Title: Functional roles and metabolic niches in the honey bee gut microbiota

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Abstract

Gut microbiota studies on diverse animals facilitate our understanding of the general principles governing microbiota-host interactions. The honey bee adds a relevant study system due to the simplicity and experimental tractability of its gut microbiota, but also because bees are important pollinators that suffer from population declines world-wide. The use of gnotobiotic bees combined with genetic tools, 'omics' analysis, and experimental microbiology has recently provided important insights about the impact of the microbiota on bee health and the general functioning of gut ecosystems.

Highlights

- Honey bees harbor a simple gut microbiota that is experimentally tractable
- The gut microbiota increases body weight gain and confers pathogen resistance
- Fermentation and breakdown of pollen wall components are major metabolic activities
- Individual community members have specialized yet overlapping metabolic capacities

Introduction

Animals have been exposed to microbes since their evolutionary origin resulting in intimate microbiota-host interactions. Consequently, diverse microbial communities colonize different host tissues, with the gastrointestinal tract harboring the most dense and diverse communities [1]. The evolution of the gut microbiota is affected by physiological (diet, metabolism, immune system, gut anatomy) and ecological (interactions, transmission, bottlenecks) processes [2–6], generating specialized communities that are taxonomically and functionally diverse [7–9]. Despite this diversity, the gut microbiota has conserved symbiotic roles across animals affecting metabolism, development, pathogen resistance and immune system maturation. Yet, we still lack a general understanding of many aspects of gut microbe-host interactions [10,11]. That is in part because

general principles can only arise from the observation of similar patterns across multiple model organisms [1,12].

The honey bee gut microbiota has recently emerged as an attractive model to understand fundamental aspects of gut microbiology due to its relatively simple community composition and experimental amenability [13]. But research on the honey bee gut microbiota is also relevant from an applied standpoint. Honey bees provide worldwide ecosystem services as crop pollinators that amount to an estimated 150 billion euros [14]. However, diverse pathogens and environmental stressors threaten bee health. Here we will summarize the latest findings that have significantly advanced our understanding of the impact of the gut microbiota on bee health and the general functioning, ecology and evolution of microbiota-host symbioses [15–19].

Composition of the honey bee gut microbiota

In most animals, the distal section of the gut (ileum and rectum in honey bees) houses most of the bacterial biomass. Dietary compounds that are not digested by the host (e.g. glycans) accumulate in these regions, becoming the main sources of energy, carbon and nitrogen available for the microbiota [20,21]. The honey bee hindgut is home to a simple, yet highly specific bacterial community [16,22]. It is composed of five core members (Table 1), which are typically present in every female worker bee across the world and differentially distributed between ileum and rectum [23]. These bacteria are also found in related corbiculate bees but have rarely been detected in other environments [16,22,24,25] suggesting longstanding and specialized symbiotic associations [16,25]. Other non-core members can be categorized into specialized honey bee gut associates that are present in every hive but not every individual (such as *F. perrara*) [26], or environmental bacteria that are found in the hive or flower environment and occasionally colonize the bee gut (Table 1). The latter group comprises diverse bacteria depending on sampling methodology, analysis depth and conceptual definition of the gut microbiota. Consequently, the reported bacterial species richness varies throughout the literature, but the consensus is that the honey bee gut microbiota is

relatively simple and conserved in terms of taxonomic composition and in comparison to the mammalian or termite gut microbiota.

Several studies have found subtle differences in community composition of the bee gut microbiota, changing with season [27], diet [28], host age [23,29], caste [30] and geography [31]. However, the relative abundance of core members differs substantially between studies, making cross-study comparisons difficult, and highlighting the needs for standardization of experimental design, primer choice, and analysis pipelines. Furthermore, all core members harbour extensive genomic diversity in terms of sequence divergence and gene content variation, which suggests that selection in the honey bee gut (similarly to the human gut) occurs at the strain level [32]. Many questions regarding the compositional dynamics and genetic diversity thus remain to be addressed.

Functions inferred from genomics

Initial genomic inferences revealed that most bee gut bacteria possess relatively small genomes that have lost core metabolic functions, but encode pathways for biofilm formation and cell-to-cell interactions [8,33–35]. Four core species dedicate an extensive amount of their gene contents to carbohydrate metabolism, but encode an incomplete citrate cycle (Table 1) suggesting fermentation is the predominant metabolism in the bee gut. In contrast, the remaining core member *S. alvi* harbors no genes for glycolysis, but instead encodes genes for carboxylate transport and an alternative citrate cycle optimized for acetate utilization, indicating a different metabolic niche [16,33]. Overall, the genomic properties of the core members reflect host-associated lifestyles and suggest adaptation to the carbohydrate-rich bee diet and colonization of distinct spatial and metabolic niches.

Functions identified by experiments

Experimental manipulations of the adult bee gut microbiota have recently become possible. Cultivable representatives and genomes are available for most community members and

gnotobiotic bees can be generated by transferring developing larvae from brood cells into laboratory hoarding cages. The resulting newly emerged bees do not harbor any of the specific gut symbionts, as they are usually acquired after adult emergence by exposure to nest mates and the hive environment [36]. We refer to these bees as microbiota-depleted because they are not axenic and can harbor environmental bacteria at low abundances [15,36]. Yet, by feeding cultured strains or gut homogenates of hives bees, individual members or the complete microbiota can be experimentally established in the gut of these bees. A number of studies have taken advantage of this experimental amenability, which has substantially advanced our understanding of the functional capabilities of bee gut bacteria and their impact on bee health as summarized below [15,17–19,37,38].

Impact on bee health and physiology

There is increasing evidence that the gut microbiota of social bees confers colonization resistance from potentially harmful microbes. In bumblebees, the presence of the gut microbiota was shown to cause a significant reduction of the gut parasite *Crithidia bombi* [39]. Three recent studies suggested a similar role in honey bees. First, interfering with the assembly of the gut microbiota in adult honey bees was shown to increase loads of the parasite *Lotmaria passim* [40]. Second, when disturbing the gut microbiota with the antibiotic tetracycline, honey bees had decreased survivorship which was linked to an increased susceptibility to the opportunistic pathogen *Serratia* [41]. Third, bees that were fed ‘aged’ pollen experienced increased mortality, higher loads of the fungal pathogen *Nosema* and severe shifts in gut microbiota composition [42]. Collectively, these results suggest a link between the honey bee gut microbiota, colonization resistance or tolerance against pathogens, and host benefits. Microbiota transplants and monocolonization experiments both showed that bee gut bacteria stimulate the host immune system [19,37]. However, to what extent these effects may serve as priming responses increasing pathogen resistance is yet to be shown.

Additional host phenotypes are affected by the gut microbiota (Figure 1). Newly emerged bees colonized with the microbiota of hive bees gained larger body and gut weights than their microbiota-depleted counterparts, but without showing any difference in survival [18]. Compared to their microbiota-depleted counterparts, these bees also showed an increased behavioral response towards sucrose and water along with increased expression levels of insulin-like peptide I in the head and vitellogenin in the abdomen [18]. These two major endocrine factors have been linked to nutrition and affect bee lifespan and foraging behaviour [43,44]. Although the underlying causes of weight gain remain to be elucidated (gut enlargement or increased food content), these findings collectively suggest that the microbiota regulates bee appetite and/or growth via increased insulin signalling.

Metabolic roles in dietary breakdown

Most symbiotic roles of gut bacteria are based on metabolic interactions with their host [21,45,46]. Therefore, studying the metabolism of the gut microbiota is key to understand how effects on the host are mediated. For example, in *Drosophila* the production of acetate by the gut commensal *Acetobacter pomorum* was shown to be responsible for elevated host growth via insulin signalling [47]. Honey bees obtain all their nutrients from a highly specific diet consisting exclusively of pollen and nectar [48]. Although little is known about pollen breakdown and catabolism in the bee gut, it is evident that the gut microbiota influences these processes. For example a metagenomic study showed that *G.apicola* is capable of degrading pectin, a major component of the inner pollen wall (intine) [49]. Recently, two studies applied untargeted metabolomics to characterize metabolic activities of the honey bee gut microbiota in a more comprehensive manner [15,18]. Zheng et al. found that galacturonate, the major pectin breakdown product, accumulates in the gut compartment predominantly colonized by *G. apicola*. The second study conducted by Kesnerova et al. revealed a plethora of pollen-derived substrates utilized by the bee gut microbiota. Prominent substrates were diverse aromatic compounds (glycosylated flavonoids, phenolamides and quinate), nucleosides,

carboxylic acids, and ω-hydroxyacids (Figure 1). Strikingly, many of these substrates are part of the recalcitrant outer pollen wall (flavonoids and phenolamides from the coat, ω-hydroxy acids from the exine). The extent to which utilization of these substrates may be beneficial for the host needs further investigation. However, these findings highlight that the bee gut microbiota has specialized on utilizing the dietary compounds that are not degraded and assimilated by the host and accumulate in the hindgut.

Both studies also revealed a large number of gut metabolites that accumulated in the presence of the microbiota [15,18]. High concentrations of organic acids (succinate, acetate and propionate) in the gut of colonized bees confirmed that fermentation is a major metabolic activity of the bee bacteria as inferred from genomics. In particular, acetate was produced in high quantities, making it tempting to speculate that a similar mechanism as in *Drosophila* may be responsible for the impact on insulin signalling and growth [18,47]. Simple sugars such as glucose and fructose present in nectar and pollen, and complex polysaccharides such as pectin from the pollen wall are apparent substrates for bacterial fermentation. A less obvious pollen-derived fermentation substrate are glycosylated flavonoids. They can make up to 4% of the pollen dry weight and constitute the majority of the flavonoids utilized by the bee gut microbiota [15]. Indeed, when growing certain honey bee gut symbionts outside the host in the presence of pollen extracts, flavonoid glycosides decreased in abundance and deglycosylated flavonoids (aglycones) accumulated, indicating that the sugar moieties are cleaved off and fermented (Figure 1). Aromatic compound degradation intermediates also accumulated in the presence of the microbiota and were produced by the same bacteria that bioconverted flavonoids [15]. This may suggest that flavonoids are not only deglycosylated, but also that the polyphenolic backbone is broken down. However, a direct link between flavonoid utilization and the production of these intermediates has not yet been established, since the same products can result from the degradation of phenolamides or aromatic amino acids.

Metabolic changes induced by the microbiota will change the physicochemical properties of the gut environment. Indeed, in the presence of the microbiota both pH and redox potential decreased throughout the bee gut and oxygen levels were reduced in the ileum [18]. However it is currently unknown which metabolites are transferred to the host, although this is central to understand how the microbiota triggers certain phenotypes (Figure 1). In mammals, for example, butyrate is transferred from the gut into other host tissues and impacts colonocyte function [50]. Similar roles in bees have been suggested [18], but are yet unconfirmed. Flavonoids antioxidant, antimicrobial or anti-inflammatory bioactivities have been reported, and their potential is largely influenced by their glycosylation state [51]. Thus, flavonoid degradation or bioconversion by the microbiota may have important functional consequences [52] that could be linked to colonization resistance and gut homeostasis.

Functional roles of individual species

The simple composition of the bee gut microbiota makes it possible to reveal the functional roles of each and every community member. This allows obtaining an integrated view on the gut ecosystem of bees and advances our general understanding of how gut communities function.

By monocolonizing bees with individual species, Kesnerova et al. [15] probed the contribution of major community members to the overall metabolic changes induced by the bee gut microbiota. This approach uncovered, among many other metabolic functions, that Firm-5 is the major flavonoid utilizer and that *B. asteroides* triggers the production of host hormones and signalling molecules (Table 1). In total ~80% of the metabolic changes induced by the complete microbiota could be attributed to individual community members. While some substrates were utilized by only a single community member and a few instances of cross-feeding were revealed, in most cases several community members were found to metabolize the same compound. This suggests that bee gut bacteria have overlapping metabolic capabilities, leading to the fundamental question of how co-evolved gut bacteria share resources during co-existence and avoid competition.

How bacterial activities in the bee gut impact the host or gut environment has so far remained elusive except for a few cases. For example, the microaerophilic core member *S. alvi* has been implicated in maintaining anoxic conditions in the ileum [18] by respiring oxygen via an alternative citrate cycle driven by acetate [23,53,54]. Interestingly, acetate is one of the major fermentation products generated by *G. apicola,* which together with *S. alvi* forms a multispecies biofilm on top of the host epithelium, from where the oxygen may be released. Another example is the local deposition of melanin on the epithelial surface of the pylorus (the so-called scab phenotype), triggered by the gammaproteobacterium *Frischella perrara* by eliciting a specific host immune response after colonization [26,37]. While neither its functional role nor the underlying mechanism have been yet elucidated, the scab phenotype shows that bee gut symbionts engage in specific and functionally distinct interactions with the host. Genetic approaches are useful to identify genes responsible for such interactions in the bee gut. For example, a genome-wide transposon-sequencing screen in *S. alvi* revealed that genes for amino acid biosynthesis, adhesion, pili formation, and iron uptake are dispensable for growth in vitro but not for colonization of the bee gut environment [17]. Comparisons of fitness factors across different community members and under different host conditions will further add to our understanding of niche separation in the gut ecosystem.

Keeping the gut microbiota simple

Considering the many parallels to the gut microbiota of mammals (e.g. transmission, metabolism, host adaptation and biogeography; see references [1,55] for further details) it seems surprising that the honey bee gut microbiota has a relative simple and stable composition [7,9]. There may be numerous reasons for this. First of all, the diet of honey bees is highly specialized, which may have facilitated the evolution of a small number of core members with streamlined metabolic activities [4,10]. However, pollen from different plant sources can differ in nutrient content and thus one may expect a larger extent of diversity in the bee gut microbiota. Indeed, when looking beyond the 16S

rRNA gene level, most bee gut symbionts show substantial strain-level diversity with divergent lineages varying in functional gene content[32,49,56,57]. This intra-species diversity may be the result of substrate specialization mirroring the nutrient diversity in floral pollen. Strong selection by the host, either through specific immune responses, epithelial receptors, growth-promoting secretions, or physiochemical gut properties may also contribute to the simple composition of the bee gut microbiota [58,59]. Interestingly, when comparing between corbiculate bees, Kwong et al. [16] found that host ecology drives microbiota diversity. Bee species with larger colonies tended to have a more diverse microbiota, which is in line with the concept of species-area relationship postulating that larger habitats harbor greater diversity [60]. However, one needs to keep in mind that this analysis was based on 16S rRNA amplicon data, which can only provide limited insights into intra-species diversity. Finally, microbe-microbe interactions are known to determine community assembly. Strong positive interactions between core members of the bee microbiota together with efficient transmission to newly emerged bees [36,61] may hinder other bacteria from invasion during community assembly. Moreover, bee gut symbionts harbor genes for toxin secretion that could target environmental bacteria [64]. These properties may have contributed to the emergence of a relatively simple community over the course of evolution.

Conclusion and perspective

The studies reviewed here expand our understanding of the roles of the gut microbiota in bee health and provide many new insights about the metabolic capacities of individual community members. The established methods will help to build on these findings; and the ease to manipulate the bee system suggests that additional methods from other microbe-host system may be readily adaptable. We have just started to understand the impact of the bee gut microbiota on the host immune system, its physiology and metabolism. RNAi or CRISPR will be useful to reveal host genes involved in the crosstalk with the microbiota, while bacterial genetics and advanced microscopy can already be applied to characterize bacterial factors underlying symbiosis in the bee gut. Future research should

also tackle how the metabolic activities in the bee gut impact the metabolism of the host and trace metabolite transfer from the gut into host tissues and vice versa.

One of the biggest challenges in the field of gut microbiology is explaining the large variability in microbial community composition between individuals. Understanding how environmental, bacterial or host factors affect community assembly and stability has thus remained difficult. The experimental approaches reviewed here offer the possibility to probe the contribution of these factors in a defined and controllable model system.

Of equal relevance is how closely related community members with similar metabolic activities can coexist in the gut. While several studies have shown that extensive strain diversity exists in honey bee gut bacteria, quantitative population genomics coupled with experimental approaches will help to identify ecologically and genetically cohesive groups within species and explain their coexistence and evolution.

Finally, while we have learned that the gut microbiota impacts bee health, it has remained elusive to what extent gut bacteria influence virus loads and pesticide resilience, two major concerns for bee health. Given the contribution of the bee gut microbiota to host nutrition and immune system stimulation, or the fact that gut bacteria of other animals have been shown to harbour pesticide degradation capacities, such functions are likely for the bee gut microbiota and may have major implications for bee management. Overall, we expect that future research on the honey bee microbiota will make further substantial contributions to better understand bee health and to implement a common theoretical framework for microbiota-host symbiosis.

Acknowledgements

This work was funded by the HFSP Young Investigator grant RGY0077/2016 and the ERC-StG 'MicroBeeOme'.

Figure legend

Figure 1. Summary of the functional roles of the honey bee gut microbiota. (A) Known host effects of the honey bee gut microbiota. Bacteria in the gastrointestinal tract are depicted according to their preferentially localization in either ileum or rectum, according to the colors in B. (B) Major activities of gut bacteria in the ileum and rectum. Most bacterial substrates originate from the host diet (honey/nectar and pollen). A schematic cross-section through a pollen grain shows the pollen wall consisting of pollen coat, exine, and intine. The intine is the inner wall containing cellulose, hemicellulose and pectin. Pectin can be degraded by *G. apicola* in the ileum. The pollen coat and exine contain ω-hydroxy acids, flavonoids, and phenolamides, all of which are utilized by different members of the bee gut microbiota. Additional pollen-derived substrates utilized by the microbiota are nucleosides, organic acids, quinate, and sugars or sugar acids. The epithelium of the pylorus (the ileum's entrance) is colonized by *F. perrara* and induces the scab phenotype, a local immune response resulting in the deposition of melanin on the epithelium's cuticle lining. The ileum is predominantly colonized by *G. apicola* and *S. alvi*. *G. apicola* produces organic acids such (pyruvate, acetate, or succinate) during fermentation, which can be respired by *S. alvi* under the utilization of oxygen presumably responsible for the decrease of oxygen levels in the colonized ileum. The rectum is dominated by *Lactobacillus* Firm-5 and Firm-4, and *Bifidobacterium asteroides*. They utilize various pollen coat-derived aromatic compounds including flavonoids and phenolamides and ω-hydroxy acids from the exine. Flavonoids are degylcosylated, the sugars fermented and the backbone possibly degraded further. As a result, aromatic compound degradation intermediates and organic acids accumulate throughout the hindgut. Which bacteria-derived metabolites are absorbed by the host is currently unkown. However, *B. asteroides* seems to trigger the production of host-derived prostaglandins (PGs) and juvenile hormone derivatives (JHs).

References

1. Kostic AD, Howitt MR, Garrett WS: **Exploring host–microbiota interactions in animal models and humans**. *Genes Dev* 2013, **27**:701–718.

2. Bashan A, Gibson TE, Friedman J, Carey VJ, Weiss ST, Hohmann EL, Liu Y-Y: **Universality of human microbial dynamics**. *Nature* 2016, **534**:259–262.

3. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA: **The Application of Ecological Theory Toward an Understanding of the Human Microbiome**. *Science* 2012, **336**:1255–1262.

4. Engel P, Moran NA: **The gut microbiota of insects – diversity in structure and function**. *FEMS Microbiol Rev* 2013, **37**:699–735.

5. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J: **Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans**. *Am J Clin Nutr* 2011, **94**:58–65.

6. Levy R, Borenstein E: **Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules**. *Proc Natl Acad Sci* 2013, **110**:12804–12809.

7. Consortium THMP: **Structure, function and diversity of the healthy human microbiome**. *Nature* 2012, **486**:207–214.

8. Engel P, Moran NA: **Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis**. *Gut Microbes* 2013, **4**:60–65.

9. Su L, Yang L, Huang S, Li Y, Su X, Wang F, Bo C, Wang ET, Song A: **Variation in the Gut Microbiota of Termites (*Tsaitermes ampliceps*) Against Different Diets**. *Appl Biochem Biotechnol* 2017, **181**:32–47.

10. Adair KL, Douglas AE: **Making a microbiome: the many determinants of host-associated microbial community composition**. *Curr Opin Microbiol* 2017, **35**:23–29.

11. Koskella B, Hall LJ, Metcalf CJE: **The microbiome beyond the horizon of ecological and evolutionary theory**. *Nat Ecol Evol* 2017, **11**:1606–1615.

12. Douglas AE: **Lessons from Studying Insect Symbioses**. *Cell Host Microbe* 2011, **10**:359–367.

13. Engel P, Kwong WK, McFrederick Q, Anderson KE, Barribeau SM, Chandler JA, Cornman RS, Dainat J, Miranda JR de, Doublet V, et al.: **The Bee Microbiome: Impact on Bee Health and Model for Evolution and Ecology of Host-Microbe Interactions**. *mBio* 2016, **7**:e02164-15.

14. Gallai N, Salles J-M, Settele J, Vaissière BE: **Economic valuation of the vulnerability of world agriculture confronted with pollinator decline**. *Ecol Econ* 2009, **68**:810–821.

15. Kešnerová L, Mars RAT, Ellegaard KM, Troilo M, Sauer U, Engel P: **Disentangling metabolic functions of bacteria in the honey bee gut**. *PLoS Biol* 2017, **15**:e2003467.

** This study revealed major metabolic activities of the honey bee gut microbiota linked to the degradation of recalcitrant pollen components and assigned these activities to individual community members. To this end, microbiota-depleted bees were colonized with either a community reconstituted from cultured strains or with individual species and subsequently analysed by metabolomics. Results obtained from these bee colonization experiments were confirmed by bacterial culture experiments.

16. Kwong WK, Medina LA, Koch H, Sing K-W, Soh EJY, Ascher JS, Jaffé R, Moran NA: **Dynamic microbiome evolution in social bees**. *Sci Adv* 2017, **3**:e1600513.

** This paper presents the most comprehensive analysis of the corbiculate bee gut microbiota, reporting distribution patterns and defining a core set of shared species.

17. Powell JE, Leonard SP, Kwong WK, Engel P, Moran NA: **Genome-wide screen identifies host colonization determinants in a bacterial gut symbiont**. *Proc Natl Acad Sci* 2016, **113**:13887–13892.

* This is the first study revealing a genome-wide catalogue of genetic determinants involved in colonization of the bee gut, applying transposon sequencing (TnSeq) to the gut symbiont *S. alvi.*

18. Zheng H, Powell JE, Steele MI, Dietrich C, Moran NA: **Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling**. *Proc Natl Acad Sci* 2017, **114**:4775–4780.

** Through a series of independent experiments with colonized and microbiota-depleted bees, the authors show that the gut microbiota induces host phenotypes linked to nutrition and metabolism. The authors further show that the gut microbiota changes physicochemical properties and metabolite profiles in different gut compartments and that saccharolytic fermentation is a dominant metabolic activity in the bee gut.

19. Kwong WK, Mancenido AL, Moran NA: **Immune system stimulation by the native gut microbiota of honey bees**. *R Soc Open Sci* 2017, **4**:170003.

20. Donaldson GP, Lee SM, Mazmanian SK: **Gut biogeography of the bacterial microbiota**. *Nat Rev Microbiol* 2015, **14**:nrmicro3552.

21. Flint HJ, Duncan SH, Louis P: **The impact of nutrition on intestinal bacterial communities**. *Curr Opin Microbiol* 2017, **38**:59–65.

22. Martinson VG, Danforth BN, Minckley RL, Rueppell O, Tingek S, Moran NA: **A simple and distinctive microbiota associated with honey bees and bumble bees**. *Mol Ecol* 2011, **20**:619–628.

23. Martinson VG, Moy J, Moran NA: **Establishment of Characteristic Gut Bacteria during Development of the Honeybee Worker**. *Appl Environ Microbiol* 2012, **78**:2830–2840.

24. Corby-Harris V, Snyder LA, Schwan MR, Maes P, McFrederick QS, Anderson KE: **Origin and Effect of Alpha 2.2 Acetobacteraceae in Honey Bee Larvae and Description of *Parasaccharibacter apium* gen. nov., sp. nov.** *Appl Environ Microbiol* 2014, **80**:7460–7472.

25. Koch H, Abrol DP, Li J, Schmid-Hempel P: **Diversity and evolutionary patterns of bacterial gut associates of corbiculate bees**. *Mol Ecol* 2013, **22**:2028–2044.

26. Engel P, Bartlett KD, Moran NA: **The Bacterium *Frischella perrara* Causes Scab Formation in the Gut of its Honeybee Host**. *mBio* 2015, **6**:e00193-15.

27. Ludvigsen J, Rangberg A, Avershina E, Sekelja M, Kreibich C, Amdam G, Rudi K: **Shifts in the Midgut/Pyloric Microbiota Composition within a Honey Bee Apiary throughout a Season**. *Microbes Environ* 2015, **30**:235–244.

28. Jones JC, Fruciano C, Hildebrand F, Hasan al Toufailia, Nicholas Balfour, Philipp Engel, Peer Bork, Francis LW Ratnieks, William OH Hughes: **Gut microbiota composition is associated with environmental landscape in honey bees**. *Ecol Evol* 2017, doi:10.1002/ece3.3597.

29. Tarpy DR, Mattila HR, Newton ILG: **Development of the Honey Bee Gut Microbiome throughout the Queen-Rearing Process**. *Appl Environ Microbiol* 2015, **81**:3182–3191.

30. Kapheim KM, Rao VD, Yeoman CJ, Wilson BA, White BA, Goldenfeld N, Robinson GE: **Caste-Specific Differences in Hindgut Microbial Communities of Honey Bees (*Apis mellifera*)**. *PLoS ONE* 2015, **10**:e0123911.

31. Ludvigsen J, Porcellato D, L'Abée-Lund TM, Amdam GV, Rudi K: **Geographically widespread honeybee-gut symbiont subgroups show locally distinct antibiotic-resistant patterns**. *Mol Ecol* 2017, **26**:6590–6607.

32. Ellegaard KM, Engel P: **Beyond 16S rRNA Community Profiling: Intra-Species Diversity in the Gut Microbiota**. *Front Microbiol* 2016, **7**.

33. Kwong WK, Engel P, Koch H, Moran NA: **Genomics and host specialization of honey bee and bumble bee gut symbionts**. *Proc Natl Acad Sci* 2014, **111**:11509–11514.

34. Kwong WK, Mancenido AL, Moran NA: **Genome Sequences of *Lactobacillus* sp. Strains wkB8 and wkB10, Members of the Firm-5 Clade, from Honey Bee Guts**. *Genome Announc* 2014, **2**:e01176-14.

35. Ellegaard KM, Tamarit D, Javelind E, Olofsson TC, Andersson SG, Vásquez A: **Extensive intra-phylotype diversity in lactobacilli and bifidobacteria from the honeybee gut**. *BMC Genomics* 2015, **16**:284.

36. Powell JE, Martinson VG, Urban-Mead K, Moran NA: **Routes of Acquisition of the Gut Microbiota of the Honey Bee *Apis mellifera***. *Appl Environ Microbiol* 2014, **80**:7378–7387.

37. Emery O, Schmidt K, Engel P: **Immune system stimulation by the gut symbiont *Frischella perrara* in the honey bee (*Apis mellifera*)**. *Mol Ecol* 2017, **26**:2576–2590.

    ***** The authors used RNA sequencing (RNAseq) to characterize the host response underlying the scab phenotype induced by *F. perrara* by analysing monocolonized honey bees. The study shows that honey bee gut symbionts that colonize the same gut compartment can trigger distinct host response.

38. Ricigliano VA, Fitz W, Copeland DC, Mott BM, Maes P, Floyd AS, Dockstader A, Anderson KE: **The impact of pollen consumption on honey bee (*Apis mellifera*) digestive physiology and carbohydrate metabolism**. *Arch Insect Biochem Physiol* 2017, **96**:e21406.

    ***** This study shows that sugar hydrolysis activity increases in the gut after pollen ingestion, and that underlying enzymes may be bacteria-, host-, and pollen-derived.

39. Koch H, Schmid-Hempel P: **Socially transmitted gut microbiota protect bumble bees against an intestinal parasite**. *Proc Natl Acad Sci* 2011, **108**:19288–19292.

40. Schwarz RS, Moran NA, Evans JD: **Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers**. *Proc Natl Acad Sci* 2016, **113**:9345–9350.

    * The authors demonstrate that the susceptibility to parasite infections is increased when interfering with the natural assembly of the gut microbiota.

41. Raymann K, Shaffer Z, Moran NA: **Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees**. *PLoS Biol* 2017, **15**:e2001861.

    ** This study showed that tetracycline a widely used antibiotics in beekeeping causes dysbiosis of the gut microbiota, increases susceptibility to pathogens, and elevates mortality of honey bees.

42. Maes PW, Rodrigues PAP, Oliver R, Mott BM, Anderson KE: **Diet-related gut bacterial dysbiosis correlates with impaired development, increased mortality and *Nosema* disease in the honeybee (*Apis mellifera*)**. *Mol Ecol* 2016, **25**:5439–5450.

    * This study shows that low quality diet can cause severe gut dysbiosis increasing parasite susceptibility and honey bee mortality.

43. Nelson CM, Ihle KE, Fondrk MK, Jr REP, Amdam GV: **The Gene vitellogenin Has Multiple Coordinating Effects on Social Organization**. *PLoS Biol* 2007, **5**:e62.

44. Ihle KE, Baker NA, Amdam GV: **Insulin-like peptide response to nutritional input in honey bee workers**. *J Insect Physiol* 2014, **69**:49–55.

45. Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, et al.: **Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children**. *Science* 2016, **351**:aad3311.

46. Hehemann J-H, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G: **Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota**. *Nature* 2010, **464**:nature08937.

47. Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, Yoon J-H, Ryu J-H, Lee W-J: ***Drosophila* Microbiome Modulates Host Developmental and Metabolic Homeostasis via Insulin Signaling**. *Science* 2011, **334**:670–674.

48. Seeley TD: *Honeybee Ecology: A Study of Adaptation in Social Life: A Study of Adaptation in Social Life*. Princeton University Press; 2014.

49. Zheng H, Nishida A, Kwong WK, Koch H, Engel P, Steele MI, Moran NA: **Metabolism of Toxic Sugars by Strains of the Bee Gut Symbiont *Gilliamella apicola***. *mBio* 2016, **7**:e01326-16.

    * Using genomics and *in vitro* experiments, this study shows that divergent strains of *G. apicola* can utilize various, potentially toxic sugars present in the bee diet.

50. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, Hammer RE, Williams SC, Crowley J, Yanagisawa M, et al.: **Effects of the gut microbiota on host adiposity are**

modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci* 2008, **105**:16767–16772.

51. Xiao J: **Dietary flavonoid aglycones and their glycosides: Which show better biological significance?** *Crit Rev Food Sci Nutr* 2017, **57**:1874–1905.

52. Thaiss CA, Itav S, Rothschild D, Meijer MT, Levy M, Moresi C, Dohnalová L, Braverman S, Rozin S, Malitsky S, et al.: **Persistent microbiome alterations modulate the rate of post-dieting weight regain**. *Nature* 2016, **540**:544–551.

53. Kwong WK, Moran NA: **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* 2013, **63**:2008–2018.

54. Kwong WK, Zheng H, Moran NA: **Convergent evolution of a modified, acetate-driven TCA cycle in bacteria**. *Nat Microbiol* 2017, **2**:17067.

    * This study revealed that *S. alvi* has evolved a modified TCA cycle, possibly as an adaptation to the production of acetate by other bee gut symbionts as a result of bacterial fermentation.

55. Kwong WK, Moran NA: **Gut microbial communities of social bees**. *Nat Rev Microbiol* 2016, **14**:374–384.

56. Engel P, Stepanauskas R, Moran NA: **Hidden Diversity in Honey Bee Gut Symbionts Detected by Single-Cell Genomics**. *PLOS Genet* 2014, **10**:e1004596.

57. Praet J, Parmentier A, Schmid-Hempel R, Meeus I, Smagghe G, Vandamme P: **Large-scale cultivation of the bumblebee gut microbiota reveals an underestimated bacterial species diversity capable of pathogen inhibition**. *Environ Microbiol* [date unknown], doi:10.1111/1462-2920.13973.

58. Näpflin K, Schmid-Hempel P: **Host effects on microbiota community assembly**. *J Anim Ecol* [date unknown], doi:10.1111/1365-2656.12768.

59. Schluter J, Foster KR: **The Evolution of Mutualism in Gut Microbiota Via Host Epithelial Selection**. *PLOS Biol* 2012, **10**:e1001424.

60. Horner-Devine MC, Lage M, Hughes JB, Bohannan BJM: **A taxa–area relationship for bacteria**. *Nature* 2004, **432**:750–753.

61. Billiet A, Meeus I, Van Nieuwerburgh F, Deforce D, Wäckers F, Smagghe G: **Colony contact contributes to the diversity of gut bacteria in bumblebees (*Bombus terrestris*)**. *Insect Sci* 2017, **24**:270–277.

62. Chen I-MA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang J, Andersen E, Huntemann M, et al.: **IMG/M: integrated genome and metagenome comparative data analysis system**. *Nucleic Acids Res* 2017, **45**:D507–D516.

63. McFrederick QS, Wcislo WT, Taylor DR, Ishak HD, Dowd SE, Mueller UG: **Environment or kin: whence do bees obtain acidophilic bacteria?** *Mol Ecol* 2012, **21**:1754–1768.

64. Steele MI, Kwong WK, Whiteley M, Moran NA: **Diversification of type IV Secretion System Toxins Reveals Ancient Antagonism among Bee Gut Microbes.** *mBio* 2017, **8**:e01630-17.

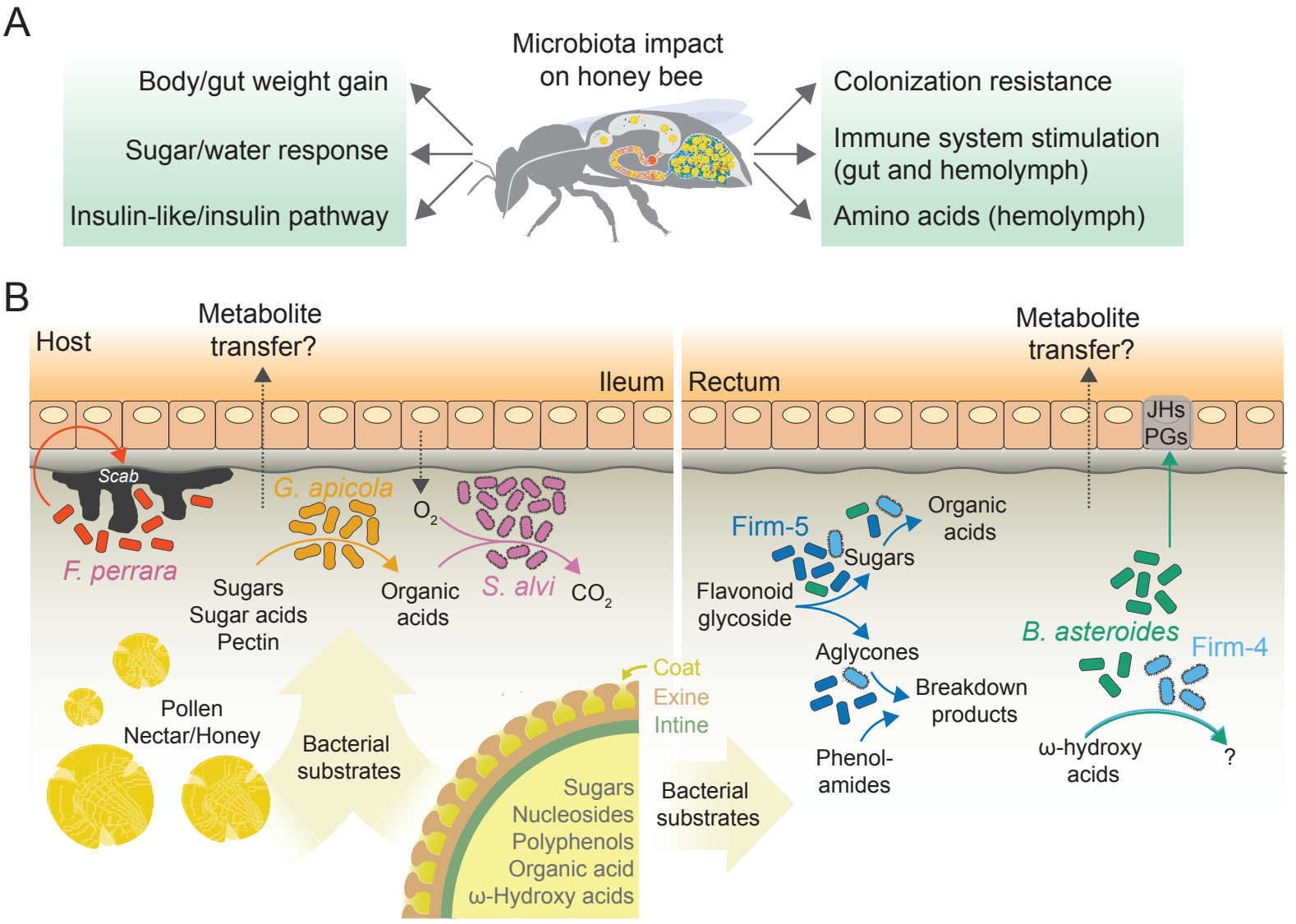
A
Microbiota impact on honey bee
Body/gut weight gain
Sugar/water response
Insulin-like/insulin pathway
Colonization resistance
Immune system stimulation (gut and hemolymph)
Amino acids (hemolymph)
B
Host
Metabolite transfer?
Ileum
Rectum
Metabolite transfer?
JHs
PGs
Scab
F. perrara
G. apicola
O2
Sugars
Sugar acids
Pectin
Organic acids
S. alvi
CO2
Pollen
Nectar/Honey
Bacterial substrates
Coat
Exine
Intine
Sugars
Nucleosides
Polyphenols
Organic acid
ω-Hydroxy acids
Bacterial substrates
Firm-5
Organic acids
Sugars
Flavonoid glycoside
Aglycones
Breakdown products
Phenol-amides
B. asteroides
Firm-4
ω-hydroxy acids
?

Table 1. Characteristics of bacteria associated with the honeybee gut.

				Inferred metabolism [4]						Metabolic activities in the bee gut[11]		
Core member	**Other names**	**Host Range[2]**	**Gut region[3]**	**E[5]**	**Cit[6]**	**Gly[7]**	**AA[8]**	**Nuc[9]**	**Vit[10]**	**Substrates**	**Products**	**Other notes**
Gilliamella apicola	Gamma-1	Corbiculate bees	Ileum	F	-	+	+	R	+	Nucleosides Quinic acid Sugars*	Organic acids* Aromatic compound degradation intermediates	Pectin degradation Cross-feeding with *S. alvi* High strain diversity
Lactobacillus Firm-5	*L. melliventris* *L. kimbladii* *L. kullabergensis* *L. helsingborgensis*	Corbiculate bees	Rectum	F	-	+	-	Y	-	Flavonoid glycoside Nucleosides Quinic acid, citrate Phenolamides Sugars*	Organic acids Aromatic compound degradation intermediates ω-hydroxy acids	Most abundant core member High strain diversity
Lactobacillus Firm-4	*L. mellis* *L. mellifer*	Corbiculate bees	Rectum	F	-	+	-	Y	-	Flavonoid glycosides Nucleosides ω-hydroxy acids Sugars	Aromatic compound degradation intermediates	Two divergent lineages
Snodgrassella alvi	Beta	Corbiculate bees	Ileum	A	+	-	+	RY	+	Carboxylic acid Orotate, Furoate	Kynurenic acid	Oxygen consumption in ileum
Bifidobacterium asteroides	Bifido	Corbiculate bees	Rectum	F	-	+	+	RY	-	Flavonoid glycoside Nucleosides ω-hydroxy acid	Prostaglandins Juvenile hormone derivatives	Two divergent lineages
Non-core member[1]												
Frischella perrara	Gamma-2	Honeybees	Pylorus	F	-	+	+	R	+	Citrate Nucleosides Fuorate	Kynurenic acid	Only found in honeybees Induces scab phenotype
Bartonella apis	Alpha-1	Honeybees	n.d.	A	+	+	+	RY	+	Orotate Quinic acid	n.d.	Only found in honeybees
Lactobacillus kunkeei	*L. apinorum*	Corbiculate bees Sweat bees	Crop Nectar	F	-	+	-	RY	-	n.d.	n.d.	Likely acquired from flower nectar

Bombella apis	Alpha-2.2 *Parasacchari-bacter apium Bombella intestini*	Honeybees Bumblebees	Midgut Crop Hive	A	+	+	+	RY	+	n.d.	n.d.	Found in queens, larvae and royal jelly
Commensali-bacter sp.	Alpha-2.1	Honeybees Stingless bees	n.d.	U	U	U	U	U	U	n.d.	n.d.	Found in queens

[1]: Only the most prominent non-core members are listed.

[2]: Based on [15,21,57]. Corbiculate bees: honeybees, stingless bees, bumblebees.

[3]: Region where found in greatest abundance, or from where type strains were isolated.

[4]: From [58] and available genomes in IMG/M [59]

[5]: Metabolism for energy production: (A)erobic respiration, (F)ermentation, (U)nknown.

[6]: Citrate cycle: (-) pathway complete, (+) pathway incomplete

[7]: Glycolysis: (-) pathway complete, (+) pathway incomplete

[8]: Amino acids: most biosynthetic pathways absent (-), or present (+)

[9]: Nucleosides: most genes present for pu(R)ine or p(Y)rimidine biosynthesis present

[10]: Vitamins : (+) most genes for B2, B3, B5, B6, B9, B12 biosynthesis present or (-) absent

[11]: Based on the most discriminatory ion changes from [14] , except for those indicated with asterisk, which come from [18].