *et al.*, 2012). Although the analysis showed a high degree of diversity in community composition, a significant positive correlation between increased abundance of *Enterobacteriaceae* and *P. falciparum* infections was found. While these data suggest that *Enterobacteriaceae* would in general increase host susceptibility to *P. falciparum* infections, an *Enterobacter* species isolated from guts of wild mosquitos conferred resistance to *P. falciparum* infection (Cirimotich *et al.*, 2011a). Rather than eliciting an immune response that reduces parasite load, experiments conducted in this study suggested a more direct mechanism of protection, potentially through ROS produced by the bacterium itself. However, the distribution and ecological role of this strain in natural populations of mosquitos remain elusive. While it is well understood that *P. falciparum* can compromise the fitness of its insect host (Tahar *et al.*, 2002; Aguilar *et al.*, 2007b), the importance of mutualistic gut bacteria for providing colonization resistance needs to be further validated. Also, it will be interesting to elucidate the mechanisms underlying protective functions. Do these bacteria have direct antagonistic properties against the parasite? Or, do they rather change the immune competence of the host via priming mechanisms or roles in host nutrition?

Microbiota-mediated effects on intraspecific and interspecific communication

In several cases, biosynthesis or catabolism carried out by gut microorganisms is known to result in the production of compounds that function in the host as pheromones or kairomones. In the swarming grasshopper, *S. gregaria*, *Pantoea agglomerans*, and other common gut bacteria produce components of the aggregation pheromone, through breakdown of dietary components (Dillon *et al.*, 2002). In *D. melanogaster*, the composition of the gut microbiota determines mating attractiveness: flies mate preferentially with individuals harboring similar microbiota (Sharon *et al.*, 2010, 2011). When *L. plantarum* isolates from fly guts were used to inoculate axenic flies, mating preferences could be reversed. In turn, the food type governed microbiota composition, with *L. plantarum* dominating in individuals feeding on high starch diets. Thus, this microbiota effect on mating could lead to divergence of host lineages that feed on different substrates, ultimately leading to speciation (Sharon *et al.*, 2010).

Because chemical communication often involves only tiny amounts of the signaling compounds, even low microbial titers may suffice for substantial effects. A striking example of this has been documented in aphids, which produce liquid feces (honey dew) containing kairomones that act as attractants to aphid predators. *Staphylococcus sciuri* inhabiting guts of aphids at titers of about 10^6 per mL, only a few thousand cells per aphid, were shown to be the source of these compounds, which are highly attractive to ovipositing hover fly females (*Syrphidae*; Leroy *et al.*, 2011). Because the resulting fly larvae then devour the aphids, the bacterial activity is deleterious to the host in this case.

Practical applications of insect gut microorganisms

Harnessing human gut bacteria to supplement and balance diets or to sustain gut homeostasis and treat diseases has been in the focus of the food industry and medicine for a long time. Likewise, gut bacteria of insects might be utilized to manage insect species of human concern. This is particularly promising for the control of so-called pest species, that is, insects with ecological characteristics that negatively affect human health, economic interests, or environmental quality. Insects function as vectors for pathogens causing severe human disease such as dengue, trypanosomiases, and malaria. With 174 million diagnoses and 655 000 million deaths in 2011, malaria is considered as one of the most important infectious diseases worldwide (WHO: World malaria report 2011). In addition, insects can be serious pests in agriculture through damage to major crop plants. Annually, half of the global crop production is lost to pests, and the majority of these losses are due to insects through their activities as herbivores or as vectors of plant pathogens (Oerke, 2006). Insects can also negatively affect natural ecosystems. For example, population explosions of wood-feeding bark beetles destroy large areas of coniferous forests in Northern America resulting in serious economic

losses and environmental degradation (Paine *et al.*, 1997; Raffa *et al.*, 2008). Current strategies for insect pest control management include the application of chemical insecticides, the dissemination of sterile insects, or the introduction of natural enemies such as predators or parasites.

A number of approaches based on bacteria have also been proposed, and some have been successfully implemented (Fig. 5). In Australia, two natural *Aedes aegypti* populations have been transformed using the intracellular symbiont *w*Mel *Wolbachia* (Hoffmann *et al.*, 2011). This bacterium suppresses dengue transmission and shortens the life span of the infected insect, thereby decreasing its ability to act as vector of this disease agent. The release of entomopathogenic bacteria producing insecticidal toxins is a common method in agricultural pest control, but can also be used in the management of mosquitoes transmitting human diseases (Becker, 2000; Ffrench-Constant & Waterfield, 2006; Ffrench-Constant *et al.*, 2007; Bravo *et al.*, 2011; Sanahuja *et al.*, 2011). Popular biological insecticides used against plant pests are *Bacillus thuringiensis*, *Photorhabdus luminescens*, and *Xenorhabdus nematophilus*. The gene encoding the insecticidal toxin of *B. thuringiensis* has been introduced into the genomes of a number of major crop species, including corn, soybean, and cotton, and this transgenic approach has proven to be highly effective against insect pests (Christou *et al.*, 2006; Sanahuja *et al.*, 2011). Transgenesis is also under investigation as a potential strategy to control vector-borne diseases. Here, the goal is to introduce genes into insects that produce proteins impairing pathogen development or transmission. Despite the existence of various transgenic germ lines of disease-transmitting insects, many hurdles still exist to successfully apply such transgenic vectors in the environment (Coutinho-Abreu *et al.*, 2010). Major challenges are the lack of transgenes, which effectively reduce pathogen load, the inefficiency of current methods for introducing and propagating transgenes in natural vector populations, and the reduced fitness of transgenic insects in the environment. Another important barrier for the development and application of such systems is public opinion, which generally is against the use of genetically modified organisms; however, this opposition may be most focused on agricultural production.

Paratransgenesis

Insect gut symbionts could help to overcome some of the limitations of transgenesis. The large diversity of bacteria associated with insect guts represents a valuable resource to identify activities, which could be harnessed for pest control. Furthermore, genetically modified symbionts can be used as vehicles to specifically express foreign traits, which interfere with pathogen development or insect fitness. This type of pest control is referred to as paratransgenesis (Fig. 5) (Olson *et al.*, 1996; Beard *et al.*, 2002; Aksoy *et al.*, 2008; Coutinho-Abreu *et al.*, 2010; Caragata & Walker, 2012). Gut bacteria are suitable for the development of such paratransgenic applications for several reasons. In contrast to host-dependent endosymbionts, gut bacteria are cultivatable and thus amenable for genetic manipulation. They can easily be re-introduced into the host insect by, for example, oral ingestion and disseminated in the environment via horizontal transfer. Furthermore, most vector-borne parasites specifically colonize the guts of their insect hosts, which directly expose them to the effector

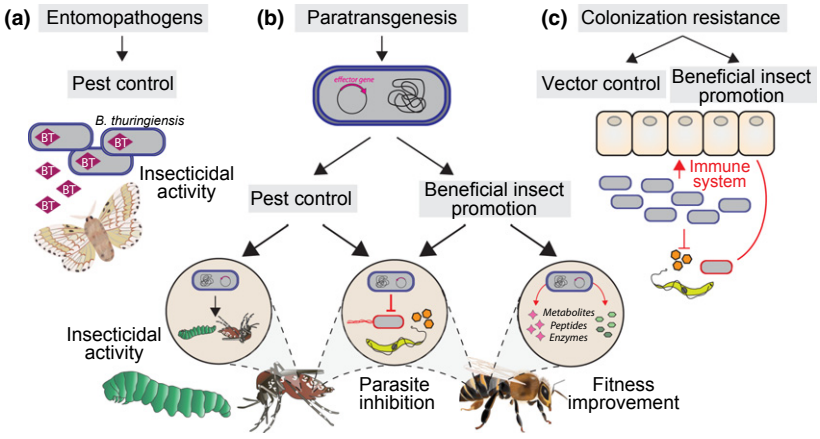

Fig. 5. Different applications of gut bacteria for the management of insects. (a) Insecticidal potential of entomopathogenic gut bacteria can be used to control pest species. (b) In paratransgenesis, bacteria are used as vehicles to express molecules in the gut, which negatively or positively affect health of the host or suppress parasite colonization. These approaches could be applied for the management of pest species and beneficial insects. (c) Alternatively, gut bacteria that naturally inhibit parasite colonization could be disseminated in insect populations, for example, to prevent the spread of human disease via insect vectors or to protect beneficial insects from parasitic diseases.

proteins of the engineered gut symbionts. Bacterial species considered for paratransgenic disease control in mosquitoes and tsetse flies include the midgut bacteria *S. glossinidius*, *Asaia* sp., and *P. agglomerans*. They potentially are suitable *in vivo* drug delivery vehicles to control vector-borne diseases, as they persist in the insect for extended time periods and are reliably transmitted via horizontal and/or vertical routes (Favia *et al.*, 2007; Aksoy *et al.*, 2008; Bisi & Lampe, 2011; Dinparast Djadid *et al.*, 2011). Further, they are widespread in populations of the corresponding host species and are genetically amenable. Promising effector proteins under investigation, for which inhibitory activities against *Plasmodium* and *Trypanosome* sp. have been reported, include AMPs of the insect immune system and antibody fragments targeting surface epitopes of the parasites (Bisi & Lampe, 2011; De Vooght *et al.*, 2012). While some of these effectors have been successfully expressed and secreted by the corresponding bacterial delivery vehicles (De Vooght *et al.*, 2012), their potential inhibition of parasite development in the insect host has not been published yet.

The most advanced project for the application of paratransgenesis is the inhibition of *Trypanosoma cruzi*, the causative agent of Chagas disease, in its host *R. prolixus* by the genetically modified gut symbionts *R. rhodnii* (Beard *et al.*, 2002). In this system, an AMP, cecropin A, and a single-chain antibody were expressed in *R. rhodnii*; and both peptides were shown to exhibit effective inhibitory activity against *T. cruzi in vivo* (Durvasula *et al.*, 1997, 1999). Furthermore, a method for the dispersal of transformed symbionts in natural populations of the insect host was developed. First instar nymphs of *R. prolixus* acquire *Rhodococcus* by probing the symbiont-contaminated feces of adults. This feeding behavior (coprophagy) was exploited by adding genetically modified symbionts to a synthetic paste called CRUZIGARD, which was then dispersed as droplets in the environment. The addition of a small amount of ammonium sulfate to the paste simulated natural feces of *R. prolixus* and stimulated feeding of aposymbiotic nymphs (Durvasula *et al.*, 1997).

Despite this progress, no transformed symbionts have yet been released for applied pest control, and the effectiveness of paratransgenic methods in the environment thus remains questionable. Risk assessment and evaluation of environmental impacts are inherent challenges associated with the release of genetically modified organisms. It is also unclear whether transformed symbionts can replace the nontransformed ones in natural insect populations, even though no fitness deficits due to genetic modification of the symbionts have been reported from laboratory experiments. Other challenges are the instability of introduced DNA in bacteria and the few insect symbionts, which are efficiently transformed to date. Effectiveness of paratransgenesis might further be hindered by the fact that many parasites can be transmitted by dozens of different vector species, which all have specific symbiotic associations in their guts. Incompatibility between bacterial delivery vehicles and host insects would therefore demand species-specific approaches.

Harnessing colonization resistance properties of resident gut bacteria

To reduce pathogen load in natural populations of vector insects, increasing the prevalence of naturally occurring inhibitory gut bacteria could be a reasonable alternative to paratransgenic approaches (Fig. 5). Several recent studies showed that composition of the commensal gut microbiota influences vector competence by modulating immune responses, competing for niches, or producing inhibitory molecules (Azambuja *et al.*, 2005; Dong *et al.*, 2006, 2009; Meister *et al.*, 2009; Cirimotich *et al.*, 2011b; Boissière *et al.*, 2012). Therefore, functional analysis of the commensal gut microbiota with the aim to better understand its interaction with the host and the parasite could yield novel and more effective strategies for the control of vector-borne diseases. Accordingly, this applies to the development of future control strategies for plant pests. A number of bacterial plant pathogens are transmitted by insect vectors, and characterization of commensal gut bacteria of these insects has been undertaken with the aim of developing strategies to counteract pathogen transmission (Bextine *et al.*, 2004, 2005; Marzorati *et al.*, 2006; Raddadi *et al.*, 2011). A good example is Pierce's disease of grapes caused by the pathogen *Xylella fastidiosa*. A bacterial symbiont, *Alcaligenes xylosoxidans*, was isolated from the sharpshooter (family *Cicadellidae*) that transmits *X. fastidiosa.* This commensal bacterium colonizes the foregut of the insect, where it co-occurs with *X. fastidiosa*. Interestingly, when the insect feeds on plant sap, *A. xylosoxidans* is translocated into the plant's xylem, which can facilitate its transmission to other insect hosts. These characteristics make *A. xylosoxidans* a candidate as a biological control agent against *X. fastidiosa* colonization by competitive niche exclusion or as paratransgenic vehicle for the delivery of anti-*Xylella* products (Miller, 2010).

Effects of gut microbiota on the activity of insecticides

The presence of gut bacteria has also been implicated in promoting insecticidal activity of *B. thuringiensis*, the most widely used biological insecticide for plant pest control (Broderick *et al.*, 2006, 2009; Johnston & Crickmore, 2009; Mason *et al.*, 2011). In gypsy moth larvae, elimination of the gut microbial community abolished

B. thuringiensis insecticidal activity, and re-introduction of a specific member of the indigenous midgut microbial community restored *B. thuringiensis*-mediated killing (Broderick *et al.*, 2009). In contrast, gut symbionts might also have the potential to protect their host from insecticides. An intriguing example is the degradation of the insecticide fenitrothion by a *Burkholderia* gut symbiont of *R. pedestris*, cited earlier. After experimental application of the pesticide, the midgut crypts of *R. pedestris* were colonized with fenitrothion-degrading *Burkholderia* which conferred resistance of the host to the insecticide (Kikuchi *et al.*, 2012). Taken together, these findings demonstrate the necessity to consider the gut microbiota of insects for the implementation of novel pest control measures.

Harnessing gut bacteria to promote populations of beneficial insects

Strategies based on gut bacteria are also under consideration to sustain or promote populations of beneficial insects, such as pollinators, natural competitors of pests, or producers of useful substances for humans (Fig. 5). An interesting example is the fitness improvement in γ-irradiated sterile male flies of the Mediterranean fruit fly *C. capitata*, achieved by feeding a bacterially enriched diet. The Mediterranean fruit fly is a common pest of fruits and vegetables worldwide, and sterilized male insects are released into the environment to compete with wild males for copulations with wild females. The result is a reduction in the total fly population in the next generation due to females mating with sterilized male flies not yielding any progeny. However, sterilized males are less competitive in mating and thus have a disadvantage compared with wild males (Lance *et al.*, 2000). Sterilization-induced shifts in the microbial gut community of the mass-reared male flies seem to contribute to this fitness decrease. Compared with wild flies, the abundance and diversity of *Enterobacteriaceae*, in particular *Klebsiella* sp., were reduced, and the abundance of potentially entomopathogenic *Pseudomonas* sp. increased (Ben Ami *et al.*, 2010). Feeding of a 'probiotic' diet enriched in *Klebsiella oxytoca* significantly increased the sexual competitiveness of γ-irradiated males, enhanced their survival, and inhibited sexual receptivity of female flies (Gavriel *et al.*, 2011). Thus, inoculation of the sterilized male flies with commensal gut bacteria can improve the effectiveness of control.

Probiotics could also be used to ensure healthy pollinator species such as bumble bees (*Bombus* sp.) and honey bees (*A. mellifera*) (Fig. 5). There is an increasing interest in such approaches, because bee populations have been declining in recent years. The losses are probably caused by a combination of different factors including environmental stresses, such as lack of food resources and the use of insecticides, and biotic stresses, such as parasites and infectious diseases. Furthermore, a syndrome referred to as colony collapse disorder (CCD), which is characterized by the rapid disappearance of the adult bee population from a colony, severely contributes to honey bee declines in the United States, Europe, and Japan (Evans & Lopez, 2004; Genersch, 2010). The multitude of factors influencing bee health represents a major challenge for management. However, an important factor in bee health could be the characteristic gut microbiota; thus, these bacteria are a focus for probiotic applications. So far, there are only limited data available about how the diversity and dynamics of these microbial communities might affect gut homeostasis and health of the host. A survey of the microbial communities of CCD hives and normal hives revealed potential minor differences in the relative abundance of bacterial phylotypes associated with adult worker bees of *A. mellifera,* although limited sampling did not allow statistical testing of these differences (Cox-Foster *et al.*, 2007). Later, a more extensive survey of microorganisms associated with healthy and CCD bee colonies found that CCD bees had higher levels of a variety of pathogens and parasites but no clear change in the gut microbiota (Cornman *et al.*, 2012). These surveys were based on the relative abundances of microorganisms present but did not document the absolute numbers, so the possibility remains that the overall size of the gut community differs in the affected bees. In bumble bees, it was shown that susceptibility to the parasite *C. bombi* was determined by the host-specific composition of the gut microbiota and was not influenced by the insect genotype, a result that substantiates the role of the microbiota of social bees in gut homeostasis and resistance against parasites and diseases (Koch & Schmid-Hempel, 2012). Dysbiosis of the community due to environmental stress could thus result in a higher susceptibility of the host. Commensal bacteria have been proposed as probiotics of honey bee larvae with the goal to protect them against the common pathogen *Paenibacillus larvae*. When larvae were administered a mix of commensal bacteria consisting of different *Lactobacillus* and *Bifidobacterium* sp., an immune response similar to the one observed upon a *P. larvae* infection was observed, suggesting that such bacteria could be used in prophylactic or therapeutic treatment against natural pathogens (Evans & Lopez, 2004). A number of bacterial isolates were also reported to exhibit direct antagonistic effects against *P. larvae in vitro*, and in one study, the inhibitory activity could be confirmed in *in vivo* experiments (Forsgren *et al.*, 2009). Despite the limited progress made so far, probiotics might be a suitable alternative to commonly used

chemical treatments in beekeeping. The social lifestyle of these insects would facilitate inoculation and dissemination of beneficial bacteria in the populations. Furthermore, members of the stable core gut microbiota could also present promising candidates for paratransgenic management strategy in beekeeping (Rangberg *et al.*, 2012).

Concluding remarks

The vast ecological and taxonomic diversity of insects makes it difficult to generalize about their gut microbiota. But several broad factors are evident. First, insects vary in the extent to which gut microorganisms are essential or even influential, with extremes represented by some sap-feeding insects, which have little or no gut microbiota but depend on intracellular symbionts for nutrients, and by termites, which have large and complex gut communities, that are essential for digesting food and producing nutrients. Most insects fall somewhere in between. Thus, insects show a wide range in their degree of dependence on gut bacteria, providing a contrast to mammals, all of which appear to harbor a prominent and distinctive gut microbiota. Therefore, we have to be careful when generally assuming similarly important roles for gut microorganisms in insects and in mammals. Microorganism–host interactions in insects are shaped by different factors than in mammals. Insects lack a 'classical' adaptive immune system, which has been hypothesized to allow mammals to foster large and complex microbial communities in their guts (McFall-Ngai 2007). In agreement with this is the finding that some insect gut communities contain relatively few bacterial species (Colman *et al.*, 2012). However, another explanation could be that most insects are tiny compared with most mammals and that their guts simply provide fewer ecological niches than do mammalian guts. Also, the retention time of bacteria in the gut of most insects may be shorter than in most mammals. Transit time of food varies among insects: grasshoppers and cockroaches void meals within 3 and 20 h, respectively (Chapman *et al.*, 2013). Insects such as scarab beetles and wood roaches that specialize on foods high in lignocellulose and have fermentative guts retain food longer and also have more diverse gut communities (Colman *et al.* 2012). Whereas all mammals have social behavior, at least in the form of prolonged contact of mothers and offspring, enabling direct host-to-host transmission of microorganisms, many insects lack reliable mechanisms for direct transmission of gut bacteria. As a result, novel bacteria enter the gut each generation, or following each molting event during development, and some insects appear to be colonized erratically by bacteria picked up from the external environment rather than by specialized residents. Clear exceptions are social insects and insects that have evolved specific mechanisms for bacterial transfer to progeny such as egg-smearing or egg capsules. In addition, insect guts display a large diversity in terms of morphology, physicochemical properties, and food content. These factors shape the gut microbiota of insects and contribute to the broad array of different community structures, which have been reported.

We are seeing a proliferation of studies that document the 16S rRNA gene profiles of gut communities, but many of these do not provide estimates of the numbers of bacterial cells present or only provide a single snapshot of the community. Estimates of absolute densities would be useful complements to diversity data to get an idea about the abundance and stability of the gut microbiota. A second point is that laboratory surveys and experiments may not yield a valid picture of gut community profiles or gut community roles as they occur in natural populations. In particular, the roles of gut microorganisms are evident only if the appropriate environmental conditions are used in experiments: dependence on gut bacteria for nutritional provisioning or pathogen protection will not be observed if the laboratory conditions provide full nutrition and exclude pathogens.

While the gut microbiota has only been sampled for a tiny number of insect taxa, studies to date make it clear that gut microorganisms are critical to the nutrition, physiology, immune responses, and pathogen resistance of many species. However, strong dependence on symbiotic associates is less likely to evolve if those associates are not reliably present.

In animals generally, the presence of a commensal or beneficial gut microbiota must be reconciled with immune responses that underlie defenses against pathogenic bacteria. In mammals, the adaptive immune system plays a key role in these processes. However, studies on *D. melanogaster* have added to evidence that gut communities play a role in modulation of innate immune systems and that these systems have been shaped by trade-offs involving compatibility with gut microbial communities as well as avoidance of overly intense and self-damaging responses (Ayres & Schneider, 2012). During the next few years, we will likely learn much more about how insects discriminate between nonpathogenic or mutualistic gut microorganisms on the one hand and harmful pathogens on the other. Such insights will help in efforts to manipulate gut microorganisms of insects to control damaging insect species or to protect beneficial ones, including pollinators. While this paper was in proof, new data were published showing that bacterial-derived uracil activates DUOX-dependent gut immunity in *D. melanogaster*, leading to intestinal ROS production (Lee *et al.,* 2013). Uracil is the first bacterial ligand other than PGN shown to activate DUOX-regulatory pathways in the gut of *D. melanogaster*. The authors of this study

also showed that exemplar pathogens and commensals differ in uracil release, which can explain the selectivity of the immune responses raised against pathogenic bacteria in the gut of *D. melanogaster*.

Acknowledgements

We thank Kim Hammond for her immense help with preparation of the figures and editing of the manuscript. We thank Fabienne Wichmann and Hauke Koch for critical reading of the manuscript.

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