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Transitions and transmission: behavior and physiology as drivers of honey bee-associated microbial communities

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Microbial communities have considerable impacts on animal health. However, only in recent years have the host factors impacting microbiome composition been explored. An increasing wealth of microbiome data in combination with decades of research on behavior, physiology, and development have resulted in the European honey bee (*Apis mellifera*) as a burgeoning model system for studying the influence of host behavior on the microbiota. Honey bees are eusocial insects which exhibit striking behavioral and physiological differences between castes and life stages. These include changes in social contact, environmental exposure, diet, and physiology: all factors which can affect microbial composition and function. The honey bee system offers an opportunity to tease apart the interactive effects of all these factors on microbiota composition, abundance, and diversity.

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Introduction

Host-associated microbial communities play diverse roles in host development, nutrition, and disease resistance [1–8]. Conversely, it is increasingly apparent that the consequences of host behavior (such as changes in diet, social contact, and environmental exposure) translate to shifts in the microbiome [9–15] and can subsequently impact host fitness [16] (Table 1). Additionally, physiological changes of the host can accompany behavioral shifts that impact microbiome composition [9,17,18]. However, establishing a causal link between host behavior and microbiome composition is challenging due to the complexity and intractability of many animal hosts and their microbiota.

The European honey bee, *Apis mellifera*, represents a manipulatable model system where both behavior and the microbiome are well characterized, providing an ideal system to explore the consequences of behavioral traits on microbiome composition. Managed honey bee colonies have distinct microbial communities conserved across geographic location [19,20•]. Furthermore, differences in host behavior and physiology, such as caste, age, and worker task dramatically alter the microbiome composition of individuals. While previous studies provide a substantial foundation describing honey bee life cycle and behavior, in recent years these traits have been explored in the context of the microbiome [21]. In this review we elaborate on the influence of changing honey bee behavior and physiology on the microbiota, and advocate for the honey bee system as a model for studying the relationship between host factors (physiology, diet, social contact, and environmental exposure) and microbial composition and function.

Caste drastically influences microbiome

Honey bees are eusocial insects with distinct behavioral, physiological, and morphological castes and clear division of reproductive labor. A honey bee colony consists of three castes: male drones, female workers, and a single queen. The queen, the sole reproductive female in the colony, mates with drones once, then lays eggs continuously to repopulate the colony. The other females, worker bees, are responsible for all remaining hive tasks, including cleaning and building comb, feeding brood, storing food, and foraging for floral resources [22]. Accompanying the striking life history differences between the female castes are radically different adult gut microbiomes (Figure 1). The adult worker gut is colonized with a distinct community of eight to nine bacterial clades, including the Proteobacterial members *Gilliamella apicola*, *Frischella perrara*, *Snodgrassella alvi*, and *Bartonella apis*, plus various species of Lactobacillus and Bifidobacterium [19,23,24]. These hindgut community members play a role in honey bee digestion, assisting in breakdown of recalcitrant dietary fibers, and participating in carbohydrate utilization [24,25]. In contrast, queen guts are primarily composed of one species: *Parasaccharibacter apium* (formerly Alpha 2.2) [26,27] and its function, if any, in the queen gut is unknown (Figure 1e). Lastly, the microbiome of male drones has been explored [26], but this review will focus on the workers and queens as they are more experimentally tractable and exhibit more striking behavioral shifts.

Table 1

Host factors shaping microbiomes across animals. The studies cited demonstrate a link between a given factor and the composition, abundance, or diversity of the microbiome in each animal system

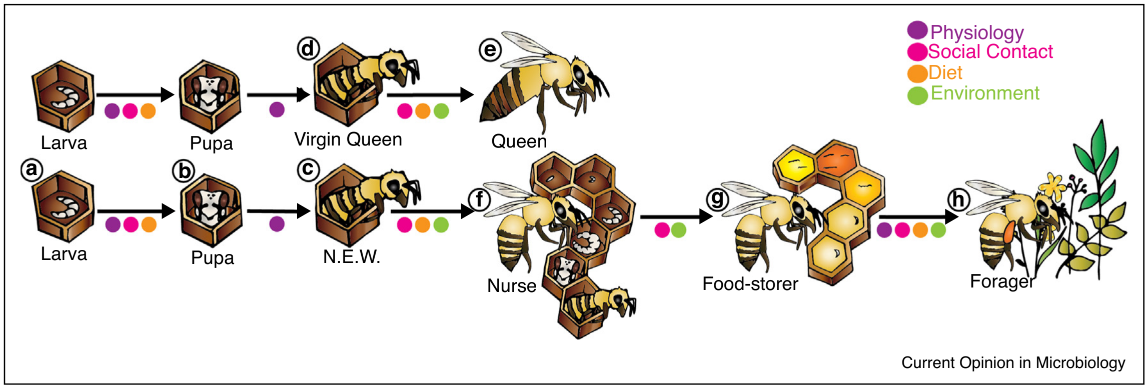
System	Physiology	Social contact	Diet	Environment	Description	Citation
Baboon		✓			Social groups and social grooming networks underlie microbiome composition	Tung *et al.*, 2015 [11]
Chimpanzee		✓	✓		Sociality and diet explain microbiome richness in an additive manner	Moeller *et al.*, 2016 [10]
Human			✓		Major, rapid shifts in gut microbiome follow transition to either plant or animal-based diets	David *et al.*, 2014 [12]
Salmon	✓			✓	Microbiome shifts accompany transitions between freshwater and marine lifestyle which coincide with shifts in life stage	Llewellyn *et al.*, 2015 [9]
Shorebird				✓	Higher abundances of Corynebacterium are consistently associated with migratory birds versus conspecific residentials	Risely *et al.*, 2018 [56]
Zebrafish	✓			✓	Microbiome varies between life stages; larval fish microbiome is similar to microbes in surrounding environment	Stephens *et al.*, 2016 [18]
Cichlid		✓	✓		Higher microbial diversity correlates with social hierarchy, which determines diet and access to mates	Singh *et al.*, 2019 [57]
Desert locust	✓	✓		✓	Hindgut bacterial composition varies between gregarious and solitary morphotypes	Lavy *et al.*, 2019 [58]
Biting midge			✓	✓	Microbiome composition varies with bloodmeal preference and environmental conditions	Díaz-Sánchez *et al.*, 2018 [59]
Pony		✓			Social group membership predicts microbiome structure; family interactions lead to more similar microbiomes	Antwis *et al.*, 2018 [60]
Koala			✓		Microbiome diversity and composition is driven strongly by diet across populations	Brice *et al.*, 2019 [61]
Salamander				✓	Geographic location and habitat type, not taxonomy, predict skin microbiome composition between species	Bird *et al.*, 2018 [62]
Crocodile lizard			✓	✓	Microbiome composition varies based on diet and captivity status	Jiang *et al.*, 2017 [63]
House sparrow	✓		✓		Gut microbiome composition and structure varies through developmental stages and changing diets	Kohl *et al.*, 2019 [64]

Acquiring the microbiota

Although worker and queen microbial communities are highly dissimilar as adults, both castes first interact with microbes at the larval stage (Figure 1a) [27,28]. After hatching, larval honey bees progress through five larval stages (instars) and are continuously fed by young workers (nurse bees). Worker-destined larvae are initially fed royal jelly before switching to worker jelly, a mixture of royal jelly, honey, and bee bread regurgitated from the nurse crop [29,30]. Queen-destined larvae, in contrast, are continuously fed royal jelly [29]. While this royal jelly-rich diet is known to induce queen development, it is not known if the diet fed to developing queens influences their microbial community. Despite being reared on microbe-rich food, larvae are devoid of most core microbial taxa [31]. The fact that many cultivable microbes can be isolated from larvae [28,32,33] suggests that the microbes present in larvae are transiently associated with larvae through their diet. It is possible that antimicrobial peptides in the royal jelly-rich larval diet inhibit or select against the growth of many microbes [30]. Indeed, in the early stages of larval development, *Parasaccharibacter apium*, a microbe known to survive royal jelly [34], can be found in association with larvae [31]. However, as worker-destined larvae mature, *Lactobacillus spp.* both from the worker gut (Firm-5) and the hive environment (*Lactobacillus kunkeei*) establish and displace *P. apium* and other acetic acid bacteria [28]. These microbial shifts of worker-destined larvae may be due to shifts in physiology (the repeated molting of the gut tract) and diet (the shift from royal jelly to worker jelly) [35,29].

Following transient microbial interactions during larval development, pre-pupae are sealed within their cells, and removed from any subsequent social contact, feeding, and microbial exposure for twelve days (Figure 1b) [22]. The

Figure 1



Host factors influence microbial shifts throughout honey bee development: Shifts in honey bee associated microbial communities are accompanied by changes in the physiology, social contact, diet, and environment of the host. Before adulthood both worker-destined and queen-destined brood undergo changes in physiology, diet, and degree of social contact **(a–b)**. Upon emergence as adults, virgin queens and newly emerged workers (N.E.W.) undergo dissimilar shifts in diet and social interaction and are established by dissimilar microbial communities **(c–e)**. Lastly, as workers transition between tasks they experience prominent shifts in physiology, social contact, diet and environmental exposure, in addition to minor shifts in microbial abundance and diversity **(f–h)**. Colored circles represent factors changing at each transition along the life cycle.

entire larval gut, along with its microbial residents, is eliminated during pupal organ remodeling, enforcing the transience of the larval gut microbiome [35]. When an adult queen emerges, she rapidly re-acquires *P. apium*. The exact route of inoculation of *P. apium* is unclear, though social contact with workers early alters both the abundance and composition of the queen's gut community, including *P. apium* (Figure 1d) [36••]. Immediately upon emergence, newly eclosed worker bees are colonized by traces of their larval microbiota or transient environmental microbes in low abundance (Figure 1c) [31,37••]. Over four to six days newly eclosed workers accumulate the characteristic worker gut microbiota. The route of inoculation of the gut community varies by bacterial taxa. Through social contact with nurse bees or their feces, new bees are colonized with *Gamma* and *Betaprotebacteria* (*G. apicola*, *F. perrara*, and *S. alvi* respectively); in the absence of nurses these taxa are present in low abundance. Firmicutes, such as the predominant *Lactobacillus* sp., Firm-5, are successfully acquired by exposure to the hive environment alone [38••]. Ultimately, a combination of social interactions, coprophagy, and contact with surfaces of the hive environment are necessary to acquire the entire community. Once the characteristic bacterial community has established, these core taxa remain in the worker gut for the rest of its life [31].

Progression through worker tasks is accompanied by microbiome shifts

Within the honey bee colony, worker tasks are temporally allocated. Workers perform in-hive tasks (nursing brood and storing food) following emergence, and out-of-hive tasks (foraging) afterwards [39]. Firstly, a new worker becomes a nurse bee, tending to brood and queen by feeding both mixtures of nectar and royal jelly. Next, the worker transitions to a food-storer, packing nectar and pollen into cells, cleaning pre-existing comb, and building out new comb. Lastly the worker transitions to life outside the hive, performing foraging flights for nectar and pollen [22].

The core gut microbiome is present in nurses, food-storers, and foragers, but the proportions of these core bacterial taxa vary with worker task. For example, the proportion of *Lactobacillus* species (e.g. Firm-5) and *Snodgrassella alvi* vary between nurse and forager lifestyles [26,40,41••]. Additionally, microbiome diversity has been reported to either increase or decrease at the onset of foraging [26,41••]. While differences in methods of sample collection and data analysis impede effective comparisons between published datasets, there are numerous examples of changes in worker task correlating with changes in the microbiota.

Although the causal agents underlying these shifts in microbial community makeup are unknown, temporal allocation of tasks coincides with changes in (i) bee physiology, (ii) diet, (iii) social contact, and (iv) environment. The transition from nurse to forager is accompanied first by changes in physiological traits including the decreased size of hypopharyngeal glands and abdominal lipid content [42,43]. Second, task-dependent physiological changes may be driven by dietary shifts rather than worker age alone [42]. Nurse bees consume high levels of protein-rich pollen and bee bread to refine into royal jelly (Figure 1f). Pollen, unlike nectar, is recalcitrant to breakdown by most microbial members. However recent metabolic work implicates *Lactobacillus* Firm-5 and *Bifidobacterium asteroides* in the catabolism of flavonoids in the outer wall of pollen grains [44•]. Additionally, *Gilliamella apicola* degrades pectin, a major component of the inner pollen wall [45]. Conversely, some microbiome members such as *Snodgrasella alvi* have reduced growth rates relative to other core microbes when grown in pollen-supplemented media [44•]. High-pollen diets in nurse bees, in contrast to the sugar-rich diets of foragers, may select for different proportions of core microbial members as a result of these differences in bacterial metabolism [25,44•,46,47•]. Thus, as the honey bee progresses from nurse to forager, changes in physiology and diet may be powerful drivers of microbial shifts.

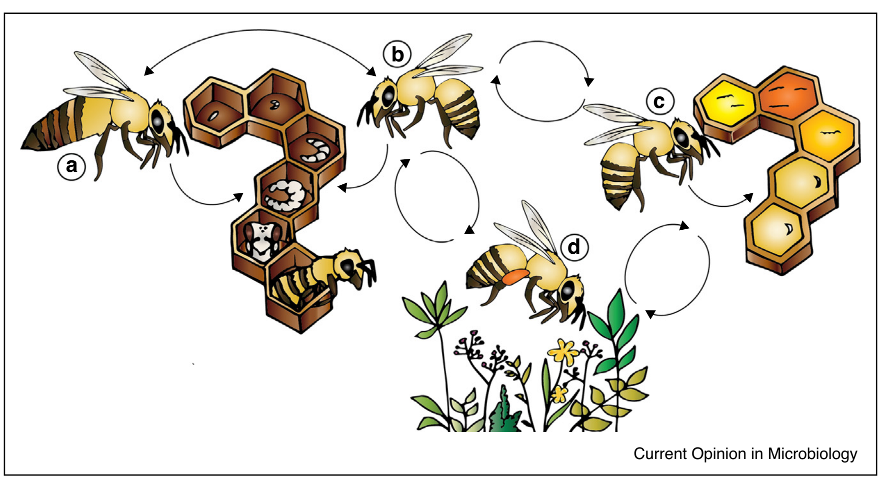
A third potential driver of microbial composition at the onset of foraging are the changing social interactions of the worker bee. Foragers and nurse bees participate in distinct social networks within a hive. Nurse bees are frequently in contact with other in-hive bees, the developing brood, and the queen (Figures 1f and 2). Foraging bees have more infrequent social contact, only participating in the transfer of floral resources, oral trophallaxis (mouth-to-mouth feeding), and social grooming when returning to the hive (Figures 1h and 2) [22].

Fourth, workers recruited to foraging are exposed to a myriad of floral environments, each with established microbial communities of their own [48,49]. Foragers face the constant challenge of these foreign microbial communities (Figures 1f–h and 2), and as a result their guts are transiently occupied with a diversity of fungal taxa absent in nurse bees [37••]. In contrast to foragers, nurses and food-storers interact with hive microbes responsible for food fermentation and preservation, but are otherwise removed from environmental microbes [50,51]. By extension, the queen, who exclusively interacts with nurse attendants, is essentially quarantined from potential environmental pathogens [22]. Overall, changes in worker tasks involve drastic shifts in physiology, diet, social networks, and environmental exposure which may shape microbial communities.

Exploring mechanisms and function of microbial communities in a tractable model system

The honey bee system lies at an interface between manipulability and ecological relevance and thus lends itself nicely to studies which seek to explore the composition and function of the microbiota across changing host behavior and physiology. Honey bees are classically housed in colonies outside of the laboratory where they forage for their own food and are in constant contact with the environment. This lifestyle likely continuously selects for a host-microbe fit allowing the honey bee to best cope with fluctuating nutritional conditions and exposure to pests and pathogens. Classic model systems, in contrast, undergo generations of rearing under laboratory conditions resulting in microbiota/host combinations and interactions removed from natural environmental selective pressures [52]. The honey bee microbiota therefore represents a system that bypasses the classical tradeoff between model system tractability and real-world applicability.

Figure 2



Interaction networks within the honey bee colony: As adults, honey bees experience different social and environmental interactions dependent on caste and task. Queens **(a)** leave the hive only to mate, returning to the hive to lay eggs and be fed by nurse bees. Nurse bees **(b)** frequently interact with the queen and brood, which they feed, and sometimes engage in social activities such as oral trophylaxis (mouth-to-mouth feeding) and allogrooming (social grooming of conspecifics) with other nestmates. Food-storers **(c)** are further removed from the queen and brood, storing floral resources brought in by foragers. Finally, foragers **(d)** spend the majority of their time outside the hive interacting with flowers and other pollinators.

Utilizing this tractability, determinants of microbiota assembly in newly emerged workers and queens have been elucidated by directly manipulating points of inoculation followed by assessment of the associated microbiomes via quantitative PCR and amplicon sequencing [38••,36••]. This experimental manipulation of adult social and environmental interactions has deepened our understanding of the factors shaping adult microbiota acquisition. However, the influence of diet and physiology on the larval microbiota, and the contribution of shifting diet, physiology, and social/environmental interactions to task-associated microbiota variation in adults remains to be established. In order to determine explicit cause and effect relationships between physiological/behavioral factors and microbiota composition, direct manipulation of these factors is needed. By transplanting larvae early in development from a honey bee colony, one can manipulate dietary, social, and environmental conditions at any point of the bee life cycle in a tightly controlled laboratory setting [53]. The culturability and genetic tractability of microbiota members associated with each caste, developmental stage, and task allow for easy manipulation of microbial communities associated with lab-reared hosts [28,54,55••].

Conclusions

The honey bee is a promising model system for exploring the influence of host physiology and behavior on the microbiota. We have just begun to understand the establishment of the microbiota and functions it may serve in the colony. For example, we know social interactions help establish the microbiota in workers but it is unclear if further social interactions among adult workers can continue to shape the microbiota. Similarly, although honey bee genetics can be manipulated, we do not yet understand the influence of host genetics on the microbiome in this system. Behavioral tasks certainly may shape the microbial community in worker bees. However, conflicting evidence so far does not point to a specific shift in microbiome composition. In addition, it is unclear how much of this shift would be due to dietary changes as bees age and change behavioral tasks in the colony. Because both host and symbiont are tractable and can be separated from each other and reconstituted in the laboratory, the honey bee system is a promising one, in which to ask these foundational questions about microbiomes at the interface of host physiology, behavior, and society.

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