# Multiorganismal Insects: Diversity and Function of Resident Microorganisms

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## Abstract

All insects are colonized by microorganisms on the insect exoskeleton, in the gut and hemocoel, and within insect cells. The insect microbiota is generally different from microorganisms in the external environment, including ingested food. Specifically, certain microbial taxa are favored by the conditions and resources in the insect habitat, by their tolerance of insect immunity, and by specific mechanisms for their transmission. The resident microorganisms can promote insect fitness by contributing to nutrition, especially by providing essential amino acids, B vitamins, and, for fungal partners, sterols. Some microorganisms protect their insect hosts against pathogens, parasitoids, and other parasites by synthesizing specific toxins or modifying the insect immune system. Priorities for future research include elucidation of microbial contributions to detoxification, especially of plant allelochemicals in phytophagous insects, and resistance to pathogens; as well as their role in among-insect communication; and the potential value of manipulation of the microbiota to control insect pests.

## INTRODUCTION

Insects are chronically colonized by microorganisms that are not overtly pathogenic and are often beneficial or even required by the insect host. Most of the cells in a healthy insect are microbial, and the microbiota accounts for up to 1–10% of the insect's biomass. As a result, an insect is fundamentally a multiorganismal entity.

The microbiology of healthy insects has become the focus of intense research interest in recent years. This heightened activity can be attributed to two linked developments: dramatic technical advances in sequencing technologies, enabling microorganisms to be identified and investigated in situ, and large consortial initiatives [e.g., Human Microbiome Project (**http://commonfund.nih.gov/hmp/index**), MetaHIT (**http://metahit.eu**)] that have successfully applied these technologies to study the resident microorganisms in humans (24) and raised awareness among biologists of the wider importance of animal-associated microbiota. Despite great interest, the study of insect-microbial interactions is still widely regarded as crossing traditional disciplinary boundaries, with the consequence that the literature is scattered among journals of microbiology, ecology, evolution, and molecular biology and physiology, as well as entomology. The purpose of this review is to synthesize this diffuse literature to provide an overview of interactions between insects and their resident microbiota.

## INSECT HABITATS

An insect comprises multiple habitats for microorganisms. The most accessible habitats for microbial colonists are the external cuticle and the gut. Microorganisms that can breach the exoskeleton or gut wall can gain access to the hemocoel and a further set of habitats provided by insect cells.

### Cuticle

Although the insect exoskeleton is correctly recognized as a vitally important physical barrier against microbial infections (116), it is also a substrate that can be colonized by various microorganisms. Up to 1,000 culturable bacterial cells are associated with the body surface of *Drosophila melanogaster*, two orders of magnitude fewer than are borne internally by flies of the same age (92). Factors limiting microbial populations on the insect cuticle can include physical disturbance (e.g., ecdysis and grooming behavior) as well as antimicrobial secretions (e.g., from the metapleural glands of ants, Hymenoptera) (122). The extent to which cuticle-associated bacteria can proliferate and form stable communities, as occurs on human skin (46), is largely unknown.

Cuticular structures that promote colonization by specific microorganisms have evolved in many insects. In particular, the mycangia, i.e., cuticular invaginations housing fungi in adult insects, can be considered as culture vessels in which fungi required by the insect's offspring are stored and protected against abiotic factors and contamination by other microorganisms. The defining feature of mycangia—that they house fungi—is somewhat artificial because at least some mycangia additionally bear bacteria (56, 101). Some cuticular modifications house bacteria exclusively. For example, solitary digger wasps of the tribe Philanthini retain *Streptomyces* spp. in cuticle-lined glandular reservoirs, in each of 5–6 antennal segments (61) (**Figure 1a**), and attine ants house actinobacteria of the genus *Pseudonocardia* in similar glandular invaginations, known as crypts or foveae, on the thorax, legs, or other locations of the body, varying with ant species (26).

### Gut

Some attributes of the insect gut are favorable for colonization by microorganisms, including ease of access for food-associated microbial cells, availability of nutrients, and protection from
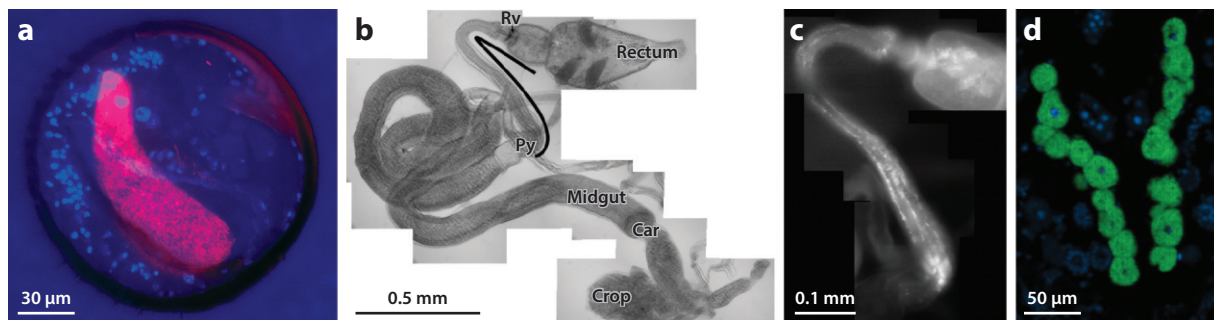
**Figure 1**

Insect habitats for microorganisms. (*a*) Antennal gland reservoir of the beewolf *Philanthinus quattuordecimpunctatus*, with "*Candidatus* Streptomyces philanthi"–specific probe SPT177-Cy3, the general eubacterial probe EUB784-FAM, and DAPI (*blue*) counterstain. "*Ca*. S. philanthi" binds both SPT177 and EUB784 probes, generating yellow fluorescence (note *red* autofluorescence of chitin at *right*) (micrograph of M. Kaltenpoth). (*b*) Dissected gut of *Drosophila putrida* [Py, pylorus; Rv, rectal valve; Car, cardia from a natural population in Rochester, NY, USA (micrograph by V. Martinson)]. (*c*) Expanded image of ileum in (*b*) showing bacteria, false color white fluorescence emission from general bacterial probe EUB338-Cy3 (micrograph by V. Martinson). (*d*) Section through embryo of black bean aphid *Aphis fabae*, showing *Buchnera* symbionts with general eubacterial probe EUB338-FITC (*green*) in bacteriocytes, and DAPI (*blue*) counterstain (micrograph by S. Chandler).

various stresses of the external environment (e.g., desiccation, ultraviolet radiation). Nevertheless, the insect gut poses multiple challenges for microorganisms ingested with the food, including unfavorable physicochemical conditions (e.g., oxygen content, pH, redox potential) in the gut lumen, secreted digestive enzymes and immune-related compounds, physical disturbance caused by peristalsis of gut contents, and loss of habitat at insect molts and metamorphosis. The conditions, resources, and hazards of the gut habitat for microorganisms vary among insect groups and with the life stage of the insect and region within the gut, reflecting the great variation in insect gut anatomy and physiology.

In many insects, the hindgut is the gut region bearing the largest microbial populations (**Figure 1*b,c***). In particular, the ileum (the region between the proximal pylorus and distal rectum) is a relatively benign environment, in that it lacks the digestive enzymes of the midgut and, for many terrestrial insects, the desiccation stress of the distal hindgut, where water is actively resorbed from the lumen into insect tissues. Microbial function and growth may also be favored by the ions and metabolites delivered to the hindgut in the filtrate from the Malpighian tubules. In many insects, the ileum displays no evident morphological or physiological adaptations to maintain microorganisms, but the ileum of some insects (e.g., termites, scarab beetles) is expanded to form an anoxic fermentation chamber in which the microbiota degrade complex plant polysaccharides into products utilizable by aerobic metabolism of the insect (14, 54). In many insect taxa, the cuticle of the hindgut is thrown into spines and plates, and microorganisms can preferentially adhere to these structures (14).

The midgut tends to be a hostile environment for microorganisms. The midgut epithelium secretes an arsenal of enzymes and is immunologically very active. For example, the *D. melanogaster* midgut produces various antimicrobial peptides (70); a suite of digestive enzymes, including lysozymes (29, 102); and a dual oxidase (DUOX: NADPH oxidase) enzyme that generates microbicidal reactive oxygen species (ROS) (49). It also includes a region of pH < 3 that likely kills many microbial cells (102). However, the strongly acidic region of the midgut in *D. melanogaster* and other cyclorraphous dipterans is unusual among insects and may be a specific adaptation to bactivory; i.e., utilizing ingested bacteria as food (70). The midgut pH of many insects is mildly

acidic to neutral (i.e., 6–7 units), which is suitable for a wide range of microorganisms, but the alkaline midgut (pH 8–12 units) of some insects, including larval lepidopterans, is likely inimical to many microorganisms (51). Compounding the various chemical barriers to the microbial colonization of the insect midgut is the physical barrier posed by the peritrophic matrix (PM), which separates the food bolus from the midgut epithelium. Many ingested microorganisms do not penetrate the PM and transit passively through the midgut with the bulk flow of food. Passage of certain microorganisms across the PM can be facilitated by chitinases of microbial or insect origin (34, 114), and some insects bear apparently benign bacterial communities in the ectoperitrophic space (between the PM and epithelial cells) (14).

For some insects, the dominant foregut habitat for microorganisms is provided by the crop, which can contain microorganisms at densities comparable to, or even exceeding, more distal gut regions (67, 100). However, the crop most commonly functions in the temporary storage of food and is evacuated regularly, raising the possibility that microorganisms may reside in this location for a relatively short period. Unusually, the dipteran olive fly *Bactrocera oleae* has an esophageal evagination, known as the cephalic bulb, which houses a dense culture of a single bacterium, "*Candidatus* Erwinia dacicola" (18). In insect vectors of plant or animal pathogens, other regions of the foregut have been identified as sites for microbial adhesion; e.g., the precibarium of the leafhopper *Graphocephala atropunctata* for the plant pathogen *Xylella fastidiosa* (85).

## Cells

Intracellular microorganisms are widespread or universal in certain insect groups and restricted to cells whose sole function appears to be to maintain and house microorganisms (35). These insect cells are known as bacteriocytes, containing bacteria (**Figure 1d**), or mycetocytes, containing yeasts. The developmental origin of these insect cells is largely obscure, but the bacteriocytes of the aphid *Acyrthosiphon pisum*, although morphologically uniform, comprise two populations that differentiate at different stages in embryonic development (10). The dominant bacteria in bacteriocytes (primary symbionts) have no access to the external environment and are transmitted vertically (**Table 1**), usually by transfer to the ovaries of the female and, thence, to the cytoplasm of the egg. In this way, the host maintains very precise control over the location and abundance of the microorganisms in transit from bacteriocytes to offspring. Many insects with primary symbionts additionally bear other bacteria, known as secondary symbionts, which are associated with the bacteriocytes and are vertically transmitted but differ from primary symbionts in several important traits (**Table 1**).

Table 1   Characteristics of primary and secondary symbionts: bacteria associated with bacteriocytes of insects[a]

| Primary symbionts | Secondary symbionts |
| --- | --- |
| Restricted to bacteriocytes | May be located in bacteriocytes, sheath cells bounding bacteriocytes, and hemolymph |
| Present in all individual insects | Intermediate prevalence |
| Vertical transmission only | Vertical and horizontal transmission |
| Required by the insect | Can confer ecologically important traits (e.g., thermal tolerance, resistance to parasitoids or fungi); may reduce or promote insect fitness under laboratory conditions |

[a]Bacteriocytes are universal or widely distributed in some insect orders (e.g., Blattodea, Phthiraptera, Hemiptera, Coleoptera) and present in a few species of Hymenoptera and Diptera, but they are absent from most other insects (35, 37).

## INSECT-ASSOCIATED MICROORGANISMS

### Taxonomic Diversity

The microbial inhabitants of insects comprise bacterial, archaeal, and eukaryotic (fungal and various unicellular eukaryotic) microorganisms. Viruses are not considered in this article. Although all insects are colonized by microorganisms, most microorganisms are not associated with insects (96). Four phyla of Bacteria (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*) are particularly strongly represented, but other phyla dominate certain insect groups; e.g., *Spirochaetae*, *Fibrobacteres*, and candidate phylum TG3 in the hindgut of the wood-feeding termites (*Nasutitermes* spp.) (67, 120). Archaea generally are not associated with animals (48), although representatives of the *Methanoarchaeota* (methanogens) and the nonmethanogenic *Thermoplasmatales* and *Halobacteriales* are known in insects and are prevalent in the hindgut of cockroaches (order Blattodea), termites (infraorder Isoptera), and larval scarab beetles (family Scarabaeidae) (5, 15). Most of the eukaryotic microorganisms described in insects are fungi, especially ascomycetes (e.g., *Clavicipitaceae*, *Saccharomycetes*). Also well studied are the flagellate protists, apparently restricted to wood roaches and lower termites, and comprising members of the phylum *Metamonada* (the order *Oxymonadida*, and trichomonads and hypermastigotes within the class *Parabasalia*). Anaerobic ciliates of the order *Clevelandellida* (including *Nyctotherus* species) are also found in cockroaches and termites (100), and hemipterans, hymenopterans, and dipterans are often infected with trypanosomatids, which are generally benign but can be opportunistic pathogens (20, 74).

### Evolutionary History and Ecological Status of Insect-Associated Microorganisms

The duration of relationships between many insects and their associated microorganisms varies across taxa. At one end of the spectrum, some insect-associated microbial taxa maintain substantial free-living populations or are closely allied to free-living microorganisms. In particular, *Pantoea* spp. (*Gammaproteobacteria*) are readily isolated from both environmental samples (e.g., water, soil, plant material) and insects, including mosquitoes (Diptera), thrips (Thysanoptera), bees (Hymenoptera), and hemipterans (115); and the *Acetobacteraceae* (*Alphaproteobacteria*), found in fruits and fermented foods and beverages, are also found in the guts of insects feeding on sugar-rich diets; e.g., bees, drosophilid fruit flies, and mosquitoes (25). At least some of these microbial populations may transfer regularly between the insect and the external environment, utilizing the insect as a route for dispersal. Other clades of microorganisms are widely distributed across insects and other animals but are unknown or rarely reported in the free-living condition—e.g., some lineages of *Rikenellaceae* and *Porphyromonadaceae* (*Bacteroidetes*), and *Clostridiaceae* and *Ruminococcaceae* (*Firmicutes*) (100, 111). At the other end of the scale are insect-specific species of bacteria, including some gut microorganisms [e.g., *Snodgrassella alvi* (*Betaproteobacteria*) and *Gilliamella apicola* (*Gammaproteobacteria*) in honey bees (*Apis mellifera*) (42, 69, 73)] and the primary and secondary symbionts associated with insect bacteriocytes (16, 35) (**Table 1**).

## ASSEMBLY OF INSECT-MICROBIAL ASSOCIATIONS

The processes shaping the composition of insect microbial communities differ substantially between open associations (i.e., subject to invasion by external microorganisms) and closed associations (isolated by location and host factors from incoming microorganisms). Microbial

communities on the cuticle and in the gut are generally open, whereas intracellular symbioses are predominantly closed.

## Open Associations

The patterns of assembly of open associations in insects have been studied in gut symbioses, focusing particularly on the variation in the microbial communities with location within the gut. Generally, the microbiota varies longitudinally in the insect gut. For example, the density of microorganisms in the hindgut of the cockroach *Shelfordella lateralis* is $1–2 \times 10^{10}$ cells/g, an order of magnitude greater than in the crop, ceca, and midgut (100); members of the bacterial phylum *Firmicutes* are abundant in the termite *Nasutitermes corniger*, but the dominant representatives of this phylum vary with gut region (*Lactobacillales* in the crop, *Lachnospiraceae* in the midgut, and *Ruminococcaceae* in the distal hindgut) (67); and the gut microbiota of larval *Spodoptera littoralis* (Lepidoptera) is dominated by *Clostridium* species in the midregion of the midgut but by *Enterococcus* spp. in more proximal and distal gut regions (110). The microbiota can also vary radially, with marked differences between the communities associated with the gut wall and the gut lumen. The gut wall community may dominate some aspects of the interactions with the host because it is persistent (not voided with bulk flow of the food). It can also define metabolite flux across the gut wall and affect the physicochemical conditions in the gut lumen (15, 67).

Diet plays a major role in structuring the gut microbial community. Effects on the microbiota have been observed in comparisons between artificial diets and natural foods, as well as between diets in which the major nutritional classes (protein, lipid, sugar, fiber) are varied (33, 64, 68, 97, 110). These studies raise important and largely unanswered questions about the processes determining the scale and direction of microbial responses to diet. In principle, diet can influence the gut microbiota directly and indirectly. With respect to direct effects, food-associated microorganisms ingested by the insect may vary with the composition of the food, and the microorganisms favored in the gut environment likely include those taxa that can best utilize food-derived nutrients in the gut lumen, including compounds intractable to host digestive enzymes. Indirect effects are mediated through the impact of food on gut anatomy, digestive function, and immunity and may be significant in the many insects where the microbiota in the gut and the food overlaps weakly (5, 107, 110). The difference between the microbiota in the gut and that in the food can be exaggerated by behavioral adaptations that further promote the dominant gut microbial taxa, including coprophagy, trophallaxis (transfer of gut fluids by anus-to-mouth or mouth-to-mouth feeding), and maternal smearing of gut microorganisms on the eggshell, which is subsequently consumed by the offspring (11, 14).

The among-individual variation in the composition of the microbiota is substantial for the gut microbiota in some insects. Some of this variation may be driven by intraspecific genetic variation of the host, although the importance of host genotype in these interactions remains to be investigated systematically. Evidence for nongenetic sources of variation come from striking differences in the gut microbiota of single *D. melanogaster* strains reared on the same food (21, 121), suggesting that the microbiota may not be shaped exclusively by deterministic factors (e.g., gut pH, oxygen tensions). Stochastic processes, including the microorganisms that happen to be ingested, proximity between competing or mutualistic microbial cells in the gut environment, and the gut wall microsite where an individual microbial cell adheres may influence the composition of the gut community and persistence of individual taxa in the gut. Determining the relative contribution of deterministic (niche-based) and stochastic (neutral) processes in the assembly of the gut microbiota is an important challenge for future research.

## Closed Associations

The bacteriocyte symbioses (**Table 1**) are predominantly closed systems, raising this question: How is the bacteriocyte protected from colonization by other microorganisms in the insect body while maintaining an intracellular environment that is suitable for the actively dividing primary symbionts? The immunological status of the bacteriocyte may be important, as is suggested by research on antimicrobial peptides (AMPs) in bacteriocytes of the weevil *Sitophilus zeamais*. AMPs are produced by the insect immune deficiency (IMD) signaling pathway, in response to the bacterial cell wall peptidoglycan (PGN) fragments, but the activity of this pathway is reduced in *S. zeamais* bacteriocytes by the high expression of an IMD-dependent PGN amidase, PGRP-LB, that degrades the immunogenic PGN fragments (6). Despite this generalized immunological suppression, one AMP (coleoptericin-A) is strongly expressed in the bacteriocytes, and when this AMP is reduced experimentally by RNAi, the symbionts overgrow the bacteriocytes and invade the insect body cavity (71). Downregulation of IMD signaling by PGRP-LB has also been demonstrated in the bacteriocytes of tsetse flies, *Glossina* spp. (119). However, this mechanism cannot explain the persistence of primary symbionts in all insects. For example, the aphid immune system lacks PGRPs, an intact IMD pathway, and recognizable AMPs expressed in bacteriocytes (47). Other candidate immune effectors may include lysozyme and small, cysteine-rich proteins, both of which are enriched in the transcriptome of aphid bacteriocytes (80, 105).

Phylogenetic analyses of intracellular symbioses in various insects reveal that many of these systems are not perfectly closed. Ancestral symbionts have been displaced by bacteria or yeasts—for example, in dryophthorid weevils (23), cerataphidine aphids (117), and philaenine spittlebugs (65).

## Interactions Among Microbial Partners

The field of microbial ecology is replete with examples of interspecific interactions among microorganisms, including multiple mechanisms by which microbes compete for resources or enter into mutualistic consortia that can exploit resources unavailable to consortium members in isolation. This raises the possibility that interactions among microorganisms can affect the composition of the microbiota associated with insects. These interactions have not yet received extensive study but have been demonstrated in several associations.

In the *D. melanogaster* gut, the prevalence of the various bacteria is generally negatively related (121), suggestive of antagonistic interactions (although the alternative explanation that different individual insects meet the habitat requirements of different bacteria cannot be excluded). However, experimental colonization studies reveal great complexity, with both positive and negative relationships between the abundance of different *Acetobacter* and *Lactobacillus* species in the *D. melanogaster* gut (84). In the gut of the mosquito *Aedes albopictus*, the prevalence of *Asaia* and *Acinetobacter* spp. is positively related (78), but in the desert locust *Schistocerca gregaria* the abundance of *Serratia marcescens* is negatively correlated with the abundance of other bacteria (33).

The role of interactions among microorganisms in shaping microbial communities is also evident from the relationship between fungal associates of some insects and antibiotic production by actinobacteria borne on the insect exoskeleton. The dominant fungus partners of the bark beetle *Dendroctonus frontalis* are *Entomocorticium* spp., which line the galleries constructed by the insect in the phloem vessels of host trees, but this association is susceptible to invasion by a related fungus, *Opisthosoma minus*, which supports poor beetle growth. Protection against the antagonistic *O. minus* is provided by actinobacteria of the genus *Streptomyces*, which secrete a polyene peroxide antimicrobial that selectively inhibits the growth of *O. minus* (101). Similarly, antibiotics produced by actinomycete symbionts protect the fungal symbiont of attine ants against the fungal parasites of the genus *Escovopsis* (27, 28).

## MICROBIAL IMPACTS ON INSECT PHENOTYPE

### Nutrition

Many insect-associated microorganisms promote insect capacity to utilize diets of low or unbalanced nutritional content by providing specific nutrients that the insect cannot synthesize, including essential amino acids and B vitamins and sterols and, for insects feeding on diets rich in plant fiber, by degrading complex plant polysaccharides.

The role of microorganisms in provisioning essential amino acids has been demonstrated most conclusively in hemipteran insects feeding on plant phloem sap. The principal sources of nitrogen in phloem sap are the free amino acids of unbalanced composition, with <20% essential amino acids (the 9/10 of 20 amino acids that contribute to protein that cannot be synthesized by animals) (36). The key evidence that the primary symbiont, *Buchnera aphidicola*, in aphids synthesizes and releases essential amino acids is threefold: (*a*) Aphids have no dietary requirement for essential amino acids (unlike most animals) and can synthesize essential amino acids de novo, but they lose these capabilities when the *Buchnera* bacteria are eliminated by antibiotic treatment (39, 44); (*b*) isolated *Buchnera* bacteria release essential amino acids at linear rates for an hour or more (93); and (*c*) the *Buchnera* genome has retained the genetic capacity for essential amino acid synthesis, despite massive genome reduction (106). Microbial involvement in essential amino acid provisioning in other plant sap–feeding insects is indicated by the apparently universal incidence of symbioses in these insects (16) and by the retention of essential amino acid biosynthesis genes in all symbionts tested (76). Microbial symbionts have also been implicated in essential amino acid provisioning in ants (45), cockroaches (95), and some wood roaches (113).

Microorganisms associated with insects can gain access to nitrogenous precursors from dietary nitrogen, insect waste nitrogen, and nitrogen fixation. Insect nitrogenous waste is recycled to essential amino acids in the ant-*Blochmannia* symbiosis, in planthopper (*Nilaparvata lugens*)-yeast associations, and in cockroaches and termites (45, 90, 95, 98) but apparently not in the aphid-*Buchnera* symbiosis (72). Persuasive evidence for nitrogen fixation by insect-associated bacteria has been obtained for some termites (87). The microbiota in various other insects includes taxa with the genetic capacity to fix nitrogen and, in some instances, with demonstrable nitrogen fixation or acetylene reduction (which is a valid proxy for nitrogen fixation) (3, 7, 79, 82, 94), but the quantitative contribution of this capability to the nitrogen economy of the insects is largely unexplored.

B vitamins have been inferred to be provided by resident microorganisms, especially in insects feeding throughout the life cycle on vertebrate blood (e.g., the tsetse flies and other Diptera Pupipara, Cimicidae bed bugs, anopluran lice) and some phytophagous and xylophagous insects, including plant sap–feeding hemipterans, and various Coleoptera spp. of the families Anobiidae and Curculionidae (4, 16, 35, 75, 106). Contributions of microbiota to insect sterol nutrition relate exclusively to eukaryotic, particularly yeast, symbionts, because bacteria lack the capacity for sterol synthesis. A fungal source of insect sterols is indicated by the fungal sterol ergosterol and related compounds in the sterol profile of anobiid beetles (Coleoptera, Anobiidae) and planthoppers with yeast symbionts (83, 86). However, sterol analysis of the wood wasp *Sirex noctilio* suggests that this xylophage derives its sterols from the diet and not the fungal symbiont (112).

Microorganisms make a critical contribution to the degradation of plant cell wall material in insects that feed on sound wood and other plant products with a high lignocellulose content (e.g., termites, wood roaches, scarab beetle larvae). The microorganisms are located in a hindgut fermentation chamber, where they mediate the slow enzymatic degradation of the cellulose and hemicellulose components of the diet to sugars, which are then fermented to short-chain fatty

acids and made available to the insect (17). Insects that feed on living plant material are largely independent of microbially mediated degradation of plant cell wall material because they subsist on the soluble carbohydrates and proteins in the plant cell contents and produce midgut glucosyl hydrolases capable of degrading plant cellulose and other plant cell wall polysaccharides (8, 17).

## Protection Against Natural Enemies

Resident microorganisms can protect their insect hosts against pathogens and other natural enemies by multiple mechanisms that are not mutually exclusive, including competition for nutrients or space, production of toxins active against the invader, and activation of insect immune system functions that are more deleterious to the invader than the resident. Some of these mechanisms are equivalent to traits of environmental microorganisms that protect a resource patch, for example in soil or the water column, with the implication that protective traits of insect-associated microorganisms are not necessarily specific adaptations to the insect habitat. Microorganisms may defend their insect habitat against competing microorganisms that happen to include insect pathogens. Nevertheless, many protective functions of insect microbiota likely involve adaptations specific to the insect habitat, such as novel microbial chemistries against parasitoids. Coevolutionary interactions between insects and their microbiota are also expected and would lead to selection for reduced toxicity of the microbial agents against the host and coordination of the timing and magnitude of microbial toxin production to optimize protection of particularly vulnerable insect life stages or tissues.

There is now persuasive evidence that resident microorganisms can dictate the outcome of insect interactions with natural enemies, but understanding of the underlying mechanisms is fragmentary. The secondary symbiont *Hamiltonella defensa* confers pea aphid resistance to the parasitoid *Aphidius ervi* (88), but not all *Hamiltonella* spp. are protective. Function has been correlated with a bacteriophage in the *Hamiltonella* spp., and specifically with phage-encoded genes for toxins, such as Shiga-like toxin, cytolethal distending toxin, and YD-repeat toxins (31). A different group of toxins, polyketides, has been implicated in the *Pseudomonas*-mediated protection of *Paederus* rove beetles against predators (89) and in an undefined protective role of *Profftella armatura*, localized in the bacteriocytes of Asian citrus psyllid *Diaphorina citri* (81). ROS produced in the insect gut by either microorganisms or the insect gut epithelium can have strong antimicrobial effects. ROS production by prevalent gut bacteria *Enterobacter* spp. in anopheline mosquitoes inhibits the development of *Plasmodium* ookinetes into oocysts (22), and *Leishmania* parasites are sensitive to ROS induced by some members of the gut microbiota in their phlebotomine sand fly vector (*Lutzomyia longipalpis*) (32).

Antimicrobial compounds are of particular importance to insects living in enclosed, humid environments, where opportunistic fungal or bacterial infections can develop rapidly. Adult females of the solitary digger wasp *Philanthus triangulum* smear the ceiling of each subterranean brood cell with an antennal secretion containing antibiotic-producing *Streptomyces* spp. (**Figure 1a**); the larva subsequently transfers the secretion to the surface of the cocoon. Survival is reduced from 80% to 10% if the *Streptomyces* bacteria are removed (60). Similarly, adults of the spruce bark beetle *Dendroctonus rufipennis* smear oral secretions containing bacteria onto the gallery walls of the trees they infest, likely conferring protection against antagonistic fungi, such as *Aspergillus* spp. (19).

From an evolutionary perspective, these beneficial effects of resident microorganisms in insects challenged by natural enemies can be attributed to strong selective overlap between the microbiota and their insect host: Persistence of the insect habitat is advantageous to the microbiota. Some microbial partners may, however, respond to pathogen/parasitoid-mediated reduction in

insect fitness by increased proliferation and dissemination from the failing insect. These microbial residents of insects are opportunistic pathogens. One possible instance of this response comes from the reduced virulence of baculovirus infecting *Spodoptera exigua* treated with antibiotic to eliminate the gut microbiota, relative to untreated caterpillars (59).

### Detoxification of Toxins: Plant Allelochemicals and Insecticides

Most described instances of detoxification in insects are intrinsic. They are mediated by capabilities encoded by the insect genome, including cytochrome P450 monooxygenases, glutathione S-transferases, and esterases. Resident microorganisms have, however, been implicated in a few systems. Elimination of the yeast-like symbiont *Symbiotaphrina kochi* from *Lasioderma serricorne* beetles depresses larval development on diets containing allelochemicals that cultured *S. kochi* can degrade (40, 104). The capacity of the mountain pine beetle *Dendroctonus ponderosae* to utilize terpene-rich trees may be facilitated by species of *Pseudomonas*, *Rahnella*, and other resident gut bacteria that have the genetic capacity to degrade terpenes (1). A laccase enzyme produced by the fungal symbiont of attine ants also mediates the detoxification of plant material brought to the nest by the worker ants (30).

Resident microorganisms have repeatedly been proposed as a source of insecticide resistance, but most claims lack proper validation. Exceptionally, the resistance of the alydid stink bug *Riptortus pedestris* to the organophosphate fenitrothion is mediated by fenitrothion-degrading *Burkholderia* bacteria that are acquired from the soil by the insects (63). Further research is required to establish whether other insects benefit from microorganisms that can utilize both the insect habitat and the wider environment in this way.

### A Source of Cues and Signals

Microorganisms associated with insects have been invoked as the source of chemicals that alter the behavior of conspecifics or other organisms (43), to the benefit or disadvantage of the insect host. To illustrate, the phenolic guaiacol in the aggregation pheromone of the desert locust *Schistocerca gregaria* is synthesized by *Pantoea agglomerans* and other *Enterobacteriaceae* in the insect gut (33); *Drosophila* prefer to mate with conspecifics that have a similar gut microbiota, and this preference is probably linked to microbiota-dependent variation in the cuticle hydrocarbon profile (103); and parasitic wasps of the bark beetle *Dendroctonus ponderosae* are attracted to logs containing the fungal partners (*Grosmannia clavigera* and *Ophiostoma montium*) of the beetle, suggesting that these parasitoids use fungal volatiles as cues to locate beetle larvae and pupae (2). Most experimental studies, however, lack definitive evidence (38), and establishing precisely the role of the microorganisms in the synthesis of insect semiochemicals is a priority for future research in insect chemical ecology.

## RESIDENT MICROORGANISMS IN ECONOMICALLY IMPORTANT INSECTS

The resident microbiota offers great potential for improved methods to manage economically important insects. Three primary opportunities are to predict the traits of insect pests, and hence efficacy of control strategies, from the composition of the microbiota, to target the microbiota for insect pest control, and to manipulate the microbiota to depress the vector competence of insects.

### Predictor of Insect Pest Traits

Traits crucial to the management of certain insect pests are dictated by their possession of particular microorganisms. Examples include the secondary symbionts that determine the resistance of

aphids to parasitoids and fungal pathogens used as biological control agents (88, 99); the pesticide-resistant *Burkholderia* strains that confer pesticide resistance to *Riptortus pedestris* (63); and bacteria that enable *Megacopta* stink bugs to utilize soybean crops (53). In these systems, the prevalence of the critical microorganisms in insect populations can be used to monitor the pest status and identify preferred control strategies. Regular monitoring would be required because the microbiota in insect populations can change rapidly (52, 58). Monitoring may be particularly valuable for exotic insect species, whose invasiveness can depend on interactions with microorganisms in the introduced range. For example, the US turpentine beetle *Dendroctonus valens* (Scolytinae) is a minor forestry pest in its native range, but it has been causing high mortality to Chinese pines since its introduction to China in the 1980s (108), partly because of its acquisition of fungi from local Chinese Scolytinae (109). By contrast, *Megacopta cribraria*, introduced from eastern Asia to the eastern United States in 2009, appears to have retained the ancestral *Ishikawaella* symbiont; and its rapid transfer to soybean crops in the United States is not linked to symbiont switching (12).

## A Target of Novel Insect-Pest-Control Strategies

The potential of insect pest control with the microbiota as the primary target is greatest for insects dependent on vertically transmitted microorganisms because the insect has no opportunity to acquire equivalent microorganisms from the environment. Many insects with vertically transmitted bacteriocyte symbioses (**Table 1**) are agricultural or medically important pests: aphids (superfamily Aphidoidea), whiteflies (family Aleyrodidae), planthoppers (infraorder Fulgoroidea), and sharpshooters (tribe Proconiini of the family Cicadellidae) feeding on plant sap; anopluran lice (suborder Anoplura), bed bugs (family Cimicidae), and tsetse flies (family Glossinidae) feeding on vertebrate blood; pests of stored products and timber (e.g., various beetles of the families Curculionidae and Anobiidae); and cockroaches (order Blattodea). The population increase of these insects is abrogated by antibiotics that eliminate the microbial symbionts. The key priority is to identify alternatives to antibiotics that are cost-effective and specific. Recent advances in understanding the cellular processes underlying vertical transmission (66) and nutrient translocation between the insect and microbial partners (91, 93) are providing candidate molecular targets for disruption of these symbioses.

## Microbially Mediated Manipulation of Insect Traits

The insects of greatest interest for microbially mediated manipulation are vectors of disease agents, especially mosquitoes. The goal is to introduce microorganisms that both suppress vector competence and promote their own dissemination through the insect population. Bacteria of the genus *Wolbachia* have long been identified as candidate microorganisms for this application. Most mosquito vector species are not infected with *Wolbachia* naturally, and the stable introductions of *Wolbachia* from *Drosophila* into *Aedes aegypti* (77) and *Anopheles stephensi* (9) are major breakthroughs. The insects bearing *Wolbachia* display enhanced resistance to dengue and chikungunya viruses and *Plasmodium* parasites, probably through heightened immunological function (9, 55, 62, 118). Field trials are investigating the fate of introduced *Wolbachia*-infected mosquitoes, with the long-term goal to release these insects to reduce disease transmission.

An alternative route for microbial manipulation of insect pests is to exploit members of the native microbiota of the insect vector by introducing gene(s) deleterious to the disease agent into a bacterial symbiont. Proof of principle has been obtained for *Trypanosoma cruzi*, the agent of Chagas disease, vectored by *Rhodnius prolixus*; when the gut symbiont, *Rhodococcus rhodnii*, was genetically modified to express the antimicrobial peptide cecropin A and then introduced to *R. prolixus*, transmission of *T. cruzi* was suppressed (41). Furthermore, *R. prolixus* populations in

domestic environments are readily infected when they feed on mock fecal pellets containing the genetically modified bacteria, a formulation that has been developed as Cruzigard. Analogous approaches are under development to suppress transmission of the *Leishmania* parasite by sand flies *Phlebotomus argentipes* (57).

In principle, multiple opportunities are available to modify the pest status of insects by promoting members of the insect's native microbiota that influence vector competence or other traits of interest (e.g., plant range of crop pests, capacity for dispersal, mate choice, oviposition preference). Effective manipulation of native microbiota in pest management, however, depends on a detailed understanding of the function of insect-associated microorganisms and the interplay of factors that shape their abundance within an insect and dissemination through insect populations.

## PERSPECTIVES AND FUTURE DIRECTIONS

Microorganisms are ubiquitous in insects and have pervasive impacts on multiple aspects of insect biology. Consequently, microorganisms should be included as candidate factors affecting virtually any aspect of insect biology. Fortunately, the tools to study these associations are increasingly available, including methods to identify and quantify microorganisms and their functions, to manipulate the composition of the microbiota, and to investigate their interactions with the nutrition, immunity, and other physiological systems of the insect.

Some aspects of insect-microbe interactions now have a firm experimental foundation, but others remain contentious. Of particular interest for future research are the mechanisms by which resident microorganisms influence insect susceptibility to pathogens, insect capacity to degrade phytotoxins, and insect capacity to vector plant viruses and medically important disease agents. Careful experimental analyses are required to assess the generality of microorganisms as determinants of insect communication (38), plant range (50), resistance to insecticides (63), and insect speciation events (13).

### SUMMARY POINTS

1. An insect represents multiple habitats, including the exoskeleton, gut lumen, and cells, that are colonized by microorganisms.

2. The composition and abundance of insect-associated microorganisms are shaped by the physicochemical conditions in the insect habitat, insect immune function, interactions among microorganisms, and transmission mechanisms of the insect.

3. Some microorganisms contribute to insect nutrition by providing nutrients or degrading plant material intractable to insect digestion.

4. Resident microorganisms protect their insect hosts against natural enemies, including viruses, bacteria, and parasitoids, by synthesizing toxins or modulating the insect immune system.

5. Microorganisms have been implicated in the detoxification of dietary compounds and insecticides, and as the source of signals and cues important to insect communication, but the incidence and general significance of these functions are largely unexplored.

6. The resident microbiota of insects has great potential in promoting effective management of insect pests, as biomarkers for insect traits, as modulators of insect vector competence, and as targets for novel strategies to control pests and manipulate their traits.

## DISCLOSURE STATEMENT

## ACKNOWLEDGMENTS

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