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Review article

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The honeybee microbiota and its impact on health and disease

Erick V. S. Motta & Nancy A. Moran

Abstract

Honeybees (*Apis mellifera*) are key pollinators that support global agriculture and are long-established models for developmental and behavioural research. Recently, they have emerged as models for studying gut microbial communities. Earlier research established that hindguts of adult worker bees harbour a conserved set of host-restricted bacterial species, each showing extensive strain variation. These bacteria can be cultured axenically and introduced to gnotobiotic hosts, and some have basic genetic tools available. In this Review, we explore the most recent research showing how the microbiota establishes itself in the gut and impacts bee biology and health. Microbiota members occupy specific niches within the gut where they interact with each other and the host. They engage in cross-feeding and antagonistic interactions, which likely contribute to the stability of the community and prevent pathogen invasion. An intact gut microbiota provides protection against diverse pathogens and parasites and contributes to the processing of refractory components of the pollen coat and dietary toxins. Absence or disruption of the microbiota results in altered expression of genes that underlie immunity, metabolism, behaviour and development. In the field, such disruption by agrochemicals may negatively impact bees. These findings demonstrate a key developmental and protective role of the microbiota, with broad implications for bee health.

Sections

Department of Integrative Biology, University of Texas, Austin, TX, USA. ✉e-mail: nancy.moran@austin.utexas.edu

Introduction

The honeybee, *Apis mellifera*, has a long history of domestication for honey and wax production, as well as for pollination. In research, honeybees have served as models for developmental plasticity[1], cognition[2] and social behaviour[3]. More recently, they have emerged as models for gut microbiota studies[4,5]. The advent of nucleotide sequencing technologies revealed a specific microbial community that inhabits the honeybee gut[6–9]. Since then, a combination of sequencing and culture-based approaches has been used to characterize the core members of the honeybee gut microbiota, their metabolic capabilities and their roles in bee health.

The honeybee gut microbiota is relatively simple, dominated by five core bacterial lineages present in all healthy worker bees. These bacteria are acquired orally after emergence from the pupal stage through social interaction and contact with hive compartments[4,10], and they correspond to clusters within the genera *Bifidobacterium*, *Bombilactobacillus* (previously called *Lactobacillus* Firm-4 (ref. 11)), *Gilliamella*, *Lactobacillus* (previously called *Lactobacillus* Firm-5 (ref. 11)) and *Snodgrassella*[4]. These core bacteria form a consistent community of about 10^8–10^9 cells, although their absolute and relative abundances vary with life stage, season and geographical location[12–18]. Other non-core bacteria are commonly present, and include *Bartonella*, *Commensalibacter* and *Frischella*[4]. Within each of these genera, between one and five species have been formally characterized[19–24], although additional closely related species may remain to be recognized[25].

Environmental (for example, *Fructobacillus* spp.) and pathogenic bacteria (for example, *Serratia marcescens*, *Hafnia alvei* and other Enterobacterales) are often present at low abundances in the adult bee gut[8]. Still other bacteria (for example, *Apilactobacillus kunkeei* and *Bombella apis*) are associated with larvae, queens and hive compartments[26,27]. Although dominated by bacteria, some honeybee guts harbour a small proportion of eukaryotes, including fungi[28], trypanosomatid parasites (*Crithidia* and *Lotmaria* species)[29] and microsporidian parasites (*Vairimorpha ceranae*, previously called *Nosema ceranae*)[30,31]. Fungal presence is erratic, and varies between geographical locations, suggesting that fungi are transient in the bee gut[32].

The five core bacterial lineages appear to have evolved with bees since the origin of the Corbiculata clade, about 80 million years ago[33]. Most corbiculate bees, including other honeybee (*Apis*) species native to eastern Asia[34], bumblebee species (genus *Bombus*) worldwide[35] and stingless bees (tribe Meliponini) in tropical regions[36], retain these core bacteria, although stingless bees have more often gained or lost certain bacteria[37–39]. Different bee species can also harbour distinct sets of bacteria, such as *Frischella perrara* and *Bartonella apis* in *Apis* spp. and *Bombiscardovia* and *Schmidhempelia* in *Bombus* spp. In general, these bacterial lineages have not been reported outside bees, although some have been detected in non-corbiculate bees, such as carpenter bees[40] and euglossine bees[39]. Thus, the corbiculate bee gut microbiota is dominated by specialized, host-restricted bacterial lineages that have evolved with one another and with hosts for long evolutionary periods.

A stable gut microbiota appears to be intrinsically associated with honeybee health. It aids in digestion[41,42] and detoxification of food components[43,44], stimulates the innate immune system[45,46] and protects against pathogens[47–49]. Additionally, the gut microbiota affects key developmental pathways, such as the endocrine signalling pathway that regulates feeding behaviour and weight gain[50–52], and olfactory learning and memory acquisition pathways[53].

Honeybees have been used as models in efforts to disentangle how gut microorganisms interact with each other, with opportunistic microorganisms, with environmental stressors and with the host[4]. Experimental approaches have been enabled by the ability to culture strains of each core species, to perform genetic manipulations on some and to reintroduce them into microbiota-deprived bees (Box 1). Earlier research, previously reviewed, has described the honeybee microbiota and metabolic capabilities of the core gut species[4,5,47,54]. Here, we assess recent research on the roles of the gut microbiota in bee health and disease. We first discuss recent findings on how beneficial bacteria interact with each other and establish themselves in the bee gut. Next, we summarize results from studies that show that the microbiota has a crucial role in several aspects of bee health. We also examine evidence that environmental stressors can compromise the microbiota, with negative consequences for bees, and preliminary evidence that probiotics might be useful to restore perturbed gut microbial communities. Finally, we identify knowledge gaps and discuss areas that require further investigation.

Microbial interactions within the gut

Members of the honeybee microbiota have adopted distinct ecological niches within the hindgut, a nutrient-poor region of the digestive tract that consists of three main sections: ileum, rectum and pylorus (Fig. 1a). *Snodgrassella alvi* and *Gilliamella* spp. are more abundant in the ileum, where they form a stable biofilm (Fig. 1c), whereas *Lactobacillus*, *Bombilactobacillus* and *Bifidobacterium* species are dominant in the rectum[4] (Fig. 1d). *F. perrara*, when present, colonizes the pylorus, a region that connects the midgut to the ileum, where it strongly activates the bee immune system[55] and induces formation of a melanized scab[56] (Fig. 1b). The pylorus, at the junction of the midgut and ileum, offers a distinctive niche, as the site where the Malpighian tubules empty host nitrogenous waste[57]. Although these core bacteria concentrate in specific gut compartments, they can also be found in other compartments with lower abundance[9,10].

Species within the honeybee gut microbiota harbour extensive strain diversity[34,58,59]. Even within a single bee gut, strains vary in gene repertoires affecting functional attributes, such as the ability to process dietary or host-derived components and to interact with related strains or species and with the host[25,41,60]. These abilities and interactions are likely to influence the composition of the microbiota, with specific strains adopting specific niches and roles. Potentially, these activities stabilize the microbiota and in turn promote the overall health of bees. Here, we summarize findings to date, noting that much remains unknown.

Diversification within the host gut

The genus *Gilliamella* has diverged into at least two genetically isolated species within the honeybee gut, *Gilliamella apis* and *Gilliamella apicola*, and these appear to be associated with distinct gut niches[57]. Genome analyses and experiments with two representative fluorescently marked strains has shown that *G. apis* is concentrated in the pylorus region and possesses urease and urea transporters, enabling it to use urea derived from bee waste (Fig. 1b), whereas *G. apicola* colonizes downstream regions of the ileum and cannot use urea[57] (Fig. 1c). Whether this metabolic difference is consistent across strains is unclear, but the incidence of *Gilliamella*-encoded urease genes is greater in the pylorus region[57].

Bee-restricted *Lactobacillus* species are abundant and diverse in honeybee guts[25] (Fig. 1d). Investigations of strains that represent the closely related species *Lactobacillus apis*, *Lactobacillus helsingborgensis*, *Lactobacillus melliventris* and *Lactobacillus kullabergensis* have

Box 1

Approaches to identify the effects of the gut microbiota on bee biology

A combination of experimental and genomic approaches has been used to identify the effects of the gut microbiota on honeybees. These approaches include the following.

- The use of microbiota-deprived bees, conventionalized bees or bees colonized with a single or several isolates, followed by examination of bee phenotypes[45,46,48,51,53,61,64,82,88,115]. In this context, microbiota-deprived bees refer to newly emerged bees that have been extracted from brood frames at the pupal stage or allowed to emerge on the frame without exposure to hive bees and raised under aseptic conditions in the laboratory. These bees are subjected to minimal exposure to microorganisms, resulting in a reduced presence of microbial colonization and specifically a lack of the usual core lineages[10]. Complete absence of microorganisms cannot be guaranteed and must be checked for each bee. On the other hand, conventionalized bees are those that have been colonized with the full microbiota obtained from gut homogenates of hive bees, resulting in a typical native and diverse gut microbial community. Effects of these microbiota treatments on gene expression patterns linked to behavioural[53,115], developmental[50,51], immunity[45,46,82,88] and metabolic pathways[61,64] have been investigated, as well as the ability of specific bee gut bacteria or the intact microbiota to prevent pathogen proliferation[45,46,48,82,88].
- Perturbation of the normal microbiota using antibiotics or other stressors, followed by phenotype examination[59,123,133,135,138,139,143]; for example, honeybees exposed to tetracycline or streptomycin exhibit perturbed gut communities and increased susceptibility to bacterial and fungal infections[86,96].
- Genetic engineering of bee gut strains to include visual markers or resistance genes[57,165,167]; for example, the expression of distinct fluorescent proteins in strains of *Gilliamella apis* and *Gilliamella apicola* has allowed visualization of spatial niche partitioning within the ileum[57].
- Heterologous expression of genes from bee gut microbiota to verify gene functions[63,143,170]; for example, a *Bifidobacterium* spp. gene that encodes a glycoside hydrolase family 3 was heterologously expressed in *Escherichia coli* to identify its role in the metabolism of amygdalin[63].
- Mark–recapture experiments with hive bees that enable examination of survivorship under natural, field conditions[96,125,140,151]; for example, honeybees were exposed under laboratory conditions to tetracycline[96] or a Roundup formulation[151], then returned to their original hives to investigate recovery rates and microbiota resilience.
- Genomic sequencing and in vitro experiments to establish symbiont metabolic and antagonism attributes[42,43,60,69]; for example, the ability of *Gilliamella* spp. strains to digest pectin and to metabolize diverse sugars was hypothesized from genome sequences and verified experimentally with cultured isolates[41,43].

shown that these species appear to reduce interspecific competition by partitioning nutrients derived from pollen, including various sugars and plant secondary metabolites[61] (Fig. 1d). All four species metabolize simple sugars and acids, but exhibit preferences based on metabolic rates. For example, *L. helsingborgensis* and *L. kullabergensis* use citrate at higher rates than do *L. apis* and *L. melliventris*, and *L. helsingborgensis* uses glucitol at higher rates than the other species[61]. Regarding plant secondary metabolites, *L. apis*, *L. melliventris* and *L. kullabergensis*, but not *L. helsingborgensis*, contribute to the metabolism of specific glycosylated flavonoids, and *L. apis* can also metabolize iridoid glycosides[61].

Potential cross-feeding interactions

The honeybee microbiota shapes the hindgut environment, for example, lowering oxygen and pH levels[50]. *S. alvi* is an obligate aerobe that forms a biofilm on the stable cuticular lining of the ileum wall (Fig. 1c), depleting oxygen and creating an anaerobic interior lumen in which other microbiota members reside. These members, including *Gilliamella*, *Lactobacillus* and *Bifidobacterium* species, produce various carbohydrate-degrading enzymes, such as pectin lyases and glycoside hydrolases[41,42] (Fig. 1c,d). These enzymes enable the breakdown of indigestible components from pollen husks (for example, hemicellulose and pectin[42,62]) and a wide range of nectar-derived metabolites, including disaccharides (for example, cellobiose[44]), monosaccharides (for example, galactose, mannose, rhamnose and xylose[43,44]) and secondary metabolites (for example, cyanogenic glycosides[63], flavonoid glycosides[61,64] and others[65]). Microbial digestion of some of these metabolites prevents intoxication[43] and promotes parasite protection in various bee species[65,66].

As by-products from bacterial metabolism within the bee gut, short-chain fatty acids (organic acids) are produced, including acetate, butyrate, formate, lactate, pyruvate and succinate[44,64]. Some of these are used as energy sources by *S. alvi*[50], which cannot use carbohydrates directly, and potentially by the host (Fig. 1c). Such cross-feeding interactions may have a role in shaping bee gut microbial communities[64]. At least some cross-feeding can occur between *Gilliamella* and *Snodgrassella* strains, which co-exist in the ileum biofilm[7] and possess complementary metabolic capabilities[67]. Carbohydrate metabolism by *G. apicola* leads to accumulation of pyruvate, and this is used, at least in part, by *S. alvi*, based on metabolomic analyses of cultures[64]. In vitro assays show that *S. alvi* growth is mildly enhanced when provided with supernatant from *G. apicola* cultures[64]. However, cross-feeding is not required as *S. alvi* can use host-derived organic acids, such as citrate, glycerate and 3-hydroxy-3-methylglutarate as carbon sources within the bee gut[68] (Fig. 1c), and mono-inoculation with *S. alvi* leads to robust colonization[33,45,48,64,67].

Metabolomics on sections of the honeybee gut reveal higher levels of aromatic amino acids in the ileum and rectum, but not in the midgut, of conventionalized bees compared with microbiota-deprived bees[50]. *S. alvi*, for example, cannot colonize the ileum without intact amino acid biosynthetic pathways[69]. *Bombilactobacillus* spp. and *Lactobacillus* spp. are auxotrophic for several amino acids[42] and may take advantage of amino acids produced by *G. apis* and *S. alvi*. However, these are not essential, as single strains of *Bombilactobacillus* spp. and *Lactobacillus* spp. can colonize the bee gut in the absence of other community members[48,64,70].

Antagonistic interactions within the gut

The ability to colonize niches in the bee gut depends on host-defined factors, but also appears to reflect the outcomes of antagonistic interactions between strains and species. These interactions can be contact dependent or contact independent. Genes that underlie antagonism are some of the most dynamic in the genomes, showing rapid evolution and gain and loss across strains[60,62,67,71].

Type 6 secretion systems (T6SSs) confer survival advantages to bacteria in microbial communities by delivering toxins that kill competing bacteria in a contact-dependent way (interference competition) and by improving acquisition of essential micronutrients (exploitation competition)[72]. On the basis of genome sequences, many Gram-negative gut symbionts of *A. mellifera* (for example, *S. alvi*, *Gilliamella* spp. and *F. perrara*), as well as of *Apis cerana* (for example, *Apibacter* spp.) and *Bombus* spp. (for example, *Schmidhempelia* spp.), carry genes that encode T6SSs, associated Rhs toxins and their respective immunity genes[60,71] (Fig. 1b,c). In some *S. alvi* strains, two independently acquired T6SSs are present and appear to differ in function, as only one is associated with the presence of Rhs toxins[60]. In a global mutagenesis study of *S. alvi*, immunity gene mutants failed to colonize guts, verifying inter-strain toxicity of the Rhs toxins, whereas some mutants in genes of the T6SS itself were favoured, indicating that production of the structure is costly[69].

In *F. perrara*, the T6SS machinery may interact with both the host and other bacteria in the bee gut[73]. The expression of T6SS genes, as well as pilus, colibactin and aryl polyene (APE) biosynthesis genes, is regulated by a DNA-binding protein, the integration host factor (IHF). Deletion of *ihf* impairs the ability of *F. perrara* to colonize the pylorus and form the scab phenotype, suggesting some direct host interaction[73]. Deletion of IHF-regulated genes leads to impaired gut colonization, and/or abolishes scab development[73]. In the presence of a defined community, *F. perrara* mutants that lack a T6SS or APE biosynthesis show reduced colonization, suggesting their advantage in interactions with other bee gut symbionts[73].

Some bee bacterial pathogens, such as *S. marcescens*, encode T6SSs that can antagonize closely related *S. marcescens* strains and *Escherichia coli* (Fig. 1c). In vitro experiments examining the effects of T6SSs from bee-associated *S. marcescens* on *Gilliamella* spp. and *S. alvi* revealed only weak impact on specific *Gilliamella* spp. strains[48]. However, *S. marcescens* T6SSs potentially target Gram-positive bacteria[74], which are abundant in the bee gut.

The roles of T6SSs in the bee gut are not fully defined. Their erratic presence and rapid evolution across strains within species are consistent with roles in ongoing antagonistic co-evolution among competing community members. Potentially, this microbial warfare contributes to the stability of the community, which may in turn provide protection for hosts against invasive pathogens[75].

Bacteriocins are small peptides that exhibit contact-independent antimicrobial properties, resulting in antagonism between bacterial strains or species and thus influencing the composition of gut microbial communities[76]. Although little is known about the roles of bacteriocins in bee gut microbial communities (Fig. 1c,d), bee-associated *Lactobacillus* spp. and *Apilactobacillus kunkeei* strains possess genes that encode bacteriocins and respective immunity genes[62,77]. Various bacteriocins are found in *Lactobacillus* spp. strains associated with bumblebees (for example, lactococcin 972 homologues) versus honeybees (for example, helveticin J homologues)[62] (Fig. 1d).

Bacteriophages can mediate antagonistic and beneficial interactions within bacterial communities, including gut communities[78]. The honeybee gut community includes phages that have co-evolved with the core bacterial lineages[79–81]. At least some of these phages are likely highly specific: for example, matching CRISPR spacers are found across *Gilliamella* spp. genomes within recombining species clusters but not across distinct clusters, such that *G. apis* and *G. apicola* do not share spacers[57]. The most abundant phages target major core members of the bee gut microbiota, such as *Bifidobacterium*, *Gilliamella* and *Lactobacillus* species, but also non-core members such as *Bartonella* species[79–81] (Fig. 1d). These include both temperate and lytic phages that represent undescribed families or genera within *Siphoviridae*, *Myoviridae* and *Podoviridae*, as well as some *Microviridae*, *Inoviridae* and *Caudovirales*. The roles of phages within the bee gut community remain to be elucidated.

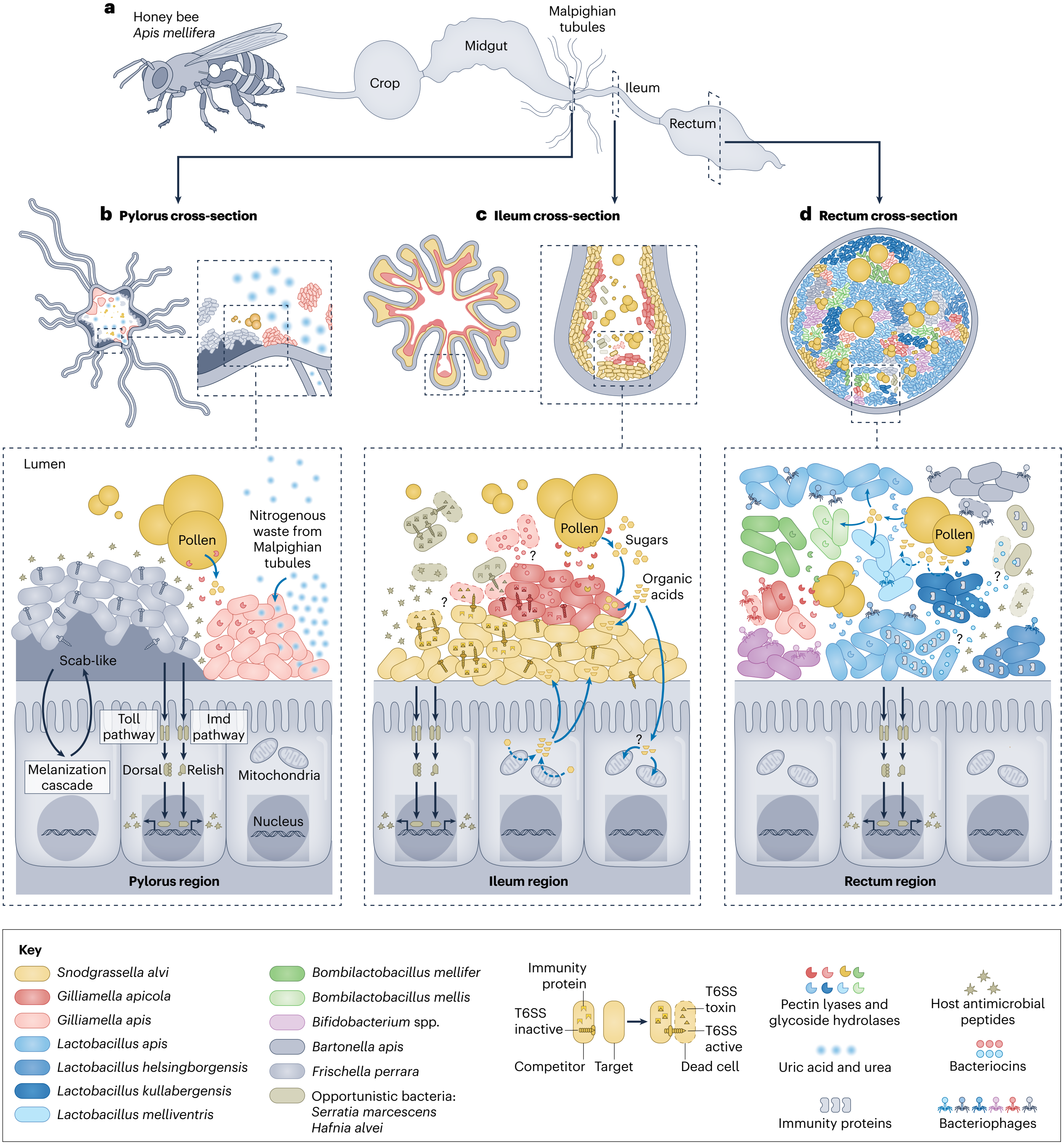
Functions in bee biology and health

Pathogen protection and immune system

Several experimental studies have demonstrated that the gut microbiota can protect honeybees against pathogens[47], including opportunistic bacterial[45,46,48,82] and fungal[83–86] pathogens and potentially against RNA viruses[87] (Fig. 2). These studies have used gnotobiotic honeybees with defined communities or bees with native microbiota disrupted by antibiotics or other agents to investigate effects of the overall community or of specific core members on susceptibility to subsequent pathogen challenge. Often the mechanisms of protection remain unidentified, but studies suggest that enhanced resistance can result from stimulation of host immune pathways[45,46,82,88], competition for space and/or nutrients[48], physical barrier protecting the gut wall from pathogen invasion[48] or production of antimicrobial metabolites[83].

The gut microbiota has a major impact on bee immunity. Colonization by the whole gut community or by single community members upregulates the expression of host immunity genes, such as those that encode antimicrobial peptides (AMPs)[45,46,55,82,88] and the melanization cascade[55]. The honeybee innate immune system provides protection against opportunistic bacteria, fungi and parasites[89] and is broadly categorized into humoral and cellular immunity. Both are initiated by pattern recognition receptors that recognize molecules such as peptidoglycan and lipopolysaccharides from the bacterial outer membrane. Humoral immunity involves the production of AMPs, such as abaecin, apidaecin, defensin and hymenoptaecin, that circulate in different body regions. Cellular immunity involves phagocytosis, nodulation and encapsulation, often accompanied by melanization. The latter involves the activation of phenoloxidase, which results in the formation of melanin able to encapsulate and kill invading microorganisms[89,90]. The details of how the bee gut microbiota influences the immune system are still unclear.

Protection against bacterial pathogens. In comparison with microbiota-deprived bees, bees colonized with a conventional microbiota, with single native gut bacterial strains or with defined communities of several native bacterial strains show improved survivorship following exposure to opportunistic bacterial pathogens, including *S. marcescens*[46,48], *H. alvei*[82,88] and potentially *E. coli*[45] (Fig. 2).



S. marcescens and *H. alvei* are broad-range pathogens that cause sepsis in animals, including humans. Although not widely recognized as bee pathogens, they can kill workers following oral ingestion or wounding[82,88,91–93]. In contrast to larval pathogens, adult pathogens are not conspicuous in hives because sick workers abandon the hive to avoid spreading disease[94,95]. However, loss of adult workers can cause colonies to collapse. Oral exposure to *S. marcescens* causes high rates of mortality in microbiota-deprived bees and in microbiota-perturbed bees but not in bees with a conventional microbiota or with a defined community of core gut bacteria[48,96]. Partial protection is observed in bees monocolonized with single core bacterial strains[48]. A conventional microbiota also limits proliferation of *H. alvei*, potentially

Fig. 1 | Microbial dynamics and spatial organization in the honeybee gut. **a**, Characteristic bacterial communities colonize the distal region of a typical worker honeybee gut (pylorus, ileum and rectum), based on fluorescence in situ hybridization, localization of fluorescently marked strains, 16S rRNA amplicon sequencing and quantitative PCR studies[9,56,57]. **b**, In the pylorus, *Frischella perrara* activates the host immune system, including humoral immunity (Toll and Imd pathways that lead to the production of antimicrobial peptides) and cellular immunity (melanization cascade that leads to the scab-like phenotype observed in this tissue)[55,56]; *Gilliamella apis* is involved in the recycling of waste nitrogen and some degradation and fermentation of polysaccharides present in pollen[57]. **c**, In the ileum, *Snodgrassella alvi* and *Gilliamella apicola* form a stable biofilm[165], activate the host immune system[45,46] and are potentially involved in cross-feeding with one another and the host[64,68]. *G. apicola* produces enzymes for digestion and fermentation of pollen wall components. *S. alvi* can use host-derived organic acids to independently colonize the bee gut[68], but it is unclear whether bees can use bacteria-derived organic acids. **d**, In the rectum, *Lactobacillus* spp. and *Bifidobacterium* spp. are the most abundant bacteria and are involved in digestion and host immune system activation[25,42,88]. A distinctive bacteriophage community is associated with specific members of the microbiota[79–81]. Gut microbiota members possess extensive mechanisms for antagonism, such as type 6 secretion systems (T6SSs) and bacteriocins, and these may fulfil a role in community dynamics and pathogen protection. Question marks indicate unconfirmed processes, such as microbial interactions between and within *S. alvi* and *G. apicola* strains and absorption of bacteria-derived organic acids by bees. Black arrows indicate activation of host immunity pathways and blue arrows indicate metabolism (degradation, uptake and/or utilization). Complexities of bee gut morphology are not depicted in this diagram.

by stimulating the bee immune system[82,88]. Specific strains of *L. apis* induce expression of genes that regulate the Toll pathway, causing increased production of host AMPs such as apidaecin, which strongly inhibits *H. alvei* in vitro[88].

Paenibacillus larvae and *Melissococcus plutonius* are the causal agents of foulbrood diseases[97,98] and are larval pathogens that can spread between hives in the guts of asymptomatic adult bees. Some non-core microbiota members found in larvae and at low abundance in the adult gut, such as *A. kunkeei*, may contribute protection against *P. larvae* and/or *M. plutonius*[77,99,100] (Fig. 2).

Protection against eukaryotic pathogens. The first experimental demonstration of a beneficial effect of bee gut microbiota was microbiota-dependent protection against the trypanosomatid *Crithidia bombi* in *Bombus terrestris*[101,102]. Similar results were later found for *Bombus impatiens*[103]. *C. bombi* has been shown to infect bumblebees by using its flagellum to attach to the ileum wall[104], raising the possibility that the biofilm formed in the ileum by *S. alvi* and *Gilliamella* spp. may serve as a protective physical barrier. In vitro studies with bumblebee- and honeybee-associated *Lactobacillus* species suggest the production of metabolites that inhibit *Crithidia* spp[49,105].

Bombella apis (previously called *Parasaccharibacter apium*), a bacterial symbiont associated with honeybee larvae, inhibits two fungal pathogens in vitro, *Beauveria bassiana* and *Aspergillus flavus*, and protects larvae against *A. flavus*[83], but not against the bacterial pathogen *M. plutonius*[106]. Fungal protection is probably achieved through the production of specific antifungal metabolites (Fig. 2).

Whether the gut microbiota protects against members of the microsporidian *Vairimorpha* genus (formerly *Nosema*), the most common eukaryotic parasite of honeybees, remains unclear. In contrast to trypanosomatids, which infect hosts through the hindgut, *Vairimorpha* spp. invade the host through the wall of the midgut, potentially limiting protection by the hindgut community. Monocolonization of honeybees with *S. alvi* strains has shown some reduction in *V. ceranae* spore loads[84] and increased bee survival[85]. Disruption of the microbiota using antibiotics has been shown to increase *V. ceranae* spore loads[86]; conversely, *V. ceranae* infection itself can lead to microbial dysbiosis[107].

Protection against viruses. RNA viruses are common and harmful pathogens of honeybees. There is limited experimental evidence to suggest that the core gut microbiota has a role in viral tolerance. Studies have shown that microbiota-deprived bees had lower survival rates than conventionalized bees when exposed to deformed wing virus[87]. However, viral titres were not affected in these bees[87]. Other studies have found correlations between viral infection and the composition or size of the gut microbiota[108,109]. Further investigation is needed to confirm and, if so, elucidate the mechanisms and dynamics that underlie microbiome-mediated protection against viruses, whether the protection primarily arises from the microbiome's role in enhancing bee health and immune system function or from direct mechanisms within the microbiome that contribute to viral tolerance or resistance.

Role in development and behaviour

Adult worker honeybees undergo distinct developmental changes after emergence from the pupal stage and these are accompanied by weight gain and behavioural shifts, which are in turn linked to changes in expression of key developmental genes, including those that affect juvenile hormone titres, insulin signalling and vitellogenin[1,3]. Recent experimental studies suggest that the gut microbiota can influence these aspects of bee biology (Fig. 3).

In some studies that compared microbiota-deprived bees with conventionalized bees, the former exhibited reduced weight gain during early adulthood[50,52] and abnormal guts characterized by elevated oxygen and pH levels. These gut changes are expected in the absence of oxygen depletion by *S. alvi* and short-chain fatty acid production by *Gilliamella* spp. and other fermenters[50]. Microbiota-deprived bees also had suppressed expression of developmental genes, including vitellogenin and genes involved in the insulin pathway in head, abdomen or whole bee body samples[50,51], and changes in levels of other hormones, such as prostaglandins and juvenile hormone III derivatives, in gut samples[64].

Although these effects are usually attributed to the complete microbiota, in some instances, they have been attributed to specific symbionts. For instance, monocolonization by *Bifidobacterium asteroides* elevates the gut concentration of juvenile hormone III derivatives[64]. Juvenile hormone III is a key regulator of insect growth, development and reproduction. In honeybees, it governs the transition from nurse bees to forager bees[1,110], a process influenced by nutrition[111] and potentially impacted by gut bacterial metabolism. Juvenile hormone III derivatives can affect insect gut functioning[112], but their roles in the bee gut are not known.

The gut microbiota also seems to influence bee behaviour. Proboscis extension response assays, which measure feeding reactions to gustatory or olfactory stimuli, have been used to study the roles of the gut microbiota on sucrose sensitivity, olfactory learning and memory abilities of honeybees. The full native gut microbiota, with its high strain diversity, appears to have a role in normal taste-related behaviour in honeybees. Conventionalized bees are more sensitive to lower doses

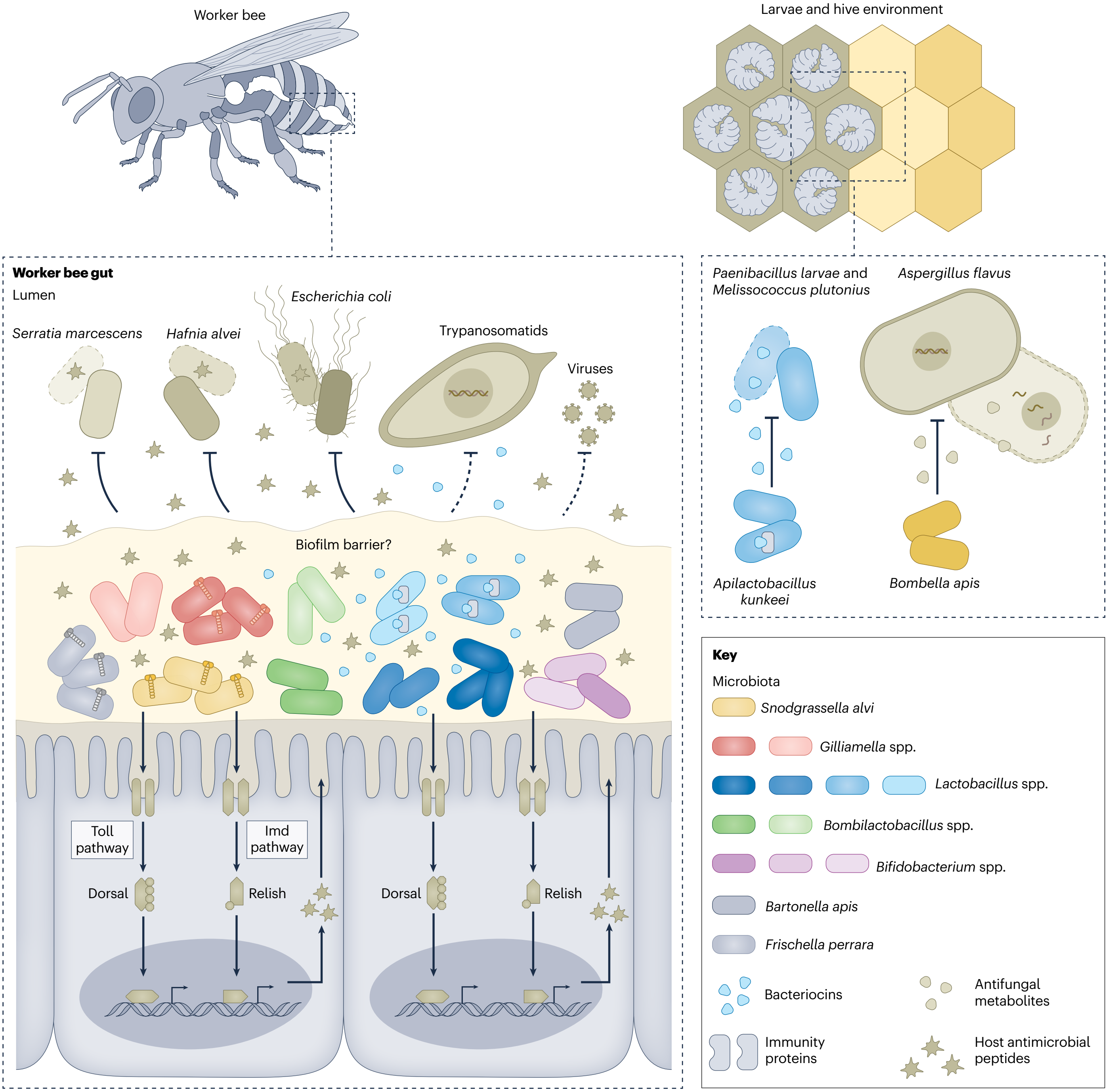


Fig. 2 | The roles of the honeybee gut microbiota in pathogen protection. Members of the microbiota protect honeybees from prokaryotic and eukaryotic pathogens. Protection may arise from activation of the bee innate immune pathway, as in the case of protection by *Snodgrassella alvi* against *Serratia marcescens*[46] and potentially *Escherichia coli*[45], and *Lactobacillus* spp. against *Hafnia alvei*[88] and potentially trypanosomatids[49]. Protection can occur also from production of antimicrobial molecules (for example, *Bombella apis* protection against *Aspergillus flavus*[83], *Apilactobacillus kunkeei* protection against *Paenibacillus larvae* and *Melissococcus plutonius*[77,100]) or from formation of a stable biofilm that forms a physical barrier on the gut wall[48]. An intact microbiota provides greater protection[48]. The top left shows a worker honeybee with a simplified image of the gut. The top right shows a piece of frame comb from a hive, in which cells have different contents, including larvae (brown), pollen (yellow) and nectar (orange). Solid arrows indicate activation of specific immunity pathways, solid lines indicate inhibition of specific pathogens and dashed lines indicate potential inhibition of specific pathogens. Complexities of bee gut morphology are not depicted in this diagram.

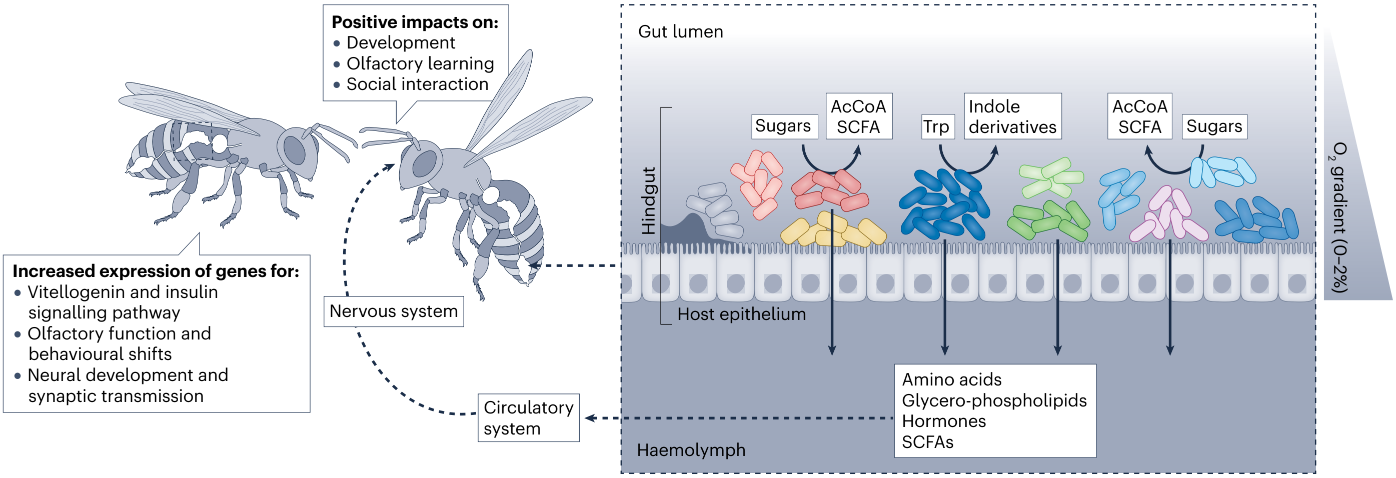


Fig. 3 | The roles of the honeybee gut microbiota in development and behaviour. An intact microbiota is associated with increased expression of genes for vitellogenin and insulin signalling pathway, olfactory functions and behavioural shifts, neural development and synaptic transmission, and increased abundance of amino acids, glycerophospholipids, hormones and short-chain fatty acids (SCFAs) in the gut, haemolymph and/or brain tissues[50,53,64,113,115]. These metabolites are associated with gut physiology, oxygen concentrations, pH, redox potential and with regulation of developmental and behavioural genes, olfactory learning and social interactions[50,53,113,115]. Curved solid arrows indicate microbial metabolism, straight solid arrows indicate host uptake of microbially derived by-products and dashed arrows indicate movement of microbially derived metabolites within the honeybee body. Colours of the bacterial taxa correspond to those in Fig. 1. AcCoA, acetyl coenzyme A; Trp, tryptophan.

of sucrose than are microbiota-deprived bees[50,113]. However, this effect was not observed for a defined community of specific native strains[70]. On the other hand, honeybees colonized with a conventional or defined microbiota of native bacterial strains exhibit higher learning rates than microbiota-deprived or antibiotic-treated bees[53,70].

In bumblebees, the gut microbiota seems to drive individual memory variation, with one study showing a positive correlation between the abundance of *L. apis* and memory retention[114]. Bumblebees supplemented with a strain of *L. apis* displayed improved long-term memory retention, based on a visual discrimination foraging test[114]. This was accompanied by increased levels of glycerophospholipids in the haemolymph, which is associated with enhanced long-term memory[114].

Some experimental evidence suggests potential routes by which the bee gut microbiota could affect brain function and thus behaviour. A direct connection between the bee gut and the nervous system could be mediated by the haemolymph metabolome, which is shaped in part by the gut microbiota[50,53,115,116] (Fig. 3). Metabolomic analyses demonstrate distinct profiles in the gut[50,64], the haemolymph and brain tissues[53,115] of microbiota-colonized bees compared with microbiota-deprived bees. These profiles show consistently increased levels of amino acids and intermediates of amino acid metabolism. For example, specific members of the microbiota may impact the metabolism of tryptophan, an essential amino acid for honeybees, in the gut and haemolymph samples[53]. When supplemented with tryptophan, a specific strain of *L. apis* promoted memory in honeybees, possibly by transforming tryptophan to indole derivatives that activate the host aryl hydrocarbon receptor[53]. Additionally, the gut microbiota appears to influence carbohydrate and glycerophospholipid metabolism in the haemolymph[113].

Bee gut bacteria have been reported to alter brain neurotransmitter levels directly. The levels of biogenic amines with inhibitory effects on sensory sensitivity, such as dopamine and serotonin, are downregulated in bees monocolonized with *Bombilactobacillus*, *Gilliamella* or *Lactobacillus* species[113].

Brain transcriptomes of conventionalized bees and bees monocolonized with *Bifidobacterium*, *Bombilactobacillus* or *Lactobacillus* species exhibit elevated expression of genes related to olfactory functions (for example, odorant binding proteins and receptors) and/or genes that affect caste determination and age polyethism, which is the phenomenon whereby an animal shows different behaviour at different ages (for example, genes that underlie the major royal jelly protein)[53,113,115]. Moreover, differentially spliced genes in the brains of bees monocolonized with *Bombilactobacillus* or *Lactobacillus* species are enriched for neural development and synaptic transmission pathways[113].

The honeybee gut microbiota also appears to modulate the colony social network, influencing interactions between nestmates through changes in chromatin accessibility and amino acid biosynthesis[115]. Bees colonized with a conventional microbiota have increased head-to-head interactions with nestmates and exhibit greater specialization and stronger social ties with specific subsets of nestmates compared with microbiota-deprived bees[115]. These effects may be linked to higher levels of specific brain metabolites, such as serine and ornithine, which are known to be involved in synaptic transmission[117] and correlated with the numbers of nestmate interactions[115]. These findings highlight a potential role of the gut microbiota in promoting and organizing social interactions within the honeybee colony[115].

It is important to note that studies on microbiota effects on bee development and behaviour are challenged by the fact that larval development occurs under varying hive conditions, which have been shown to affect adult phenotypes[118]. Moreover, we note that only some of these results have been replicated, so their generality among bee genotypes, microbiota strains and environmental conditions is not yet certain.

Role in nutrition and detoxification

The microbiota primarily colonizes the bee hindgut (ileum and rectum) (Fig. 4). Readily accessible nutrients, such as sugars in nectar and amino acids in pollen germ cells, are processed and absorbed in the midgut, leaving primarily refractory components of the pollen coat to enter the ileum (Fig. 4a), along with the nitrogenous waste products of the Malpighian tubules (Fig. 1b). Genomic analyses have demonstrated that specific members of the bee gut microbiota have extensive capabilities for digestion of polysaccharides and for transport and metabolism of the released sugars. Genes for pectin lyases, glycoside hydrolases and sugar transport and use are found in bee-restricted *Bifidobacterium* spp., *Bombilactobacillus* spp., *Gilliamella* spp. and *Lactobacillus* spp., and the presence of these genes varies between strains[41,42,62] (Fig. 4b). Specific strains of these four bacterial genera can uptake and metabolize mannose, arabinose, xylose and rhamnose, sugars known to be toxic for bees if accumulated in the gut[43,44]. Genes that underlie these capabilities were probably acquired from members of the phylum Bacillota (formerly called Firmicutes) through horizontal gene transfer[43].

The ability of the gut microbiota to metabolize plant polysaccharides and other dietary components has potential consequences for both bee nutrition and detoxification. At least some of the short-chain fatty acids released from bacterial metabolism are taken up by hosts, and these, especially butyrate, dominate in the bee haemolymph[50]. However, the extent to which bacterial digestion of pollen coats contributes to bee nutrition is currently unknown.

Protein is often limited in bee diets[119], and the microbiota has the potential to contribute to the bee nitrogen budget through the recycling of nitrogenous waste that enters from the Malpighian tubules at the midgut–ileum junction. *S. alvi* and *G. apis* have genes for urea utilization[57], and several bee gut bacteria have complete pathways for amino acid biosynthesis[42]. Although uptake of amino acids in the hindgut is not documented in bees, absorption could occur through unknown mechanisms such as backflow into the midgut extraperitrophic space. In comparisons between microbiota-deprived bees and conventionalized bees, the latter usually exhibit increases in amino acids and/or amino acid derivatives in both the hindgut[50,64] and the haemolymph[50,53,115].

Bee gut bacteria have been shown to play a part in metabolizing recalcitrant plant secondary metabolites, including flavonoid glycosides[64], cyanogenic glycosides[63] and others[65], primarily by deglycosylation of these metabolites (Fig. 4b). The consequences of the release of aglycones are understudied, but some studies point to

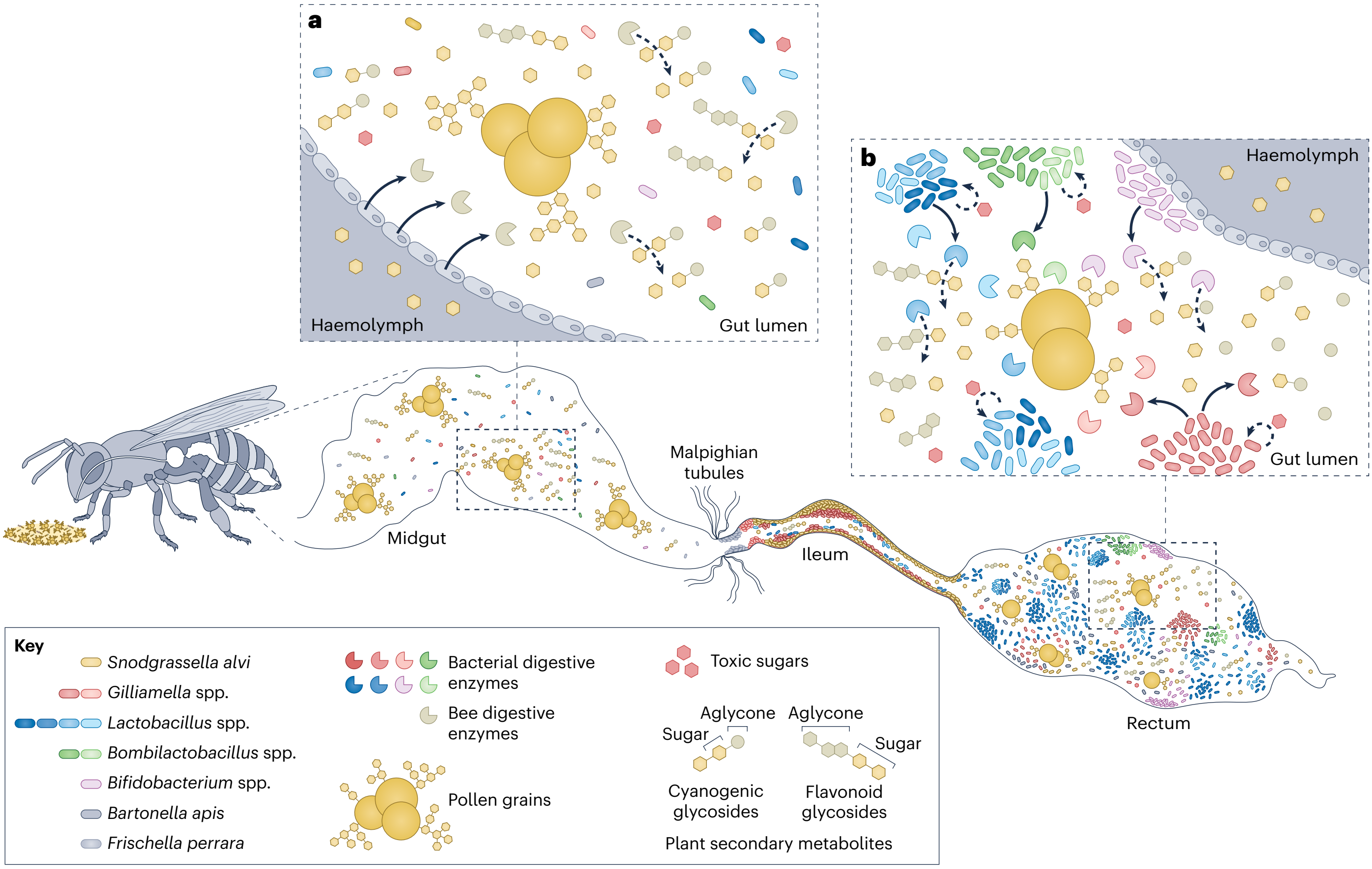


Fig. 4 | The roles of the honeybee gut microbiota in digestion and detoxification. a, Easy-to-digest components from nectar (for example, some polysaccharides and simple sugars) and pollen (for example, amino acids, lipids, vitamins) are absorbed or metabolized by bee enzymes in the midgut. Metabolism of polysaccharides from the pollen coat releases several simple sugars that may be metabolized (for example, fructose and glucose) or not (for example, arabinose, galactose, mannose and rhamnose) by bee enzymes. **b**, Hard-to-digest components, including refractory polysaccharides[41], toxic sugars[43,44] and plant secondary metabolites such as flavonoid and cyanogenic glycosides[61,63,65], are primarily metabolized by specific strains of major members of the native microbiota (for example, *Gilliamella* spp., *Bifidobacterium* spp., *Bombilactobacillus* spp. and *Lactobacillus* spp.) in the ileum and rectum, through the production of pectin lyases and glycoside hydrolases[42,64]. Solid arrows indicate production and release of digestive enzymes, and dashed arrows indicate microbial metabolism of toxic sugars and plant secondary metabolites.

activation or deactivation of these bioactive products. For example, the full metabolism of amygdalin, a cyanogenic glycoside found in honeybee-pollinated almond trees, is only possible in the presence of the microbiota[63]. Although bee enzymes can metabolize amygdalin into an intermediate, prunasin, this intermediate accumulates in the bee gut only if the microbiota is absent. Microbial metabolism of prunasin leads to the release of hydrogen cyanide, a toxic chemical for aerobic organisms. Whether this full metabolism is toxic for bees deserves investigation, but it seems that naturally occurring amygdalin concentrations in nectar and pollen do not affect the microbiota and may even prevent parasite proliferation under hive conditions[66].

Other nectar secondary metabolites, such as tiliaside from linden trees and unedone from strawberries, are known to be metabolized (glycosylated or deglycosylated) by bumblebees and their gut microbiota[65]. This metabolism leads to activation or inactivation of activity against *C. bombi*. For example, deglycosylation of tiliaside by host or microbial enzymes is required for activity during gut passage[65]. The aglycone unedone, on the other hand, has both in vitro and in vivo antiparasitic activity. Bee enzymes glycosylate and therefore inactivate unedone in the midgut, and microbial enzymes deglycosylate and reactivate it in the hindgut[65].

Potentially, the microbiota interacts with the host to promote or limit processing of dietary components and other chemicals. For example, microbiota-deprived and antibiotic-treated bees show reduced expression of cytochrome P450 genes in the midgut and increased accumulation of pesticides in their bodies[120]. Overall, there is still limited evidence on how the bee gut microbiota plays a part in detoxifying xenobiotics.

Impact of agricultural practices

Honeybees are often exposed to agrochemicals used in beekeeping (for example, antibiotics and acaricides) or in agriculture (for example, pesticides). Sometimes these chemicals, particularly insecticides, directly harm bees, whereas others may have sublethal impacts, including impacts mediated by disruption of the microbiota.

Beekeeping practices

Early studies on the effects of agrochemicals on the bee gut microbiota concerned antibiotic exposure[121]. Antibiotics are used in beekeeping for the prevention or treatment of larval infections, such as those that cause foulbrood diseases. However, owing to their broad spectrum of action, antibiotics can also impact the adult or larval gut microbiota. For instance, tetracycline, widely used in beekeeping in some countries since the 1950s, has been shown to decrease the abundance of core gut bacteria, including *S. alvi* and species of *Bifidobacterium*, *Lactobacillus* and *Bombilactobacillus* (Fig. 5), and increase mortality rates within the hive environment and susceptibility to *S. marcescens*[96]. Other studies have corroborated the impacts of tetracycline on the adult bee microbiota[122,123]. These impacts can occur despite high levels of tetracycline resistance in some core bee gut bacteria[121].

Tylosin is another antibiotic commonly used in beekeeping that has detrimental effects on the bee gut microbiota in both laboratory[124] and hive conditions[123,125,126], and increases susceptibility to *S. marcescens* in the laboratory[125]. Additionally, mixtures of penicillin and streptomycin lead to increased susceptibility to *V. ceranae* and downregulate the expression of host AMPs such as abaecin, defensin 1 and hymenoptaecin under laboratory conditions[86].

Not surprisingly, antibiotic treatment reduces bacterial loads in the larval gut and impacts nutrient metabolism, body weight gain, development and immune competence of larvae[127]. The expression of host AMPs abaecin, apidaecin, defensin 1 and hymenoptaecin, for example, is reduced at specific stages of larval development upon exposure to penicillin–streptomycin[127].

Antibiotics potentially have direct negative effects on bees[126,128], which can be difficult to distinguish from those arising owing to impacts on the microbiota. However, in a control experiment on microbiota-deprived bees in the lab, tetracycline had no negative impact on bee survival[96]. Also, the increased susceptibility to *S. marcescens* caused by antibiotic exposure echoes that of microbiota-deprived bees[48], consistent with a role of microbiota perturbation.

Acaricides, such as flumethrin, are commonly used in beekeeping for the treatment and/or prevention of infestation by mites, primarily *Varroa destructor*. *V. destructor* attaches to the bee exoskeleton and feeds on fat bodies and haemolymph, thereby spreading viruses such as deformed wing virus, which also impact bee health[129]. Flumethrin exposure leads to overexpression of immune- and detoxification-related genes, and decreases microbial abundance and diversity in the larval gut[130], but it seems to have limited effects on the adult gut microbiota composition[131]. Other acaricides used to control *V. destructor*, such as coumaphos and *tau*-fluvalinate, can affect microbial diversity associated with adult honeybees[132,133]. Potential safer alternatives for combating *V. destructor* include the use of menthol, thymol and oxalic acid[134], found naturally in honey and plants. Oxalic acid, however, has been shown to inhibit growth of specific *Lactobacillus* species in vitro[134] and impact microbial composition, including reduction in strain richness, in adult honeybees[135].

Agrochemicals encountered by foragers

In addition to exposure to chemicals used in beekeeping, foragers can be exposed to agrochemicals, such as insecticides, herbicides and fungicides used on crops (Fig. 5). Foragers deliver these back to the hive, where they can accumulate in food stores, thus exposing larvae and young bees.

Insecticides affect bees primarily through direct toxicity, but sublethal effects including gut microbial perturbations have also been detected. Neonicotinoids are widely used broad-spectrum neurotoxic insecticides[136] that are less toxic to mammals than are long-standing insecticides such as carbamates, organophosphates and pyrethroids[137]. Neonicotinoids, such as acetamiprid, sulfoxaflor and thiacloprid, can affect microbial diversity in the bee gut, although these effects may reflect other impacts on bee physiology, as exposure reduces survivorship and appetite[135,138,139]. Other experimental studies on both honeybees and bumblebees found no impacts of imidacloprid on the gut microbiota and little or no ability of the microbiota to metabolize imidacloprid[140,141] (Fig. 5).

Some herbicides have antimicrobial properties and can indirectly affect bees through effects on the microbiota. Glyphosate, the most used herbicide globally, inhibits an enzyme in the shikimate pathway (5-enolpyruvylshikimate-3-phosphate synthase) that is required for the production of essential amino acids in plants and most microorganisms. Experimental studies have shown that some core bee gut bacteria are susceptible to glyphosate. The gut microbiota species most consistently impacted by glyphosate exposure is *S. alvi*, with this effect being dose dependent[124,142–145] (Fig. 5). Impacts on gut microbiota composition are observed when honeybees are exposed to glyphosate concentrations documented in nectar and pollen of recently exposed plants[146], and can also occur in bumblebees, in which effects appear milder and less persistent[147–149].

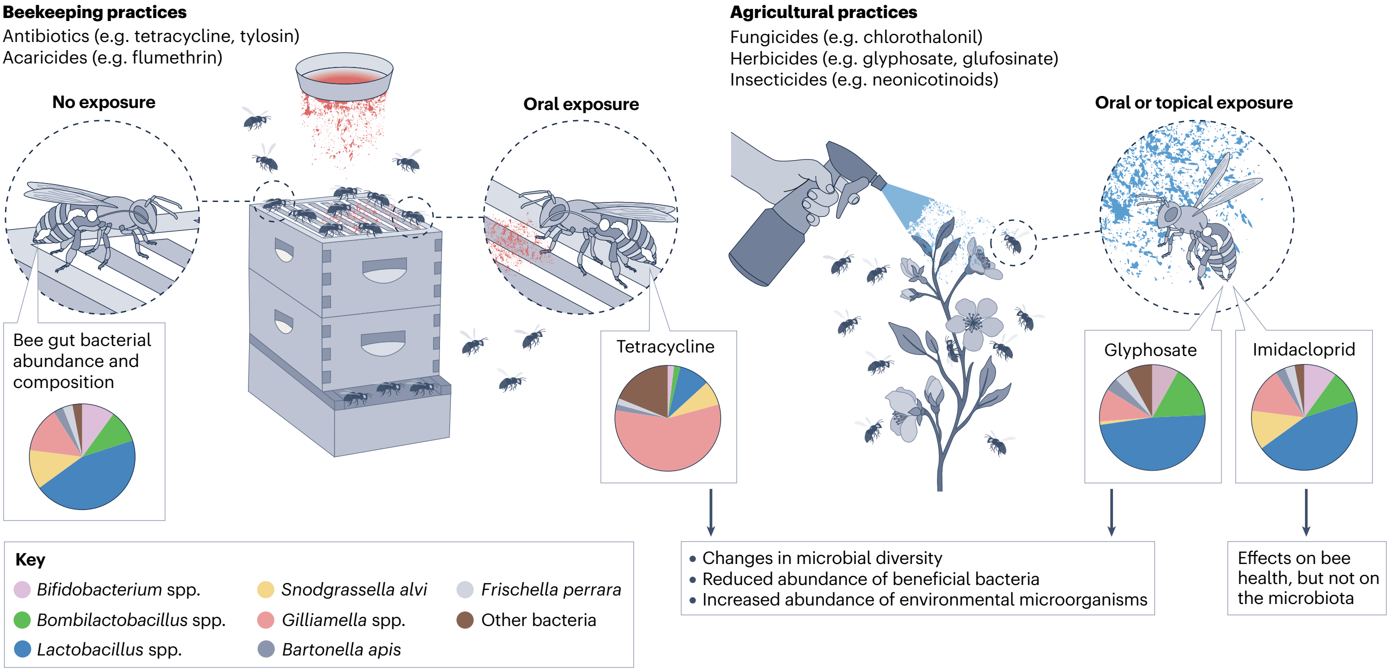


Fig. 5 | Beekeeping and agricultural practices that affect honeybee gut communities. In beekeeping, the overuse of antibiotics and acaricides for the treatment of larval infections and mite infestation, respectively, can negatively impact the abundance and composition of beneficial bacteria in the adult worker bee microbiota[125,130], with potential consequences for bee health, such as increased susceptibility to infections and higher mortality rates[125]. Similarly, the indiscriminate use of fungicides, herbicides and insecticides in agriculture can negatively impact the adult worker bee microbiota, but effects are highly variable depending on the compounds involved and exposure level[138,140,151]. From left to right, pie charts illustrate the relative abundance of bee gut bacteria under normal conditions[4] and under exposure to tetracycline[96], glyphosate[151] or imidacloprid[140].

Glyphosate exposure associated with microbiota disruption can impact the expression of host AMPs, including apidaecin, defensin and hymenoptaecin[150] and lead to increased susceptibility to *S. marcescens*[143,151], but not to *V. ceranae*[142,144]. Exposure also promotes deformed wing virus replication and decreases vitellogenin expression[144]. Additionally, glyphosate exposure impacts bee physiology, including antioxidant and detoxification systems, learning and memory, and behaviour, which has been extensively reviewed[152]. The extent to which these effects are direct or mediated by the gut microbiota is unknown.

Fungicides can also affect the honeybee gut microbiota. Chlorothalonil, a non-systemic organochlorine fungicide and one of the most used fungicides in agriculture, can perturb gut bacterial communities of adult bees[132] and increase susceptibility to *V. ceranae* infection[153]. Chronic exposure to field-realistic concentrations of azoxystrobin, a broad-spectrum fungicide commonly used in agriculture, impacts both fungal and bacterial communities in the honeybee gut and can result in an increase in the relative abundance of *Serratia*[154].

Antibiotics are used not only in beekeeping but also in agriculture to control bacterial pathogens in plant crops, and bees can be exposed to antibiotics during foraging activities. A study that compared antibiotic resistance genes in gut bacteria of honeybees from the United States and Norway revealed a high incidence of streptomycin resistance in the US samples, where streptomycin is sprayed on fruit trees to protect against fire blight (*Erwinia amylovora*) but not in samples from Norway, where streptomycin is not used[155].

Nutritional impacts on microbiota

Diet is widely documented to affect the composition of animal gut microbiota. Honeybees experience extensive variation in diet owing to varying availability of flowering plant species or to artificial dietary supplementation by beekeepers. Several studies show that these dietary variables can affect the honeybee gut microbiota and increase susceptibility to pathogens. For example, sucrose supplementation appears to lower the abundance of core gut species in relation to potentially pathogenic *Serratia* spp. in bee guts[156]. Bees fed nutritionally poor-quality pollen exhibit lower abundance of *Bombilactobacillus*, *Lactobacillus* and *Bifidobacterium* species, and higher abundance of the non-core species *Bartonella apis*, than do bees fed polyfloral pollen[157]. Feeding on poor-quality pollen also results in lower expression of vitellogenin and immunity genes, and increased proliferation of *Vairimorpha*[157].

Beekeepers often provide hives with protein supplements that contain products such as soy protein or casein that are absent from the natural bee diet. In a recent study, young adult bees with conventional microbiota were given either dietary supplements or pollen for 14 days, then sampled to examine microbiota size and composition as well as expression of genes involved in development and immunity[158]. In bees given the artificial diets, gut communities were larger in absolute numbers of bacteria, but showed lower diversity of sequence variants, lower evenness and higher incidence of bacteria atypical for bee guts, such as *Streptococcus* spp. and *Staphylococcus* spp. The artificial diet also resulted in lower expression of juvenile hormone esterase and vitellogenin and in higher susceptibility to the pathogen *S. marcescens*. These results were largely consistent across hives at two locations.

Potential for probiotics in bees

The use of probiotics that aim to treat or prevent microbial infections in hives is common in beekeeping. Recent reviews have summarized the studies in the bee probiotics field[51,159]. Most commercially available bee probiotics consist of non-native microorganisms, including bacteria and fungi from the food industry, which are marketed as promoting bee health, although they do not stably colonize bees[51,160]. An alternative approach involves probiotics that consist of native microorganisms that colonize and persist in the bee gut[51]. Orally delivered gut homogenates are one way to transfer bacteria from healthy worker bees to bees that lack microbiota or those with perturbed microbiota. Gut homogenate treatments lead to stable colonization in young bees under laboratory conditions, but potentially introduce pathogens from donor bees. Defined communities of isolates of native core bacteria are another approach[48,70,82,125]. Such defined communities can counteract perturbations caused by agrochemicals and other environmental stressors and prevent the proliferation of opportunistic pathogens that often follows perturbation[48,82,125]. However, these studies have been primarily conducted in laboratory settings, and further hive-level studies are necessary to evaluate the efficacy of probiotics for beekeeping.

Probiotic approaches may be effective ways to prevent or treat *P. larvae* in hives. In a study of two control hives and two hives treated with a bacterial consortium consisting of *A. kunkeei*, *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus*, the treated hives seemed to have lower levels of *P. larvae* and less immune dysregulation[161]. However, treating *P. larvae*-infected hives with a probiotic mixture of hive- and gut-associated *Lactobacillus* spp. and *Bifidobacterium* spp. strains, with or without antibiotic treatment, did not improve colony fitness[162,163]. These varying outcomes between studies may reflect differences in experimental design, execution or the condition of the study hives. Delivery methods may also influence the impact of probiotics on hive fitness[164].

Engineered bee gut strains offer an alternative strategy to improve bee health[165,166]. *S. alvi* was engineered to express double-stranded RNA targeting deformed wing virus or *V. destructor* through the bee or mite RNA interference pathways[167]. In bees colonized with the engineered *S. alvi* strain in the laboratory, viral proliferation was suppressed, and mites suffered elevated mortality[167]. Similarly, *S. alvi* was engineered to express double-stranded RNA targeting the microsporidian parasite *V. ceranae*, with different essential genes selected in two independent studies[84,85]. In both studies, bees monocolonized with the engineered *S. alvi* strain in the laboratory had reduced *V. ceranae* spore loads.

Although some results are promising, the potential for using probiotics in honeybees is still unclear, particularly under field conditions.

Summary and future directions

Studies to date support a substantial influence of the honeybee gut microbiota on host digestion, detoxification, behaviour, pathogen protection and immune system. Bees deprived of their normal microbiota and bees in which the microbiota is disrupted by chemicals show a range of health deficits including changes in feeding behaviour, greater susceptibility to pathogens and higher mortality in the hive itself. Experimental colonization of gnotobiotic hosts with single or multiple microbiota members can restore at least some benefits of the full bee microbiota.

Although considerable evidence points to benefits of gut symbionts for bees, the molecular mechanisms behind these effects are largely unknown. For example, specific members of the gut microbiota have been shown to prevent pathogen proliferation and to protect hosts from pathogen-induced mortality, but it is unknown whether protection results from host immune responses and/or direct interactions between microorganisms. Final effectors of immunity pathways (for example, AMPs) are upregulated in specific bee body tissues, but the identities of the microbial effectors that trigger these pathways are unknown. Biofilm formation, as observed for *S. alvi* in the ileum, appears to be a crucial component of successful colonization[69], but the triggers for biofilm formation and whether and how biofilm blocks pathogens have not been determined. Genomic analyses have shown the potential abilities of the core bacteria to interact with each other by contact-dependent (for example, T6SS) or contact-independent (for example, bacteriocins) ways. Future studies should focus on elucidating these molecular mechanisms.

Studies of the roles of the bee microbiota in toxin metabolism are still incipient. Although some studies have investigated how xenobiotics, including agrochemicals and specific plant secondary metabolites, are metabolized, the consequences of such metabolism for bee health are largely unknown. The impacts of agrochemicals on gut microbial communities may stem from bee mechanisms (for example, cytochrome P450s) or from metabolic capabilities (for example, hydrolases) of specific gut symbionts.

Another recent research direction involves the gut–brain axis. Honeybees have long been used as models to study behaviour, ranging from cognition to social interactions, and behavioural assays are well developed. Recent studies have taken advantage of these behavioural assays and gnotobiotic bees to explore the roles of the microbiota in taste, olfactory learning and colony social network, and in shaping transcriptomic and metabolomic profiles in different compartments of the bee body. Results suggest that members of the native microbiota act together to shape bee behaviour. Linking effects of the microbiota on behaviours to changes in gene expression and metabolites in the haemolymph and brain tissues[115] is a promising next step to fill the causation gap in this emerging field.

For both fundamental and applied research goals, one challenge is the variability among the bees themselves. Although an advantage of studying honeybees is their global distribution and the opportunity to study them under natural hive conditions, these same factors introduce complications. *A. mellifera* varies genetically, with different breeds or subspecies in different regions. It also varies according to environmental conditions, such as nectar and pollen sources and quantities, season, climate and weather, and exposure to environmental toxins and pathogens. Hives from the same apiary often differ in genetics and physiological condition. For example, nutrient scarcity during larval development, which occurs when floral resources are limited, can have major consequences for the metabolism, behaviour and development of the resulting adult workers[118,168]. Researchers use honeybee colonies typical for their geographical area and perform experiments during different seasons. In the future, it will be important to replicate results for bees from different genetic and environmental backgrounds to understand how these variables affect the roles of the gut microbiota.

Honeybees are exposed to environmental stressors encountered in hives and their surroundings. These stressors, including anthropogenic chemicals and long-distance transport, often impact the gut microbiota. However, most studies to date have limitations. Usually, they examine only relative abundance of gut community members, whereas measures of absolute abundance, using quantitative PCR or other approaches, are needed for robust interpretations. Moreover, agrochemicals are usually deployed along with co-formulants, but these are rarely investigated, although they sometimes exert stronger impacts than the active ingredient. For example, pure glyphosate does not lead to increased susceptibility to *Vairimorpha*, but a glyphosate-based formulation does[169]. Future studies aimed at evaluating impacts should consider both active chemicals and co-formulants. Another question rarely examined in these studies is community resilience, that is, how long detrimental impacts persist after perturbation. Moreover, identifying impacts on gut microbiota is not meaningful without examining whether these extend to effects on bee health.

Most research on the bee microbiota has focused on the honeybee, *A. mellifera*, with a more limited number of studies on the commercially available bumblebees, *B. impatiens* and *B. terrestris*. Little is known about factors that affect the microbiota of other bee species, many of which are declining in numbers. More research on microbiomes of a diversity of bee species will undoubtedly lead to new discoveries and potentially contribute to the conservation of wild pollinators.

References

1. Robinson, G. E., Page, R. E. Jr, Strambi, C. & Strambi, A. Hormonal and genetic control of behavioral integration in honey bee colonies. *Science* **246**, 109–112 (1989).
2. Menzel, R. The honeybee as a model for understanding the basis of cognition. *Nat. Rev. Neurosci.* **13**, 758–768 (2012).
3. Zayed, A. & Robinson, G. E. Understanding the relationship between brain gene expression and social behavior: lessons from the honey bee. *Annu. Rev. Genet.* **46**, 591–615 (2012).
4. Kwong, W. K. & Moran, N. A. Gut microbial communities of social bees. *Nat. Rev. Microbiol.* **14**, 374–384 (2016).
5. Romero, S., Nastasa, A., Chapman, A., Kwong, W. K. & Foster, L. J. The honey bee gut microbiota: strategies for study and characterization. *Insect Mol. Biol.* **28**, 455–472 (2019).
6. Cox-Foster, D. L. et al. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**, 283–287 (2007).
7. Martinson, V. G. et al. A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol.* **20**, 619–628 (2011).
8. Moran, N. A., Hansen, A. K., Powell, J. E. & Sabree, Z. L. Distinctive gut microbiota of honey bees assessed using deep sampling from individual worker bees. *PLoS ONE* **7**, e36393 (2012).
9. Martinson, V. G., Moy, J. & Moran, N. A. Establishment of characteristic gut bacteria during development of the honeybee worker. *Appl. Environ. Microbiol.* **78**, 2830–2840 (2012).
10. Powell, J. E., Martinson, V. G., Urban-Mead, K. & Moran, N. A. Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl. Environ. Microbiol.* **80**, 7378–7387 (2014).
11. Zheng, J. et al. A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *Int. J. Syst. Evol. Microbiol.* **70**, 2782–2858 (2020).
12. D'Alvise, P. et al. The impact of winter feed type on intestinal microbiota and parasites in honey bees. *Apidologie* **49**, 252–264 (2018).
13. Corby-Harris, V., Maes, P. & Anderson, K. E. The bacterial communities associated with honey bee (*Apis mellifera*) foragers. *PLoS ONE* **9**, e95056 (2014).
14. Rothman, J. A., Carroll, M. J., Meikle, W. G., Anderson, K. E. & McFrederick, Q. S. Longitudinal effects of supplemental forage on the honey bee (*Apis mellifera*) microbiota and inter- and intra-colony variability. *Microb. Ecol.* **76**, 814–824 (2018).
15. Kapheim, K. M. et al. Caste-specific differences in hindgut microbial communities of honey bees (*Apis mellifera*). *PLoS ONE* **10**, e0123911 (2015).
16. Wu, J. et al. Honey bee genetics shape the strain-level structure of gut microbiota in social transmission. *Microbiome* **9**, 225 (2021).
17. Kešnerová, L. et al. Gut microbiota structure differs between honeybees in winter and summer. *ISME J.* **14**, 801–814 (2020).
18. Jones, J. C. et al. Gut microbiota composition is associated with environmental landscape in honey bees. *Ecol. Evol.* **8**, 441–451 (2018).
19. Olofsson, T. C., Alsterfjord, M., Nilson, B., Butler, È. & Vásquez, A. *Lactobacillus apinorum* sp. nov., *Lactobacillus mellifer* sp. nov., *Lactobacillus mellis* sp. nov., *Lactobacillus melliventris* sp. nov., *Lactobacillus kimbladii* sp. nov., *Lactobacillus helsingborgensis* sp. nov. and *Lactobacillus kullabergensis* sp. nov., isolated from the honey stomach of the honeybee *Apis mellifera*. *Int. J. Syst. Evol. Microbiol.* **64**, 3109–3119 (2014).
20. Engel, P., Kwong, W. K. & Moran, N. A. *Frischella perrara* gen. nov., sp. nov., a Gammaproteobacterium isolated from the gut of the honeybee, *Apis mellifera*. *Int. J. Syst. Evol. Microbiol.* **63**, 3646–3651 (2013).
21. Kešnerová, L., Moritz, R. & Engel, P. *Bartonella apis* sp. nov., a honey bee gut symbiont of the class Alphaproteobacteria. *Int. J. Syst. Evol. Microbiol.* **66**, 414–421 (2016).
22. Ludvigsen, J., Porcellato, D., Amdam, G. V. & Rudi, K. Addressing the diversity of the honeybee gut symbiont *Gilliamella*: description of *Gilliamella apis* sp. nov., isolated from the gut of honeybees (*Apis mellifera*). *Int. J. Syst. Evol. Microbiol.* **68**, 1762–1770 (2018).
23. Milani, C. et al. Genomic encyclopedia of type strains of the genus *Bifidobacterium*. *Appl. Environ. Microbiol.* **80**, 6290–6302 (2014).
24. Kwong, W. K. & Moran, N. A. Cultivation and characterization of the gut symbionts of honey bees and bumble bees: description of *Snodgrassella alvi* gen. nov., sp. nov., a member of the family Neisseriaceae of the Betaproteobacteria, and *Gilliamella apicola* gen. nov., sp. nov., a member of Orbaceae fam. nov., Orbales ord. nov., a sister taxon to the order 'Enterobacteriales' of the Gammaproteobacteria. *Int. J. Syst. Evol. Microbiol.* **63**, 2008–2018 (2013).
25. Ellegaard, K. M. & Engel, P. Genomic diversity landscape of the honey bee gut microbiota. *Nat. Commun.* **10**, 446 (2019).
    Shotgun metagenomics allows the assessment of honeybee gut microbiota diversity, showing that previously described phylotypes contain sequence-discrete populations or species, which tend to co-exist in individual bees and show age-specific abundance profiles.
26. Parish, A. J., Rice, D. W., Tanquary, V. M., Tennessen, J. M. & Newton, I. L. G. Honey bee symbiont buffers larvae against nutritional stress and supplements lysine. *ISME J.* **16**, 2160–2168 (2022).
27. Powell, J. E., Eiri, D., Moran, N. A. & Rangel, J. Modulation of the honey bee queen microbiota: effects of early social contact. *PLoS ONE* **13**, e0200527 (2018).
28. Callegari, M. et al. Compartmentalization of bacterial and fungal microbiomes in the gut of adult honeybees. *NPJ Biofilms Microbiomes* **7**, 42 (2021).
29. Gómez-Moracho, T. et al. Experimental evidence of harmful effects of *Crithidia mellificae* and *Lotmaria passim* on honey bees. *Int. J. Parasitol.* **50**, 1117–1124 (2020).
30. Tokarev, Y. S. et al. A formal redefinition of the genera *Nosema* and *Vairimorpha* (Microsporidia: Nosematidae) and reassignment of species based on molecular phylogenetics. *J. Invertebr. Pathol.* **169**, 107279 (2020).
31. Grupe, A. C. II & Quandt, C. A. A growing pandemic: a review of *Nosema* parasites in globally distributed domesticated and native bees. *PLoS Pathog.* **16**, e1008580 (2020).
32. Decker, L. E. et al. Higher variability in fungi compared to bacteria in the foraging honey bee gut. *Microb. Ecol.* **85**, 330–334 (2023).
33. Kwong, W. K. et al. Dynamic microbiome evolution in social bees. *Sci. Adv.* **3**, e1600513 (2017).
    The distribution and phylogenies of the core bacterial lineages in the microbiota of social bees suggest that these lineages have co-evolved with bee hosts since the origin of the Corbiculata clade, about 80 million years ago.
34. Ellegaard, K. M., Suenami, S., Miyazaki, R. & Engel, P. Vast differences in strain-level diversity in the gut microbiota of two closely related honey bee species. *Curr. Biol.* **30**, 2520–2531.e7 (2020).
35. Hammer, T. J., Le, E., Martin, A. N. & Moran, N. A. The gut microbiota of bumblebees. *Insectes Soc.* **68**, 287–301 (2021).
36. Sarton-Lohéac, G. et al. Deep divergence and genomic diversification of gut symbionts of neotropical stingless bees. *MBio* **14**, e0353822 (2023).
37. Cerqueira, A. E. S. et al. Extinction of anciently associated gut bacterial symbionts in a clade of stingless bees. *ISME J.* **15**, 2813–2816 (2021).
38. Figueroa, L. L., Maccaro, J. J., Krichilsky, E., Yanega, D. & McFrederick, Q. S. Why did the bee eat the chicken? Symbiont gain, loss, and retention in the vulture bee microbiome. *MBio* **12**, e0231721 (2021).
39. Kueneman, J. G., Bonadies, E., Thomas, D., Roubik, D. W. & Wcislo, W. T. Neotropical bee microbiomes point to a fragmented social core and strong species-level effects. *Microbiome* **11**, 150 (2023).
40. Holley, J.-A. C., Jackson, M. N., Pham, A. T., Hatcher, S. C. & Moran, N. A. Carpenter bees (*Xylocopa*) harbor a distinctive gut microbiome related to that of honey bees and bumble bees. *Appl. Environ. Microbiol.* **88**, e0020322 (2022).
41. Engel, P., Martinson, V. G. & Moran, N. A. Functional diversity within the simple gut microbiota of the honey bee. *Proc. Natl Acad. Sci. USA* **109**, 11002–11007 (2012).
42. Zheng, H. et al. Division of labor in honey bee gut microbiota for plant polysaccharide digestion. *Proc. Natl Acad. Sci. USA* **116**, 25909–25916 (2019).
    Honeybee gut symbionts, including *Bifidobacterium*, *Gilliamella* and *Lactobacillus* strains, encode specific pectate lyases and glycoside hydrolases that are highly variable between strains and are involved in the degradation of recalcitrant components within pollen husks.
43. Zheng, H. et al. Metabolism of toxic sugars by strains of the bee gut symbiont *Gilliamella apicola*. *MBio* **7**, e01326-16 (2016).
44. Lee, F. J., Miller, K. I., McKinlay, J. B. & Newton, I. L. G. Differential carbohydrate utilization and organic acid production by honey bee symbionts. *FEMS Microbiol. Ecol.* **94**, fiy113 (2018).
45. Kwong, W. K., Mancenido, A. L. & Moran, N. A. Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* **4**, 170003 (2017).
46. Horak, R. D., Leonard, S. P. & Moran, N. A. Symbionts shape host innate immunity in honeybees. *Proc. Biol. Sci.* **287**, 20201184 (2020).
    The honeybee symbiont *S. alvi* activates the expression of host AMPs, such as apidaecin, defensin and hymenoptaecin, but live *S. alvi* cells appear to suppress some parts of innate immune signalling.
47. Raymann, K. & Moran, N. A. The role of the gut microbiome in health and disease of adult honey bee workers. *Curr. Opin. Insect Sci.* **26**, 97–104 (2018).

48. Steele, M. I., Motta, E. V. S., Gattu, T., Martinez, D. & Moran, N. A. The gut microbiota protects bees from invasion by a bacterial pathogen. *Microbiol. Spectr.* **9**, e0039421 (2021).
49. Palmer-Young, E. C., Markowitz, L. M., Huang, W.-F. & Evans, J. D. High temperatures augment inhibition of parasites by a honey bee gut symbiont. *Appl. Environ. Microbiol.* **89**, e01023 (2023).
50. Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C. & Moran, N. A. Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc. Natl Acad. Sci. USA* **114**, 4775–4780 (2017).
51. Motta, E. V. S., Powell, J. E., Leonard, S. P. & Moran, N. A. Prospects for probiotics in social bees. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **377**, 20210156 (2022).
    This paper summarizes recent research on probiotics for bees and presents new evidence that the use of defined native gut bacteria is a promising way to restore perturbed microbial communities in bees exposed to agrochemicals.
52. Wang, X. et al. High-fat diets with differential fatty acids induce obesity and perturb gut microbiota in honey bee. *Int. J. Mol. Sci.* **22**, 834 (2021).
53. Zhang, Z. et al. Honeybee gut *Lactobacillus* modulates host learning and memory behaviors via regulating tryptophan metabolism. *Nat. Commun.* **13**, 2037 (2022).
54. Engel, P. et al. The bee microbiome: impact on bee health and model for evolution and ecology of host–microbe interactions. *MBio* **7**, e02164-15 (2016).
55. Emery, O., Schmidt, K. & Engel, P. Immune system stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*). *Mol. Ecol.* **26**, 2576–2590 (2017).
56. Engel, P., Bartlett, K. D. & Moran, N. A. The bacterium *Frischella perrara* causes scab formation in the gut of its honeybee host. *MBio* **6**, e00193-15 (2015).
57. Li, Y., Leonard, S. P., Powell, J. E. & Moran, N. A. Species divergence in gut-restricted bacteria of social bees. *Proc. Natl Acad. Sci. USA* **119**, e2115013119 (2022).
    This study describes the use of genome sequences from isolates of *S. alvi* and *Gilliamella* spp. to identify 'populations' defined by evidence for homologous exchange and therefore representing biological species.
58. Bobay, L.-M., Wissel, E. F. & Raymann, K. Strain structure and dynamics revealed by targeted deep sequencing of the honey bee gut microbiome. *mSphere* **5**, e11694-20 (2020).
59. Raymann, K., Bobay, L.-M. & Moran, N. A. Antibiotics reduce genetic diversity of core species in the honeybee gut microbiome. *Mol. Ecol.* **27**, 2057–2066 (2018).
60. Steele, M. I., Kwong, W. K., Whiteley, M. & Moran, N. A. Diversification of type VI secretion system toxins reveals ancient antagonism among bee gut microbes. *MBio* **8**, e01630-17 (2017).
61. Brochet, S. et al. Niche partitioning facilitates coexistence of closely related honey bee gut bacteria. *eLife* **10**, e68583 (2021).
62. Ellegaard, K. M. et al. Genomic changes underlying host specialization in the bee gut symbiont *Lactobacillus* Firm5. *Mol. Ecol.* **28**, 2224–2237 (2019).
63. Motta, E. V. S. et al. Host-microbiome metabolism of a plant toxin in bees. *eLife* **11**, e82595 (2022).
64. Kešnerová, L. et al. Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS Biol.* **15**, e2003467 (2017).
    This study combines metabolomics and gnotobiotic bees to investigate the metabolic contributions of individual members of the bee gut microbiota.
65. Koch, H. et al. Host and gut microbiome modulate the antiparasitic activity of nectar metabolites in a bumblebee pollinator. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **377**, 20210162 (2022).
    This study disentangles the contributions of host and gut microbial enzymes to the metabolism of some plant secondary metabolites.
66. Tauber, J. P. et al. Colony-level effects of amygdalin on honeybees and their microbes. *Insects* **11**, 783 (2020).
67. Kwong, W. K., Engel, P., Koch, H. & Moran, N. A. Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proc. Natl Acad. Sci. USA* **111**, 11509–11514 (2014).
68. Quinn, A. et al. Foraging on host synthesized metabolites enables the bacterial symbiont *Snodgrassella alvi* to colonize the honey bee gut. Preprint at *bioRxiv* https://doi.org/10.1101/2023.01.23.524906 (2023).
69. Powell, J. E., Leonard, S. P., Kwong, W. K., Engel, P. & Moran, N. A. Genome-wide screen identifies host colonization determinants in a bacterial gut symbiont. *Proc. Natl Acad. Sci. USA* **113**, 13887–13892 (2016).
70. Cabirol, A. et al. A defined community of core gut microbiota members promotes cognitive performance in honey bees. Preprint at *bioRxiv* https://doi.org/10.1101/2023.01.03.522593 (2023).
71. Steele, M. I. & Moran, N. A. Evolution of interbacterial antagonism in bee gut microbiota reflects host and symbiont diversification. *mSystems* **6**, e00063-21 (2021).
72. Chen, C., Yang, X. & Shen, X. Confirmed and potential roles of bacterial T6SSs in the intestinal ecosystem. *Front. Microbiol.* **10**, 1484 (2019).
73. Schmidt, K. et al. Integration host factor regulates colonization factors in the bee gut symbiont *Frischella perrara*. *eLife* **12**, e76182 (2023).
74. Le, N.-H., Pinedo, V., Lopez, J., Cava, F. & Feldman, M. F. Killing of Gram-negative and Gram-positive bacteria by a bifunctional cell wall-targeting T6SS effector. *Proc. Natl Acad. Sci. USA* **118**, e2106555118 (2021).
75. Coyte, K. Z., Schluter, J. & Foster, K. R. The ecology of the microbiome: networks, competition, and stability. *Science* **350**, 663–666 (2015).
76. Heilbronner, S., Krismer, B., Brötz-Oesterhelt, H. & Peschel, A. The microbiome-shaping roles of bacteriocins. *Nat. Rev. Microbiol.* **19**, 726–739 (2021).
77. Zendo, T. et al. Kunkecin A, a new nisin variant bacteriocin produced by the fructophilic lactic acid bacterium, *Apilactobacillus kunkeei* FF30-6 isolated from honey bees. *Front. Microbiol.* **11**, 571903 (2020).
78. Shkoporov, A. N., Turkington, C. J. & Hill, C. Mutualistic interplay between bacteriophages and bacteria in the human gut. *Nat. Rev. Microbiol.* **20**, 737–749 (2022).
79. Bonilla-Rosso, G., Steiner, T., Wichmann, F., Bexkens, E. & Engel, P. Honey bees harbor a diverse gut virome engaging in nested strain-level interactions with the microbiota. *Proc. Natl Acad. Sci. USA* **117**, 7355–7362 (2020).
    This study shows that honeybee guts harbour bacteriophage communities that have co-evolved with core bacterial symbionts and may fulfil crucial roles in mediating antagonistic and beneficial interactions within the bee gut microbiota.
80. Busby, T. J., Miller, C. R., Moran, N. A. & Van Leuven, J. T. Global composition of the bacteriophage community in honey bees. *mSystems* **7**, e0119521 (2022).
81. Deboutte, W. et al. Honey-bee–associated prokaryotic viral communities reveal wide viral diversity and a profound metabolic coding potential. *Proc. Natl Acad. Sci. USA* **117**, 10511–10519 (2020).
82. Wang, J. et al. Stably transmitted defined microbial community in honeybees preserves *Hafnia alvei* inhibition by regulating the immune system. *Front. Microbiol.* **13**, 1074153 (2022).
83. Miller, D. L., Smith, E. A. & Newton, I. L. G. A bacterial symbiont protects honey bees from fungal disease. *MBio* **12**, e0050321 (2021).
    This study shows that the symbiont *B. apis* protects honeybee larvae against the fungal pathogen *A. flavus* by producing specific antifungal molecules.
84. Lang, H. et al. Engineered symbiotic bacteria interfering *Nosema* redox system inhibit microsporidia parasitism in honeybees. *Nat. Commun.* **14**, 2778 (2023).
85. Huang, Q., Lariviere, P. J., Powell, J. E. & Moran, N. A. Engineered gut symbiont inhibits microsporidian parasite and improves honey bee survival. *Proc. Natl Acad. Sci. USA* **120**, e2220922120 (2023).
86. Li, J. H. et al. New evidence showing that the destruction of gut bacteria by antibiotic treatment could increase the honey bee's vulnerability to *Nosema* infection. *PLoS ONE* **12**, e0187505 (2017).
87. Dosch, C. et al. The gut microbiota can provide viral tolerance in the honey bee. *Microorganisms* **9**, 871 (2021).
88. Lang, H. et al. Specific strains of honeybee gut *Lactobacillus* stimulate host immune system to protect against pathogenic *Hafnia alvei*. *Microbiol. Spectr.* **10**, e0189621 (2022).
89. Evans, J. D. et al. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Mol. Biol.* **15**, 645–656 (2006).
90. Nappi, A. J. & Christensen, B. M. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. *Insect Biochem. Mol. Biol.* **35**, 443–459 (2005).
91. Raymann, K., Coon, K. L., Shaffer, Z., Salisbury, S. & Moran, N. A. Pathogenicity of *Serratia marcescens* strains in honey bees. *MBio* **9**, e01649-18 (2018).
92. Burritt, N. L. et al. Sepsis and hemocyte loss in honey bees (*Apis mellifera*) infected with *Serratia marcescens* strain Sicaria. *PLoS ONE* **11**, e0167752 (2016).
93. El Sanousi, S. M., El Sarag, M. S. A. & Mohamed, S. E. Properties of *Serratia marcescens* isolated from diseased honeybee (*Apis mellifera*) larvae. *Microbiology* **133**, 215–219 (1987).
94. Evans, J. D. & Spivak, M. Socialized medicine: individual and communal disease barriers in honey bees. *J. Invertebr. Pathol.* **103**, S62–S72 (2010).
95. Baracchi, D., Fadda, A. & Turillazzi, S. Evidence for antiseptic behaviour towards sick adult bees in honey bee colonies. *J. Insect Physiol.* **58**, 1589–1596 (2012).
96. Raymann, K., Shaffer, Z. & Moran, N. A. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol.* **15**, e2001861 (2017).
97. Forsgren, E. European foulbrood in honey bees. *J. Invertebr. Pathol.* **103**, S5–S9 (2010).
98. Genersch, E. American foulbrood in honeybees and its causative agent, *Paenibacillus larvae*. *J. Invertebr. Pathol.* **103**, S10–S19 (2010).
99. Kačániová, M., Gasper, J. & Terentjeva, M. Antagonistic effect of gut microbiota of honeybee (*Apis mellifera*) against causative agent of American foulbrood *Paenibacillus larvae*. *J. Microbiol. Biotechnol. Food Sci.* **9**, 478–481 (2019).
100. Al-Ghamdi, A. et al. In vitro antagonistic potential of gut bacteria isolated from indigenous honey bee race of Saudi Arabia against *Paenibacillus larvae*. *J. Apic. Res.* **59**, 825–833 (2020).
101. Koch, H. & Schmid-Hempel, P. Socially transmitted gut microbiota protect bumble bees against an intestinal parasite. *Proc. Natl Acad. Sci. USA* **108**, 19288–19292 (2011).
102. Koch, H. & Schmid-Hempel, P. Gut microbiota instead of host genotype drive the specificity in the interaction of a natural host–parasite system. *Ecol. Lett.* **15**, 1095–1103 (2012).
103. Mockler, B. K., Kwong, W. K., Moran, N. A. & Koch, H. Microbiome structure influences infection by the parasite *Crithidia bombi* in bumble bees. *Appl. Environ. Microbiol.* **84**, e02335-17 (2018).
104. Koch, H., Woodward, J., Langat, M. K., Brown, M. J. F. & Stevenson, P. C. Flagellum removal by a nectar metabolite inhibits infectivity of a bumblebee parasite. *Curr. Biol.* **29**, 3494–3500.e5 (2019).
105. Palmer-Young, E. C., Raffel, T. R. & McFrederick, Q. S. pH-mediated inhibition of a bumble bee parasite by an intestinal symbiont. *Parasitology* **146**, 380–388 (2019).
106. Floyd, A. S. et al. Microbial ecology of European foul brood disease in the honey bee (*Apis mellifera*): towards a microbiome understanding of disease susceptibility. *Insects* **11**, 555 (2020).
107. Rubanov, A., Russell, K. A., Rothman, J. A., Nieh, J. C. & McFrederick, Q. S. Intensity of *Nosema ceranae* infection is associated with specific honey bee gut bacteria and weakly associated with gut microbiome structure. *Sci. Rep.* **9**, 3820 (2019).

108. Guo, J. et al. Characterization of gut bacteria at different developmental stages of Asian honey bees, *Apis cerana*. *J. Invertebr. Pathol.* **127**, 110–114 (2015).
109. Kim, C. et al. Analysis of the gut microbiome of susceptible and resistant honeybees (*Apis cerana*) against sacbrood virus disease. *J. Appl. Entomol.* **146**, 1078–1086 (2022).
110. Sullivan, J. P., Fahrbach, S. E. & Robinson, G. E. Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* **37**, 1–14 (2000).
111. Toth, A. L., Kantarovich, S., Meisel, A. F. & Robinson, G. E. Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* **208**, 4641–4649 (2005).
112. Bajgar, A., Jindra, M. & Dolezel, D. Autonomous regulation of the insect gut by circadian genes acting downstream of juvenile hormone signaling. *Proc. Natl Acad. Sci. USA* **110**, 4416–4421 (2013).
113. Zhang, Z., Mu, X., Shi, Y. & Zheng, H. Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiol. Spectr.* **10**, e0243821 (2022).
114. Li, L. et al. Gut microbiome drives individual memory variation in bumblebees. *Nat. Commun.* **12**, 6588 (2021).
115. Liberti, J. et al. The gut microbiota affects the social network of honeybees. *Nat. Ecol. Evol.* **6**, 1471–1479 (2022).
This study shows that microbiota-colonized bees, when compared with microbiota-deprived bees, exhibit increased levels of brain metabolites, such as serine and ornithine, and increased specialized head-to-head interactions between nestmates.
116. Cabirol, A., Moriano-Gutierrez, S. & Engel, P. Neuroactive metabolites modulated by the gut microbiota in honey bees. *Mol. Microbiol.* https://doi.org/10.1111/mmi.15167 (2023).
117. Billard, J.-M. D-Amino acids in brain neurotransmission and synaptic plasticity. *Amino Acids* **43**, 1851–1860 (2012).
118. Scofield, H. N. & Mattila, H. R. Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS ONE* **10**, e0121731 (2015).
119. Brodschneider, R. & Crailsheim, K. Nutrition and health in honey bees. *Apidologie* **41**, 278–294 (2010).
120. Wu, Y. et al. Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. *Microb. Biotechnol.* **13**, 1201–1212 (2020).
121. Tian, B., Fadhil, N. H., Powell, J. E., Kwong, W. K. & Moran, N. A. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. *MBio* **3**, 00377-12 (2012).
122. Soares, K. O. et al. Tetracycline exposure alters key gut microbiota in africanized honey bees (*Apis mellifera scutellata* x spp.). *Front. Ecol. Evol.* **9**, 716660 (2021).
123. Baffoni, L. et al. Honeybee exposure to veterinary drugs: how is the gut microbiota affected? *Microbiol. Spectr.* **9**, e0017621 (2021).
124. Motta, E. V. S. & Moran, N. A. Impact of glyphosate on the honey bee gut microbiota: effects of intensity, duration, and timing of exposure. *mSystems* **5**, e00268-20 (2020).
125. Powell, J. E., Carver, Z., Leonard, S. P. & Moran, N. A. Field-realistic tylosin exposure impacts honey bee microbiota and pathogen susceptibility, which is ameliorated by native gut probiotics. *Microbiol. Spectr.* **9**, e0010321 (2021).
126. Ortiz-Alvarado, Y. et al. Antibiotics in hives and their effects on honey bee physiology and behavioral development. *Biol. Open* **9**, bio053884 (2020).
127. Duan, X. et al. Antibiotic treatment decrease the fitness of honeybee (*Apis mellifera*) larvae. *Insects* **12**, 301 (2021).
128. Raymann, K. in *Honey Bee Medicine for the Veterinary Practitioner* (eds Kane, T. R. & Faux, C. M.) 125–134 (Wiley, 2021).
129. Ramsey, S. D. et al. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. *Proc. Natl Acad. Sci. USA* **116**, 1792–1801 (2019).
130. Yu, L. et al. Honey bee *Apis mellifera* larvae gut microbial and immune, detoxication responses towards flumethrin stress. *Environ. Pollut.* **290**, 118107 (2021).
131. Qi, S. et al. Acaricide flumethrin-induced sublethal risks in honeybees are associated with gut symbiotic bacterium *Gilliamella apicola* through microbe–host metabolic interactions. *Chemosphere* **307**, 136030 (2022).
132. Kakumanu, M. L., Reeves, A. M., Anderson, T. D., Rodrigues, R. R. & Williams, M. A. Honey bee gut microbiome is altered by in-hive pesticide exposures. *Front. Microbiol.* **7**, 1255 (2016).
133. Rouzé, R., Moné, A., Delbac, F., Belzunces, L. & Blot, N. The honeybee gut microbiota is altered after chronic exposure to different families of insecticides and infection by *Nosema ceranae*. *Microbes Environ.* **34**, 226–233 (2019).
134. Diaz, T., Del-Val, E., Ayala, R. & Larsen, J. Alterations in honey bee gut microorganisms caused by *Nosema* spp. and pest control methods. *Pest Manag. Sci.* **75**, 835–843 (2019).
135. Cuesta-Maté, A. et al. Resistance and vulnerability of honeybee (*Apis mellifera*) gut bacteria to commonly used pesticides. *Front. Microbiol.* **12**, 717990 (2021).
136. Hladik, M. L. et al. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. *Environ. Pollut.* **235**, 1022–1029 (2018).
137. Buszewski, B., Bukowska, M., Ligor, M. & Staneczko-Baranowska, I. A holistic study of neonicotinoids neuroactive insecticides—properties, applications, occurrence, and analysis. *Environ. Sci. Pollut. Res. Int.* **26**, 34723–34740 (2019).
138. Liu, Y.-J. et al. Thiacloprid exposure perturbs the gut microbiota and reduces the survival status in honeybees. *J. Hazard Mater.* **389**, 121818 (2020).
139. Castelli, L., Branchiccela, B., Zunino, P. & Antúnez, K. Insights into the effects of sublethal doses of pesticides glufosinate-ammonium and sulfoxaflor on honey bee health. *Sci. Total Environ.* **868**, 161331 (2023).
140. Raymann, K. et al. Imidacloprid decreases honey bee survival rates but does not affect the gut microbiome. *Appl. Environ. Microbiol.* **84**, e00545-18 (2018).
141. Rothman, J. A., Russell, K. A., Leger, L., McFrederick, Q. S. & Graystock, P. The direct and indirect effects of environmental toxicants on the health of bumblebees and their microbiomes. *Proc. Biol. Sci.* **287**, 20200980 (2020).
142. Blot, N., Veillat, L., Rouzé, R. & Delatte, H. Glyphosate, but not its metabolite AMPA, alters the honeybee gut microbiota. *PLoS ONE* **14**, e0215466 (2019).
143. Motta, E. V. S., Raymann, K. & Moran, N. A. Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl Acad. Sci. USA* **115**, 10305–10310 (2018).
144. Castelli, L. et al. Impact of chronic exposure to sublethal doses of glyphosate on honey bee immunity, gut microbiota and infection by pathogens. *Microorganisms* **9**, 845 (2021).
145. Almasri, H., Liberti, J., Brunet, J.-L., Engel, P. & Belzunces, L. P. Mild chronic exposure to pesticides alters physiological markers of honey bee health without perturbing the core gut microbiota. *Sci. Rep.* **12**, 4281 (2022).
146. Thompson, H. M. et al. Evaluating exposure and potential effects on honeybee brood (*Apis mellifera*) development using glyphosate as an example. *Integr. Environ. Assess. Manag.* **10**, 463–470 (2014).
147. Motta, E. V. S. & Moran, N. A. The effects of glyphosate, pure or in herbicide formulation, on bumble bees and their gut microbial communities. *Sci. Total Environ.* **872**, 162102 (2023).
148. Cullen, M. G., Bliss, L., Stanley, D. A. & Carolan, J. C. Investigating the effects of glyphosate on the bumblebee proteome and microbiota. *Sci. Total Environ.* **864**, 161074 (2023).
149. Helander, M. et al. Glyphosate and a glyphosate-based herbicide affect bumblebee gut microbiota. *FEMS Microbiol. Ecol.* **99**, fiad065 (2023).
150. Motta, E. V. S., Powell, J. E. & Moran, N. A. Glyphosate induces immune dysregulation in honey bees. *Anim. Microbiome* **4**, 16 (2022).
151. Motta, E. V. S. et al. Oral or topical exposure to glyphosate in herbicide formulation impacts the gut microbiota and survival rates of honey bees. *Appl. Environ. Microbiol.* **86**, 116–126 (2020).
152. Tan, S. et al. Effects of glyphosate exposure on honeybees. *Environ. Toxicol. Pharmacol.* **90**, 103792 (2022).
153. Pettis, J. S. et al. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *Nosema ceranae*. *PLoS ONE* **8**, e70182 (2013).
154. Al Naggar, Y., Singavarapu, B., Paxton, R. J. & Wubet, T. Bees under interactive stressors: the novel insecticides flupyradifurone and sulfoxaflor along with the fungicide azoxystrobin disrupt the gut microbiota of honey bees and increase opportunistic bacterial pathogens. *Sci. Total. Environ.* **849**, 157941 (2022).
155. Ludvigsen, J., Amdam, G. V., Rudi, K. & L'Abée-Lund, T. M. Detection and characterization of streptomycin resistance (strA-strB) in a honeybee gut symbiont (*Snodgrassella alvi*) and the associated risk of antibiotic resistance transfer. *Microb. Ecol.* **76**, 588–591 (2018).
156. Huang, S. K. et al. Influence of feeding type and *Nosema ceranae* infection on the gut microbiota of *Apis cerana* workers. *mSystems* **3**, e00177-18 (2018).
157. Castelli, L. et al. Impact of nutritional stress on honeybee gut microbiota, immunity, and *Nosema ceranae* Infection. *Microb. Ecol.* **80**, 908–919 (2020).
158. Powell, J. E. et al. The microbiome and gene expression of honey bee workers are affected by a diet containing pollen substitutes. *PLoS ONE* **18**, e0286070 (2023).
159. Chmiel, J. A., Pitek, A. P., Burton, J. P., Thompson, G. J. & Reid, G. Meta-analysis on the effect of bacterial interventions on honey bee productivity and the treatment of infection. *Apidologie* **52**, 960–972 (2021).
160. Damico, M. E., Beasley, B., Greenstein, D. & Raymann, K. A need for stronger regulation: commercially sold probiotics for honey bees do not live up to their claims. Preprint at *bioRxiv* https://doi.org/10.1101/2023.09.13.557574 (2023).
161. Daisley, B. A. et al. Novel probiotic approach to counter *Paenibacillus larvae* infection in honey bees. *ISME J.* **14**, 476–491 (2020).
162. Stephan, J. G. et al. Honeybee-specific lactic acid bacterium supplements have no effect on American foulbrood-infected honeybee colonies. *Appl. Environ. Microbiol.* **85**, e00606–e00619 (2019).
163. Lamei, S. et al. Feeding honeybee colonies with honeybee-specific lactic acid bacteria (Hbs-LAB) does not affect colony-level Hbs-LAB composition or *Paenibacillus larvae* spore levels, although American foulbrood affected colonies harbor a more diverse Hbs-LAB community. *Microb. Ecol.* **79**, 743–755 (2020).
164. Daisley, B. A. et al. Delivery mechanism can enhance probiotic activity against honey bee pathogens. *ISME J.* **17**, 1382–1395 (2023).
165. Leonard, S. P. et al. Genetic engineering of bee gut microbiome bacteria with a toolkit for modular assembly of broad-host-range plasmids. *ACS Synth. Biol.* **7**, 1279–1290 (2018).
166. Lariviere, P. J., Leonard, S. P., Horak, R. D., Powell, J. E. & Barrick, J. E. Honey bee functional genomics using symbiont-mediated RNAi. *Nat. Protoc.* **18**, 902–928 (2023).
167. Leonard, S. P. et al. Engineered symbionts activate honey bee immunity and limit pathogens. *Science* **367**, 573–576 (2020).
168. Wang, Y., Kaftanoglu, O., Brent, C. S., Page, R. E. Jr & Amdam, G. V. Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *J. Exp. Biol.* **219**, 949–959 (2016).
169. Faita, M. R., Cardozo, M. M., Amandio, D. T. T., Orth, A. I. & Nodari, R. O. Glyphosate-based herbicides and *Nosema* sp. microsporidia reduce honey bee (*Apis mellifera* L.) survivability under laboratory conditions. *J. Apic. Res.* **59**, 332–342 (2020).
170. Kwong, W. K., Zheng, H. & Moran, N. A. Convergent evolution of a modified, acetate-driven TCA cycle in bacteria. *Nat. Microbiol.* **2**, 17067 (2017).

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Author contributions

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Competing interests

N.A.M. is an author on a patent application (US20220152128A1) for using native bee gut bacteria as bee probiotics. E.V.S.M. declares no competing interests.

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