

Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling

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Social bees harbor a simple and specialized microbiota that is spatially organized into different gut compartments. Recent results on the potential involvement of bee gut communities in pathogen protection and nutritional function have drawn attention to the impact of the microbiota on bee health. However, the contributions of gut microbiota to host physiology have yet to be investigated. Here we show that the gut microbiota promotes weight gain of both whole body and the gut in individual honey bees. This effect is likely mediated by changes in host vitellogenin, insulin signaling, and gustatory response. We found that microbial metabolism markedly reduces gut pH and redox potential through the production of short-chain fatty acids and that the bacteria adjacent to the gut wall form an oxygen gradient within the intestine. The short-chain fatty acid profile contributed by dominant gut species was confirmed *in vitro*. Furthermore, metabolomic analyses revealed that the gut community has striking impacts on the metabolic profiles of the gut compartments and the hemolymph, suggesting that gut bacteria degrade plant polymers from pollen and that the resulting metabolites contribute to host nutrition. Our results demonstrate how microbial metabolism affects bee growth, hormonal signaling, behavior, and gut physicochemical conditions. These findings indicate that the bee gut microbiota has basic roles similar to those found in some other animals and thus provides a model in studies of host-microbe interactions.

honeybee | gut microbiota | short-chain fatty acids | insulin | metabolomics

Honey bees (*Apis mellifera*) provide a critical link in global food production as pollinators of agricultural crops (1), and their economic value is over \$15 billion annually in the United States alone (2). Honey bee populations have undergone elevated colony mortality during the last decade in the United States, Canada, and Europe (3). A potential role of gut microbial communities in the health of honey bees has recently become more widely appreciated (4). Perturbation of the gut microbiota leads to higher mortality within hives and greater susceptibility to a bacterial pathogen, suggesting a crucial role of normal microbiota in bee health (5). Honey bees are associated with specific intestinal microbiota that is simpler than the microbiota found in mammals but shares some features, including host specificity and social transmission (6) and a shared evolutionary history of bacterial and host lineages (7, 8). The bee gut is dominated by eight core bacterial species that are spatially organized within specific gut regions. Few bacteria colonize the crop and midgut; the hindgut (ileum and rectum) harbors the greatest abundance of bacteria. The ileum, a narrow tube with six longitudinal folds, is dominated by two Gram-negative bacterial species: *Snodgrassella alvi*, a nonsugar fermenter forms a layer directly on the gut wall, together with *Gilliamella apicola*, a sugar fermenter that resides more toward the center of the lumen (9). The distal rectum is dominated by the Gram-positive *Lactobacillus* spp (10).

In both insects and mammals, the gut microbiota can possess a large repertoire of metabolic capabilities and can contribute substantively to dietary carbohydrate digestion within the intestinal ecosystem (11). Short-chain fatty acids (SCFAs), namely acetate, propionate, and butyrate, produced by the gut microbiota as main

fermentation products of dietary fiber accumulate in the human colon in concentrations up to 80–130 mM (12) and serve as a major energy source for intestinal epithelial cells (13) or as the main respiratory substrate of the host (14). Moreover, microbial metabolites can have a profound effect on gut physiology; for example, their effects on oxygen concentration, pH, and redox potential can be essential for host health (15). As neuroactive compounds, SCFAs produced by the gut microbiota can affect neural and immune pathways of the host and thereby influence brain function and behavior (16).

In the case of honey bees, genome-based investigations showed that *G. apicola* strains potentially digest complex carbohydrates (i.e., pectin from pollen cell wall) that are otherwise indigestible by the host (17). A recent study documents that *G. apicola* strains can also use several sugars that are harmful to bees (18). However, how the bee gut microbiota affects host physiology and the gut microenvironment has not yet been described. Hence, we compared germ-free (GF) honey bees to those with a conventional gut community (CV) to identify how the gut microbiota affects weight gain, expression of genes underlying hormonal pathways, gut physicochemical conditions, and metabolite pools in the gut and hemolymph.

Results and Discussion

Gut Microbiota Promotes Host Body and Gut Weight Gain. To observe effects of the microbiota on growth of individual hosts, we performed serial measurements of whole-body wet weight in the presence and absence of the gut microbiota. GF and CV bees were obtained from pupae that were removed from hives, allowed to emerge in sterile laboratory conditions, and then fed either

Significance

Honey bees are globally important plant pollinators. Guts of adult workers contain specialized bacteria not found outside bees. Experimental results show that gut bacteria increase weight gain in young adult bees, affect expression of genes governing insulin and vitellogenin levels, and increase sucrose sensitivity. Gut bacteria also shape the physicochemical conditions within the gut, lowering pH and oxygen levels. Peripheral resident bacteria consume oxygen, thus maintaining anoxia, as required for microbial activity. Additionally, gut bacteria produce short-chain fatty acids, with acetate and propionate as the major metabolites, as in guts of human and other animals. This study demonstrates how bacteria in the honey bee gut affect host weight gain and improves our understanding of how gut symbionts influence host health.

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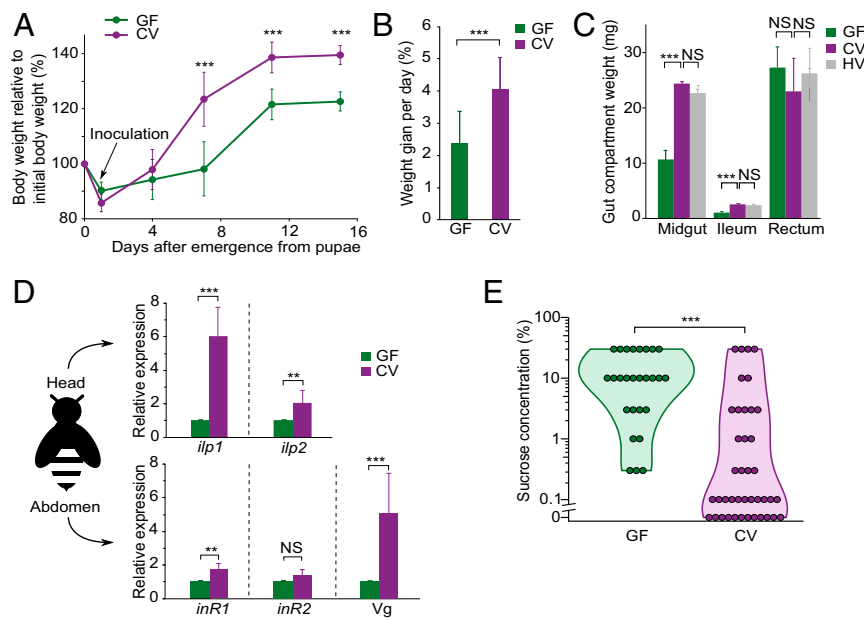


Fig. 1. Gut microbiota increases honey bee whole-body weight, gut weight, hormonal signaling, and sucrose sensitivity. (A) Whole-body wet weight growth curves of GF ($n = 45$) and CV bees ($n = 49$) originated from four different hives (the colony of origin was not statistically significant). (B) Daily weight gains of GF and CV bees after feeding with sterilized food or food supplemented with hindgut samples of hive nurse bees (day 1 to day 15). (C) Weights of different gut compartments of GF, CV, and hive nurse bees ($n = 25$). Bars indicate mean values of 10–15 pooled guts of bees from different hives (GF, $n = 4$; CV, $n = 4$) and hive bees from different hives ($n = 3$). (D) Differential expression of *ilp*, *inR*, and *Vg* genes in the head or abdomen of GF and CV bees originating from different hives. $n = 3$. (E) Distribution of sucrose-response thresholds of GF ($n = 27$) and CV ($n = 41$) bees shown as a violin plot. The colony of origin was not statistically significant. Each circle indicates a bee response to the provided concentration of sucrose. In A–D, $**P < 0.01$, $***P < 0.001$ (Mann–Whitney u test for the indicated comparisons); error bars indicate SD. In E, $***P < 0.001$ (χ^2 test). NS, not significant.

sterilized food or sterilized food inoculated with the gut content of conventional adult nurse bees. This procedure yields GF bees with $<10^5$ bacteria per gut consisting of an erratic mix of bacterial species versus CV bees with $>10^8$ bacteria per gut and consisting primarily of core bee gut species, as in naturally inoculated hive bees (19, 20). Although GF and CV bees showed similar survivorship in laboratory experiments, CV bees attained greater body weight (Fig. 1A) and achieved a weight gain 82% higher than that

of GF bees (Fig. 1B). After 15 d, the wet weights of both midgut and ileum were also larger in CV bees than in GF bees and were similar to those of nurse bees collected from hives (Fig. 1C). In contrast, rectum weights were not significantly different and were more variable, probably because of variable levels of pollen and waste accumulation in this gut compartment (Fig. 1C). These results indicate that the presence of conventional gut microbiota is required for normal body and gut weight gain during the days

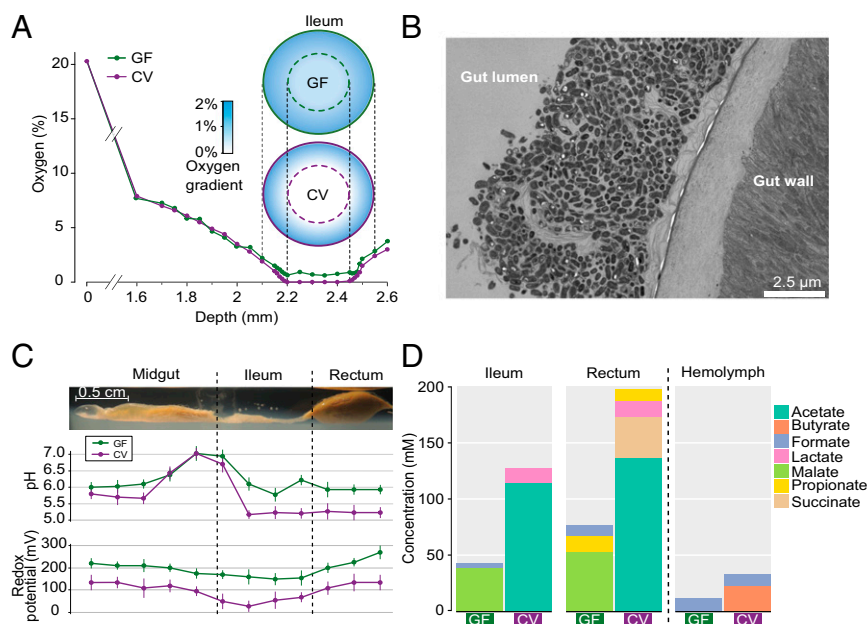


Fig. 2. Physicochemical conditions and SCFA profiles in the guts of GF and CV bees. (A) Radial profiles of oxygen concentration in the ileum of GF ($n = 3$) and CV ($n = 3$) honey bees from different hives. The central regions of CV ileums are always absolutely anoxic (0% oxygen). Deviation for each value is typically less than 0.2% oxygen. Depth refers to the distance between the electrode tip and the surface of the agarose. The schematic representation of the oxygen concentration gradient shows a microoxic periphery around the anoxic center in the CV bee ileum, whereas oxygen is present even in the center of the GF bee ileum. (B) Transmission electron micrographs of the CV ileum epithelium with a bacterial layer. (C) Microelectrode profiles of pH and redox potential along the GF and CV gut axis. The gut was embedded in a microchamber with agarose. Samples are originated from different hives, but colony of origin was not statistically significant. Error bars indicate SD ($n = 4$). (D) Concentrations of SCFA in the ileum, rectum, and hemolymph of GF and CV honey bees. Samples are originated from different hives; colony of origin was not statistically significant. A full list of concentration values is shown in Table S1.

microbiota impacts host physiology. Because the redox potentials are always positive, we did not detect hydrogen production in the guts. In all compartments of the GF gut, the homogenates contain high concentrations of glucose and fructose. However, these sugars are much lower in the homogenates of CV ileums and rectum, indicating that they are consumed by the symbiotic bacteria. Hemolymph metabolite profiles also differ between CV and GF bees, suggesting that gut bacteria affect host metabolism. Although trace amounts of formate were detected in hemolymph of both CV and GF bees, butyrate (22.8 mM) was detected only in the CV hemolymph. In mammals, butyrate is an important fermentation product produced by the gut microbiota (48) and has been shown to serve as the main energy source for colonocytes (49). The absence of butyrate in the bee gut suggests that it is absorbed and used by the host.

The different gut fermentation profiles between GF and CV bees might be shaped by oxygen status, as shown in cockroaches (50). Indeed, in *in vitro* cultures of the two most abundant bee gut fermenters, *G. apicola* and *Lactobacillus* sp., fermentation products were strongly dependent on oxygen status (Tables S2 and S3), clarifying the differences in gut SCFA profiles (Fig. 2D). The major fermentation product of *G. apicola* under ambient air is malate, but with decrease of O₂, *G. apicola* shifts to producing more acetate and propionate (Table S2), as is consistent with the *in situ* results. Although *Lactobacillus* species are considered to be lactic acid producers (51), they produce substantial amounts of acetate when headspace contains 2% of O₂ (Table S3). The shift from lactate to acetate formation with the presence of O₂ has been documented in *Enterococcus* strain RfL6 from termite gut (52). Our results indicate that the resident gut bacteria contribute to the major SCFAs detected in the hindgut.

Metabolomics. For a more in-depth understanding of the effects of gut microbiota, we compared metabolite levels in separate gut compartments and hemolymph for CV and GF bees using untargeted metabolomics by GC-MS analysis. The principal component analysis (PCA) showed that the gut microbiota has a striking effect on the gut metabolome, especially in the rectum and ileum (Fig. 3A and E), where most gut bacteria reside (10). By contrast, hemolymph samples were not clearly distinct between CV and GF bees. In the ileum and rectum, aminovaleric acid is the compound most elevated in CV bees in both the ileum (319-fold) and rectum (84-fold) (Fig. 3B and C and Dataset S2, A and B). Aminovaleric acid is also a major product of microbial activity in the human gut (53). Another notable enrichment in the CV ileum is for galacturonic acid (Fig. 3C), the main constituent of pectin, indicating pectin degradation by gut bacteria and corroborating the presence of genes encoding enzymes that target pollen wall polysaccharides (17). Galacturonic acid was not enriched in the CV rectum, suggesting that it is degraded by the dense bacterial community. This result is consistent with the ability of *G. apicola* strains to use products of pectin degradation (18). In CV bees, adenosine was elevated within the midgut, despite the very low levels of bacteria colonizing this gut compartment (Fig. 3E and Dataset S2C). Potentially gut colonization triggers endogenous production of adenosine, which may reduce gut inflammation (54).

Although the impact on hemolymph is less than that on gut compartments, when we analyzed the hemolymph samples separately the metabolite profiles also show a clear effect of gut microbiota (Fig. 3D). Several amino acids are elevated in CV

hemolymph (Dataset S2D), suggesting that they are absorbed by the host. Based on a previous mutational screen (43), biosynthetic pathways for amino acids are crucial for host colonization, suggesting that gut bacteria might contribute amino acids absorbed by the host. Alternatively, host pathways for vitamin and amino acid biosynthesis are among those significantly up-regulated in CV bees (Fig. S3), suggesting that the presence of gut microbiota stimulates host metabolism. The increased quantities of amino acids in the CV hemolymph might reflect the stimulation of the IIS pathway and subsequent weight gain, which has been linked to an amino acid-supplemented food in honey bees (26). Thus, gut symbionts may supply sufficient protein to affect hormonal signaling and to promote weight gain of the hosts.

Conclusions

Our results show that the gut microbiota of young adult honey bees promotes weight gain and that this effect is accompanied by the enhanced expression of genes affecting hormonal titers and by an increased sensitivity to sugar (Fig. 4). In addition, spatially organized microbial communities maintain an oxic–anoxic gradient in the gut with lowered pH and redox potential, effects similar to those demonstrated for the human gut microbiota (11). Our observations of the involvement of gut bacteria in the maintenance of gut physicochemical characteristics, SCFA production, and the digestion of complex dietary components (e.g., pectin) reveal further similarities between the gut microbiota of humans and honey bees and provide perspectives for future studies using the bee gut model for investigating host–microbe interactions. Further experimental work is needed to determine the extent to which similar cellular mechanisms underlie the observed similarities of gut microbiota in these different animal hosts.

Materials and Methods

Detailed protocols are available in *SI Materials and Methods*. GF and CV bees were obtained using the protocol described by Powell et al. (19). We obtained GF bees by removing pupae from brood frames of four different hives and allowing bees from each hive to emerge in a separate, sterile dish. CV bees were obtained by feeding newly emerged bees with homogenates of hindguts of nurse bees from their original hive. This inoculation method was chosen because it yields CV bees with robust gut communities similar in size and composition to those of normal bees sampled from hives (19, 20); however, we cannot entirely rule out effects of other components of the homogenized guts on the treatment bees. Bees were immobilized at 4 °C, and the whole-body wet weight was measured with an electric balance. The expression levels of genes encoding ILP, InR, and Vg were determined by quantitative PCR from RNA extracted from the heads or abdomens of GF and CV bees obtained as described above. Primers used in this study are listed in Table S4. The responsiveness of individual bees to sucrose was assessed by presenting sucrose solutions to the antennae. Examples of response or lack of response are shown in Movie S1. SCFA concentrations within different gut compartments and hemolymph were measured by HPLC. The gut O₂ concentrations, pH, and redox potential were measured using microsenors connected to a four-channel amplifier (Unisense). Metabolomic profiles were determined from extracted gut compartments at the University of California, Davis using GC-MS as described by Fiehn and Kind (55).

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