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## Gut Microbial Communities of Social Bees

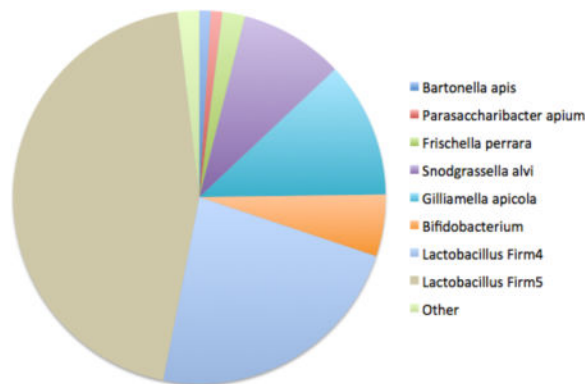
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### Preface

The gut microbiota can have profound effects on hosts, but studying these relationships in humans presents challenges. The specialized gut community of honey bees parallels the mammalian microbiota, as both are mostly composed of host-adapted, facultative anaerobes and microaerophiles. The bee gut community, however, is far simpler, being dominated by only nine bacterial species clusters that are only associated with bees and that are transmitted through social interactions between individuals. Recent developments, including the discovery of extensive strain-level variation, evidence of protective and nutritional functions, and reports of eco-physiological or disease-associated community perturbations, have drawn attention to the microbiota's role in bee health, as well as its potential as a model for studying gut symbiont ecology and evolution.

### Graphical abstract



### Introduction

The microorganisms within animal guts can benefit their hosts, by helping to digest food, detoxifying harmful molecules, providing essential nutrients, protecting against invasion by pathogens and parasites, and modulating development and immunity<sup>1–3</sup>. But, while the importance of gut microbial communities is increasingly appreciated, the processes that govern these communities are far from clear. Gut community composition varies enormously within and between species. For example, individual humans can harbour dramatically different gut communities<sup>4,5</sup>, and humans as a host species have communities

distinct from those of our closest relatives, the chimpanzees and gorillas<sup>6</sup>. Neither the causes nor the consequences of these differences are well understood, although certain shifts in the gut microbiota are correlated with disease states and with diet<sup>7</sup>.

The human gastrointestinal tract contains hundreds of bacterial species, making it difficult to reliably characterize the community roles in hosts, using both inferences from genomic data and results from *in vitro* microbiological assays and *in vivo* experiments in live bees. Although the focus is on the honey bee gut community, where applicable, we also discuss pertinent insights gleaned from related bee species and point out parallels to mammalian systems. Our review is limited to Bacteria, the primary constituents of the normal bee gut microflora, but pathogenic microorganisms, including trypanosomids, fungi, and viruses, sometimes also infect bees<sup>16,17</sup>.

The honey bee, *Apis mellifera*, provides a particularly useful model of a host-adapted gut community: its microbiota exhibits close parallels to those of mammals but is simpler in composition. Honey bees live in large colonies consisting of thousands of non-reproductive female workers and a single reproductive queen (Box 1). Guts of adult workers contain a distinctive and specialized microbiota, dominated by only nine bacterial species clusters<sup>10</sup> each representing a complex of related strains. Some evidence exists for roles in digestion of food and protection against parasites<sup>11,12</sup>. As for humans, these gut communities are dominated by host-adapted species that are largely intolerant of atmospheric oxygen levels, and transmission occurs through social interactions between hosts<sup>13,14</sup> (see Box 2 for comparison of the bee and human microbiota). However, in contrast to mammalian microbiomes, all of the species in the bee gut can be cultured in the laboratory and used to inoculate symbiont-free bees, allowing for powerful experimental studies<sup>15</sup>.

In this review, we summarize recent findings on the composition and transmission of gut communities in honey bees. We then consider evidence for their roles in hosts, using both inferences from genomic data and results from *in vitro* microbiological assays and *in vivo* experiments in live bees. Although the focus is on the honey bee gut community, where applicable, we also discuss pertinent insights gleaned from related bee species and point out parallels to mammalian systems. Our review is limited to Bacteria, the primary constituents of the normal bee gut microflora, but pathogenic microorganisms, including trypanosomids, fungi, and viruses, sometimes also infect bees<sup>16,17</sup>.

## Characterizing the bee gut microbiota

### Community composition

Guts of honey bee workers are dominated by nine bacterial species clusters that comprise 95% to 99.9% of the bacteria in almost all individuals, based on 16S ribosomal RNA community surveys<sup>10,18–22</sup> (Fig. 1A) and on metagenomics of total DNA in gut samples<sup>12</sup>. The two ubiquitous Gram-negative species are *Snodgrassella alvi* and *Gilliamella apicola*, members of the phylum Proteobacteria<sup>23</sup>. Among Gram-positives, two species clusters within phylum Firmicutes are also ubiquitous and abundant; these have been referred to as the *Lactobacillus* Firm-4 and *Lactobacillus* Firm-5 groups<sup>19,20</sup>. Although often lower in abundance, the *Bifidobacterium asteroides* species cluster<sup>24,25</sup> belonging to phylum

Actinobacteria, is also found in most adult individuals. These five bacteria form the core of the honey bee gut community.

Less numerous and of lower prevalence are the Proteobacteria species *Frischella perrara*<sup>26</sup>, *Bartonella apis*<sup>27</sup>, *Parasaccharibacter apium*<sup>28</sup>, and a *Gluconobacter*-related species group designated Alpha2.1<sup>20</sup>. These species have restricted niches in the bee gut (*F. perrara*) or are generalists that are also found in the hive environment (*P. apium*, Alpha2.1), which may explain their comparatively lower frequency in honey bee gut surveys. Together, the five core bacteria and the four rarer members constitute the dominant set of what can be found in the honey bee gut. While other bacteria may occasionally be present (see below), these nine represent bacterial lineages particularly adapted to life alongside their bee hosts. Differences among these species in abundance and prevalence are likely due to highly specialized localizations or metabolic niches within the gut, as is common in other animals<sup>29</sup>.

Such organization within the bee gut community has been examined in honey bee workers (Fig. 1B). Few bacteria occur in the crop, which is used for the storage and transport of nectar for feeding larvae and honey production<sup>13</sup>. Correspondingly, the limited numbers of crop bacteria mostly consist of species, such as Enterobacteriaceae, *Lactobacillus kunkeei*, and *P. apium*, that occur in nectar and hive materials and that can be cultured under atmospheric oxygen concentrations<sup>28,30,31</sup>. There are also few bacteria in the midgut<sup>13</sup>. The insect midgut, which functions in food digestion and absorption, does not provide a stable substrate for bacterial colonization, as it is lined by a continuously shed chitinous material, the peritrophic matrix<sup>1</sup>.

In contrast, the hindgut is lined with a stable layer of cuticle and contains a large bacterial community of between  $10^8$  and  $10^9$  cells, comprising >99% of the bacteria in adult workers, based on 16S rRNA profiling<sup>13</sup>. The hindgut is divided into two discrete regions, the ileum and the rectum, with distinct community compositions. The ileum, a narrow tube with six longitudinal folds, is dominated by the main Gram-negative species: within the lumen of the folds, *S. alvi* forms a layer directly on the gut wall, on top of which is a layer of *G. apicola*<sup>13,14</sup>. This community extends into the pylorus, a small region at the junction of the midgut, ileum, and Malpighian tubules<sup>32</sup>. Here, *F. perrara* can be abundant, localizing adjacent to the gut epithelium<sup>32</sup>.

The distal hindgut region, the rectum, is where faecal waste is stored before defaecation, and may function in reabsorption of water and salts as well. The community here is dominated by the fermentative Gram-positive bacteria: *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, and *Bifidobacterium*. The two *Lactobacillus* species groups also occur within the lumen of the ileum, but are most frequent in the rectum<sup>13,14</sup>. So far, the molecular mechanisms that enable colonization of the bee gut have not been elucidated, but genes implicated in adhesion and biofilm formation are prevalent in the genomes of several gut species<sup>12,33</sup>.

### Specificity to host species

Besides the common Western honey bee (*Apis mellifera*), related corbiculate bees also harbour stable gut communities consisting of many of the same bacterial species groups. These include the eusocial bumble bees (*Bombus* spp.) and other *Apis* species; however,

solitary bees and non-corbiculate social bees have thus far not been found to possess this gut community<sup>20,34–36</sup>. Most of the five core honey bee (*A. mellifera*) gut species clusters can also be found in multiple *Bombus* and *Apis* species<sup>20,37–44</sup>. A bee-specific Bacteroidetes species, *Apibacter adventoris*<sup>45</sup>, has been found in both *Apis* and *Bombus* hosts, though its abundance in *A. mellifera* is consistently very low<sup>19,21,39,46,47</sup>. Other microbiota lineages appear more host-restricted: *B. apis* and *F. perrara* have only been found in *Apis* spp., while the gut symbionts *Schmidhempelia bombr*<sup>48</sup> related to *G. apicola*, and *Bombiscardovia coagulans*<sup>49</sup> have mostly been found in *Bombus* species. Distinct species of *Bifidobacterium* also occur in *Apis* versus *Bombus* species<sup>50–55</sup>.

Different host species can harbour different strains of gut symbionts, and related hosts tend to harbour related strains, suggestive of possible co-diversification and co-adaptation over deep evolutionary timescales<sup>40</sup>. This specificity may be reinforced by an inability for divergent symbiont strains to cross host boundaries: for example, transplantation experiments on strains of *S. alvi* showed that strains from honey bees cannot colonize bumble bees, and vice-versa<sup>33,56</sup>. However, the contributions of specialist bacteria versus generalists (those able to colonize many bee species as well as the broader environment) to overall gut microbiome structure and function are yet unclear. A comparative genomic study of *L. kunkeei*, which is common in nectar and in hive materials but rare in the gut, revealed that it frequently moves between host species and shows no evidence of co-diversification with bee hosts<sup>57</sup>. Species of Acetobacteraceae, which include *P. apium* and Alpha2.1, can sometimes be abundant in adult guts of multiple bee species, but they are also present in nectar, pollen, hive materials, and larvae, and appear to be aerotolerant<sup>20,22,31,28,58,59</sup>.

The dedicated, core gut bacterial species clusters have not been consistently found in environments outside the bee gut. This niche restriction is likely enforced in part by the inability of these bacteria to replicate under atmospheric oxygen concentrations. They are primarily facultative anaerobes or microaerophiles<sup>15,23</sup> and hence depend largely on social interactions for transmission among hosts (Box 3). Such interactions are analogous to the routes by which mammals acquire their microbiota, and this is reflected in the pattern of development of the gut microbiota through the life of the bee.

### Variation with age, caste, and season

New honey bee larvae are devoid of bacteria, but are fed by workers (first royal jelly, then honey, nectar, and pollen) throughout their development. These interactions may lead to an accumulation in their closed guts of bacteria present in hive materials, as well as of some species usually found in adult guts; however, both the composition and abundance of this larval gut microbiota appears erratic<sup>13,37,47,60,61</sup>. The discrepancies in larval gut communities between studies and among sites may reflect variation in colony condition or diet. During metamorphosis, the gut lining is shed: newly emerged adult bees have few or no gut bacteria and are colonized by the normal gut community within the first few days of adult life, before leaving the hive<sup>13,14,47</sup> (Fig. 2, Box 3).

In addition to numerous female worker bees, a single queen and multiple males (drones) are also present in a typical colony. 16S rRNA profiles of adult queen gut communities shows striking differences from those of workers; they vary in size and composition and often lack

certain distinctive species of the worker gut<sup>62,63</sup>. Queen guts are often dominated by Acetobacteraceae, including *P. apium* and Alpha2.1. The differences in gut community composition are probably due to the unique physiology and diet of queens, which feed exclusively on highly nutritious secretions produced by attendant workers. Community profiling using 16S rRNA genes shows that drones have a microbiota composition more similar to that of workers, although more variable and with higher abundance of *Lactobacillus* spp.<sup>47,62</sup>; the reason for these differences is not yet clear.

Adult workers have a relatively stable set of gut bacterial species compared to males or queens<sup>47,62,63</sup> and compared to other insects generally<sup>1,64</sup>. Nonetheless, even workers of the same age within a colony can harbour very different proportions of the core gut species<sup>10,14,62</sup>. Colonies might also undergo age-related or seasonal shifts in relative proportions of the core species<sup>47,65</sup>. The extent to which these shifts are specific to particular geographic regions or conditions is not clear, partly because it is not possible to directly compare community profiles generated by different laboratories using different nucleic acid extraction and amplification protocols. Older foraging workers may exhibit a lower abundance of the core bacteria than young adult bees<sup>47</sup>. Some studies using 16S rRNA gene profiling have reported slight differences in gut community composition between colonies from different locations<sup>10,47,66</sup>. Regarding seasonal trends, a longitudinal study of worker midguts found microbiome shifts over the course of 6 months<sup>65</sup>; however, midguts may give erratic results since they contain so few bacteria. Another study reported minimal differences in communities from whole guts between fall and spring foragers<sup>22</sup>.

### Disruption of the bee gut microbiota

Occasionally in individual worker adults, there is substantial deviation from the characteristic composition of the gut community. This shift often involves a higher content of opportunistic environmental bacteria, including Enterobacteriaceae (e.g., species within the genera *Klebsiella*, *Pantoea*, *Enterobacter*, *Serratia*, and *Hafnia*) and other Gammaproteobacteria<sup>10,21,22,63</sup>. Potentially these shifts reflect disruption or disease; in some ways, they parallel the invasion of the human gut by opportunistic Proteobacteria, a phenomenon characteristic of several disease states including Crohn's disease<sup>67</sup>. Bumble bees appear to be more prone than honey bees to such shifts. In a study of gut community composition in three native bumble bee species in New Jersey, USA, all species carried *S. alvi*, *G. apicola*, *Lactobacillus* Firm-4, and *Lactobacillus* Firm-5<sup>46</sup>. However, some individual bees contained disrupted communities, with erratic compositions featuring low incidence of bee-specific species and high levels of environmental Acetobacteraceae and Enterobacteraceae. These perturbed communities were found in all three host species but varied in frequency among them<sup>46</sup> and were positively correlated with the presence of eukaryotic intestinal parasites<sup>46,68</sup>. Similarly, in a 16S rRNA survey of 28 bumble bee species in China, gut communities fell into two distinct compositional types, distributed at different frequencies across species<sup>43</sup>. One type was the normal corbiculate bee-specific community represented by *S. alvi*, *G. apicola*, *Lactobacillus* Firm-4 and *Lactobacillus* Firm-5. The other was dominated by a variety of Enterobacteriaceae and other Proteobacteria, and by environmental *Lactobacillus* spp.

In-depth studies using experimental colonies of a European bumble bee, *Bombus terrestris*, suggest that the shift to non-core species occurs in bees exposed to stress and environmental sources of invasive colonizers, as wild bees were more likely to contain non-core gut Enterobacteriaceae than bees from indoor colonies<sup>69,70</sup>. Together, these studies indicate that the characteristic gut symbionts are acquired by young adults within the colony, yielding a relatively stable ‘normal’ community of co-adapted bacteria, but that individual communities can eventually be overtaken by opportunistic bacteria. The cause of such perturbations is unclear. In honey bees, the immune responsiveness of gut epithelia was not found to be different between young nurse bees, which are confined to the hive, and older foragers, which encounter diverse microbes in the surrounding environment<sup>71</sup>, although this does not necessarily rule out community shifts due to nutritional or other stressors.

Another possible disruptor is the use of antibiotics, which has become widespread in many parts of the global commercial honey bee industry. Beekeepers in the United States have used oxytetracycline since the 1950’s to combat *Paenibacillus larvae* and *Melissococcus plutonius*, the causative agents of larval foulbrood disease<sup>72</sup>. Correspondingly, analysis of the characteristic gut microbiota from US honey bee colonies, via functional screening of cloned metagenomic libraries, revealed high frequencies of multiple tetracycline resistance genes<sup>73</sup>. These resistance genes are present in both Gram-positive and Gram-negative gut bacteria and are sometimes associated with mobile elements<sup>73,74</sup>. Resistance loci were detected in all US colonies, although they were fewer in feral colonies and colonies managed without antibiotic exposure for several years; in contrast, they were absent or rare in wild-caught bumble bees and in honey bees from several countries that ban antibiotic use in apiculture. Thus, antibiotic treatments can be a strong selective force in bees, paralleling the antibiotic-driven selection proposed to be acting in the human gut microbiota<sup>75</sup>.

### Diversification of strains within and between hosts

We define the nine major groups of bacteria that form the bee gut community as “species clusters” – interrelated strains that are largely monophyletic and which occupy the same general ecological niche. In 16S rRNA surveys, these clusters mostly coalesce into single operational taxonomic units at 97% identity, indicative of their close relationship. Nonetheless, each cluster can span considerable genomic diversity, with great sequence divergence in protein-coding genes and distinct gene repertoires between strains<sup>12,33</sup>. Extensive strain-level variation, even within a single host individual, also occurs within characteristic species of the human gut community<sup>5</sup>.

That strain variation is the norm for the core species clusters of the bee gut is revealed by full length 16S rDNA sequences for *S. alvi* and *G. apicola*<sup>10</sup>, by metagenomic datasets<sup>12</sup>, and by single cell genomic sequencing<sup>76</sup>. Some studies have assigned separate species names for the different strains within bee gut species clusters, such as those within *Lactobacillus* Firm-4 and Firm-5<sup>77–79</sup>. Drawing taxonomic boundaries should be done cautiously, however, given the poorly characterized genomic and functional diversity in the bee gut community. A more fundamental challenge will be to understand the processes that drive the diversification and maintenance of strains, and the consequential biological

relevance. As summarized in the next sections, this genomic variation involves capabilities that are likely important for the ecology and nutrition of bee hosts.

## Functions and metabolism in hosts

### Effects on bee health

Direct evidence for a role of gut microbiota in bee health was obtained for a European bumble bee, *Bombus terrestris*. Workers experimentally deprived of gut symbionts (through antibiotic treatment or aseptic rearing) showed elevated susceptibility to *Crithidia bombi*, a common trypanosomatid parasite, compared to workers colonized by the normal gut community through exposure to nestmate faeces<sup>11</sup>. This protective effect was supported indirectly by surveys of three North American *Bombus* species, where parasite presence was negatively correlated with *G. apicola*<sup>46</sup>. Furthermore, inter-colony microbiota transplant experiments show that different gut communities may differ in the ability to resist *Crithidia* strains, suggesting that variation in microbiota composition or bacterial strain diversity can impact host health<sup>80</sup>. Other roles in defending hosts are suggested by microbiological assays showing that some bee-derived bacteria (including *Lactobacillus* and *Bifidobacterium*) can inhibit other microorganisms on culture plates, possibly via anti-microbial compounds<sup>78,81–83</sup>.

One route through which the gut microbiota might affect host susceptibility to pathogens or parasites is by activation of the innate immune system<sup>84</sup>. Honey bees possess similar signaling pathways and effectors to those first characterized for the innate immune system of *Drosophila melanogaster*<sup>85</sup>. While it is known that gut bacteria induce immune responses in *D. melanogaster*<sup>86</sup> this has yet to be investigated in honey bees. However, the gut bacteria in bees should not be assumed to be entirely beneficial to hosts. Experiments on *F. perrara* indicate that it induces melanization in the pylorus region of the gut<sup>32</sup>; melanization is an insect innate immunity response typically associated with tissue damage and pathogen invasion. *F. perrara* can also cause DNA damage in eukaryotic cells. Assays in mammalian cell culture show that this is due to an encoded biosynthetic gene cluster homologous to that for colibactin, a small molecule metabolite produced by certain *E. coli* strains in the human gut, which causes double-stranded DNA breaks associated with tumor induction<sup>87</sup>. *F. perrara* is found in a majority of sampled honey bees<sup>21,32</sup>, and its overall impact on bee health has not yet been determined.

Potentially, the honey bee gut bacteria have roles in neutralizing dietary toxins, biosynthesis of needed nutrients, or digestion of food components, including fermentation of complex carbohydrates and sugars indigestible by the bee itself. For example, some strains of *G. apicola* possess genes for the degradation of pectin, a component of the cell wall of pollen grains. Pollen is an essential part of the bee diet, and this activity, which can be shown *in vitro*<sup>12</sup>, may help in its digestion, since bees and most other animals cannot produce pectinases themselves. Other roles in processing food or neutralizing ingested toxins have limited experimental evidence but are plausible on the basis of recent genomic studies<sup>12,33,88</sup>, which are beginning to reveal the metabolic activities and inter-bacterial interactions within the bee gut community.

## Metabolic characterization

Metagenomic and metatranscriptomic datasets, as well as complete genome sequences for most members of the honey bee gut microbiota, are now available, giving insight into their lifestyles and potential functional roles<sup>12,55,88,89</sup>. As in the human gut, a substantial fraction of the bee microbiota carries out fermentation of dietary carbohydrates (Fig. 3). These include bee-specific bacterial species within *Lactobacillus* and *Bifidobacterium*, two genera with representatives commonly found in mammalian guts.

The bee *Bifidobacterium* group encodes highly abundant and diverse genes for carbohydrate utilization, compared to both its relatives and to other bee gut members; experimental tests have verified this metabolic versatility<sup>25,55</sup>. Phylogenetically, the bee-associated *Bifidobacterium* strains branch basally to the major groups of mammal-associated *Bifidobacterium*, and thus present an evolutionary parallel with which to examine gut microbe adaptation to hosts<sup>52</sup>. Indeed, striking differences, such as the ability for aerobic respiration in bee-derived *Bifidobacterium*<sup>25,55</sup> may be indicative of distinct selective forces acting in the bee gut environment (e.g. different oxic conditions).

Bee-associated *Lactobacillus* are also distinct from their mammal-associated relatives, and generally fall into two phylogenetically separate clades, Firm-4 and Firm-5 (a third, Firm-3, is occasionally found<sup>19</sup>). Recent sequencing of their genomes<sup>55,89</sup> revealed numerous phosphotransferase systems involved in the uptake of sugars, particularly in *Lactobacillus* Firm-5. Both bee-associated *Bifidobacterium* and *Lactobacillus* possess large putative cell surface proteins of unknown function, but which may be related to adhesion or degradation of plant compounds<sup>55,89</sup>. Bee-associated *Bifidobacterium* and *Lactobacillus* Firm-5 also possess gene clusters for biosynthesis and utilization of trehalose, a disaccharide used for energy storage in insects. In contrast, mammals utilize glycogen instead of trehalose for energy storage, and mammalian *Bifidobacterium* and *Lactobacillus* typically have genes for glycogen biosynthesis and degradation, which are lacking in the bee-associated species<sup>55</sup>.

Another dominant fermenter in the honey bee gut is *G. apicola*, a member of a recently described bacterial order, Orbales, that appears largely associated with insects<sup>23</sup>. Genomic analysis of *G. apicola* strains uncovered a large repertoire of genes for the uptake and fermentation of sugars, but an incomplete tricarboxylic acid cycle and a degenerate aerobic respiratory chain<sup>33</sup>. Two other related Orbales found in bees, *S. bombi* and *F. perrara*, are similarly carbohydrate fermenters<sup>26,48,87</sup>.

Considering the carbohydrate-rich diet of bees (nectar, honey, pollen), it is perhaps unsurprising that the gut microbiota comprises members that have evolved to thrive on this resource. *G. apicola*, *F. perrara*, *Lactobacillus* Firm-4, *Lactobacillus* Firm-5, and bee-associated *Bifidobacterium* are all able to utilize glucose and fructose, the most abundant sugars in the bee diet<sup>26,55,77</sup>. Furthermore, certain strains carry genes for utilization of scarcer sugars, including some (e.g. mannose, arabinose, raffinose, galactose, and lactose) that are indigestible by the bee and that can be toxic to bees<sup>90</sup>. The ability to assimilate these more exotic carbohydrates depends on having the appropriate transporters and enzymes, and presence of these varies widely among strains within each group of fermenters<sup>33,55,76</sup>. In each of the fermenter species, a substantial proportion of the pan-genome comprises



accessory genes (those not found in every strain), of which the largest classifiable fraction (~20–40%) are genes involved in carbohydrate metabolism<sup>55,76</sup>. This dynamic genome composition suggests that gaining or losing the ability to utilize diverse substrates may not be difficult, and also points to the potential for different fermenter strains to specialize to distinct niches within the bee gut<sup>12</sup>, and for the gut community as a whole to adapt to changing dietary conditions.

The end products of microbial fermentation in the bee gut vary depending on the species (Fig. 3), but usually include lactic acid and acetate. Metagenomic studies based on both DNA and RNA sequencing reveal high representation and expression of genes for fermentation<sup>12,88</sup>, and this activity has been supported by culture-based assays<sup>26,55,77,88,91</sup>. In many mammals, fermentation products, including short chain fatty acids (acetate, propionate, butyrate), are absorbed and oxidized, substantially contributing to host nutrition<sup>92</sup>. Hindgut absorption of microbially produced short chain fatty acids occurs in some insects<sup>93</sup>; however, this possibility has not been tested in bees.

The final core member of the honey bee microbiota is *S. alvi*, a member of the family Neisseriaceae and an obligate microaerophile. The localization of *S. alvi* to the periphery of the lumen (Fig. 1B) is consistent with dependence on aerobic respiration, as oxygen concentrations in insect guts are usually highest at the epithelial surface<sup>93,94</sup>. Remarkably, *S. alvi* has lost all pathways for the uptake and glycolytic breakdown of carbohydrates, and instead relies on the aerobic oxidation of carboxylates (citrate, malate, acetate, lactic acid) for energy production<sup>23,33</sup>. The utilization of separate sets of resources appears to be a case of niche partitioning within the microbial community, enabling *S. alvi* and the fermentative bacterial species to stably co-exist in the same gut environment. These metabolic differences are also suggestive of a syntrophic interaction, as some of the substrates used by *S. alvi* (lactic acid, acetate, formate) are those provided in abundance by the fermentation of carbohydrates.

*P. apium* is likely another bacterium with a specialized niche. Although rare in the adult worker gut, *P. apium* appears relatively abundant in hive food stores, larvae, and queen guts<sup>28,31,63</sup>. Interestingly, it can thrive on royal jelly, an environment toxic to most bacteria, and can also be found in the royal jelly-producing glands of worker bees<sup>28</sup>. Evidence from its genome<sup>95</sup> and from the phenotypic traits of close relatives<sup>59,96</sup> suggests that, although a member of the family Acetobacteraceae, *P. apium* cannot produce acetic acid from the oxidation of sugars and alcohols. However, it seems well adapted to tolerate the aerobic conditions, acidity, and high sugar osmolarities found in royal jelly, nectar, and honey.

## Conclusions and Future Questions

This review summarizes a resurgence of research into the bee microbiome, first starting with early culture-independent analyses<sup>16,18,19,60</sup>, and followed in recent years by genomics-enabled studies. In this time, the core constituents of the honey bee gut community have been identified. At least 5 bacterial species groups (*S. alvi*, *G. apicola*, *Bifidobacterium* spp., *Lactobacillus* Firm-4, and *Lactobacillus* Firm-5) are ubiquitous in almost all adult workers, and also occur in related bee species, such as bumble bees. Several other bacteria, including

*F. perrara*, *B. apis*, and certain species of Acetobacteraceae and Bacteroidetes, also appear uniquely associated with bees, but may vary in abundance with particular hosts or ecological conditions. Together, these bacteria represent a specialized microbial community that has coevolved and diversified with its bee hosts over millions of years.

Genomic, transcriptomic, and culture-based studies have begun to reveal the extensive adaptations permitting this unique microbial consortium to thrive in the bee gut environment. Most striking are metabolic shifts towards the breakdown and fermentation of sugars in many gut species, enabling them to take advantage of the hosts' carbohydrate-rich diet<sup>12,33,88</sup>. Within the fermenter species, different strains possess distinct carbohydrate degradation capabilities; meanwhile, in the non-fermenter *S. alvi*, the ability to use sugars has been completely lost<sup>33</sup>. This niche partitioning through differential utilization of resources indicates a level of self-organization in the microbiota that may enhance the stability and persistence of the characteristic bee gut community.

However, this community can also be disrupted, through invasion by environmentally derived bacteria or opportunistic pathogens<sup>10,43</sup>. The gut community also differs between castes and may change with the age of the individual and the colony, likely reflecting the effects of host physiology, diet, and the environment in shaping microbiome composition<sup>14,62,63,68,69,97</sup>. A similarly dynamic gut microbiome is evident in many animals, and hence should be considered the norm rather than the exception<sup>67</sup>. Bees provide an excellent case for studying the forces that influence microbiome assembly and composition.

As an emerging system with extensive parallels to human and other mammalian microbiomes, studies of the bee gut community are poised for rapid breakthroughs in the near future. This system presents an intriguing model with a number of advantages – both practical and for the development of symbiotic theory (Box 2). As in many mammals, sociality is central for the continuous passage of the microbiota from one generation to the next. This reliable form of transmission has likely allowed the bee gut microbiota to diversify and specialize to particular host species<sup>40,56</sup> as well as to distinct ecological niches within gut microhabitats<sup>12</sup>. Thus far, studies into the role of host sociality in microbiota transmission and evolution have been mainly correlative<sup>98–100</sup>; these questions can now be investigated empirically in the corbiculate bee system. All bee gut bacteria can be cultivated *in vitro*, and substantial progress has been made in developing gnotobiotic experimental setups to study specific host-microbe interactions<sup>11,33</sup>. There is also the prospect of genetically manipulating these bacteria to directly test hypotheses and, perhaps eventually, to engineer them for host benefit<sup>101,102</sup>.

Honey bees, and their bumble bee cousins, are of global importance due to their pollination of much of the world's crops and wild flowering plants. Recent bee population declines have drawn attention to potential agents affecting their health, including their microbiota<sup>16,103,104</sup>. Indeed, there is evidence that the bee gut bacteria help defend against pathogen infection, in ways that are still poorly understood<sup>11,80</sup>. Considering the uniqueness and stability of the bee gut microbiome, it should be considered a fundamental part of bee biology. As such,

understanding this community will undoubtedly offer novel insights towards improving bee health and, more generally, crucial unresolved aspects of host-microbe symbiosis.

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## Glossary

<b>Species cluster</b>	group containing closely related strains or species
<b>Cuticle</b>	the outer layer of insect integument, primarily composed of chitin
<b>Malpighian tubules</b>	part of the insect excretory system, moving waste from the body cavity to the digestive tract for excretion
<b>Eusocial</b>	a type of social organization exemplified by cooperative brood care, reproductive division of labour, and cohabitation of overlapping generations
<b>Foulbrood</b>	bacterial disease caused by infection of the gut of honey bee larvae, resulting in death of the brood
<b>Royal jelly</b>	a highly nutritious secretion produced by worker bees, and initially fed to all larvae continued feeding after 3 days results in development of queens.
<b>Corbiculate bees</b>	a related group of bees that are characterized by the presence of corbicula (pollen baskets) on their hind limbs, and which include the social honey bees, bumble bees, and stingless bees
<b>Core microbiome</b>	the set of microbial species that is present in most members of a host species
<b>Pan genome</b>	the entire gene set of a group of related bacteria, such as that of a bacterial ‘species’
<b>Syntrophy</b>	cross-feeding, whereby metabolic interactions between two organisms enhance each other’s growth
<b>Trophallaxis</b>	transfer of food, fluids, or secretions between individuals through direct contact
<b>Gnotobiotic</b>	an organism in which the strains of microorganisms present are fully known

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**Box 1****Biology of Bees and their Microbiota**

The Western honey bee, *Apis mellifera*, was domesticated in Egypt and the Levant over 4000 years ago for honey and wax production<sup>105,106</sup> and was subsequently spread worldwide. Honey bees are a critical element in global food production, as pollinators of many crops. Their economic value in the United States alone is estimated at \$17 billion annually<sup>107</sup>. Other bee species also pollinate crops and wild plants; these include bumble bees (genus *Bombus*) and other *Apis* species, which are also social and which contain gut communities dominated by some of the same bacterial species clusters as those found in *A. mellifera*. Since 2006, honey bee colonies have been suffering high mortality, and many wild bumble bee populations are declining as well<sup>103,108</sup>. Deteriorating bee health appears to stem from multiple causes, including toxins, poor nutrition, and disease<sup>109</sup>. The movement of viral, bacterial and eukaryotic pathogens between bee species as a result of anthropogenic species introductions may be another critical factor<sup>110</sup>. All of these stressors on bees might be modulated by their gut microorganisms. Thus, besides offering general insights into gut community processes relevant to understanding the human microbiota, research on the honey bee microbiota may help to elucidate why bee populations are declining.

**Box 2**

**Some parallels and contrasts between the gut microbiota of honey bees and humans**

<b>Similarities between human and honey bee gut communities</b>	<b>Citation</b>
Transmission between hosts through social interactions	honey bees <sup>13,14</sup> ; bumble bees <sup>40,111</sup>
Dominated by host-adapted species that are not typically found outside the gut	20–22,31
Grow best under oxygen concentrations lower than those in air	23,26,112
Extensive strain diversification within resident bacterial species	10,12,55,76
Spatially organized community, with greatest abundance in the distal regions of the gut, past the area of most host digestive activity	13
Impact of chronic antibiotic exposure on resistance levels	73
<b>Features of bee gut communities that differ from those of humans</b>	
Simple community, with <10 bacterial species clusters comprising over 95% of the community	10,16,20–22,37,62
All major species cultivable in the laboratory	15,23,24,26,27,77
Relative ease of experimentally manipulating gut communities in live hosts	honey bees <sup>14,32,33</sup> ; bumble bees <sup>11,80</sup>

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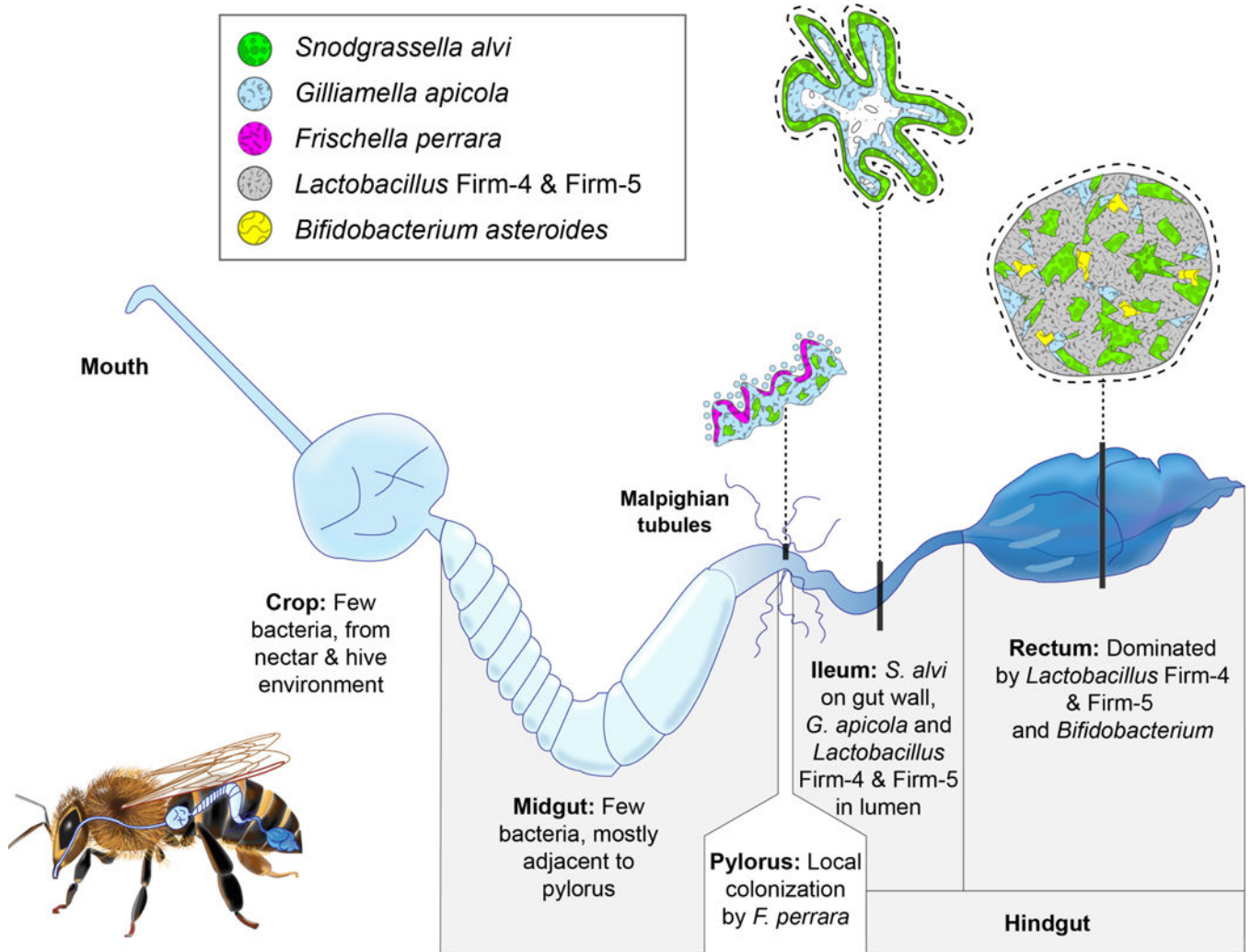
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**Box 3****Transmission of the honey bee microbiota**

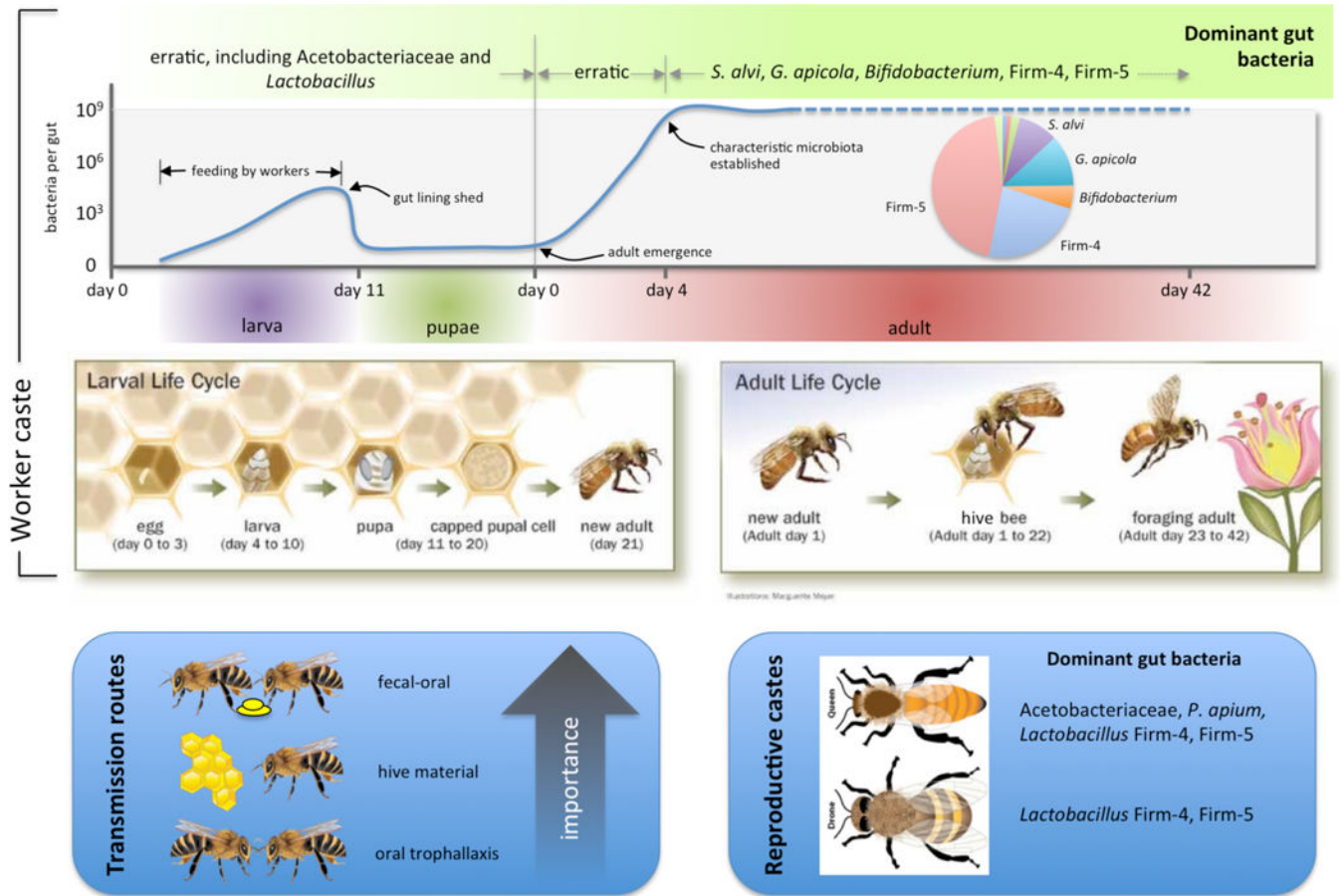
Sociality, including the sharing of the hive environment by a social group, is central to between-host transmission of the bee gut microbiota (Fig. 2). After pupating in capped cells within colonies, honey bee adults emerge with germ-free guts<sup>14,33</sup>. If removed manually from the cells and maintained under sterile lab conditions, their guts do not acquire substantial communities, as estimated using qPCR with universal bacterial primers. Newly emerged bees that chew out on their own may be inoculated by residual gut symbionts on the frame surface.

The stages of development of the gut microbiota have been characterized by sampling marked cohorts of workers from colonies and using amplicons of 16S rDNA to estimate the size and composition of distinct gut regions<sup>13,14</sup>. Initially the community is small and erratic in composition, dominated by environmental bacteria and lacking differentiation between gut regions. By day 3, the communities contain  $>10^7$  bacteria mostly from the characteristic bee gut species, and the ileum and rectum begin displaying “normal” community compositions. These communities plateau at about  $10^9$  bacteria by day 8. Once established, gut communities are generally stable as workers transition through different behavioural stages<sup>62</sup>. This shift from an initial erratic community to one dominated by “adult” bacteria, largely mirrors the development of the gut microbiota observed in human infants<sup>113</sup>.

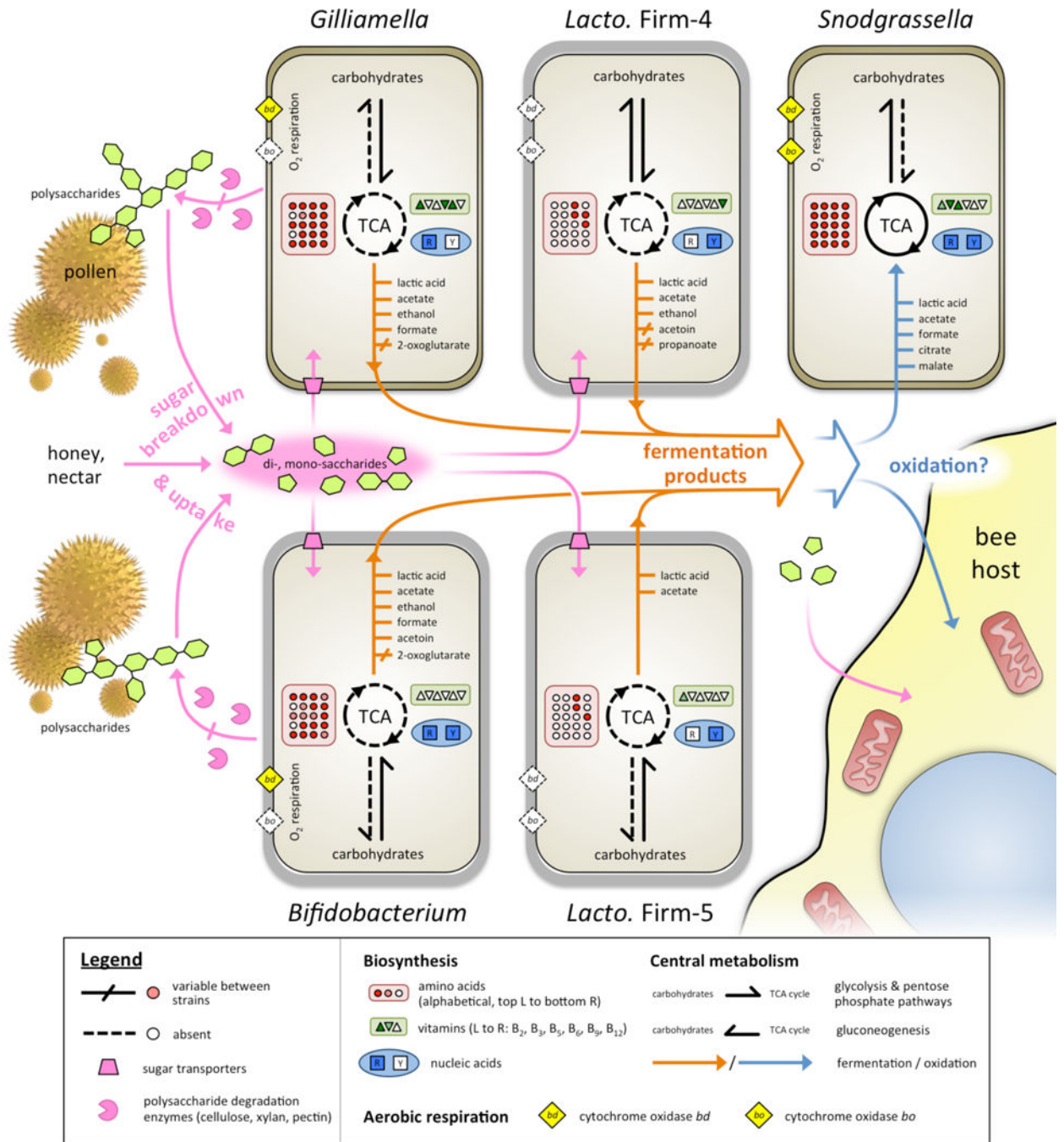
Establishment of a stable community occurs before workers leave the hive, implying transmission through nest mates or hive components, such as wax surfaces. In experimental tests, the most typical gut communities developed when bees were exposed directly to older bees or were fed their macerated hindguts<sup>14</sup>. Based on experiments manipulating potential transmission sources, oral trophallaxis, a common behaviour for communication and food transfer, is not a major route for transmission, consistent with observations that the foregut harbours few bacteria<sup>13,22</sup>. A faecal route appears important<sup>14</sup>, particularly for *S. alvi*, *G. apicola* and *F. perrara*, although members of the gut community also may be acquired through contact with hive components that have been in recent contact with live bees<sup>14,114</sup>. Acetobacteraceae strains might be transmitted through the stored pollen food supply<sup>31</sup>.



**Figure 1.** Composition and spatial organization of bacterial communities within the honey bee gut. (A) The microbiota composition of a typical adult worker, based on an averaged community profile using 16S rRNA gene amplicons from whole guts<sup>10</sup>. (B) Localization of different bacteria within the gut compartments, based on fluorescent *in situ* hybridization and qPCR studies.



**Figure 2.** Honey bee life history and associated changes in the gut microbiota. The microbiota of the worker caste (top) is best understood; studies of reproductive castes suggest their microbiota composition differs from that of workers. Naïve workers harbour no or very few bacteria and acquire the normal gut microbiota primarily through contact with faecal material, although other transmission routes may play a role as well (see Box 3). Total numbers of bacteria at any given life stage, estimated using qPCR with universal bacterial primers, vary between studies; given numbers are based on reasonable maximal estimates. Likewise, abundances of dominant species may differ among individual bees and sampling methods.



**Figure 3.** Metabolic activities of the core bee gut microbiota, as inferred from genomic, metagenomic, and metatranscriptomic data, and from experimentally verified activities from cultured bacterial strains. Fermenters (*G. apicola*, *Bifidobacterium*, *Lactobacillus*) dominate; some can break down plant polysaccharides found in pollen, such as pectin and xylan. Sugars are fermented to various end products (lactic acid, acetate, etc.), depending on the species and strain. *De novo* biosynthetic capabilities for these fermenters are limited. Fermentation products may be taken up and oxidized by *Snodgrassella alvi* for energy and carbon.

Plausibly, the bee host also gains access to products of microbe-assisted carbohydrate breakdown.

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