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Toward an Integrated Understanding of the Lepidoptera Microbiome

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Lepidoptera, microbiome, symbiosis, bacteria, diversity, multitrophic interactions

Abstract

Research over the past 30 years has led to a widespread acceptance that insects establish widespread and diverse associations with microorganisms. More recently, microbiome research has been accelerating in lepidopteran systems, leading to a greater understanding of both endosymbiont and gut microorganisms and how they contribute to integral aspects of the host. Lepidoptera are associated with a robust assemblage of microorganisms, some of which may be stable and routinely detected in larval and adult hosts, while others are ephemeral and transient. Certain microorganisms that populate Lepidoptera can contribute significantly to the hosts' performance and fitness, while others are inconsequential. We emphasize the context-dependent nature of the interactions between players. While our review discusses the contemporary literature, there are major avenues yet to be explored to determine both the fundamental aspects of host–microbe interactions and potential applications for the lepidopteran microbiome; we describe these avenues after our synthesis.

Endosymbiont:

an organism whose habitat is inside the host

Ectosymbiont:

an organism that colonizes surfaces exposed to the environment, such as the root epidermis, mammalian skin, insect exoskeletons, or animal guts

Symbiosis:

an association between dissimilar organisms that have some degree of physical association, regardless of the implications for the fitness of either organism

1. INTRODUCTION

Insects form ubiquitous associations with microorganisms, and knowledge of microbial impacts on ecological and physiological processes is rapidly expanding (22, 25, 100). Lepidoptera (butterflies and moths), which is the second largest insect order and includes important pollinators and agricultural pests, represents one of the most complex and ubiquitous symbiotic systems in the animal kingdom (63, 78, 94). High-throughput technologies have uncovered considerable new information on microbial diversity, transmission, and impact on lepidopterans (15, 71, 117), representing multiple relationships from pathogenesis to obligate mutualism.

Much of the early Lepidoptera microbiome literature focused on insect–pathogen relationships, but a recent surge in research has emphasized the roles of symbionts in mediating host interactions. Several excellent reviews address pathogens (8, 14, 86); our synthesis instead focuses on nonpathogenic microorganisms. The first goal of this review is to assess the diversity and general associations of microbes in Lepidoptera, first addressing intracellular endosymbionts and then discussing extracellular microorganisms, ectosymbionts, particularly those in guts. An impressive number (>100) of lepidopteran species have had their microbiomes surveyed with sequence-based approaches, but in many instances, the microbiome function is unresolved. The second aim of our review is to describe the potential impacts of microbes on their lepidopteran hosts. Finally, we draw attention to the applications of these symbionts in agriculture and biotechnology. We expect that, through critical exploration, evaluation, and synthesis, this review will not only reflect the salient and critical studies in this research area, but also provide future directions and analysis of microbial symbiosis in broader aspects of entomology.

2. DIVERSITY OF THE LEPIDOPTERA MICROBIOME

Lepidoptera occupy different dietary niches and consume dramatically different substrates across their holometabolous development. Both larvae and adults associate with microorganisms, either inside the host (endosymbionts) or on their body or in their gut (ectosymbionts). The unique endosymbionts in any one host are limited to one or two taxa, while there is often a high diversity of ectosymbionts. Host microbiomes are not simply static menageries but are affected by various factors. The environment is essential for continuously supplying microorganisms and shaping insect–microbe interactions, but interindividual transmission can also occur in the same generation (horizontal transmission) or across generations (vertical transmission). In this section, we summarize the taxonomic composition and structural diversity of the Lepidoptera microbiomes, including bacteria, fungi, and viruses, and discuss host and nonhost factors influencing the gut microbiome.

2.1. Endosymbionts

Endosymbiotic bacteria are widespread in insects. It has been estimated that 51% of Hexapoda species (insects and relatives) are infected with *Wolbachia* (Alphaproteobacteria), 22% with *Rickettsia* (Alphaproteobacteria), and 8% with *Cardinium* (Cytophagia) (116). To date, endosymbiont frequencies found in lepidopterans have been concordant with these general surveys (**Figure 1a**).

The majority of research undertaken on lepidopteran endosymbionts concerns *Wolbachia* (27). A global survey of 300 Lepidoptera species suggests that approximately 80% of lepidopteran species are infected with *Wolbachia* (2). *Wolbachia* is an ancient symbiont that was introduced to Lepidoptera approximately 22.6–4.7 Mya (3). *Wolbachia* lineages (supergroups) differ substantially in their biology and host distribution, but they are all classified as *Wolbachia pipientis* (38). *Wolbachia* strains carried by Lepidoptera are nearly all from supergroups A and B, with B-group *Wolbachia* having a higher frequency of association (3). *Wolbachia* are normally transmitted maternally in

nature, but horizontal transmission may also take place and can be mediated by shared food sources and natural enemies such as parasitoid wasps (3). The second most common endosymbiotic bacteria are *Spiroplasma*, detected in 4–7% of examined host species, particularly in the *Danaus* butterflies (**Figure 1a**). The majority of *Spiroplasma* symbionts have been considered commensal and have received less attention than *Wolbachia* (11). Despite *Rickettsia* being widely distributed among arthropods, only one *Rickettsia* species (*Rickettsia felis*) has been reported in Lepidoptera (7). Since *R. felis* has a diverse host range, further investigation is necessary to confirm whether the association of this strain is host specific or accidental. Symbiotic associations with *Arsenophonus* and *Sodalis* bacteria have recently been found through metagenomic approaches (33) (**Figure 1a**), but the breadth of these associations is unclear. To the best of our knowledge, *Cardinium* species have not been naturally found in Lepidoptera. Similarly, several common endosymbiotic bacteria such as *Hamiltonella* have never been observed; thus, their incidences, should they exist at all in Lepidoptera, may be very rare.

Fungal endosymbionts are also rare compared to those of other orders (105). Interestingly, an intracellular yeast-like endosymbiont *Purpureocillium* sp. (Ascomycota) was recently detected in the *Thitarodes* moth that might be vertically transmitted into the offspring via the ovary (65).

Metagenomic sequencing has led to increased recognition of the nonpathogenic viral endosymbionts of insects. Viral endosymbionts are transmitted through both eggs and sperm, rapidly spread through host populations, and have complex effects on their hosts (67). Recently, sigma-like viruses (negative-strand RNA viruses) were reported in the nymphalid butterfly *Pararge aegeria* (mean prevalence of 74%), although the effects of sigma-like viruses on the host were undetermined (66). In *Homona magnanima*, a novel double-stranded RNA virus establishes benign infections in females and is transmitted through eggs, but in males, it kills larvae late in their development (87). A widespread densovirus (single-stranded DNA virus) found in wild populations of *Helicoverpa armigera* (>67% prevalence) may benefit its host, although the mechanism is unknown (123).

2.2. Gut Microbiome

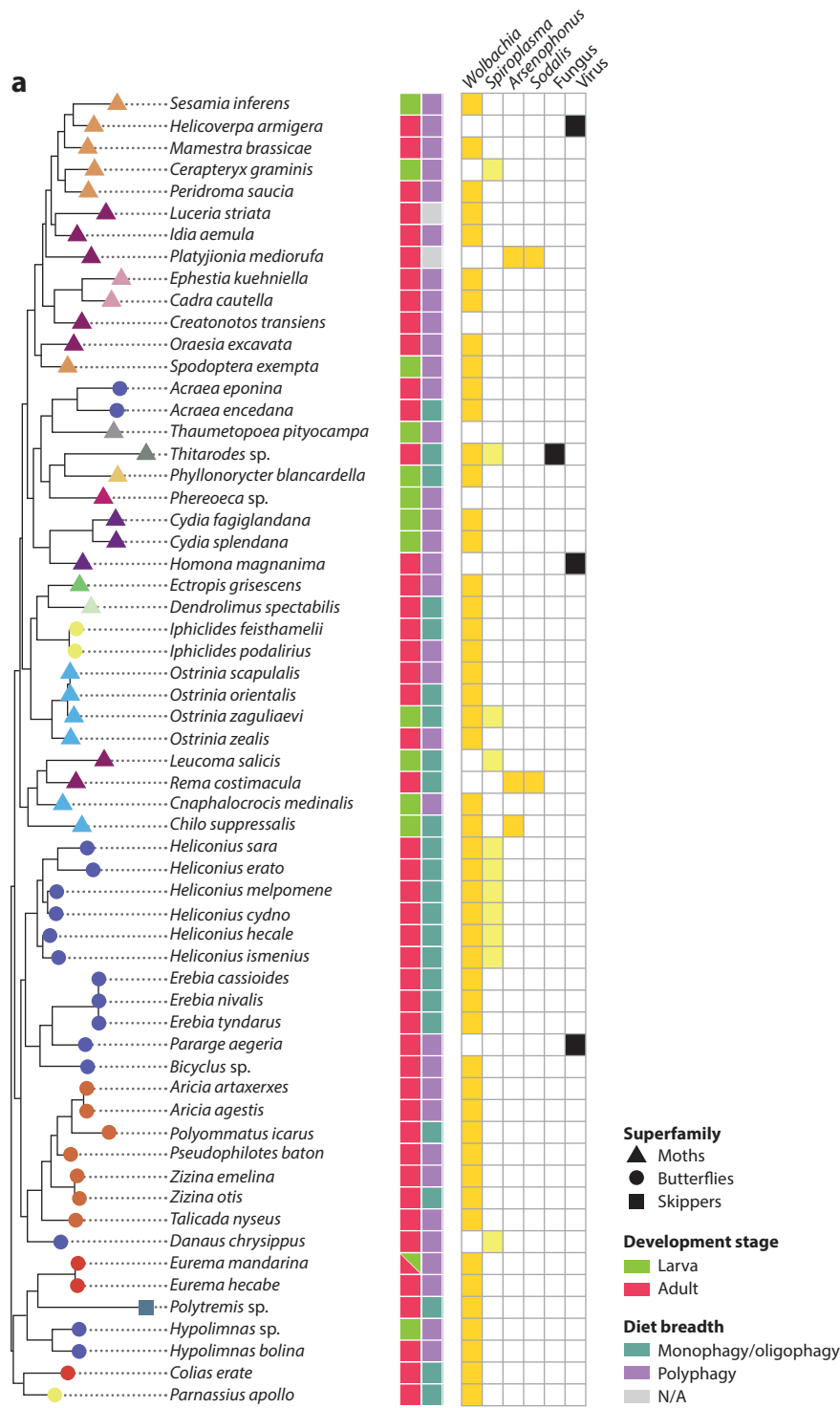
Ectosymbiotic microorganisms can efficiently colonize various host tissues including the cuticle, hemolymph, and gut epithelium. The main emphasis of ectosymbiont research has been on the digestive system (29). The larval lepidopteran gut is often relatively simple, without any specialized structures or complex morphology, and comprises the majority of the body cavity. Notably, metamorphosis radically remodels the morphology and biochemistry of the digestive system, and the hosts consume a dramatically different diet before and after metamorphosis. Most lepidopteran larvae feed on plant tissues, while adults consume nectar from flowers, fruits, or tree sap. Over the past decade, amplicon sequencing of single marker genes has been used to analyze lepidopteran microbial communities, with bacteria receiving the most attention (**Figure 1b**).

Most investigations of gut microbial titers have focused on larvae. The larval gut is a hostile environment for microbial growth due to high alkalinity, rapid food transit, constantly replacing peritrophic matrix, and an array of host-encoded antimicrobial peptides. Nonetheless, the gut harbors abundant bacteria (from 10^7 to 10^{13} colony-forming units per larva), as determined by traditional culturing techniques and direct analysis of gut tissues (5, 13, 106). A separate study determined frass concentrations to be low titer [approximately 10^4 16S ribosomal RNA (rRNA) copies per gram] and predominantly diet derived (42). More recently, it has been revealed that bacterial abundance in adults can be relatively high. Total bacterial load in adult guts ranged from 5×10^5 to 1×10^{11} 16S rRNA copies per butterfly, with a median of 7.5×10^8 (94).

Some studies suggest that larval gut microbes are generally transient, and continuous ingestion of diet is needed to maintain their presence (42). Low-abundance transient microbes simply pass

Figure 1

(a,b) Taxonomic diversity of (a) endosymbionts and (b) the gut bacterial microbiome in Lepidoptera. Stacked bars in panel b represent changes in the relative abundances of bacterial taxa. (c) Scanning electron microscope image of the luminal surface of the hindgut of *Hyles euphorbiae* larvae shows a dense biofilm of cocci. Image courtesy of Cristina Vilanova. (d) Fluorescence in situ hybridization with an *Enterococcus*-specific probe (green) shows a high density of bacterial cells adhering to the mucus layer lining the gut epithelium of *Spodoptera littoralis* larvae. Image courtesy of Yongqi Shao.



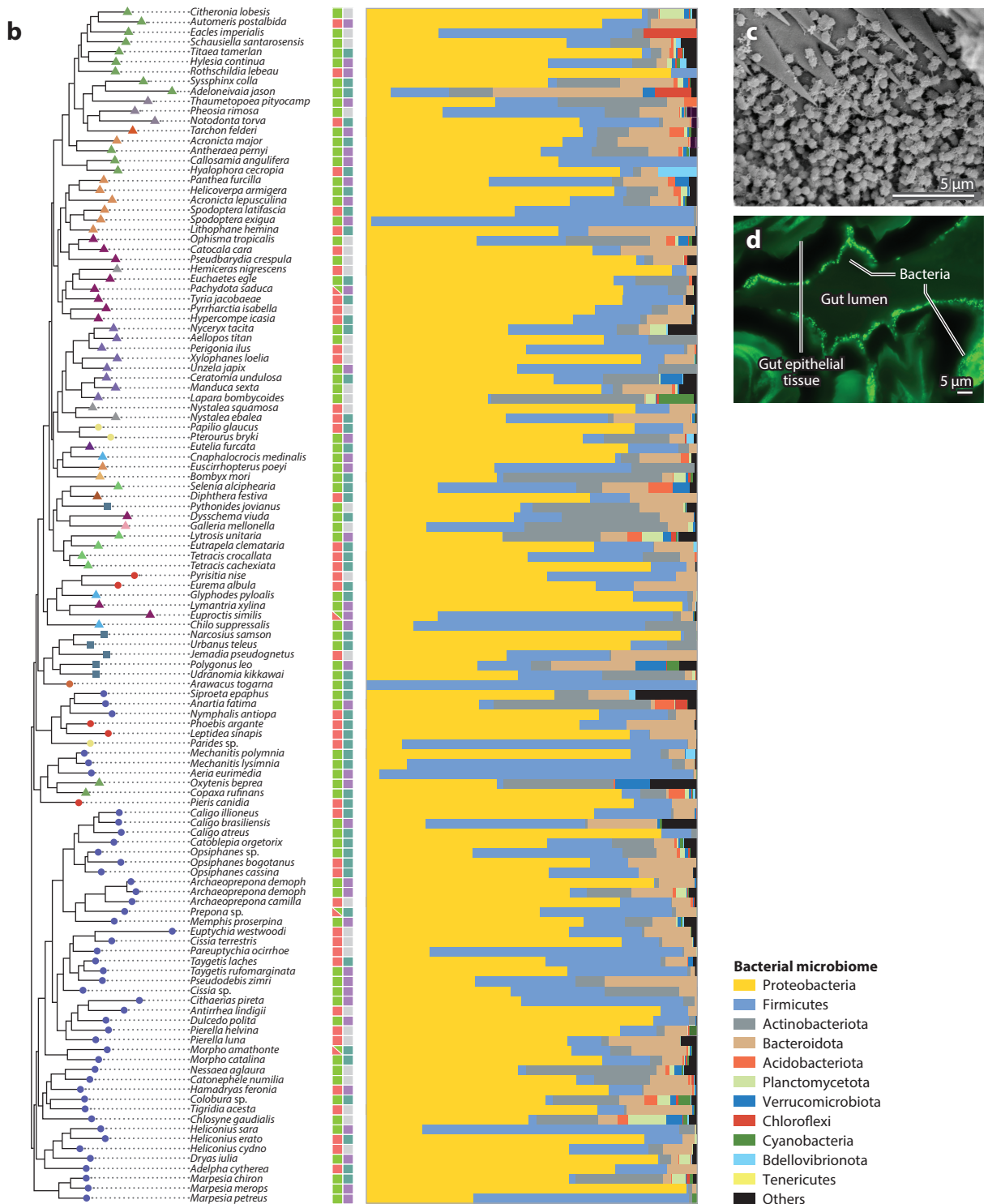


Figure 1

(Continued)

through the gut, fail to establish residence, and excrete in frass. There are conflicting studies that suggest that several microbes have residence across larval development (104, 117). Furthermore, manipulative experiments using sterile diets and controlled microbial introductions demonstrated that certain bacteria colonize and proliferate in the gut (16, 73). Gut bacteria are likely incorporated into the peritrophic matrix along the gut surface (17, 112), as exemplified by results from microscopy detailing dense bacterial layers (**Figure 1c,d**). Initial colonization of a host and biofilm formation provide a foundation for community development and support microbial processes. Wild larvae possess variable communities that reflect local habitat, yet simultaneously, they can have highly stable microbiomes (37, 43). Finally, there is evidence that some species consistently associate with the same bacteria, independent of diet (4, 10, 35–37, 39, 47, 82, 85, 88, 93, 98). While there are undoubtedly transient associates in lepidopterans, they may comprise a small portion of the important components of the microbiome in some species. The fact that some lepidopterans have a resident gut microbiome highlights interspecific differences in microbiota residency within the same insect group.

Digestive systems are selective; thus, microbial colonization is presumably nonrandom. Obtaining microbiota from food, water, and soil may be commonplace across an array of insects. For instance, *Tyria jacobaeae* larvae acquire specific microbial communities during each generation (37). Adult Lepidoptera may acquire their characteristic gut microbiota from flowers (41). Similar results are reported for some plant-feeding insects in other orders (e.g., Heteroptera) (56), suggesting that transmission from environmental sources is compatible with a high specificity of gut microbial communities. There is also potential for the spread of microbes among individuals through social interactions (72). Communal defecating and feeding sites of gregariously feeding larvae could facilitate host-to-host transmission and swap of microbiota. Acquiring new facultative symbionts may allow for adaptive potential (45). Extracellular gut symbionts and food-associated microbes often possess dynamic genomes that can rapidly gain and lose functional genes, as is typical for most free-living bacterial taxa. Symbioses can evolve easily and rapidly inside hosts, and new symbionts could potentially bring other novel functions to their host (58).

Environmental transmission between generations may be less reliable, compromising the evolutionary stability of microbial contributions to host fitness. There are several examples of vertical transmission of residents (12, 30, 70, 82, 93, 109). For example, for green fluorescent protein-labeled *Enterococcus* in *Spodoptera littoralis*, there was consistent survival of the bacteria in the gut for all life stages across generations following ingestion (109). Microbial associations with eggs are common. Newly hatched larvae ingest these microbes while consuming the eggshell, and the microbes subsequently multiply inside the host (12). Additionally, it was demonstrated in *Galleria mellonella* that bacteria in the larval gut pass across the gut epithelium into the hemocoel and can be translocated into the eggs (30). An important observation in this study is that bacteria accumulate in the ovaries and ultimately in the chorion (and to a lesser extent the yolk) of the developing eggs.

Lepidopteran guts are composed of a mélange of transient microbes, flexibly present taxa, and resident symbionts. Ecological conditions and transmission mechanisms contribute to potential recurrent patterns regarding microbiota structure and composition. Unfortunately, the localization and transmission route of gut symbionts is not well elucidated in Lepidoptera, nor is how vertical and horizontal transmission intersect. An expanded understanding of the dynamics between residents and transients, what drives population and community changes, colonization priority effects, and the conflicts between microorganisms within hosts is necessary to resolve the discourse involving the stability of the lepidopteran gut microbiome.

2.2.1. Taxonomic diversity of gut bacteria. An array of major lepidopteran families has been investigated using modern molecular techniques (**Figure 1b**). Meta-analysis revealed that gut bacterial diversity was significantly lower in herbivorous hosts compared to omnivores (127). The majority of Lepidoptera gut bacteria belong to the Proteobacteria (56.98% of 16S rRNA gene sequences) and Firmicutes (22.15%), followed by Bacteroidota (8.59%) and Actinobacteriota (7.99%); this is similar to the gut bacteria of other insects (25, 127). The natural gut flora normally contains only a small proportion of other bacterial phyla.

Gram-negative Proteobacteria are often detected in lepidopteran microbiomes; based on the data shown in **Figure 1b**, at a class level, the Alpha- and Gammaproteobacteria represent 25.36% and 31.51% of the total sequences, respectively (**Supplemental Table 1**). Bacterial families include Enterobacteriaceae, Beijerinckiaceae, and Acetobacteraceae. At the genus level, *Methylobacterium*, *Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Klebsiella*, and *Enterobacter* were most prevalent. Notably, many common bacteria identified in the larval gut (e.g., *Methylobacterium* and *Pseudomonas* spp.) are also features of the plant phyllosphere.

Gram-positive Firmicutes are the second most prevalent bacterial phylum in Lepidoptera. The classes Bacilli and Clostridia represented 16.29% and 5.61% of the sequences, respectively (**Supplemental Table 1**). Genera including *Enterococcus*, *Staphylococcus*, *Lactococcus*, *Lactobacillus*, *Streptococcus*, and *Bacillus* are widespread across lepidopterans (21, 47, 48, 112). These taxa are less common as plant associates, suggesting host filtering and selection of symbionts.

For the Gram-negative Bacteroidota (synonym Bacteroidetes), the most significant members belong to the class Bacteroidia (**Supplemental Table 1**). Some lepidopterans also possess Actinomycetota (synonym Actinobacteria), a diverse group of Gram-positive organisms; among these, Actinomycetia (7.27%) and Thermoleophilia (0.49%) are the most dominant classes (**Supplemental Table 1**).

2.2.2. Taxonomic diversity of gut fungi. Fungi (Ascomycota and Basidiomycota) may also colonize insect guts (25). Fungi have been understudied in Lepidoptera, potentially because they are more transient than bacteria (103) but also because they are less tractable with sequence-based profiling techniques. However, some guts host a surprisingly rich diversity of fungi, especially yeasts (Saccharomycetales) (94). Some of these fungi may play an active role in the gut (99, 118), while others may simply use insects as a means of dispersal (44).

2.2.3. Toward uncovering a core microbiome. Identifying core constituents of animal microbiomes can lead to inferences related to the physiological ecology and evolution of host-microbe interactions (96). There is evidence that some lepidopteran species may harbor a core microbiome, especially bacterial core components such as *Enterobacter*, *Pseudomonas*, *Enterococcus*, or *Acinetobacter* (39). These members are generally adapted to the lepidopteran gut through enriched pathways to tolerate alkaline stress, formation of biofilms and two-component signaling systems for quorum sensing, and resistance to oxidative stress (80). They may have been increasingly recruited by the host from the environment or conspecifics over the course of evolution and have high colonization efficiency in the gut. However, these assumptions have been tested in only a few systems (109). Field-collected *T. jacobaeae* larvae possess consistent, dominant bacteria in distantly located habitats (37). A core community, consisting of *Enterococcus*, *Lactobacillus*, and *Clostridium*, was identified in *S. littoralis* and *H. armigera* larvae (108). Similarly, *Enterococcus* and *Pseudomonas* were identified as core components across different *Spodoptera frugiperda* strains and from different field populations (89). Adult lepidopterans may harbor a more specific gut consortia. Phylogenetically conserved gut microbiotas have been found in *Heliconius* butterflies (41). Notably, a core of bacteria was shared across eggs, larvae, and adults in *Plodia interpunctella*, most of which were assigned to the genus *Burkholderia*, a diverse group whose members can be mutualistic to insects

(82). Similarly, 29 genera were conserved throughout the three developmental stages of *Brithys crini*, mainly belonging to the Proteobacteria phylum (39).

Besides the phylogenetic core microbiome members, efforts to understand the functional core microbiome aim to identify sets of microbes and their genes that are important for host biological function (96). Given the importance of functional traits to understanding the mechanisms by which microbes impact host biology, the functional core measured at the gene rather than taxonomic level deserves more attention.

2.3. Factors That Influence the Gut Microbiome

Despite core microbiome signatures in some species, there is usually variation in gut microbial community composition among individuals of the same species in Lepidoptera, as in other animals (94). The larval microbiome exhibits more variation compared to that of adults, although there is overlap in the taxa present in both (40–42). Environmental and genetic factors, such as diet, habitat, phylogeny, and ontogeny, can contribute to this variation (53).

2.3.1. Diet and the environment. Lepidopterans acquire gut microbes from feeding. Given that food is not sterile, this may give rise to transient–resident conflicts. Upon hatch, larvae immediately consume their eggshell. Microbial diversity of eggs can be high in some instances (15, 44) and serve as an inoculant. As larvae consume plants, they may encounter new microbes that can supplant the initial colonizers as residents, as well as taxa that are expelled from the system. Adults consume a variety of sugary substrates, some in the form of nectar. Nectar may also be a source of microbiota, but whether these microbiota establish and overtake existing members warrants additional attention. In a broad survey, the feeding guild explained only 23% of the variation in gut flora (94), highlighting that other nondietary factors must shape the variable microbiomes of Lepidoptera.

Habitat also contributes to microbiome variation. Plants host diverse microbial communities that are impacted by changing environmental conditions, and temporal and spatial variation in microbiomes of diet plants directly affect their insect consumers (47). Additionally, some lepidopteran microbiomes are affected by the soil; for example, individual cabbage moths, *Mamestra brassicae*, acquired their microbiomes largely from the soil rather than from their host plant (43).

2.3.2. Host phylogeny. Host phylogeny plays an important role in mediating associations with microbial taxa. For example, three mulberry-feeding Lepidoptera species reared under identical conditions differed greatly in their gut microbiomes (15). Similarly, it was found that one-fourth to one-third of gut community variation in butterflies can be attributed to host species (94). Undoubtedly, physiology, behavior, and other characteristics conserved across host phylogeny drive phylogenetic signals in gut microbial membership. For vertebrates, gut physiology has been suggested as the most important driver of gut microbiome biodiversity (95), so it stands to reason that the same is true for invertebrates.

Behaviorally mediated microbiome associations are also likely to be phylogenetically conserved and may affect community composition. For instance, habitat preferences between species can expose hosts to different microbial pools—species that favor the forest understory could be exposed to more soil and wood decay fungi than those that prefer the canopy (94). Similarly, the diurnal behavior of some lepidopterans in terms of feeding and movement between resources can result in accessing different microbial pools (128).

2.3.3. Developmental changes. Comparisons of microbiota spanning lepidopteran ontogeny are relatively scarce. The larval gut microbial communities often shift among instars (18). Bacterial

diversity tends to change over larval development. Field-based surveys often involve older-instar larvae, which may bias interpretations of functions and importance of taxa.

Transitions between the larval and adult stages can involve loss and replacement of gut microbiota. At the close of the final instar, larvae clear gut contents for pupation. Metamorphosis offers the opportunity to alter gut microbiota and facilitate ecological niche shifts. After pupal emergence, adults may acquire novel microorganisms. However, microbiomes of larvae and adults are not always fully decoupled; transmission of a certain set of microbes during pupation has also been reported (57).

2.3.4. Captivity- and rearing-induced changes. Like other insects, insectary-maintained lepidopterans harbor gut microbiota that diverge from field-collected insects. Microbial communities in laboratory-reared insects are generally very simple and dominated by a few species, in contrast to communities of field populations, which have greater diversity (36). While overlap among some bacterial taxa exists, the simplicity in these communities can lead to challenges in understanding their ecological and physiological functions in the hosts.

Collectively, microbiome surveys in Lepidoptera suggest a complex array of factors shaping the gut microbiota. Gut microbiota exhibit interindividual instability at the species level but conservation at the phylum level. Further research is needed to understand how different traits such as dietary specialization, habitat, and the potential environmental microbial pool govern the origins of holometabolous insects' gut flora. Beyond microbial diversity analysis, discrimination between transient and persistent microbes and their levels in the gut is needed (69). As among the most diverse insect orders, such plasticity in gut microbiomes could be beneficial if the ability to harbor a dynamic microbiome increases opportunities for environmental adaptation or if the microbiota provide redundant functional benefits to the host. These hypotheses require further examination.

3. IMPACTS OF MICROBES ON THEIR LEPIDOPTERAN HOST

Despite the recent interest in lepidopteran microbiomes, broad aspects of functionality remain a mystery. Historically, endosymbionts were viewed primarily as reproductive manipulators (for a detailed review, see 27), but some beneficial phenotypic effects have also been described, and their interactions with the insect host can evolve rapidly. For example, Weeks et al. (115) demonstrated that *Wolbachia* in natural populations of *Drosophila* changed from being parasitic to being more mutualistic within two decades. For an inherited endosymbiont, the trade-off between virulence and transmission can lead to a reduction in its pathogenicity toward the host and evolution toward mutualism.

The gut microbiome is often considered an integral component of the host phenotype— influencing important biological traits from nutrition to immunity to behavior—but has barely been studied in Lepidoptera. There is an indication that functional relationships exist between larvae and bacteria (101, 117). Metamorphosis may allow different functional microbiomes to exist in adult developmental stages (94). Many ingested microbes are likely commensal, providing no particular benefit for the host under optimal conditions; however, this does not necessarily mean that these microbes have no impact on their hosts, for instance, by contributing to host immunity (126). Moreover, beneficial effects may also occur independently of any gut colonization; even bacterial lysates improve host immunometabolic homeostasis (51). In this section, we summarize some of the identified and predicted functions of lepidopteran microbial symbionts.

3.1. Biological Significance of Endosymbionts

Fitness benefits provided by endosymbionts include increasing host survival or fecundity, protecting the host against natural enemies, and even promoting speciation. The best-characterized

mutualistic association is the *Wolbachia*-mediated green-island phenotype in the leaf-mining moth *Phyllonorycter blancardella*: *Wolbachia* indirectly affects larval nutrition by manipulating the host plant hormones to create photosynthetically active green patches in otherwise senescent yellow leaves (54). This phenotype thus allows larval development in a nutritionally constrained stage of the life cycle. A *Wolbachia* strain (wHm-c) associated with *H. magnanima* also shortened larval development time and increased pupal weight, which was beneficial for host survival and reproduction (6). Similarly, *Wolbachia* strain wGri from *Ectropis grisea* could enhance the fecundity of its host (131).

Wolbachia from *Parnassius apollo* protected its host from deleterious factors affecting wing development (68). *Wolbachia* also reduced the susceptibility of *Chilo suppressalis* to two insecticides (61). Interestingly, a viral symbiont (HaDNV-1) was demonstrated to protect *H. armigera* against pathogenic baculovirus and *Bacillus thuringiensis* (Bt) biopesticides (123). Through pre- or postmating isolation mechanisms, endosymbionts are also proposed to play a role in speciation processes, such as within the *Bicyclus* genus (26). The horizontal gene transfer from an endosymbiont to a Lepidoptera species is rarely identified. To date, there is only one report of this process, which found a 350-bp-long *Wolbachia* gene insert in the genome of the butterfly *Melitaea cinxia* (3); its functionality is unknown.

The intriguing discoveries of positive host fitness effects enlarge our knowledge of endosymbionts, which could function as both reproductive manipulators and mutualists. However, in contrast to the well-studied reproductive manipulations (27), the extent of beneficial effects and underlying mechanisms are still to be determined in Lepidoptera.

3.2. Putative Beneficial Relationships Between Lepidoptera and Their Gut Microorganisms

The gut microbiome has the potential to substantially impact lepidopterans, particularly in terms of host nutrition and protection (Figure 2). Extracellular gut symbionts may augment hosts' enzymatic repertoires by secreting products into the gut lumen to break down complex compounds or detoxify toxins. Symbionts from the phyla Proteobacteria and Firmicutes possess various plant cell wall degrading enzymes, including cellulases, hemicellulases, and pectinases (Supplemental Table 2), which potentially aid in digesting plant material. Moreover, gut bacteria in *Anticarsia gemmatilis* synthesize trypsin, which contribute significantly to protein digestion (92). The dominant fungus *Aureobasidium* of the soil-borne *Thitarodes* larvae also produces useful enzymes (e.g., amylases, lipases, and proteases) (65). Interestingly, adult gut microbiomes specialize in digestion of compounds abundant in the host diets (94). Gut microbiota can benefit their hosts by providing valuable nutrients; for example, *Plutella xylostella* gut bacteria synthesize histidine and threonine, which are essential for the host (121). *Botrytis cinerea* is a widespread plant pathogen but also a mutualist of some Tortricidae, affecting their life cycle by synthesizing sterols (97). Furthermore, the gut microbiome contributes to faster host development and higher survival rate and body weight in *Pieris canidia* (114). Participation in host metabolism appears to be a general function of the gut microbiome.

Gut microbiota also detoxify plant toxins and xenobiotics. *Acinetobacter* can metabolize multiple classes of phenolics in culture (75) and has been linked to improvements in *Lymantria dispar* growth in response to dietary phenolics (74). Similarly, gut bacteria of specialist lepidopterans can tolerate and metabolize toxic alkaloids and latex in their host plants (112). Recently, *Enterococcus* (16) and *Enterobacter* (77) isolates were shown to enable *S. frugiperda* usage of an intractable diet, enhancing larval performance and survival. Evidence from molecular-based analyses indicates the presence of detoxification-related genes in the gut metagenomes of *Spodoptera litura*, *S. frugiperda*,

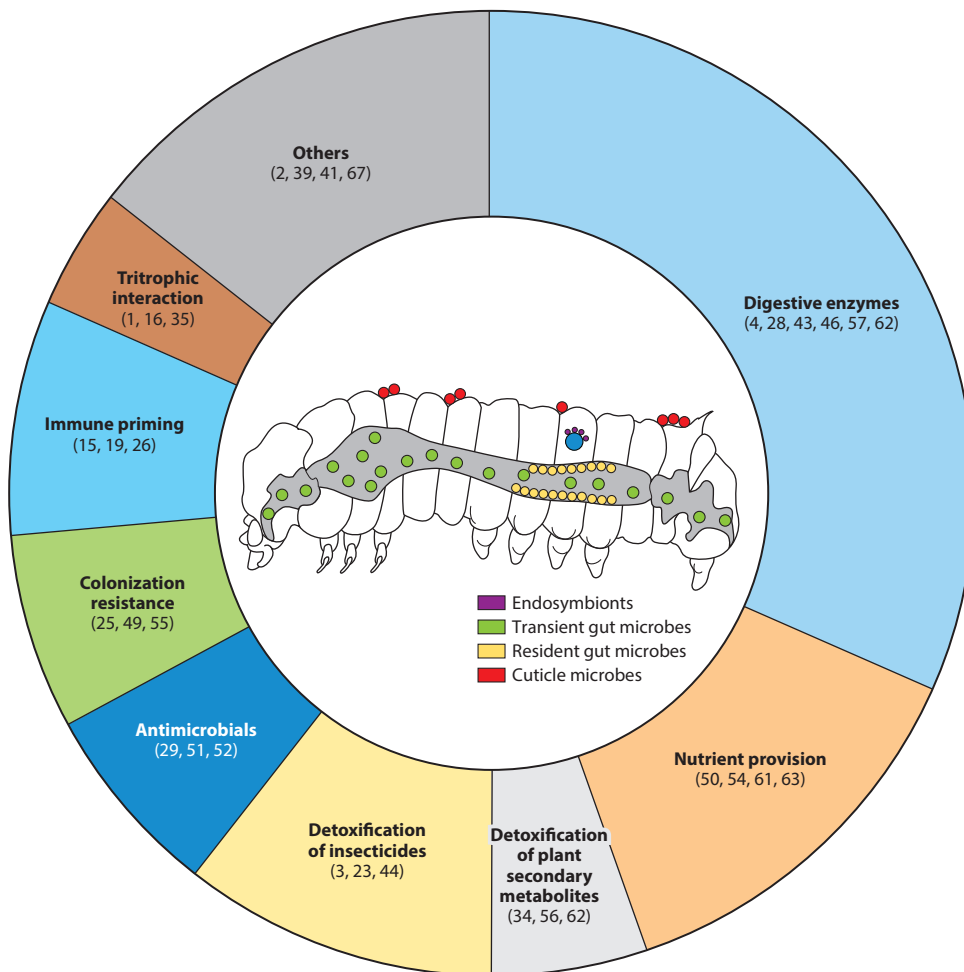


Figure 2

Functional diversity of the Lepidoptera microbiome. The inset indicates two coexisting symbiotic systems, consisting of both endosymbionts and ectosymbionts including gut microbes (transient and resident) and cuticle microbes. Numbers indicate key references in **Supplemental Table 2**.

Supplemental Material >

and *P. xylostella* (107, 121). *Enterococcus* isolates from *S. littoralis* also possess catalase and superoxide dismutase, which can mitigate oxidative stress caused by gut bacteria (80). Moreover, enrichment of *Enterococcus* bacteria enhances insecticide (methomyl) metabolism, leading to increased insecticide tolerance (48). Thus, gut microbiota of insecticide-resistant lines are a rich resource for the isolation of microbes capable of degrading insecticides (**Supplemental Table 2**).

Several studies highlight the importance of the gut microbiome in immunity and protection against entomopathogens. Shao et al. (101) demonstrated that *Enterococcus mundtii* of *S. littoralis* selectively clears pathobionts from the host gut lumen by secreting a stable antimicrobial peptide, which facilitates the normal development of gut microbiota and reduces risks of infection via the gut. Notably, multiple Lepidoptera species have intimate interactions with *Enterococcus*, a diverse group that is widespread across different environments and whose members can be mutualistic or pathogenic (52, 79). Microbes present on the cuticle may also serve as protective guards. A recent

study explored the transient microbe population on *Citrus* butterfly wings and found that many wing isolates display antibacterial, antifungal, and biosurfactant properties; interestingly, microbes inhabit these niches before the butterfly has contact with the environment (55). The antimicrobial screening of insect symbionts has led to the discovery of a diverse array of new active biomolecules, mainly peptides and polyketides (111).

Another protective effect is conferred by microbiota outcompeting pathogens, known as colonization resistance (24). Aseptically reared *H. magnanima* larvae supported 20 times greater growth of *B. thuringiensis* than larvae with a gut microbiota, which suggests that gut bacteria suppress the pathogen growth (106). Yeast (*Metschnikowia* spp.) promotes codling moth, *Cydia pomonella*, survival by reducing the incidence of fungal infestations in the diet (118). Lactic acid bacteria acidify the gut environment and potentially reduce the alkaline activation of Bt toxins (21). Colonizing gut microbes may act as a protective barrier, reducing opportunities for pathogen colonization. Importantly, colonization resistance can readily evolve even in symbioses lacking strong host-microbe specificity; for example, gut bacteria widely shared among *Heliconius* and other heliconiine butterflies may interact primarily with parasites (41).

Gut microbiomes can boost host immunity by immune priming. Egg surface sterilization blocked the vertical transmission of symbionts in silkworm, *Bombyx mori*, which further resulted in the perturbation of the host immune response (62). Increases in midgut microbiota load induced immune priming in *Spodoptera exigua* and increased tolerance to *B. thuringiensis* (46). Symbionts do not necessarily even need to be physically present to affect host life history traits. For instance, early microbe-mediated immune priming in larvae influenced colonization of beneficial symbionts in later adult life stages (52), demonstrating carryover effects shown in other insect systems (e.g., *Aedes aegypti*) (23). Priming *G. mellonella* larvae with heat-killed bacteria enhanced immune protection against pathogenic *Photobacterium luminescens*; the immune priming phenomenon of *G. mellonella* has low specificity, which was achieved mainly by regulating major innate immune effectors such as hemocytes, antimicrobial peptides, and enzymes (lysozyme and phenoloxidase) (120). Overall, transient gut microbes have received little attention; however, recent studies indicate that they play a more important role in Lepidoptera than was previously assumed (81, 126).

Notably, not all organisms rely on gut symbionts for specialized functional purposes or well-being under certain conditions. To address how gut bacteria may be useful to the model species *Manduca sexta*, these microbes were suppressed by antibiotic treatment, but the caterpillars showed no apparent negative consequences in a short-term bioassay (42). Similarly, introduction of bacteria to mid-instar, axenic *S. frugiperda* did not yield any differences in performance, even though the same bacteria introduced in early instars were crucial for robust growth and development under the same conditions (16, 76). Ontogeny and dietary context may be critical for determining microbiome-mediated effects on some lepidopteran species. Later-stage larvae are typically more resilient to toxins, pathogens, and low-quality diets, so they may derive less assistance from gut symbionts.

3.3. Multitrophic Interactions Mediated by Lepidopteran Microbes

Recent evidence is revealing that the Lepidoptera gut microbiome can alter the magnitude of plant defenses in various ways (83). As larvae consume plants, they mechanically disrupt and deposit various elicitors from their oral secretions (OSs), which the plant can perceive to initiate defense responses. In some instances, gut microbiota may confound the plant perception of the herbivore to attenuate responses. In *S. frugiperda*, reducing microbial populations from field-collected larvae by antibiotic feeding increased plant responses to caterpillar feeding and OSs (1). The bacteria isolated from the OSs of field-collected larvae, such as *Pantoea*, *Rabnella*, and

Enterobacter, suppressed peroxidase and protease inhibitor activities in tomato (1). Similar patterns were observed for *S. litura* interactions with *Arabidopsis* (124). OSs from axenically reared larvae initiated higher antiherbivore defense responses than those from larvae reared conventionally in the laboratory; the addition of *Staphylococcus epidermidis* to the OSs of axenic larvae recapitulated the suppressive ability of *S. litura* OSs (124). An important consideration for microbial mediation of plant defenses is that the suppression of responses is contextual and may depend on the plant species (and presumably genotype) involved in the interaction (1).

Parasitoids are major natural enemies of lepidopteran larvae, and parasitism has been shown to abruptly shift the lepidopteran gut microbiome (20, 113, 130). While this phenomenon is interesting, we currently cannot determine if microbial changes facilitate or attenuate parasitism. Notably, the endosymbionts *Wolbachia* and *Spiroplasma* can improve host defense against parasitoids in *Drosophila* (31, 122), and other symbionts confer similar advantages for aphids (90). An expanded understanding of multitrophic interactions mediated by lepidopteran microbes is needed.

4. APPLICATION POTENTIAL OF LEPIDOPTERA-ASSOCIATED MICROBES

Lepidoptera-associated microbes represent an important source of novel chemical compounds and enzymes with potential biotechnological applications in medicine, industry, bioremediation, and agriculture. The silkworm gut bacterium *Streptomyces* produces small-molecule bombyxamycins, which have significant antibacterial and antiproliferative effects against several human pathogens (102). Similarly, the peptide mundticin, identified as an antibiotic modulating the gut microbiota, was discovered from a *S. littoralis* gut symbiont. The core gut fungus *Phoma* isolated from *Thitarodes* moths also can produce antibiotics and economically useful secondary metabolites (65).

Lepidoptera is a well-studied insect group in relation to the reported enzyme-producing microbial partners (9). A wide range of commercially significant enzymes, such as amylase, cellulase, protease, and lipase, could be harvested from this microbial source. *Bacillus* and *Enterococcus* spp. commonly produce enzyme cocktails useful in the starch processing, textile, paper, and baking industries (34, 64). Lipase, another important digestive enzyme with applications in bioremediation, is produced by *Pseudomonas* spp. (32). Notably, numerous plastic-degrading microbes have been isolated from lepidopterans (125, 129). A recent study isolated a *Klebsiella* strain from *S. frugiperda* that secretes the key enzymes methylhydroxylase and alkyl hydroperoxide reductase, which degrade polyvinyl chloride—the world's third-most widely produced synthetic polymer of plastic (132).

Gut microbiomes contain plant-growth-promoting bacteria (PGPB) that could have enormous agricultural value. Several gut bacteria isolated from the *P. xylostella* larval gut showed PGPB features, including the ability to fix nitrogen and produce salicylic and indole-3-acetic acids (49, 50). *Enterobacter ludwigii* isolated from the *Helicoverpa zea* digestive tract, when applied to tomato seeds, promoted plant growth and yield without compromising antiherbivore defenses (91). These studies demonstrate the translational potential of innovative microbiome science.

5. CONCLUSIONS AND OUTLOOK

Recently, the lepidopteran microbiome has received an impressive amount of attention. Much of the emphasis has been on unveiling the patterns of association and general ecology. Interestingly, while host-associated microbiomes are not necessarily simple and static communities, similar microbial taxa populate within Lepidoptera. Our understanding of the impacts of microbes on their lepidopteran hosts is still in its infancy; fundamental information is lacking for many associations, such as the localization of putative microbial symbionts in the host and their potential metabolic activities in vivo. Studies on discernible functions are often complicated by dynamic communities

and the occurrence of transients in the community. Moreover, the outcomes of microbial interactions with hosts are likely context dependent; for instance, a benefit from honeybee larval gut microbes would likely be measurable only when (opportunistic) pathogens are encountered (59). This scenario could be the case for lepidopterans as well. It is also not surprising that antibiotics, applied to healthy animals, have no adverse effects on the host under optimal conditions (110). To progress the field from description to explanation of function and, eventually, application, further exploration using new strategies and tools is needed to characterize microbe–host relations.

First, we propose employing promising model organisms to demonstrate symbiotic associations. *Drosophila melanogaster* has long been used as a model system to study host interactions with gut bacteria; such studies uncovered the first true resident gut symbiont in *Drosophila* (69). The inconstant gut microbiota of *Drosophila* species has not held the fruit fly back from contributing to host–microbiota interaction research (119). Similarly, studies on budding Lepidoptera model organisms, for example, the silkworm, *B. mori*; fall armyworm, *S. frugiperda*; and tobacco hornworm, *M. sexta*, have increased our fundamental understanding of symbioses. Due to their fully sequenced genomes, elaborate molecular genetic tools, and well-understood biology, it is possible to obtain detailed insights into symbiont-related aspects of their physiology and behavior. Second, germ-free animals have proved powerful in elucidating the causal relationships between hosts and their microbial residents. However, there is little research regarding the exploitation of microbiome-free lepidopterans. In one example, Chen et al. (19) provided convincing evidence of the metabolic functions of the core silkworm microbiome. This simple but powerful tool will be important for assessing the relative roles of symbionts in Lepidoptera. Third, many surveys employing DNA-based polymerase chain reaction approaches are limited. Identification of biologically active taxa, such as by using transcriptomics, is necessary to confirm their functional significance for the hosts. Thus, incorporating high-throughput sequencing of DNA, RNA, or proteins provides insight into not only which microbial partners are present, but also what they are doing. Fourth, it is now increasingly recognized that strain-level variation may be an important contributor to host fitness. Even bacteria with identical 16S rRNA sequences may differ greatly in function. For instance, the honeybee gut microbiota consists of a handful of core bacteria, which themselves consist of multiple, functionally diverse strains (28). Since strain-level diversity is likely correlated with functional diversity, culturing interesting symbionts using efficient culturomics may lead to important discoveries (60). Finally, emphasizing multispecies interactions and environmental variation is critical for determining the functions of potentially facultative symbionts.

The most recent authoritative summary of lepidopteran diversity recognizes 157,424 extant species in 43 superfamilies and 133 families (84). Deciphering the true nature of microbial associations is an important direction for the field. Future investigations that adopt these complementary approaches and principles would facilitate hypothesis testing and a mechanistic understanding of host–microbe relationships across this complex, abundant insect group.

FUTURE ISSUES

1. The incidence of endosymbionts varies in lepidopterans, which are particularly common hosts for *Wolbachia*; *Spiroplasma* infections are also common, but infections of *Rickettsia*, *Cardinium*, and others are not. Why host species vary in the frequency of their interaction with inherited endosymbionts remains one of the most challenging questions.
2. Whole-genome sequencing of lepidopteran specimens has inadvertently sequenced genomes of endosymbionts; however, we know little about the functions of these

associates in nature. Using genomic resources to discover and develop models would illuminate whether these associates exhibit reproductive pathologies and/or protect the host from potential attackers.

3. How do hosts and microbes maintain commensal relationships? While the answer to the question of whether lepidopterans require a microbiome is unclear, the fact remains that these relationships exist and must be accounted for. Currently, we do not have a grasp on what initiates and promotes colonization of microorganisms and the adaptations that microorganisms possess to tolerate the digestive tract of lepidopterans. Development of manipulative axenic models and systematic colonization of guts using gnotobiotic communities and varying isolates would better reveal the mechanisms of both associates in stabilizing the relationship. Greater access to high-quality host genomes and affordable sequence-based approaches will only facilitate answering these questions.
4. Are symbiont strains interchangeable? Recently, we observed that beneficial functions of gut bacteria were not only strain specific, but also host specific. Considering that similar taxa are often featured in the gut of lepidopteran larvae, this result begs the question of whether all gut bacteria colonize equally and confer like functions. Reciprocal transplants of isolates into different axenic hosts would help address concepts of phylosymbiosis that are lacking in these systems.
5. Are pathobionts a broader feature of Lepidoptera, and how are they constrained? Pathobionts are microorganisms that function commensally in healthy hosts but can cause illness or pathologies when the host is stressed. Many of the taxa present in the guts of field-collected lepidopteran larvae can also be virulent, so how these microorganisms overtake hosts and under what conditions they do so are important questions to understand for potential pest management applications.
6. Do microbial interactions in early life stages of hosts translate to prolonged effects? Microbial acquisitions early in development can have long-lasting outcomes. Understanding how larval microbiome functions change over time is important for determining ultimate functions. Additionally, larval microbiomes have received a greater amount of attention compared to adult microbiomes. Greater understanding of how larval microbiomes affect adult fitness and foraging behaviors is needed.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review. The findings and conclusions in this publication are those of the authors and should not be construed to represent any official US Department of Agriculture (USDA) or US Government determination or policy. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The USDA is an equal opportunity provider and employer.

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LITERATURE CITED

1. Highlighted the importance of Lepidoptera-associated microbes in mediating insect-plant interactions and the different ways in which microbes contribute to these interactions.

18. First systematic survey of metabolically active gut microbes across the full moth life cycle.

19. Established a germ-free rearing protocol to avoid the influence of natural gut microbiota and antibiotics.

1. Acevedo FE, Peiffer M, Tan C-W, Stanley B, Stanley A, et al. 2017. Fall armyworm-associated gut bacteria modulate plant defense responses. *Mol. Plant-Microbe Interact.* 30:127–37
2. Ahmed MZ, Araujo-Jnr EV, Welch JJ, Kawahara AY. 2015. *Wolbachia* in butterflies and moths: geographic structure in infection frequency. *Front. Zool.* 12:16
3. Ahmed MZ, Breinholt JW, Kawahara AY. 2016. Evidence for common horizontal transmission of *Wolbachia* among butterflies and moths. *BMC Evol. Biol.* 16:118
4. Allonsius CN, Van Beeck W, De Boeck I, Wittouck S, Lebeer S. 2019. The microbiome of the invertebrate model host *Galleria mellonella* is dominated by *Enterococcus*. *Anim. Microbiome* 1:7
5. Anand AA, Vennison SJ, Sankar SG, Prabhu DI, Vasan PT, et al. 2010. Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. *J. Insect Sci.* 10:107
6. Arai H, Hirano T, Akizuki N, Abe A, Nakai M, et al. 2019. Multiple infection and reproductive manipulations of *Wolbachia* in *Homona magnanima* (Lepidoptera: Tortricidae). *Microb. Ecol.* 77:257–66
7. Araujo FS, Barcelos RM, Mendes TAO, Mafra C. 2021. Molecular evidence of *Rickettsia felis* in *Phereocaea* sp. *Rev. Bras. Parasitol. Vet.* 30:e015620
8. Arthurs S, Dara SK. 2019. Microbial biopesticides for invertebrate pests and their markets in the United States. *J. Invertebr. Pathol.* 165:13–21
9. Banerjee S, Maiti TK, Roy RN. 2022. Enzyme producing insect gut microbes: an unexplored biotechnological aspect. *Crit. Rev. Biotechnol.* 42:384–402
10. Berman TS, Laviad-Shitrit S, Lalar M, Halpern M, Inbar M. 2018. Cascading effects on bacterial communities: Cattle grazing causes a shift in the microbiome of a herbivorous caterpillar. *ISME J.* 12:1952–63
11. Bolanos LM, Servin-Garciduenas LE, Martinez-Romero E. 2015. Arthropod-*Spiroplasma* relationship in the genomic era. *FEMS Microbiol. Ecol.* 91:1–8
12. Brinkmann N, Martens R, Tebbe CC. 2008. Origin and diversity of metabolically active gut bacteria from laboratory-bred larvae of *Manduca sexta* (Sphingidae, Lepidoptera, Insecta). *Appl. Environ. Microbiol.* 74:7189–96
13. Broderick NA, Raffa KF, Goodman RM, Handelsman J. 2004. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. *Appl. Environ. Microbiol.* 70:293–300
14. Bulla LA. 1975. Bacteria as insect pathogens. *Annu. Rev. Microbiol.* 29:163–90
15. Chen B, Du K, Sun C, Vimalanathan A, Liang X, et al. 2018. Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. *ISME J.* 12:2252–62
16. Chen B, Mason CJ, Peiffer M, Zhang D, Shao Y, Felton GW. 2022. Enterococcal symbionts of caterpillars facilitate the utilization of a suboptimal diet. *J. Insect Physiol.* 138:104369
17. Chen B, Sun C, Liang X, Lu X, Gao Q, et al. 2016. Draft genome sequence of *Enterococcus mundtii* SL 16, an indigenous gut bacterium of the polyphagous pest *Spodoptera littoralis*. *Front. Microbiol.* 7:1676
18. Chen B, Teh BS, Sun C, Hu S, Lu X, et al. 2016. Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Sci. Rep.* 6:29505
19. Chen B, Zhang N, Xie S, Zhang X, He J, et al. 2020. Gut bacteria of the silkworm *Bombyx mori* facilitate host resistance against the toxic effects of organophosphate insecticides. *Environ. Int.* 143:105886
20. de Oliveira N, Consoli FL. 2020. Beyond host regulation: Changes in gut microbiome of permissive and non-permissive hosts following parasitization by the wasp *Cotesia flavipes*. *FEMS Microbiol. Ecol.* 96:fiz206
21. Deguenon JM, Dhammi A, Ponnusamy L, Travanty NV, Cave G, et al. 2021. Bacterial microbiota of field-collected *Helicoverpa zea* (Lepidoptera: Noctuidae) from transgenic Bt and non-Bt cotton. *Microorganisms* 9:878

22. Dicke M, Cusumano A, Poelman EH. 2020. Microbial symbionts of parasitoids. *Annu. Rev. Entomol.* 65:171–90
23. Dickson LB, Jiolle D, Minard G, Moltini-Conclois I, Volant S, et al. 2017. Carryover effects of larval exposure to different environmental bacteria drive adult trait variation in a mosquito vector. *Sci. Adv.* 3:e1700585
24. Dillon RJ, Dillon VM. 2004. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 49:71–92
25. Douglas AE. 2015. Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol.* 60:17–34
26. Duploux A, Brattstrom O. 2018. *Wolbachia* in the genus *Bicyclus*: a forgotten player. *Microb. Ecol.* 75:255–63
27. Duploux A, Hornett EA. 2018. Uncovering the hidden players in Lepidoptera biology: the heritable microbial endosymbionts. *PeerJ* 6:e4629
28. Ellegaard KM, Suenami S, Miyazaki R, Engel P. 2020. Vast differences in strain-level diversity in the gut microbiota of two closely related honey bee species. *Curr. Biol.* 30:2520–31.e7
29. Engel P, Moran NA. 2013. The gut microbiota of insects—diversity in structure and function. *FEMS Microbiol. Rev.* 37:699–735
30. Freitak D, Schmidtberg H, Dickel F, Lochnit G, Vogel H, Vilcinskas A. 2014. The maternal transfer of bacteria can mediate trans-generational immune priming in insects. *Virulence* 5:547–54
31. Fytrou A, Schofield PG, Kraaijeveld AR, Hubbard SF. 2006. *Wolbachia* infection suppresses both host defence and parasitoid counter-defence. *Proc. R. Soc. B* 273:791–96
32. Gandotra S, Kumar A, Naga K, Bhuyan PM, Gogoi DK, et al. 2018. Bacterial community structure and diversity in the gut of the muga silkworm, *Antheraea assamensis* (Lepidoptera: Saturniidae), from India. *Insect Mol. Biol.* 27:603–19
33. Ghanavi HR, Twort VG, Duploux A. 2021. Exploring bycatch diversity of organisms in whole genome sequencing of Erebidae moths (Lepidoptera). *Sci. Rep.* 11:24499
34. Gharsallah H, Ksentini I, Abdelhedi N, Naayma S, Hadj Taieb K, et al. 2019. Screening of bacterial isolates related to olive orchard pests in Tunisia using 16S ribosomal RNA and evaluation of their biotechnological potential. *J. Appl. Microbiol.* 126:489–502
35. Gohl P, LeMoine CMR, Cassone BJ. 2022. Diet and ontogeny drastically alter the larval microbiome of the invertebrate model *Galleria mellonella*. *Can. J. Microbiol.* 68:594–604
36. Gomes AFF, Omoto C, Consoli FL. 2020. Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. *J. Pest Sci.* 93:833–51
37. Gomes SIF, Kielak AM, Hannula SE, Heinen R, Jongen R, et al. 2020. Microbiomes of a specialist caterpillar are consistent across different habitats but also resemble the local soil microbial communities. *Anim. Microbiome* 2:37
38. Gomes T, Wallau GL, Loreto ELS. 2022. Multiple long-range host shifts of major *Wolbachia* supergroups infecting arthropods. *Sci. Rep.* 12:8131
39. Gonzalez-Serrano F, Perez-Cobas AE, Rosas T, Baixeras J, Latorre A, Moya A. 2020. The gut microbiota composition of the moth *Brithys crini* reflects insect metamorphosis. *Microb. Ecol.* 79:960–70
40. Hammer TJ, Bowers MD. 2015. Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecologia* 179:1–14
41. Hammer TJ, Dickerson JC, McMillan WO, Fierer N. 2020. Heliconius butterflies host characteristic and phylogenetically structured adult-stage microbiomes. *Appl. Environ. Microbiol.* 86:e02007–20
42. Hammer TJ, Janzen DH, Hallwachs W, Jaffe SP, Fierer N. 2017. Caterpillars lack a resident gut microbiome. *PNAS* 114:9641–46
43. Hannula SE, Zhu F, Heinen R, Bezemer TM. 2019. Foliar-feeding insects acquire microbiomes from the soil rather than the host plant. *Nat. Commun.* 10:1254
44. Harrison JG, Urruty DM, Forister ML. 2016. An exploration of the fungal assemblage in each life history stage of the butterfly, *Lycaeides melissa* (Lycaenidae), as well as its host plant *Astragalus canadensis* (Fabaceae). *Fungal Ecol.* 22:10–16

42. Demonstrated that *Manduca sexta* is symbiont independent; but see also Reference 117.

43. Demonstrated that Lepidoptera microbiomes are enriched in particular beneficial microbes to an extent disproportionate to their relative presence in soils.

52. Demonstrated that symbiotic gut microbes pass through Lepidoptera metamorphosis and benefit the resulting adult host.

54. Demonstrated that Lepidoptera leaf-miners rely on *Wolbachia* to cope with nutritional constraints in senescent leaves.

63. Demonstrated that Lepidopterans acquired, on average, the highest number of horizontal gene transfer-acquired genes in insects, mostly from bacteria.

45. Hauffe HC, Barelli C. 2019. Conserve the germs: the gut microbiota and adaptive potential. *Conserv. Genet.* 20:19–27
46. Hernandez-Martinez P, Naseri B, Navarro-Cerrillo G, Escriche B, Ferre J, Herrero S. 2010. Increase in midgut microbiota load induces an apparent immune priming and increases tolerance to *Bacillus thuringiensis*. *Environ. Microbiol.* 12:2730–37
47. Higueta Palacio MF, Montoya OI, Saldamando CI, Garcia-Bonilla E, Junca H, et al. 2021. Dry and rainy seasons significantly alter the gut microbiome composition and reveal a key *Enterococcus* sp. (Lactobacillales: Enterococcaceae) core component in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn strain from northwestern Colombia. *J. Insect Sci.* 21:10
48. Hu L, Sun Z, Xu C, Wang J, Mallik AU, et al. 2022. High nitrogen in maize enriches gut microbiota conferring insecticide tolerance in lepidopteran pest *Spodoptera litura*. *iScience* 25:103726
49. Indiragandhi P, Anandham R, Madhaiyan M, Poonguzhali S, Kim G, et al. 2007. Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, *Plutella xylostella* and their potential for, antagonism towards entomopathogenic fungi and host insect nutrition. *J. Appl. Microbiol.* 103:2664–75
50. Indiragandhi P, Anandham R, Madhaiyan M, Sa T. 2008. Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). *Curr. Microbiol.* 56:327–33
51. Jensen BAH, Holm JB, Larsen IS, von Burg N, Derer S, et al. 2021. Lysates of *Methylococcus capsulatus* Bath induce a lean-like microbiota, intestinal FoxP3⁺RORγ⁺IL-17⁺ Tregs and improve metabolism. *Nat. Commun.* 12:1093
52. Johnston PR, Rolff J. 2015. Host and symbiont jointly control gut microbiota during complete metamorphosis. *PLOS Pathog.* 11:e1005246
53. Juottonen H, Moghadam NN, Murphy L, Mappes J, Galarza JA. 2022. Host's genetic background determines the outcome of reciprocal faecal transplantation on life-history traits and microbiome composition. *Anim. Microbiome* 4:67
54. Kaiser W, Huguet E, Casas J, Commin C, Giron D. 2010. Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proc. Biol. Sci.* 277:2311–19
55. Kamala Jayanthi PD, Vyas M. 2022. Exploring the transient microbe population on citrus butterfly wings. *Microbiol. Spectr.* 10:e0205521
56. Kikuchi Y, Hosokawa T, Fukatsu T. 2007. Insect-microbe mutualism without vertical transmission: A stinkbug acquires a beneficial gut symbiont from the environment every generation. *Appl. Environ. Microbiol.* 73:4308–16
57. Kingsley VV. 1972. Persistence of intestinal bacteria in the developmental stages of the Monarch butterfly (*Danaus plexippus*). *J. Invertebr. Pathol.* 20:51–58
58. Koga R, Moriyama M, Onodera-Tanifuji N, Ishii Y, Takai H, et al. 2022. Single mutation makes *Escherichia coli* an insect mutualist. *Nat. Microbiol.* 7:1141–50
59. Kowallik V, Mikheyev AS. 2021. Honey bee larval and adult microbiome life stages are effectively decoupled with vertical transmission overcoming early life perturbations. *mBio* 12:e0296621
60. Lagier JC, Dubourg G, Million M, Cadoret F, Bilen M, et al. 2018. Culturing the human microbiota and culturomics. *Nat. Rev. Microbiol.* 16:540–50
61. Lei S, Zhang F, Yun YL, Zhou WH, Peng Y. 2020. *Wolbachia* bacteria affect rice striped stem borer (*Chilo suppressalis*) susceptibility to two insecticides. *Bull. Insectol.* 73:39–44
62. Li G, Cai M, Zheng X, Xie X, Zhu Y, Long Y. 2022. Impact of disinfectants on the intestinal bacterial symbionts and immunity of silkworm (*Bombyx mori* L.). *Environ. Sci. Pollut. Res. Int.* 29:79545–54
63. Li Y, Liu Z, Liu C, Shi Z, Pang L, et al. 2022. HGT is widespread in insects and contributes to male courtship in lepidopterans. *Cell* 185:2975–87.e10
64. Liang X, Sun C, Chen B, Du K, Yu T, et al. 2018. Insect symbionts as valuable grist for the biotechnological mill: an alkaliphilic silkworm gut bacterium for efficient lactic acid production. *Appl. Microbiol. Biotechnol.* 102:4951–62
65. Liang Y, Hong Y, Mai Z, Zhu Q, Guo L. 2019. Internal and external microbial community of the *Thitarodes* moth, the host of *Ophiocordyceps sinensis*. *Microorganisms* 7:517

66. Longdon B, Day JP, Schulz N, Leftwich PT, de Jong MA, et al. 2017. Vertically transmitted rhabdoviruses are found across three insect families and have dynamic interactions with their hosts. *Proc. Biol. Sci.* 284:20162381
67. Longdon B, Jiggins FM. 2012. Vertically transmitted viral endosymbionts of insects: Do sigma viruses walk alone? *Proc. Biol. Sci.* 279:3889–98
68. Lukasiewicz K, Sanak M, Wegrzyn G. 2016. A lack of *Wolbachia*-specific DNA in samples from Apollo butterfly (*Parnassius apollo*, Lepidoptera: Papilionidae) individuals with deformed or reduced wings. *J. Appl. Genet.* 57:271–74
69. Ma D, Leulier F. 2018. The importance of being persistent: the first true resident gut symbiont in *Drosophila*. *PLOS Biol.* 16:e2006945
70. Ma Q, Cui Y, Chu X, Li G, Yang M, et al. 2021. Gut bacterial communities of *Lymantria xyliana* and their associations with host development and diet. *Microorganisms* 9:1860
71. Marin-Miret J, Gonzalez-Serrano F, Rosas T, Baixeras J, Latorre A, et al. 2022. Temporal variations shape the gut microbiome ecology of the moth *Brithys crini*. *Environ. Microbiol.* 24:3939–53
72. Martinson VG, Carpinteyro-Ponce J, Moran NA, Markow TA. 2017. A distinctive and host-restricted gut microbiota in populations of a cactophilic *Drosophila* species. *Appl. Environ. Microbiol.* 83:e01551–17
73. Mason CJ, Clair AS, Peiffer M, Gomez E, Jones AG, et al. 2020. Diet influences proliferation and stability of gut bacterial populations in herbivorous lepidopteran larvae. *PLOS ONE* 15:e0229848
74. Mason CJ, Couture JJ, Raffa KF. 2014. Plant-associated bacteria degrade defense chemicals and reduce their adverse effects on an insect defoliator. *Oecologia* 175:901–10
75. Mason CJ, Lowe-Power TM, Rubert-Nason KF, Lindroth RL, Raffa KF. 2016. Interactions between bacteria and aspen defense chemicals at the phyllosphere–herbivore interface. *J. Chem. Ecol.* 42:193–201
76. Mason CJ, Peiffer M, Chen B, Hoover K, Felton GW. 2022. Opposing growth responses of lepidopteran larvae to the establishment of gut microbiota. *Microbiol. Spectr.* 10:e0194122
77. Mason CJ, Peiffer M, Felton GW, Hoover K. 2022. Host-specific larval lepidopteran mortality to pathogenic *Serratia* mediated by poor diet. *J. Invertebr. Pathol.* 194:107818
78. Mason CJ, Ray S, Shikano I, Peiffer M, Jones AG, et al. 2019. Plant defenses interact with insect enteric bacteria by initiating a leaky gut syndrome. *PNAS* 116:15991–96
79. Mason KL, Stepien TA, Blum JE, Holt JF, Labbe NH, et al. 2011. From commensal to pathogen: translocation of *Enterococcus faecalis* from the midgut to the hemocoel of *Manduca sexta*. *mBio* 2:e00065–11
80. Mazumdar T, Teh BS, Murali A, Schmidt-Heck W, Schlenker Y, et al. 2021. Transcriptomics reveal the survival strategies of *Enterococcus mundtii* in the gut of *Spodoptera littoralis*. *J. Chem. Ecol.* 47:227–41
81. McMillan LE, Adamo SA. 2020. Friend or foe? Effects of host immune activation on the gut microbiome in the caterpillar *Manduca sexta*. *J. Exp. Biol.* 223:jeb226662
82. Mereghetti V, Chouaia B, Limonta L, Locatelli DP, Montagna M. 2019. Evidence for a conserved microbiota across the different developmental stages of *Plodia interpunctella*. *Insect Sci.* 26:466–78
83. Minard G, Kahilainen A, Biere A, Pakkanen H, Mappes J, Saastamoinen M. 2022. Complex plant quality-microbiota-population interactions modulate the response of a specialist herbivore to the defence of its host plant. *Funct. Ecol.* 36:2873–88
84. Mitter C, Davis DR, Cummings MP. 2017. Phylogeny and evolution of Lepidoptera. *Annu. Rev. Entomol.* 62:265–83
85. Montagna M, Mereghetti V, Gargari G, Guglielmetti S, Faoro F, et al. 2016. Evidence of a bacterial core in the stored products pest *Plodia interpunctella*: the influence of different diets. *Environ. Microbiol.* 18:4961–73
86. Myers JH, Cory JS. 2016. Ecology and evolution of pathogens in natural populations of Lepidoptera. *Evol. Appl.* 9:231–47
87. Nakanishi K, Hoshino M, Nakai M, Kunimi Y. 2008. Novel RNA sequences associated with late male killing in *Homona magnanima*. *Proc. Biol. Sci.* 275:1249–54
88. Noel G, Serteyn L, Sare AR, Massart S, Delvigne F, Francis F. 2022. Co-diet supplementation of low density polyethylene and honeybee wax did not influence the core gut bacteria and associated enzymes of *Galleria mellonella* larvae (Lepidoptera: Pyralidae). *Int. Microbiol.* 26:397–409
89. Oliveira NC, Rodrigues PA, C nsoli FL. 2023. Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the Western Hemisphere. *Microbial. Ecol.* 85:1552–63

90. Oliver KM, Higashi CHV. 2019. Variations on a protective theme: *Hamiltonella defensa* infections in aphids variably impact parasitoid success. *Curr. Opin. Insect Sci.* 32:1–7
91. Pan Q, Shikano I, Hoover K, Liu T-X, Felton GW. 2019. *Enterobacter ludwigii*, isolated from the gut microbiota of *Helicoverpa zea*, promotes tomato plant growth and yield without compromising anti-herbivore defenses. *Arthropod-Plant Interact.* 13:271–78
92. Pilon FM, da Rocha Silva C, Visôto LE, de Almeida Barros R, Rodrigues da Silva Júnior N, et al. 2017. Purification and characterization of trypsin produced by gut bacteria from *Anticarsia gemmatalis*. *Arch. Insect Biochem. Physiol.* 96:e21407
93. Pinto-Tomas AA, Sittenfeld A, Uribe-Lorio L, Chavarria F, Mora M, et al. 2011. Comparison of midgut bacterial diversity in tropical caterpillars (Lepidoptera: Saturniidae) fed on different diets. *Environ. Entomol.* 40:1111–22
94. **Ravenscraft A, Berry M, Hammer T, Peay K, Boggs C. 2019. Structure and function of the bacterial and fungal gut microbiota of Neotropical butterflies. *Ecol. Monogr.* 89:e01346**
95. Reese AT, Dunn RR. 2018. Drivers of microbiome biodiversity: a review of general rules, feces, and ignorance. *mBio* 9:e01294-18
96. Risely A. 2020. Applying the core microbiome to understand host-microbe systems. *J. Anim. Ecol.* 89:1549–58
97. Rizvi SZ, Raman A. 2015. *Epiphyas postvittana* (Lepidoptera: Tortricidae)-*Botrytis cinerea* (Helotiales: Sclerotiniaceae)-*Vitis vinifera* (Vitales: Vitaceae) interaction: the role of *B. cinerea* on the development of *E. postvittana* in synthetic nutritional media. *J. Econ. Entomol.* 108:1646–54
98. Robinson CJ, Schloss P, Ramos Y, Raffa K, Handelsman J. 2010. Robustness of the bacterial community in the cabbage white butterfly larval midgut. *Microb. Ecol.* 59:199–211
99. Rosa E, Minard G, Lindholm J, Saastamoinen M. 2019. Moderate plant water stress improves larval development, and impacts immunity and gut microbiota of a specialist herbivore. *PLOS ONE* 14:e0204292
100. Salem H, Kaltenpoth M. 2022. Beetle-bacterial symbioses: endless forms most functional. *Annu. Rev. Entomol.* 67:201–19
101. Shao Y, Chen B, Sun C, Ishida K, Hertweck C, Boland W. 2017. Symbiont-derived antimicrobials contribute to the control of the Lepidopteran gut microbiota. *Cell Chem. Biol.* 24:66–75
102. Shin YH, Beom JY, Chung B, Shin Y, Byun WS, et al. 2019. Bombyxamycins A and B, cytotoxic macrocyclic lactams from an intestinal bacterium of the silkworm *Bombyx mori*. *Org. Lett.* 21:1804–8
103. Sigut M, Pyszek P, Sigutova H, Visnovska D, Kostovcik M, et al. 2022. Fungi are more transient than bacteria in caterpillar gut microbiomes. *Sci. Rep.* 12:15552
104. Sigutova H, Sigut M, Pyszek P, Kostovcik M, Kolarik M, Drozd P. 2023. Seasonal shifts in bacterial and fungal microbiomes of leaves and associated leaf-mining larvae reveal persistence of core taxa regardless of diet. *Microbiol. Spectr.* 11:e0316022
105. Suh SO, Noda H, Blackwell M. 2001. Insect symbiosis: derivation of yeast-like endosymbionts within an entomopathogenic filamentous lineage. *Mol. Biol. Evol.* 18:995–1000
106. Takatsuka J, Kunimi Y. 2000. Intestinal bacteria affect growth of *Bacillus thuringiensis* in larvae of the oriental tea tortrix, *Homona magnanima diakonoff* (Lepidoptera: tortricidae). *J. Invertebr. Pathol.* 76:222–26
107. Tang R, Liu F, Lan Y, Wang J, Wang L, et al. 2022. Transcriptomics and metagenomics of common cutworm (*Spodoptera litura*) and fall armyworm (*Spodoptera frugiperda*) demonstrate differences in detoxification and development. *BMC Genom.* 23:388
108. Tang X, Freitak D, Vogel H, Ping L, Shao Y, et al. 2012. Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. *PLOS ONE* 7:e36978
109. Teh BS, Apel J, Shao Y, Boland W. 2016. Colonization of the intestinal tract of the polyphagous pest *Spodoptera littoralis* with the GFP-tagged indigenous gut bacterium *Enterococcus mundtii*. *Front. Microbiol.* 7:928
110. van der Hoeven R, Betrabet G, Forst S. 2008. Characterization of the gut bacterial community in *Manduca sexta* and effect of antibiotics on bacterial diversity and nematode reproduction. *FEMS Microbiol. Lett.* 286:249–56
111. Van Moll L, De Smet J, Cos P, Van Campenhout L. 2021. Microbial symbionts of insects as a source of new antimicrobials: a review. *Crit. Rev. Microbiol.* 47:562–79

112. Vilanova C, Baixeras J, Latorre A, Porcar M. 2016. The generalist inside the specialist: Gut bacterial communities of two insect species feeding on toxic plants are dominated by *Enterococcus* sp. *Front. Microbiol.* 7:1005
113. Wang J, Mason CJ, Ju X, Xue R, Tong L, et al. 2021. Parasitoid causes cascading effects on plant-induced defenses mediated through the gut bacteria of host caterpillars. *Front. Microbiol.* 12:708990
114. Wang Y, Zhu J, Fang J, Shen L, Ma S, et al. 2020. Diversity, composition and functional inference of gut microbiota in Indian cabbage white *Pieris canidia* (Lepidoptera: Pieridae). *Life* 10:254
115. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. 2007. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLOS Biol.* 5:e114
116. Weinert LA, Araujo-Jnr EV, Ahmed MZ, Welch JJ. 2015. The incidence of bacterial endosymbionts in terrestrial arthropods. *Proc. Biol. Sci.* 282:20150249
117. Windfelder AG, Muller FHH, Mc Larney B, Hentschel M, Bohringer AC, et al. 2022. High-throughput screening of caterpillars as a platform to study host-microbe interactions and enteric immunity. *Nat. Commun.* 13:7216
118. Witzgall P, Proffitt M, Rozpedowska E, Becher PG, Andreadis S, et al. 2012. “This is not an apple”—yeast mutualism in codling moth. *J. Chem. Ecol.* 38:949–57
119. Wong AC, Chaston JM, Douglas AE. 2013. The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *ISME J.* 7:1922–32
120. Wu G, Zhao Z, Liu C, Qiu L. 2014. Priming *Galleria mellonella* (Lepidoptera: Pyralidae) larvae with heat-killed bacterial cells induced an enhanced immune protection against *Photobabdus luminescens* TT01 and the role of innate immunity in the process. *J. Econ. Entomol.* 107:559–69
121. Xia X, Gurr GM, Vasseur L, Zheng D, Zhong H, et al. 2017. Metagenomic sequencing of diamondback moth gut microbiome unveils key holobiont adaptations for herbivory. *Front. Microbiol.* 8:663
122. Xie J, Vilchez I, Mateos M. 2010. *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. *PLOS ONE* 5:e12149
123. Xu P, Liu Y, Graham RI, Wilson K, Wu K. 2014. Dengvovirus is a mutualistic symbiont of a global crop pest (*Helicoverpa armigera*) and protects against a baculovirus and Bt biopesticide. *PLOS Pathog.* 10:e1004490
124. Yamasaki Y, Sumioka H, Takiguchi M, Uemura T, Kihara Y, et al. 2021. Phytohormone-dependent plant defense signaling orchestrated by oral bacteria of the herbivore *Spodoptera litura*. *New Phytol.* 231:2029–38
125. Yang J, Yang Y, Wu WM, Zhao J, Jiang L. 2014. Evidence of polyethylene biodegradation by bacterial strains from the guts of plastic-eating waxworms. *Environ. Sci. Technol.* 48:13776–84
126. Yoon SA, Harrison JG, Philbin CS, Dodson CD, Jones DM, et al. 2019. Host plant-dependent effects of microbes and phytochemistry on the insect immune response. *Oecologia* 191:141–52
127. Yun JH, Roh SW, Whon TW, Jung MJ, Kim MS, et al. 2014. Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Appl. Environ. Microbiol.* 80:5254–64
128. Zeng JY, Vuong TM, Guo JX, Shi JH, Shi ZB, et al. 2020. Diel pattern in the structure and function of the gut microbial community in *Lymantria dispar asiatica* (Lepidoptera: Lymantriidae) larvae. *Arch. Insect Biochem. Physiol.* 104:e21691
129. Zhang J, Gao D, Li Q, Zhao Y, Li L, et al. 2020. Biodegradation of polyethylene microplastic particles by the fungus *Aspergillus flavus* from the guts of wax moth *Galleria mellonella*. *Sci. Total Environ.* 704:135931
130. Zhang S, Huang J, Wang Q, You M, Xia X. 2022. Changes in the host gut microbiota during parasitization by parasitic wasp *Cotesia vestalis*. *Insects* 13:760
131. Zhang Y, Liu S, Jiang R, Zhang C, Gao T, et al. 2021. *Wolbachia* strain wGri from the tea geometrid moth *Ectropis grisea* contributes to its host's fecundity. *Front. Microbiol.* 12:694466
132. Zhang Z, Peng H, Yang D, Zhang G, Zhang J, Ju F. 2022. Polyvinyl chloride degradation by a bacterium isolated from the gut of insect larvae. *Nat. Commun.* 13:5360
117. Revealed a previously unknown protective function of the *Manduca sexta* resident gut microbiome.