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Honey bees as models for gut microbiota research

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Abstract

The gut microbiota of the honey bee (*Apis mellifera*) offers several advantages as an experimental system for addressing how gut communities affect their hosts and for exploring the processes that determine gut community composition and dynamics. A small number of bacterial species dominate the honey bee gut community. These species are restricted to bee guts and can be grown axenically and genetically manipulated. Large numbers of microbiota-free hosts can be economically reared and then inoculated with single isolates or defined communities to examine colonization patterns and effects on host phenotypes. Honey bees have been studied extensively, due to their importance as agricultural pollinators and as models for sociality. Because of this history of bee research, the physiology, development, and behavior of honey bees is relatively well understood, and established behavioral and phenotypic assays are available. To date, studies on the honey bee gut microbiota show that it affects host nutrition, weight gain, endocrine signaling, immune function, and pathogen resistance, while perturbation of the microbiota can lead to reduced host fitness. As in humans, the microbiota is concentrated in the distal part of the gut, where it contributes to digestion and fermentation of plant cell wall components. Much like the human gut microbiota, many bee gut bacteria are specific to the bee gut and can be directly transmitted between individuals through social interaction. Although simpler than the human gut microbiota, the bee gut community presents opportunities to understand the processes that govern the assembly of specialized gut communities as well as the routes through which gut communities impact host biology.

Introduction

Complex microbial communities are found in virtually every site on the human body[1–3], but the microbial communities associated with the gastrointestinal tract, home to the vast majority of microbes in most animals[4], are of particular interest due to their diverse impacts on host health. For example, the human gut microbiota aids in food digestion, modulates the immune system, and bolsters resistance against pathogens[5–7]. Sequencing and sequence analysis have been used to identify correlations between microbiota composition and a variety of diseases[8,9], but experimental approaches are critical in order to move beyond correlations and address cause and effect relationships. As ethical and practical

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considerations constrain experiments in humans, good model systems are essential for experimental study of gut microbiotas. In this review, we present the gut microbiota of the honey bee (*Apis mellifera*) as an experimentally tractable model system that offers numerous parallels to the human gut microbiota (Figure 1).

Over the past decade, sequencing and culture-based approaches have revealed a characteristic and relatively stable bacterial community present in the guts of all healthy adult worker honey bees. This community makes essential contributions to digestion, gut development, weight gain, and resistance to pathogens[10–13] and is dominated by a limited number of bacterial lineages that live only in bee guts[14–16]. These species can be cultured[17] and genetically manipulated[18].

Here, we focus on recent publications that illustrate the utility of the bee gut microbiota as a model system for addressing general questions about microbiota functions. We provide an overview of the advantages of the honey bee as a model organism for microbiota studies, and review the composition and organization of the bee gut microbiota, recent developments in bee gut microbiota research, and parallels between the bee and human gut microbiotas. We emphasize experimental studies that use gnotobiotic hosts with defined gut communities. The diversity of the bee gut microbiota and its role in digestion of food are discussed only briefly, as these subjects have been reviewed elsewhere (e.g., [17,19,20]).

Honey bees as model organisms for microbiota studies

One significant advantage of the honey bee gut microbiota as a model system is the wealth of existing knowledge of honey bee biology. For hundreds of years, humans have kept domesticated honey bees for honey and wax production and for pollination of agricultural crops. Honey bees have long been studied as models of social behavior[21,22] and developmental plasticity[23], and more recently as models for aging[24], and behavioral disorders[25]. Their economic value, mainly from pollination services, is estimated in the billions of dollars annually[26]. Global concern over recent high seasonal mortality rates of bee hives[27] has motivated research into ecological factors affecting bee health, including nutrition, toxins, pathogens, and parasites. The honey bee genome has been sequenced[28], and genomic variation within the species has been surveyed[29,30].

Another key advantage of honey bees as a model system for microbiota research is the availability of microbiota-free hosts, enabling investigation into how the microbiota influences host phenotypes including disease states[31,32]. Microbiota-free mammals can only be obtained by Caesarean section and must be maintained in specialized housing. In some insects, germ-free individuals can be generated by chemical surface sterilization of eggs[33,34]. However, the honey bee life cycle can be exploited to obtain large numbers of microbiota-free hosts without the use of antibiotics.

Honey bee workers undergo four distinct developmental phases: egg, larva, pupa, and adult[35]. The absolute abundance of gut-associated bacteria changes dramatically across these stages[36]. Each hive contains a single queen, able to lay 1,000–2,000 eggs per day under favorable conditions[37]. A fertilized egg is deposited into an individual worker cell, where it

hatches into a larva that is fed by adult workers, called nurse bees[38]. Larval guts possess few bacterial cells, often too few to be detectable using standard PCR protocols or microscopy using fluorescent *in situ* hybridization[36]. Several bacterial species characteristic of adults have been detected from larval guts through culture-dependent or high-throughput analyses[39,40], but larval gut bacteria appear to be largely acquired from food provisioned by the adult nurse bees[41], and likely represent transients rather than stable colonizers. At the end of the larval stage, nurse bees construct a wax cap that seals the cell before pupation. Before the start of pupation, a septum that separated the midgut and hindgut during the larval stage is eliminated[38,42]. At the beginning and end of pupation[42], the exoskeleton including the gut lining is shed (a process called ecdysis), eliminating any bacteria that may have been present in the larval midgut prior to pupation. Upon the completion of metamorphosis, the adult bee chews through the wax cap. Newly-emerged adult worker bees are nearly free of bacteria[36,43,44] though some bacteria may be acquired as they chew[45]. The characteristic gut microbiota is established through social interactions with other workers during the first three days after emergence. The number of bacteria in the gut expands logarithmically until the community stabilizes at 10^8–10^9 bacterial cells around 4 days after emergence[46].

To generate gnotobiotic bees (Figure 2), hive frames with mature pupae (capped brood) are removed from the hives and transported to the laboratory, where pupae with pigmented eyes but lacking movement are aseptically removed and placed in sterile dishes. Pupae kept under sterile conditions will emerge as microbiota-free adult workers within three days[46,47]. Alternatively, microbiota-free workers can also be reared in the lab by rearing larvae manually[48], though this approach requires more elaborate experimental infrastructure and may yield bees that are less robust. Microbiota-free workers can be inoculated orally with specific bacterial strains or with whole communities to investigate the roles of the gut microbiota in bee health, as well as the mechanisms by which microbes interact with their hosts and with one another (e.g., [46,47]).

Honey bees are also amenable to experiments on the effects of perturbation on an established gut microbiota. Honey bee colonies are inexpensive to maintain[49] and, with 30,000–80,000 adult workers per colony[37], readily provide large numbers of individuals for experiments with large sample sizes (See Box 1 for the availability and cost of beekeeping). In studies of microbiome perturbation, adult workers are collected and marked, treated in the lab with antibiotics or chemicals, and then returned to their hive of origin[12]. This approach can be used to study the resilience of the bee gut microbiota to perturbation and to understand the impacts of xenobiotics, including chemicals applied to crops that may be transported back to the hive by forager bees[50,51], on bee behavior and health. Furthermore, isolates of bacterial pathogens, originating from sick honey bees, can be used to assess the role of the bee gut microbiota in colonization resistance[12]. In summary, a honey bee hive can provide hundreds to thousands of bees for experiments, including conventional and microbiota-free bees.

The honey bee gut microbiome

A primary advantage of the honey bee as a model system for microbiota research is that healthy workers have a simple and specific gut microbiota that is present in honey bees collected worldwide[52,53]. This community is dominated by five to nine taxa, each corresponding to a species or a cluster of closely related species (Table 1), which together account for >98% of bacterial 16S rRNA gene sequences in the gut of a typical adult worker[14–16]. The dominance of these core taxa is consistent across bees from different hives and even different continents[15,52,54–56]. Once established in the adult gut, the composition of the microbiota changes little, despite seasonal changes and shifts in diet, behavior, and gene expression that occur as workers transition from nurse bees into foragers[36,57]. Compositional surveys using 16S rRNA genes reveal variation in the abundance of core taxa. Typically, a cluster of *Lactobacillus* strains collectively referred to as the Firm-5 phylotype is most abundant, followed by *Lactobacillus* Firm-4, *Bifidobacterium* spp., *Gilliamella apicola*, and *Snodgrassella alvi* (Table 1). *Frischella perrara*, *Bartonella apis*, *Apibacter adventoris*, and *Parasaccharibacter apium* are sometimes present at variable levels[56,58]. The occurrence of *P. apium* in honey bees is sporadic[14,59], but it is routinely isolated both from bee guts and from other environments, including queen bee guts, honey and beebread[60]. While *Lactobacillus* Firm-4 and Firm-5 are true gut symbionts and rarely detected outside of bee guts, other *Lactobacillus* species can be found within the hive and on hive materials (e.g., *Lactobacillus kunkeei*). These *Lactobacillus* spp. belong to distantly related phylogenetic clusters, and genetic markers and methods should be designed to differentiate core species from environmental species when analyzing the *Lactobacillus* phylotypes[61–64]. Multiple strains from all major taxa of bee gut bacteria have been isolated (Table 1) and cultured. The relative ease of culturing these species facilitates genome sequencing and analysis to identify strain-level gene repertoires, as well as the development of genetic tools, further enabling insight into the evolution and functional roles of members of the gut microbiota[11,65].

Recently developed genetic tools for manipulating bee gut microbes facilitate investigation of the molecular basis of host-microbe and microbe-microbe interactions in this system. Leonard *et al.* published genetic methods for heterologous gene expression and targeted disruption of multiple genes in Gram-negative bee gut microbes, including gut symbionts *S. alvi*, *G. apicola*, *B. apis, and P. apium,* and the opportunistic pathogen *Serratia marcescens*[18]. These methods comprise a toolkit for combinatorial assembly of broad-host-range replicative plasmids that can be transferred by conjugation into recipient bacteria, multiple functional antibiotic cassettes, inducible synthetic promoters, and CRISPR-interference. Furthermore, strains engineered to produce fluorescent proteins readily colonize microbiota-free bees in mono- and co- inoculation and can be directly imaged in the digestive tract of the bee. This methodology makes it possible to study the localization of bacteria within the bee gut without the time-consuming sample preparation and expensive probes required for fluorescence *in situ* hybridization. No genetic tools have been reported for the Gram-positive members of the bee gut microbiota, *Bifidobacteria* and *Lactobacillus.* However, Rangberg *et al.* introduced a recombinant plasmid into the hive-associated bacterium *L. kunkeei* and showed that engineered strains had no negative effects on bee health[66,67]. This was done to

validate the potential of *L. kunkeei* for "paratransgenesis," that is, the ability of engineered gut bacteria to improve host health and pathogen resistance. As interest in microbiome engineering for human health expands[68], the bee gut microbiome provides a useful model to study the stability and function of engineered bacteria in the gut context.

Experimental evidence for roles of bee gut bacteria

The field of microbiota research is young, but the honey bee model system has already been used to investigate several important questions relating to the function and evolution of host-associated gut communities (Figure 3). Here we present an overview of significant findings in this system.

Effects on endocrine signaling and behavior

A study comparing microbiota-free to conventional honey bee workers has shown that the gut microbiota is required for normal weight gain[69] and that the midgut and ileum of conventional bees are heavier than those of microbiota-free bees (Figure 3). This effect on weight gain is associated with shifts in endocrine signaling and gene expression[70,71], including changes in insulin/insulin-like signaling[69] (also observed in *Drosophila*[72]) and increased levels of vitellogenin, a nutritional status regulator in honey bees[73]. Kešnerová *et al.* discovered that *Bifidobacterium asteroides* stimulates the production of host-derived prostaglandins and juvenile hormone derivatives known to impact bee development[74]. Interestingly, Schwarz *et al.*[45] demonstrated downregulation of vitellogenin expression in gnotobiotic workers mono-inoculated with *S. alvi* and subsequently infected with a trypanosomatid parasite (*Lotmaria passim*) under hive conditions. As vitellogenin also regulates development of social behaviors in honey bees[73], these observations suggest a role for the gut microbiota in influencing bee social behavior.

To date, few experiments have addressed the possible links between the honey bee gut microbiota and behavior. Gut microbes may affect host behavior by altering levels of biogenic amines, such as octopamine, dopamine and serotonin. Levels of these amines in worker brains vary seasonally, and are higher in the summer when foraging activity is highest[75]. Levels are significantly lower in brains of newly-emerged, microbiota-free bees relative to brains of older, conventional adults[75]. Conventional and microbiota-free bees do behave differently: conventional bees respond to sucrose more readily and thus feed more, which is consistent with observed shifts in insulin signaling[69]. These results provide compelling evidence that the gut microbiota can alter host behavior and hormonal signaling.

Intersection of host and microbiota metabolism

Gut microbes associated with humans and many other plant-eating animals utilize recalcitrant dietary glycans and complex carbohydrates as substrates for growth[76,77]. Pollen is a key component of the bee diet, and the only source of amino acids, fat, vitamins and minerals[78]. Most of these nutrients are absorbed by the host midgut, leaving only the compounds that are most difficult to digest–including pollen cell wall components such as cellulose, hemicellulose, and pectin from pollen cell walls[79]–to be broken down by the microbial community in the hindgut. Recent studies have illuminated the role of the bee gut

microbiota in breaking down such components of the host diet and, in some cases, have identified metabolic activities associated with specific members of this community. For example, metagenomic and genomic analyses attributed the genes enabling pectin degradation to *G. apicola*[10,11], a result that was confirmed when metabolomics analysis documented increased galacturonic acid in honey bee ileums colonized by conventional microbiota (mainly *G. apicola* and *S. alvi*) compared to ileums of microbiota-free bees[69]. Guts of conventional bees also contained large amounts of acetate and other short-chain fatty acids (SCFAs) relative to microbiota-free individuals[69], indicating that these SCFAs are largely produced via microbial fermentation (Figure 3). In the bee gut, anoxia is maintained by *S. alvi*, which associates with the gut wall and uses acetate as the electron donor for consumption of O_2 *in vitro* [69] (Figure 3). This is significant, as alteration of the gut microenvironment can strongly affect metabolic activities. Among bee gut bacteria known to produce SCFAs, *Lactobacillus* Firm-5 is the main producer of fermentation products succinate and pimelate, while *B. asteroides* is the main producer of valerate[74]. Using gnotobiotic bees inoculated with defined communities, Kešnerová *et al.*[74] found that *Bifidobacterium* and *Lactobacillus* Firm-4 and Firm-5 can digest other pollen components, including flavonoids and compounds in the outer pollen wall and coat, such as ω-hydroxy acids and phenolamides. *Lactobacillus* Firm-5 and *G. apicola* release ω-hydroxy acids from the outer pollen wall, likely facilitating utilization of these compounds by *Lactobacillus* Firm-4 and *B. asteroides*[74].

Though not all microbe-microbe interactions can be inferred from the abundance of particular compounds in mono-colonized models, many examples support the cross-feeding of essential metabolites between bee gut bacteria. Kešnerová *et al.*[74] showed several complementary pathways suggestive of such cross-feeding. These involve the production and use of pyruvate, which accumulates in guts of bees mono-colonized with *G. apicola*, but which decreases in concentration in bee guts colonized by *S. alvi*, a species which lacks a functional glycolysis pathway[47]. The observation that the growth of *S. alvi* in culture is improved by the addition of supernatant from *G. apicola* cultures further supports the premise that these species engage in cross-feeding interactions[74]. All members of the bee gut community salvage nucleosides from the gut environment, except for *S. alvi* and *B. apis,* which encode a functional nucleoside biosynthetic pathway[17,47,74] and may produce nucleosides utilized by other bacteria. *S. alvi* and *B. apis* also convert carboxylic acids and keto acids–malate, fumarate, citrate, and α-ketoglutarate–through the TCA cycle[17,47,80].

Colonization determinants of specialized gut bacteria

Comparison of host and microbe phylogenies suggests that the assemblages of *S. alvi* and *G. apicola* strains found in social bees reflect a long evolutionary history of co-diversification with hosts and limited host switching[52,81]. Native strains easily outcompete non-native strains[47] during colonization of microbiota-free hosts, although some strains can also colonize non-native hosts closely related to their native host species[52]. The gut communities of different, co-occurring *Apis* species remain distinct[52], though some evidence supports transmission of pathogens and parasites between different bee species[82]. A study of *S. alvi* strain distributions in field-collected bees using fine-scale markers showed that no

movement occurs between *Apis* and *Bombus*[83], which is consistent with laboratory experiments on host specificity.

Environmental bacteria are generally unable to colonize the honey bee gut, indicating that gut-associated bacteria have adaptations which enable them to tolerate or evade the host immune system and other stressors within the gut. An analysis of *S. alvi* genes required to establish within the host gut utilized transposon mutagenesis and high-throughput sequencing to identify nearly 400 genes that contribute to fitness during colonization of the bee gut[84]. These included genes in pathways required for colonization in mammalian models[85]. Among these were type IV-pili and adhesion factors, which are likely to be important for localization of *S. alvi* to the gut wall, amino acid biosynthesis pathways, and DNA repair and stress response pathways. These findings provide insight into the challenges that *S. alvi* experiences during colonization of the bee gut, including nutrient scarcity in the ileum and competition for resources.

Effects on immune functioning

Gut microbes can modulate host immune function, which could indirectly affect other microbes and may affect host fitness. Colonization by a conventional microbiota or by a single *S. alvi* isolate results in upregulation of the antimicrobial peptides apidaecin and hymenoptaecin in gut epithelial cells[86]. A more dramatic immune response occurs when *F. perrara,* a bacterium present in many but not all honey bees, colonizes the honey bee pylorus[46], where the midgut transitions into the ileum. Colonization by *F. perrara* triggers the development of a ‘scab’ phenotype consisting of a dark ring around part of the gut circumference[87]. This dark ring is produced by melanization, a honey bee immune response[88]. Although *F. perrara* interacts with the honey bee immune system, it is not yet clear whether this interaction is harmful or beneficial, for example, via immune priming[89].

Perturbation of the native microbiota

Perturbation of the normal, established gut community, using antibiotics or other disruptors, provides further insight into microbiota function. Raymann *et al.*[12] measured the effects of treatment with tetracycline, a broad-spectrum antibiotic, on the size and composition of the honey bee gut microbiota and on host fitness. Antibiotic-treated bees showed altered relative abundance and diversity of core microbial taxa[90], elevated abundance of non-core taxa, decreased survival in the hive, and increased mortality when exposed to the opportunistic pathogen *S. marcescens* kz11. However, none of the dominant members of the microbiota were fully eliminated after antibiotic treatment. Perturbation of the gut microbiota was harmful even in the absence of opportunistic pathogens, as conventional bees exhibited increased mortality after antibiotic treatment in sterile laboratory conditions, relative to microbiota-free bees treated with antibiotic. In another study, Li *et al.*[91] found that perturbation of the worker gut microbiota with antibiotics lowered immune responses and elevated susceptibility to a major microsporidian parasite (*Nosema ceranae*) that invades through the midgut epithelium.

The honey bee gut microbiota shows parallels with the human gut microbiota

Although the importance of bee health is itself a major motivation for studying the bee gut microbiota, this system also offers the advantage of having numerous parallels to the human gut microbiota.

Specificity and evolutionary adaptation to hosts:

As in the human gut microbiota, most dominant members of the honey bee gut community are found solely in the host gut environment. Gut bacteria in both honey bees and humans are likely to be specifically adapted to these habitats, as they have coevolved with their hosts over millions of years[52,92]. The five most abundant bacterial species associated with the guts of modern corbiculate (social) bee hosts, which include species of *Apis* (Asian honey bees), *Bombus* (bumble bees), and *Meliponini* (stingless bees), most likely descend from a core community present in the shared ancestor of these bees, with subsequent strain divergence and gains and losses of taxa producing the gut communities found today[52].

Transmission through social interactions:

Both bee and primate gut bacteria are primarily transmitted through social interactions[46,92,93]. In bees, phylogenetic analyses of bacterial strains present across corbiculate bee hosts suggest that their acquisition coincided with the transition to social lifestyles[52]. Core taxa of social bees are not found in solitary bees or other related insects such as wasps, nor have they been isolated from other environments[14]. In contrast, many invertebrate gut communities have erratic compositions dominated by bacteria from environmental sources (e.g., [94–97]).

Strain variation:

Although the bee gut microbiota possesses a limited number of bacterial species, each component species exhibits extensive strain variation, which is also true of the human gut microbiota[98]. Deep sequencing of single-copy protein coding genes revealed high levels of *S. alvi* and *G. apicola* strain diversity in bee guts[52,83,90]. These two species also have large pools of accessory genes, which are not present in all strains. For example, many of the accessory genes in *G. apicola* strains are involved in carbohydrate metabolism[47,99], but only some strains encode genes for utilization of components of pollen cell walls[100] or monosaccharides that are toxic to the bee host[11], suggesting that strains of the same species differ in contributions to host nutrition. Different strains have distinct assortments of Type VI Secretion System (T6SS)-associated toxin and antitoxin genes[101], which may influence which combinations of strains are capable of co-colonizing a single host. Additionally, some *Apibacter* strains encode a T6SS similar to the T6SS used in interbacterial antagonism by *Bacteroidetes* species in the human gut[102,103].

Pathogen resistance:

Perturbation of gut microbial communities can have negative repercussions for host health. In humans, dysbiosis, or abnormal composition or function of the microbiota, is associated

with numerous diseases and can result from antibiotic treatment, poor diet, and other disturbances. For example, destabilization of the gut microbiota due to antibiotic treatment increases susceptibility to *Clostridium difficile* infections in humans[104]. Similarly, perturbation of the bee gut microbiota by antibiotics or other chemicals increases susceptibility to infection by *S. marcescens*[12].

Role in fermentation and SCFA production:

As in humans and other animals[105,106], the gut microbiota of honey bees is concentrated in the distal gut where it contributes to the digestion and fermentation of complex carbohydrate polymers derived from plant cell walls. This role of the honey bee gut microbiota contrasts with the gut communities of some other insects. For example, in *Drosophila*, the gut community occupies the midgut and is not implicated in digesting plant cell wall components, though it is important in immune and developmental signaling[107]. Even herbivorous lepidopteran larvae, which consume only plant material, appear not to rely on gut microbiota for digestion or nutrition[96]. Oxygen availability within the gut can influence patterns of colonization and can affect the mutualistic interactions between gut microbes[108,109]. Most herbivorous insects' guts are nearly anoxic[110], unlike the gut of *Drosophila* which contains oxygen and is dominated by aerobes[111]. Anoxia in the bee ileum is maintained by the respiration of *S. alvi*, a bacterium that associates with the ileum wall, which is fueled by acetate, the most abundant SCFA in the gut (Figure 3)[69].

History of antibiotic exposure:

Long-term antibiotic use may have impacted the diversity within human gut communities and has resulted in high frequencies of resistance determinants[112]. Likewise, antibiotic exposure has affected gut communities of honey bees, particularly in the United States and other countries where beekeepers have used antibiotics since the late 1940's to control or prevent larval bacterial diseases known as foulbrood[113–115]. This practice has resulted in high frequencies of antibiotic resistance determinants in core gut bacteria isolated from bees in the United States, in contrast to gut bacteria of honey bees from countries which do not permit the use of antibiotics in beekeeping[116,117]. In both human and honey bee gut communities, resistance determinants have been exchanged among community members through horizontal transfer[118]. Furthermore, antibiotic exposure has an immediate impact on the size and diversity of honey bee gut communities[12,90].

Limitations of the honey bee as a model for gut microbiota

We have described the utility of the honey bee as a model for microbiota research, but this system does have some drawbacks and important differences from humans. As with any model system, the honey bee microbiota is most valuable as a tool for understanding general principles of microbe-microbe and microbe-host interactions. Care should be taken in any attempt to directly apply findings from the bee system to the human gut microbiota, as physiology and diet differ markedly between bees and humans and the bee gut microbiota, which has comparatively low biomass and diversity, may not reflect all of the processes that occur in the human gut microbiota[119].

Practical considerations when planning honey bee experiments include the seasonal life cycle of the honey bee and the challenges posed by bee genetics. We have considered rate of brood production as an advantage of the honey bee system because of the potential to perform experiments with large sample sizes. However, honey bee brood production is seasonal and the queen's oviposition rate is drastically reduced in winter, when the number of workers in the colony can drop below 10,000 bees. Thus, newly emerged, microbiota-free workers are not available from outdoor hives during winter months in most geographic regions. Methods exist for *in vitro* rearing of honey bees in the laboratory through artificial feeding of larvae[48,120], and for maintaining whole colonies indoors, but these add to the complexity and expense of rearing, and bees produced through these approaches may have developmental abnormalities.

Seasonal shifts also subject bees to fluctuations in environmental conditions, food availability, and nutritional requirements [121], which can affect gut community composition and potentially influence experimental results[122]. Abundances of honey bee pathogens also vary seasonally[123]. Both pathogens and gut bacteria alter expression of immune genes, including antimicrobial peptides[86,124], so studies of the microbiota could be confounded by the presence of a pathogen or vice versa. Another consideration is that, while the method of obtaining microbiota-free bees (discussed above) is effective for obtaining workers with little to no bacteria in their guts, it does not prevent these workers from developing viral infections. Honey bee viruses, such as Deformed Wing Virus (DWV), are common and can be transmitted to bees by parasitic *Varroa destructor* mites during pupation[124].

Honey bees are not amenable to genetic manipulation or crossing experiments, due to their reproductive division of labor. A single queen can mate with multiple males, or can be artificially inseminated with a single male's sperm[125], so workers from the same hive are genetically similar as half- or full-sisters. Workers from different hives may have different genetic backgrounds, so experiments should control for source hive. The only tool currently available for genetic manipulation of honey bees is an injectable *piggyBac*-derived transposon cassette[126], and its use is not widespread. Generation of transgenic honey bees using CRISPR/Cas9 is an alternative, but to date only one study has reported successful production of mutants with this approach[127]. Maintaining lines generated using either strategy would likely be more difficult than in other models, such as *Drosophila*, due to the need to propagate queens.

In the absence of stable transgenic lines, the use of RNA-interference (RNAi) to modulate expression of target genes is widespread in insect research and established in honey bees[128,129]. Feeding or injecting bees with double stranded RNA (dsRNA) results in gene knockdown, though, as with other insects, the magnitude and effectiveness of knockdown is variable[130]. Honey bees also possess a sequence-non-specific antiviral response, which requires the inclusion of appropriate controls in RNAi studies[131]. Regardless, RNAi approaches will allow researchers to selectively downregulate honey bee genes, such as those encoding immune effectors or immune signaling pathways, to assess their functions in structuring the gut microbial community.

Conclusions and future outlook

The studies summarized in this review demonstrate the many advantages of the honey bee model system for microbiota research. These advantages include intrinsic properties of the gut microbiota, such as its simple and specific composition, and established methods for cultivating and genetically manipulating the core gut bacterial species. Other benefits of this system stem from the biology of the host, such as the ability to experimentally colonize large numbers of gnotobiotic host animals and the ease of rearing bees economically. Years of bee research in multiple fields of study has built a knowledge base that includes genomic, ecological, behavioral, developmental, and physiological information as well as experimental protocols.

One motivation for studying the bee gut microbiota is the increasing evidence for its role in bee health: such studies may enable improvements in beekeeping that help to prevent damaging colony losses and preserve these important pollinators. Additionally, the bee gut microbiota has numerous similarities to that of humans (Figure 1), and may be useful for establishing general rules of microbiome function.

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Box 1. The cost and feasibility of beekeeping.

Honey bee colonies can be established in almost every habitat inhabited by humans, wherever flowering plants are available for foraging, which makes bees broadly accessible as model organisms. The costs of standard hive equipment and bees are low, approximately $200-$300 per hive in the United States, and new hives can be started from healthy hives. Beekeeping methods are readily learned from local beekeepers, beekeeping clubs, or from various “how-to” manuals. Once established, most honey bee hives last for years and are largely self-sustaining.

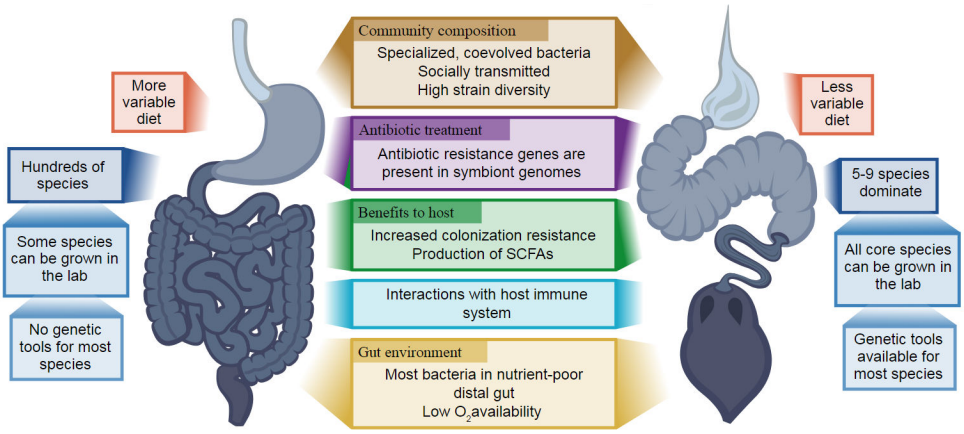

Figure 1.

Similarities (center) and differences (right and left sides) between the gut microbiota of humans and the gut microbiota of honey bees. SCFA, short chain fatty acids.

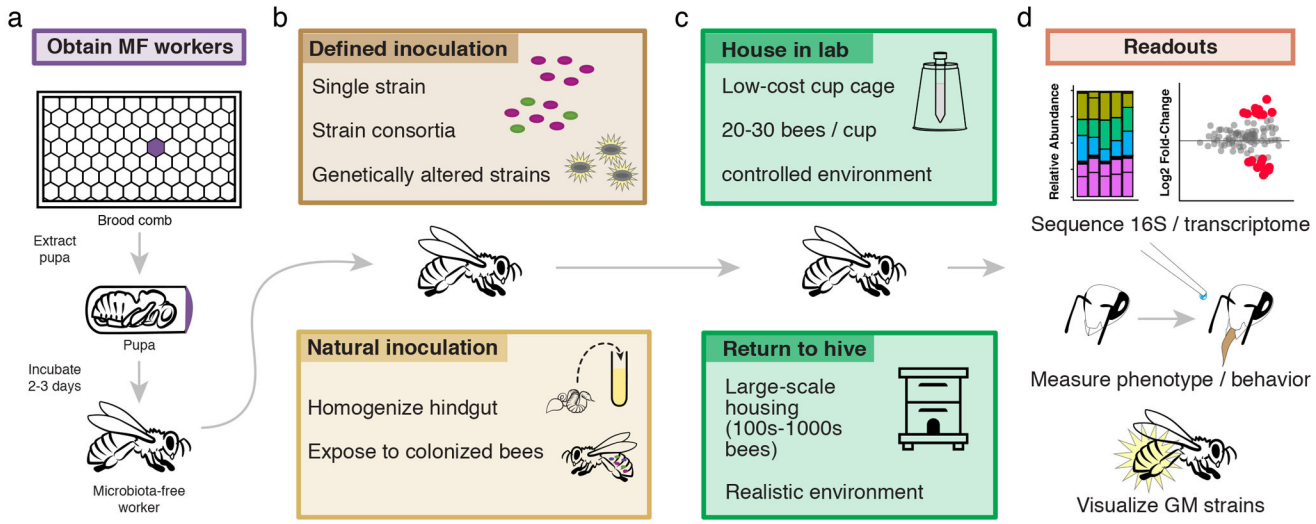

Figure 2.

Design of gnotobiotic honey bee studies. Microbiota-free (MF) hosts emerge in the lab (a) where they can be inoculated with isolated gut symbiont strains, genetically modified symbionts, or natural communities (b). These bees may be maintained in laboratory conditions or marked and returned to the hive environment (c). Destructive sampling of bees and sequencing allows for analyses of community composition and function. Alternatively, phenotypic (e.g., behavioral) assays, can reveal effects of defined gut communities on bees, while genetically modified (GM) fluorescent strains facilitate *in situ* imaging of bacteria (d).

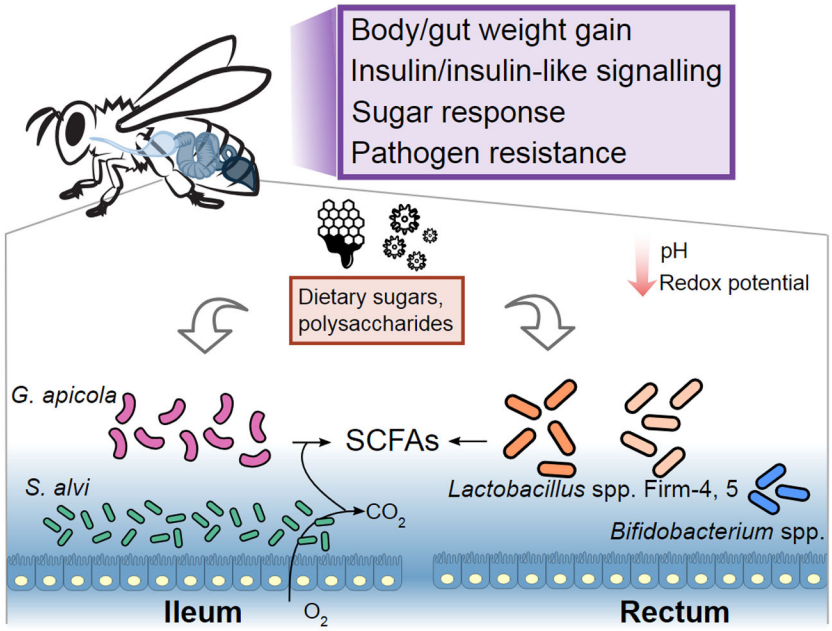

Figure 3.

Summary of the effects of the honey bee gut microbiota on host and the gut microbial metabolism. The dominant members in the ileum and rectum ferment sugars and polysaccharides from the host diet (honey and pollen) to SCFAs. Oxygen consumption by the species *S. alvi,* which forms a layer attached to the inner gut wall, maintains an anoxic gut environment. The presence of gut bacteria can also reduce the gut pH and redox potential. Moreover, gut bacteria have various effects on the weight gains, insulin signaling, behaviors, and pathogen resistance of the hosts.

Table 1.

Bacteria occurring in honey bee guts and in the hive environment.

Taxa	Phylum	Primary location	References
Ubiquitous gut-restricted taxa			
Lactobacillus sp. Firm-5	*Firmicutes*	Hindgut (ileum, rectum)	61,132
L. apis			
L. helsingborgensis			
L. kimbladii			
L. kullabergensis			
L. melliventris			
Lactobacillus sp. Firm-4	*Firmicutes*	Hindgut (rectum)	61
L. mellifer			
L. mellis			
Bifidobacterium sp.	*Actinobacteria*	Hindgut (rectum)	133-135
B. asteroides			
B. coryneforme			
B. indicum			
Snodgrassella alvi	*Proteobacteria*	Hindgut (ileum wall)	136
Gilliamella apicola	*Proteobacteria*	Hindgut (ileum lumen)	136
Frischella perrara	*Proteobacteria*	Hindgut (pylorus, ileum)	137
Bartonella apis	*Proteobacteria*	Hindgut, variably present	138
Commensalibacter sp.	*Proteobacteria*	Hindgut, variably present	14,139
Other common taxa			
Apibacter adventoris	*Bacteroidetes*	Adult gut	103,140
Parasaccharibacter apium	*Proteobacteria*	Larval gut, adult crop, queen gut, hive	57,141
Lactobacillus kunkeeii	*Firmicutes*	Larval and adult gut, hive, nectar	142
Fructobacillus fructosus	*Firmicutes*	Larval and adult gut, hive	143
Saccharibacter spp.	*Proteobacteria*	Bee stomach, honey, pollen	144
Opportunistic pathogens			
Serratia marcescens	*Proteobacteria*	Adult gut	12,145
Hafnia alvei	*Proteobacteria*	Adult gut	146