

COMMENTARY

The buzz within: the role of the gut microbiome in honeybee social behavior

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ABSTRACT

Gut symbionts influence the physiology and behavior of their host, but the extent to which these effects scale to social behaviors is an emerging area of research. The use of the western honeybee (*Apis mellifera*) as a model enables researchers to investigate the gut microbiome and behavior at several levels of social organization. Insight into gut microbial effects at the societal level is critical for our understanding of how involved microbial symbionts are in host biology. In this Commentary, we discuss recent findings in honeybee gut microbiome research and synthesize these with knowledge of the physiology and behavior of other model organisms to hypothesize how host–microbe interactions at the individual level could shape societal dynamics and evolution.

KEY WORDS: Collective dynamics, *Apis mellifera*, Neuroendocrinology, Evolution

Introduction

Animals have an intimate relationship with the microorganismal communities that colonize them (Bordenstein and Theis, 2015). In fact, microbial symbionts are necessary for host development and physiology, as the perturbation of these symbionts causes a breadth of host dysbiosis (see Glossary; Gesù et al., 2022; McAnulty et al., 2023; Raymann et al., 2017; Sgritta et al., 2019). Thus, animal–microbe symbioses and the factors that govern this fundamental relationship are currently of high interest. Microbes that colonize the host's intestinal lining, or 'gut microbiota', are of particular interest as they contribute to many aspects of internal host biology (Liberti et al., 2022; Poutahidis et al., 2013; Sgritta et al., 2019; Wu et al., 2021). In its most well-documented role, the gut microbiome aids in host digestion and essential nutrient production by liberating nutrients from complex dietary fibers that are otherwise inaccessible to the host (LeBlanc et al., 2013; Shreiner et al., 2015; Silva et al., 2020). Transcending digestion, the presence of the gut microbiome is critical to proper development of host physiology, including that of brain regions dedicated to social behaviors (Cryan and Dinan, 2012; Wu et al., 2021; Zheng et al., 2021). Indeed, host physiology and behavior can affect the gut microbiome, and the gut microbiome can affect host physiology and behavior (Archie and Tung, 2015; Cryan and Dinan, 2012; Nagpal and Cryan, 2021; Shropshire and Bordenstein, 2016). As we have built a considerable foundation on the importance of symbiotic microbes, we are now able to consider their impacts on host social behavior. Socially relevant behavioral consequences are important

to study because of the ubiquity of sociality across life: ultimately, individual-level behaviors can scale to affect population dynamics (Berdahl et al., 2013; Grueter et al., 2020; Krause et al., 2002).

Western honeybees are rapidly emerging as models in which to study the gut microbiome because of their short generation time, expression of many complex behaviors, and reliance on gut microbial symbionts (Douglas, 2019; Engel et al., 2016; Zheng et al., 2018). Honeybees are also eusocial animals, living in extensive societies that rely on the coordination of all workers to maintain optimum function (Wilson and Hölldobler, 2005). The structure of honeybee societies, as well as the amenability of honeybees to experimental manipulation (Engel et al., 2016; Zheng et al., 2018), allows researchers to ask nuanced questions about the relationship between microbes and societal conditions. Understanding how the products and responses of an individual's gut microbiome scale to affect colony-level characteristics in honeybees could help provide insights into how microbiomes drive evolutionary change within animal populations.

In this Commentary, we review the most recent findings of the roles that microbes play in honeybee neurophysiology and behavior, and consider how hypothesized mechanisms found in other animal models may apply to the complexity of honeybee biology. We use these findings to speculate how individual host–microbe interactions could ultimately impact colony-level outcomes. It is our goal to urge scientists to investigate causal links between gut microbiota and social behavior, with an eye towards population-level impacts.

The honeybee gut microbiome

Bacteria are an inherent part of a honeybee's adult life; however, gut symbionts have rarely been detected in larvae, which shed their gut lining when molting and metamorphosing (Manthey et al., 2022; Powell et al., 2014; Zheng et al., 2018). Immediately after eclosion (see Glossary), new honeybee workers are exposed to what will become their core gut microbes through social interactions with their nestmates and exposure to the colony environment (Fig. 1; Anderson et al., 2022; Kwong and Moran, 2016; Martinson et al., 2012; Powell et al., 2014). The honeybee gut microbiome is mainly found in the hindgut and comprises five core bacterial phylotypes (see Glossary) that make up over 95% of the community: *Lactobacillus* Firm-5 is most abundant, followed by *Lactobacillus* Firm-4, *Bifidobacterium* spp., *Gilliamella apicola* and *Snodgrassella alvi* (Fig. 1; Bonilla-Rosso and Engel, 2018; Brochet et al., 2021; Cox-Foster et al., 2007; Martinson et al., 2011, 2012; Moran, 2015; Zheng et al., 2018). *Frischella perrara*, *Bartonella apis* and *Parasaccharibacter apium* are sometimes identified in the worker gut community but vary in their abundance across individuals (Zheng et al., 2018). The core members are spatially organized in the honeybee gut, with *Snodgrassella* and *Gilliamella* dominating the ileum, and *Lactobacillus* and *Bifidobacterium* in the rectum (Fig. 1; Kešnerová et al., 2017;

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Glossary

Acceptance assay

Experiment in which worker honey bees from other colonies are presented to a tested colony and are observed to see whether they are accepted or rejected by the colony.

Behavioral castes

Eusocial insect societies are categorized into divisions of labor based on task performance; namely, queens are the reproductive caste whereas workers are the non-reproductive caste. Within the worker caste, workers are further divided into behavioral castes, which have specific jobs within the colony.

Collective-level behavior

A behavior that emerges at the colony level that is the result of many individuals working together; for example, the ability of a colony to defend itself depends on the guards and soldiers that do the defending.

Dysbiosis

A term used to describe a disruption or an imbalance in the gut microbiome, usually associated with disease.

Eclosion

The process by which an insect emerges from an egg or pupal state.

Head-to-head interactions

Interactions that consist of two individuals touching their heads.

Horizontal transmission

The transmission of microbial organisms between two individuals within an ecosystem. This is the alternative to vertical transmission, where microbes are passed between parent and offspring, such as during live births.

Hypopharyngeal glands

Paired glands in the heads of worker hymenopterans that secrete food for larvae.

Mono-colonization

The inoculation of a single microbe, usually in a germ-free organism. Used in microbiology to tease apart the individual role of each microbe in each microbiome.

Nucleus colonies

Small colonies that have only a couple of hundred workers and their queen.

Phylotype

A class of organisms that have a specific, similar phylogenetic relationship to each other. This term is frequently used in microbiology, where the idea of species is blurred in a class of organisms that freely exchange genetic material with each other and the environment.

Moran, 2015). The establishment of gram-negative bacteria *Snodgrassella* and *Gilliamella* in newly emerged workers largely depends on social interactions with their nestmates, whereas gram-positive bacteria *Bifidobacterium* and *Lactobacillus* are established through exposure to hive environment and diet of nectar and pollen (Fig. 1; Anderson et al., 2022; Powell et al., 2014). Within the main phylotypes, there is high strain-level diversity and variation that is maintained through niche partitioning of metabolites found in the pollen diet of honeybees (Bonilla-Rosso and Engel, 2018; Brochet et al., 2021). For instance, four species of honeybee-associated *Lactobacillus* were shown to upregulate host genes targeting different pollen-derived compounds, allowing for their coexistence in the honeybee gut (Bonilla-Rosso and Engel, 2018; Brochet et al., 2021; Zheng et al., 2018).

Perturbation of the honeybee core microbiome through pesticide, antibiotic or experimental manipulation causes numerous physiological impacts, ranging from increasing disease susceptibility (Motta et al., 2018; Raymann and Moran, 2018; Raymann et al., 2017) to delaying behavioral development (Ortiz-Alvarado et al., 2020). Thus, honeybee gut symbionts are essential to the proper functioning of honeybee physiology (Schwarz et al., 2016), including their metabolism (Moran, 2015), immune system

function (Kwong et al., 2017) and developmental processes such as weight gain by stimulating host pathways that synthesize key hormones such as juvenile hormone and vitellogenin (Kešnerová et al., 2017; Zhang et al., 2022b; Zheng et al., 2017).

These observations are particularly interesting, as juvenile hormone and vitellogenin are major regulators of maturation and behavioral changes in honeybee workers as they age (Amdam et al., 2012; Fluri et al., 1982; Guidugli et al., 2005; Nelson et al., 2007; Robinson, 1987). *Bifidobacterium asteroides* in particular seems to stimulate the production of juvenile hormone derivatives (Kešnerová et al., 2017), while *Snodgrassella alvi* appears to regulate vitellogenin expression (Schwarz et al., 2016), both through unknown mechanisms. These correlative links between behaviorally relevant hormones and gut symbiont abundance (Kešnerová et al., 2017; Liberti et al., 2022; Zhang et al., 2022b; Zheng et al., 2017, 2018) suggest that gut bacteria could regulate honeybee behavior. Studying how honeybee gut symbionts impact honeybee behavior has therefore become a new research frontier.

Bacteria and behavior

Honeybee workers display a wide range of complex behaviors at different ages to organize themselves within their labor castes temporally and spatially (Seeley, 1982; Seeley and Kolmes, 1991). This organization depends on environmental and social contexts to maintain optimal conditions for colony success across seasons (Beshers et al., 2001; Huang and Robinson, 1992; Knoll et al., 2020; Robinson, 1992; Rowland and McLellan, 1987; Wilson and Hölldobler, 2005; Winston, 1991). It is well established that this seasonally fluctuating task specialization is regulated by hormones (Huang and Robinson, 1995; Knoll et al., 2020); however, the gut microbiome may be another mechanism contributing to this process. Recently, Kešnerová et al. (2020) discovered that the abundance of core gut symbionts also fluctuates with the seasons. Therefore, honeybee physiology, environmental contexts and the gut microbiome could all intersect to regulate honeybee behavior (Copeland et al., 2022b). Despite the stability of their core gut microbiome, worker behavioral castes (see Glossary) are marked by variations in the abundance of certain core symbionts that distinguish them from other castes (Anderson et al., 2016; Copeland et al., 2022b; Corby-Harris et al., 2014; Jones et al., 2018; Kapheim et al., 2015; Martinson et al., 2012). These variations could contribute to honeybee division of labor (Jones et al., 2018; Kapheim et al., 2015). In the following subsections, we explore the effects of the gut microbiome on the behavior of different colony castes.

Nurses

The youngest caste, nurses, have significantly more *Lactobacillus* Firm-4, Firm-5 and *Bifidobacterium* than the eldest worker caste, foragers (Jones et al., 2018; Kapheim et al., 2015). As *Lactobacillus* and *Bifidobacterium* play large roles in digesting pollen (Lee et al., 2015; Moran, 2015), possessing high numbers of these microbes could be beneficial to role of nurse honeybees in feeding and caring for the brood. Particularly, nurses feed on pollen, which develops their hypopharyngeal glands (see Glossary) for producing larval food (Crailsheim et al., 1992; Lucchetti et al., 2018). Additionally, the establishment of *Gilliamella* in the ileum could be important for nurses to develop into foragers that can withstand the extreme oxidative physiological cost of foraging (Copeland et al., 2022b). For example, failure of *Gilliamella apicola* to establish in nurses is associated with lowered nutrient metabolism capability, which could cause nurses to transition into precocious foragers and explain

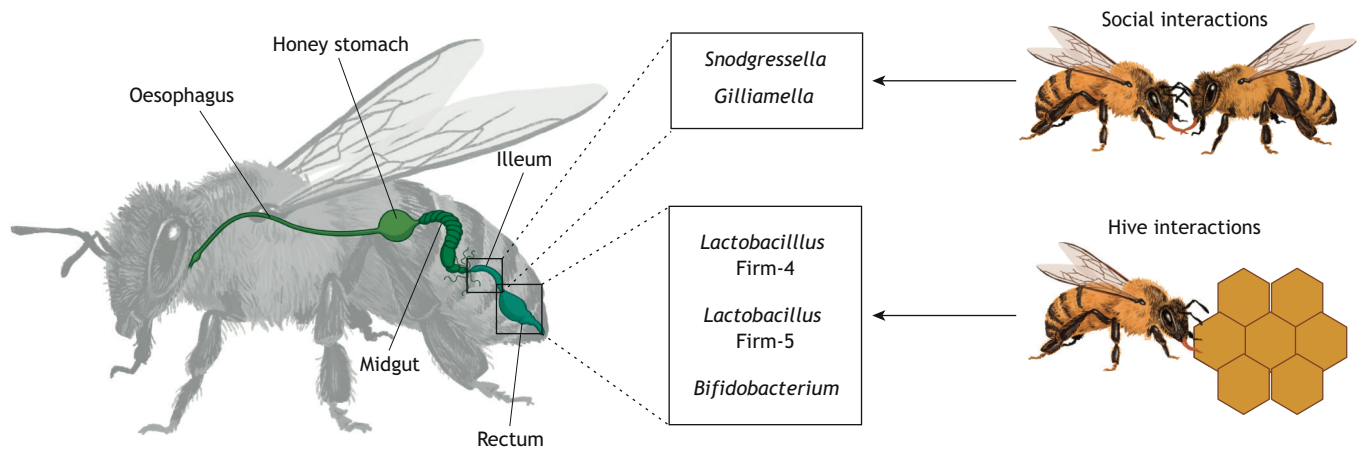


Fig. 1. The honeybee gut microbiota is spatially structured and is gained through social and environmental interactions. Regions of the hindgut where core members reside are indicated in the boxed areas. Core members of the mature honeybee gut microbiome consist of *Lactobacillus* Firm-5 and Firm-4, *Bifidobacterium* spp., *Gilliamella apicola* and *Snodgressella alvi*, with transiently found members including *Frischella perrara*, *Bartonella apis* and *Parasaccharibacter apium*. *Snodgressella* and *Gilliamella* are transmitted to newly emerged workers through social interactions with older nestmates and are concentrated in the ileum, whereas *Lactobacillus* and *Bifidobacterium* are acquired through diet and exposure to the hive environment. As a result, the honeybee gut microbiome is inherited horizontally (see Glossary) and not vertically as is common in mammals. Figure made with BioRender; honeybee illustrations by Impact Media Lab.

their poor foraging ability (Copeland et al., 2022b). It is possible that *Lactobacillus*, *Bifidobacterium* and *Gilliamella* all regulate proper development of nurses into eventual foragers, but the causal intricacies of whether and how these symbionts directly influence nurse physiology and brood-rearing behavior remain a mystery.

Foragers

Foragers are a commonly studied behavioral caste. Sugar sensitivity is a key aspect of honeybee behavioral maturation, as it influences olfactory learning, memory and foraging preference (Ament et al., 2008; Page et al., 1998; Simcock et al., 2018). In fact, nectar foragers are less sensitive to sucrose than pollen foragers, driving their ability to find highly rewarding nectar sources from the environment (Arenas and Kohlmaier, 2019; Scheiner et al., 2004). Sugar sensitivity is regulated by the insulin-like signaling pathway within the honeybee brain (Ament et al., 2008; Mott and Breed, 2012). Honeybee gut microbes appear to contribute to sucrose sensitivity by enhancing insulin production, which has broad implications for forager behavior (Mott and Breed, 2012; Zheng et al., 2017). Namely, insulin-like peptide genes *ilp1* and *ilp2* are downregulated in microbiota-depleted bees, and behaviorally, microbiota-depleted bees are less responsive to sugar rewards (Zheng et al., 2017). Yet, the specific microbes and mechanism by which the gut community interacts with the insulin-like signaling pathway in honeybees is unclear.

Because olfactory learning is associated with sugar sensitivity (Page et al., 1998; Simcock et al., 2018), a recent study investigated the effect of honeybee gut microbiota on learning behavior (Zhang et al., 2022a). The study found that *Lactobacillus apis* mono-colonization (see Glossary) in microbiota-depleted bees, in conjunction with dietary tryptophan supplementation, promoted learning behavior by regulating tryptophan metabolism (Zhang et al., 2022a). Tryptophan supplementation was required to rescue learning because *Lactobacillus apis* metabolizes tryptophan through the aromatic amino acid aminotransferase enzyme and generates indoleacetic acid and indolealdehyde – metabolites implicated in regulating the gut–brain axis (Roager and Licht, 2018; Zhang et al., 2022a). A similar finding was seen in

bumblebees (*Bombus terrestris*), where *L. apis* supplementation correlated with increased glycerophospholipid lysophosphatidic acid, which promotes long-term memory and is also implicated in the gut microbiome's modulation of the gut–brain axis (Li et al., 2021; Zheng et al., 2021). These findings are unsurprising, as studies in *Drosophila* and rodent models demonstrate that the gut microbiome influences host physiology to impact olfactory learning (Ma et al., 2021; Silva et al., 2021; Slankster et al., 2019). Thus, the honeybee gut microbiome could influence the foraging decisions and preferences of forager bees. However, most honeybee gut microbiome and behavior studies are correlative, and causation studies are required to better understand the intricate mechanisms involved.

Middle-aged castes

Although we know much about the gut communities of nurses and foragers, little is known about the gut microbiome of middle-aged bees and its part in regulating the differentiation of tasks within this age group. The behaviors and tasks of the middle-aged caste can vary substantially, ranging from guarding behavior to thermoregulation (Breed et al., 2004; Stabentheiner et al., 2010). For instance, guards and soldiers protect the colony from outside invasion (Breed et al., 2004). Guard bees have higher juvenile hormone titers and are more aggressive than individuals with low juvenile hormone (Pearce et al., 2001). The presence of *Bifidobacterium asteroides* is associated with increased juvenile hormone (Kešnerová et al., 2017), but whether *B. asteroides* is present at higher abundances in guard bees and whether it plays any role in their aggression is unknown. A link between aggression and symbiotic bacteria has been identified in other organisms (Jia et al., 2021; Rohrscheib et al., 2015; Teseo et al., 2019). Specifically, infection by a *Wolbachia* strain correlated with how frequently a male *Drosophila* fly would initiate an aggressive encounter, which could be facilitated through octopamine signaling (Jia et al., 2021; Rohrscheib et al., 2015). Additionally, significant changes in the abundance of an alphaproteobacterium in the gut was positively correlated with aggression in leaf-cutter ants (Teseo et al., 2019). Therefore, a similar relationship between gut symbionts and

aggression may exist in honeybees. As for the wide range of other behaviors middle-aged bees perform, it is unknown whether the gut microbiome affects hygienic behaviors, waggle-dance communication and thermoregulation; all important jobs that contribute to the well-being of the colony. Gut symbionts are known to be associated with temperature preference and tolerance in *Drosophila*, mice and lizards (Bongers et al., 2023; Henry and Colinet, 2018; Moeller et al., 2020; Suito et al., 2022); perhaps gut symbionts are similarly associated in honeybees.

Queens

The factors that regulate honeybee societies cannot be fully understood without the consideration of their reproductive individuals. As eusocial animals, honeybee colonies have a single queen responsible for producing workers and a small drone population responsible for spreading genetic material during the summer (Wilson and Hölldobler, 2005). The core gut microbiome of queens is remarkably distinct from the gut microbiome of workers (Kapheim et al., 2015; Powell et al., 2018; Tarpy et al., 2015). The guts of queens aged 4 days old are dominated by enteric bacteria such as *Escherichia* and *Gilliamella*, and by 14 days old, their gut communities become dominated by *Parasaccharibacter apium* and its close relative *Alphaproteobacteria* strains that are associated with the hypopharyngeal glands of workers (Kapheim et al., 2015; Powell et al., 2018; Tarpy et al., 2015). This correlation is to be expected, as queens are fed royal jelly produced by the hypopharyngeal glands of workers their entire lives (Tarpy et al., 2015), and this specialized diet could contribute to the composition of their gut community. However, to our knowledge, no studies have looked at the metabolic capabilities of the queen gut microbiome, which may be interesting as it might contribute to her egg-laying capabilities by providing the energy needed for such costly labor (Nieuwdorp et al., 2014), as recently suggested in chickens (Ricke et al., 2022; Zhu et al., 2019). Additionally, no studies have extended their investigation beyond a few weeks to look at the development of the queen microbiome as she ages, as queens live for several years (Corona et al., 2005, 2007; Jemielity et al., 2005). It is possible that the queen gut microbiome can impact oogenesis, as shown in mice and *Drosophila* (Elgart et al., 2016; Gnainsky et al., 2021; Xie et al., 2016), which might have implications for her reproductive success. The role that the queen's simplistic microbiome plays in her behavior – such as the ability to function as a successful reproductive within a colony – remains unknown. Lack of progress in these areas of honeybee queen research is most likely due to the difficulties of rearing many queens outside of a colony environment.

Drones

The drone gut microbiome is the least studied of all castes, likely because of their perceived limited role in the colony and our bias for studying the behaviorally diverse worker population. Drone guts appear to be dominated by *Lactobacillus* Firm-4 and Firm-5 species (Kapheim et al., 2015), but the metabolic capabilities of these simple gut communities are unclear. Drones are also fed a larval diet by nurses (Haydak, 1970), but whether this influences the drone gut microbiome like in queens is unknown. Our knowledge of the role the drone gut microbiome has on drone biology or behavior is therefore also limited. Recent research has shown that gut microbiome health is correlated with spermatogenesis in mice (Ding et al., 2020; Wang and Xie, 2022) and *Drosophila* (Dou et al., 2023), so the gut microbiome may influence drone reproductive success by regulating sperm quality and providing the stamina

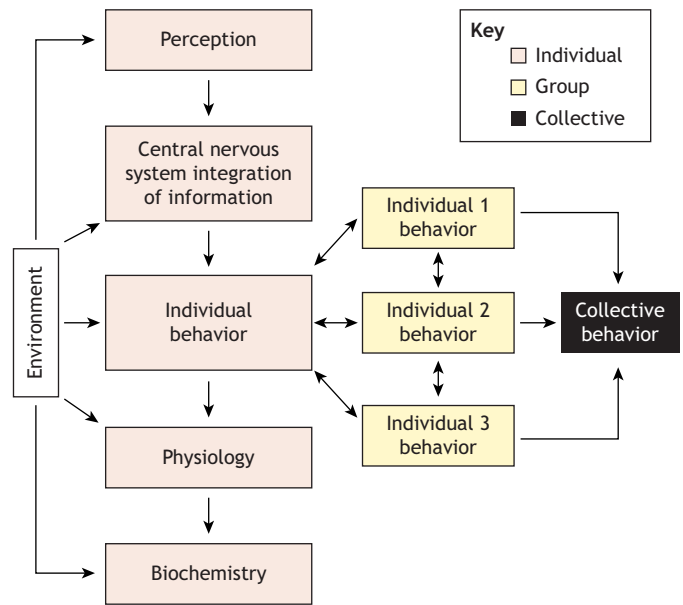


Fig. 2. Individual physiology and behavior scales to affect group-level dynamics. Environmental contexts can uniquely modulate multiple aspects of individual physiology, ultimately affecting behavior depending on the perception of that context. Individual behavior can then influence the behavior of others via communication, and once this communication disseminates to the entire group, the behavior of the individual can regulate how the group behaves. Figure made with BioRender.

needed for his mating flights (Gmeinbauer and Crailsheim, 1993). Future studies could focus on studying the change and development of the drone gut microbiome as they age and mature, as drones have a similar maturation timeline to workers (Zayed et al., 2012), to better understand the intricacies of the drone microbiome.

Bacteria in a honeybee world: colony-level implications

Investigating individual honeybee behavior in the lab is useful to gain primary insights. Applying such findings to group-level studies would provide more ecologically relevant contexts to these behaviors, as individual behaviors can scale to dictate group decisions (Fig. 2; Bicca-Marques and Garber, 2005; Cook and Breed, 2013; Cook et al., 2020; King and Cowlshaw, 2007). More importantly, it would implicate the gut microbiome in forces that drive selection (Miller et al., 2021). Such pursuits are critical to unraveling the role of the gut microbiome in evolutionary processes.

Studies in *Drosophila* and *Caenorhabditis elegans* have demonstrated that gut microbes can impact foraging decisions and mating behavior (O'Donnell et al., 2020; Qiao et al., 2019; Sharon et al., 2010; Slankster et al., 2019; Wong et al., 2017; Trevelline and Kohl, 2022; Shu et al., 2021). *Drosophila* prefer to mate with flies that are reared on the same diet (Sharon et al., 2010). Antibiotic treatment abolishes this phenotype, whereas inoculation of *Lactobacillus* cultures isolated from fly media promotes the phenotype by changing the cuticular hydrocarbon sex pheromones (Sharon et al., 2010). As a result, the microbiome likely has evolutionary consequences that impact populations, species and ecosystems. We propose that applying these ideas to the honeybee may elucidate the impact microbial symbionts have on animal societies and populations. Our breadth of knowledge in the genetic and physiological mechanisms that drive individual behavior in social insects (Beshers and Fewell, 2001; Bonabeau et al., 1997; Brian, 2012; Leonhardt et al., 2016; Oster and Wilson,

1978) will be advantageous as we move towards considering the relationship between the gut microbiome and collective-level behavior (see Glossary).

Gut microbes impact foundational aspects of behavior within honeybee worker types (i.e. sucrose sensitivity, olfactory learning and memory; Zhang et al., 2022a; Zheng et al., 2017). Such impacts in a highly social animal can affect its interactions with other individuals and cascade into consequences with implications for the entire colony, such as colony survival and fitness (Fig. 3). For instance, Zhang et al. (2022a,b) found that antibiotic-treated colonies with lower relative abundance of several *Lactobacillus* Firm-4 and Firm-5 species had an impaired ability to rear brood. This could be due to the antibiotic treatment perturbing key bacteria that contribute to memory and learning behaviors in young bees, rendering them unable to care for the brood properly. These observations have broad implications for colony-level success, as a colony that cannot rear brood will likely fail. Furthermore, disturbances in learning, memory and sucrose sensitivity through gut dysbiosis can scale to ultimately affect the ability of a forager to find and bring resources (e.g. nectar) back to the colony. Differences in forager learning phenotypes can influence the foraging location preference of whole colonies (Cook et al., 2020). If we consider the gut microbiome as another mechanism regulating these learning phenotypes, we can gain a nuanced understanding of how honeybee societies work.

Odors from cuticular hydrocarbon (CHC) signals within social insect groups allow for discrimination between nestmates and non-nestmates, which protects social insects from exploiters

(van Zweden and d'Ettorre, 2010). Vernier et al. (2020) found that different honeybee colonies have distinct gut microbiome profiles, which contribute to colony-specific CHC signals. When subjected to an acceptance assay (see Glossary), honeybees are more likely to be accepted by bees that possess similar gut communities, analogous to how *Drosophila* prefer to mate with individuals on the same diet (Sharon et al., 2010; Vernier et al., 2020). However, bees inoculated with an opportunistic environmental bacteria, *Lonsdalea quercina*, are less likely to be accepted by other bees (Vernier et al., 2020). These results suggest that the gut microbiome is key in defining odors that shape group membership and have broad ramifications for group selection (Fig. 3).

Perhaps the most compelling evidence comes from Liberti et al. (2022), who video tracked whole nucleus colonies (see Glossary) consisting of microbiota-depleted honeybees. Such bees have reduced head-to-head interactions (see Glossary) compared with colonies consisting of bees with their normal gut communities, despite having similar activity levels to a control colony (Liberti et al., 2022). Furthermore, the interactions of microbiota-depleted bees were more varied than those of honeybees with a normal microbiota, indicating that the interactions were more random and less specialized (Liberti et al., 2022). Social interactions drive much of the communication that occurs within animal societies (Ward and Webster, 2016); therefore, gut dysbiosis could have consequences for necessary colony-level communication, especially that involved in nestmate recognition, thermoregulation and foraging. Further investigation into the extent to which dysbiosis could affect social

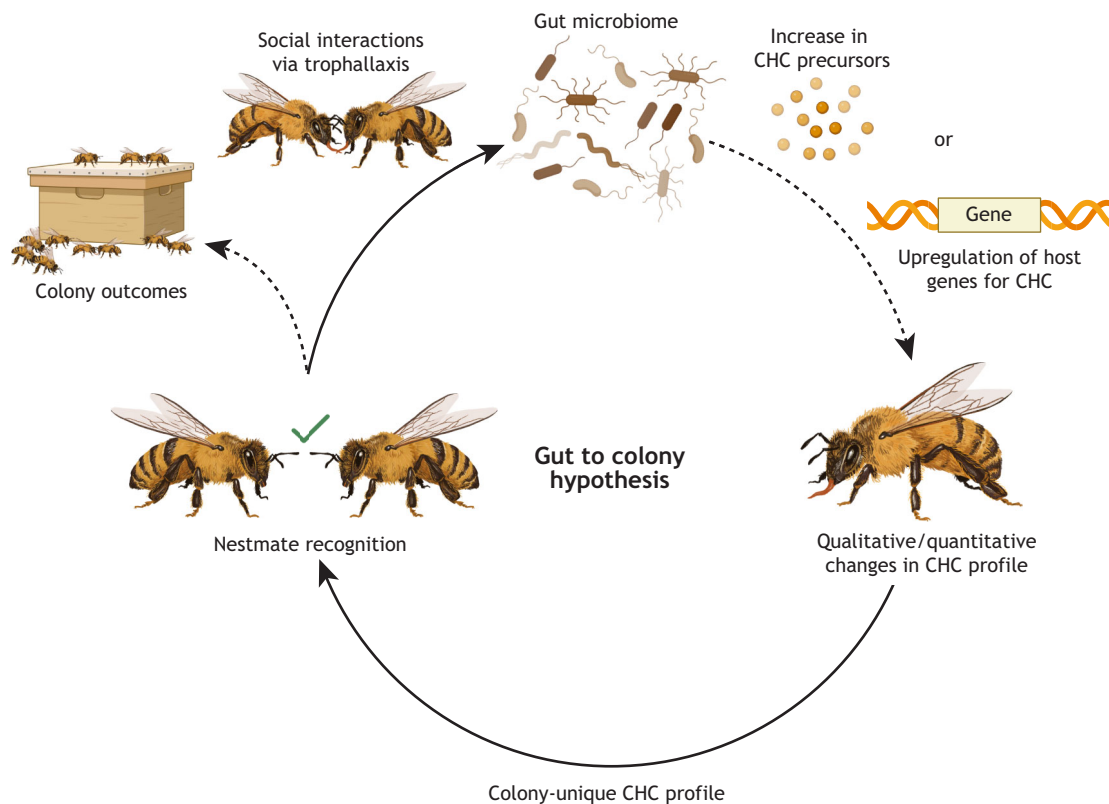


Fig. 3. Hypothesized gut effects to colony outcomes using nestmate recognition. Solid lines represent known relationships, whereas dashed lines represent hypothesized effects and mechanisms. Each honeybee colony has a unique gut microbiome profile that is also associated with their cuticular hydrocarbon (CHC) profile. Although the mechanism behind these findings is still unknown, it is possible that the gut microbe community either directly contributes to CHC precursors or regulates the expression of host genes for CHC. We hypothesize that because the gut microbiome can influence a critical part of social living – nestmate recognition – gut microbiome effects on individuals can scale and have broad significance for colony outcomes. Figure created with BioRender. Honeybee illustrations by Impact Media Lab.

interactions could shed light on the intricacies of these microbial effects. Whether social interactions depend on the whole gut community, or a few key players, is unknown. Causative research is needed to identify the mechanisms by which gut microbes as a community and as individuals can affect such large-scale change in their hosts and the groups to which they contribute. We propose that honeybees as a model would be advantageous for this research (Douglas, 2019; Engel et al., 2016; Zheng et al., 2018).

Future directions

Studying honeybees and their gut symbionts presents an opportunity to study host–microbe interactions at multiple levels of social organization – from individual to the collective (Fig. 3). However, several unknowns at the individual and collective levels hinder our ability to fully grasp the extent of host–microbe interactions within the honeybee model and should be the focus of future studies.

Foremost, despite the myriad of interesting correlations between individual physiology and gut microbiome in the literature, how gut microbes enact changes in individual honeybee physiology and behavior remains a significant gap in current knowledge. In the case of a honeybee's CHC profile (Vernier et al., 2020), whether the microbial community directly increases CHC precursors or changes host physiology involved in producing CHC to impact the odor profile is unclear (Fig. 3). Gut microbiome presence is associated with an upregulation of amino acids related to social interactions in the brain (Liberti et al., 2022), but how exactly the microbiome interacts with host physiology to enact these changes is unknown.

We posit that microbial endocrinology is a promising avenue for future researchers interested in testing the mechanisms behind gut microbial effects as done in other studies (Feng et al., 2022; O'Donnell et al., 2020; Silva et al., 2020; Varian et al., 2017). Microbial endocrinology, the microbial production of and response to neuroactive compounds used by organisms in their biology, has been proposed as a mechanism behind gut bacteria modulation of host physiology and behavior (Lyte, 2011; Silva et al., 2020). In other words, symbiont metabolic activity in the gut can produce compounds that can directly and indirectly stimulate neurophysiological systems responsible for behavior in the host. Candidate molecules include short-chain fatty acids, particularly propionate, acetate and butyrate, and neurotransmitters, such as GABA and serotonin (Silva et al., 2020; Strandwitz, 2018). We believe the microbial endocrinology hypothesis holds the greatest potential to unraveling causation between bacteria and honeybee behavior because of recent findings in other models. Indeed, from *C. elegans* (O'Donnell et al., 2020) to zebrafish (Hill et al., 2022) and *Anopheles* mosquitoes (Feng et al., 2022), gut communities produce key compounds that induce physiological changes in their hosts and subsequently affect their host's behavior. As a result, using microbial endocrinology as a lens for future research may be an interesting avenue to pursue the causative mechanisms that drive the relationship between behavior and gut symbionts in honeybees.

If the gut microbiome does influence individual honeybee worker physiology to affect behavior as in other organisms (Agranyoni et al., 2021; O'Donnell et al., 2020; Poutahidis et al., 2013; Qiao et al., 2019; Wu et al., 2021), then the implications for social dynamics in the colony are massive (Fig. 3). Changes in physiology may impact critical collective behaviors, such as foraging and nest defense. When examining honeybee societies, the gut microbiome of workers may shape how individuals within the colony communicate, as it impacts the number of social interactions workers will have with others (Liberti et al., 2022). Inadequate

social interactions could lead to disrupted waggle-dance communication, such as less recruitment of other foragers, or could affect waggle-dance quality, impacting collective foraging decisions (Cook et al., 2020). Future studies could investigate how effectively microbiota-depleted workers waggle dance to relay potential food sources, if at all, or how microbiota-depleted workers respond to a wagging worker. Moreover, because the gut microbiome influences nestmate recognition (Vernier et al., 2020), it could also impact how well a colony can protect itself from intruders. Outside of defending valuable resources, preventing intruders is important for regulating colony health, as robber honeybees are often heavily infected with parasites and pathogens (Kuszevska and Woyciechowski, 2014). Future studies could measure how likely a microbiota-depleted colony is to be robbed compared with a control colony. Thus, the effects of the gut microbiome have potential ramifications for colony survival.

Changes in the gut microbiome may even drive colony fitness. For one, the ability of a colony to rear high-quality queens and drones depends on several environmental conditions – including resource availability – that workers must monitor to decide whether to invest in rearing drones (Boes, 2010; Gilley et al., 2003). If workers cannot effectively forage owing to communication breakdown caused by gut dysbiosis (Liberti et al., 2022), their colony may be unable to invest in producing new reproductive individuals. Similarly, workers that cannot protect their colony from intruders may be unable to rear high-quality queens or drones, and such colonies might suffer from higher parasite or pathogen load (Kuszevska and Woyciechowski, 2014). High-quality queens and drones could also depend on the health of their gut microbiome, which could directly impact their respective reproductive capabilities by driving cell differentiation that can inhibit or enhance the gonadal development of their host, as recently shown in other invertebrates (Belcaid et al., 2019; McNulty et al., 2023). Queen microbiome composition is sensitive to rearing environment (Copeland et al., 2022a); therefore, any environmental changes within the colony as already discussed may alter her ability to attract drones (Sharon et al., 2010). It could also alter the queen's ability to signal her own fertility and inhibit worker reproduction within the hive environment (Hoover et al., 2003; Keller and Nonacs, 1993; Oi et al., 2015). These outcomes all have the potential to have real fitness consequences for honeybee colonies, shaping the evolutionary trajectories of all organisms involved in this complex symbiosis in a multi-kingdom manner (Miller et al., 2021; Shropshire and Bordenstein, 2016). Such hypotheses remain untested. Future research could test for correlations of colony gut microbiome with queen and drone quality and production to begin investigating these possibilities.

Conclusions

We are only beginning to scale our understanding of the effects of the gut microbiome from the individual honeybee to the superorganism that is the honeybee colony (Fig. 3). It is essential to fill these crucial gaps in knowledge to eventually understand how the gut microbiome shapes host evolution (Shropshire and Bordenstein, 2016). The microbiome clearly interacts with host physiology to drive behavior (Agranyoni et al., 2021; Qiao et al., 2019; Wu et al., 2021), and this can scale to shape collective dynamics. But the impacts of the microbiome go beyond behavioral mechanisms in individuals and groups. As we have discussed, the microbiome shapes host fitness in many ways: (1) producing vital nutrients not acquired by diet (Conly and Stein, 1992; LeBlanc et al., 2013), (2) shaping mate choice (Arbuthnott et al., 2016;

Sharon et al., 2010), (3) regulating the development of reproductive organs and subsequent production of gametes (Dou et al., 2023; Gnainsky et al., 2021; McNulty et al., 2023) and (4) influencing the health and behavior of offspring across several generations (Buffington et al., 2016; Gesù et al., 2022). As such, the honeybee microbiome is likely to play a significant role in the evolution of its host and is an enticing model system to understand selection at many different levels in a society.

In nature, host–microbe interactions rarely exist within a vacuum; these relationships need to be examined within the context of the social environment, especially given that social living has independently evolved multiple times across the tree of life. It is possible that the gut microbiome has evolutionary consequences that impact populations, species and ecosystems. Such a nuanced comprehension of host–microbe interactions will require the usage of interdisciplinary techniques spanning microbiology, physiology and behavioral ecology. To this end, studying the eusocial honeybee and their gut symbionts underneath the framework laid out in other model systems will prove useful as we look to the future of gut microbiome, behavioral ecology and evolutionary research.

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Competing interests

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ECR Spotlight

This article has an associated ECR Spotlight interview with Justine Nguyen.

References

- Agranyoni, O., Meninger-Mordechay, S., Uzan, A., Ziv, O., Salmon-Divon, M., Rodin, D., Raz, O., Koman, I., Koren, O., Pinhasov, A. et al. (2021). Gut microbiota determines the social behavior of mice and induces metabolic and inflammatory changes in their adipose tissue. *NPJ Biofilms Microbiomes* **7**, 1–14. doi:10.1038/s41522-021-00193-9
- Amdam, G. V., Fennern, E. and Havukainen, H. (2012). Vitellogenin in honey bee behavior and lifespan. In *Honeybee Neurobiology and Behavior* (ed. C. G. Galizia, D. Eisenhardt and M. Giurfa), pp. 17–29. Dordrecht: Springer Netherlands.
- Ament, S. A., Corona, M., Pollock, H. S. and Robinson, G. E. (2008). Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proc. Natl. Acad. Sci. USA* **105**, 4226–4231. doi:10.1073/pnas.0800630105
- Anderson, K. E., Rodrigues, P. A. P., Mott, B. M., Maes, P. and Corby-Harris, V. (2016). Ecological succession in the honey bee gut: shift in lactobacillus strain dominance during early adult development. *Microb. Ecol.* **71**, 1008–1019. doi:10.1007/s00248-015-0716-2
- Anderson, K., Ricigliano, V. A., Copeland, D. C., Mott, B. M. and Maes, P. (2022). Social interaction is unnecessary for hindgut microbiome transmission in honey bees: the effect of diet and social exposure on tissue-specific microbiome assembly. *Microb. Ecol.* **85**, 1498–1513. doi:10.1007/s00248-022-02025-5
- Arbuthnott, D., Levin, T. C. and Promislow, D. E. L. (2016). The impacts of *Wolbachia* and the microbiome on mate choice in *Drosophila melanogaster*. *J. Evol. Biol.* **29**, 461–468. doi:10.1111/jeb.12788
- Archie, E. A. and Tung, J. (2015). Social behavior and the microbiome. *Curr. Opin. Behav. Sci.* **6**, 28–34. doi:10.1016/j.cobeha.2015.07.008
- Arenas, A. and Kohlmaier, M. G. (2019). Nectar source profitability influences individual foraging preferences for pollen and pollen-foraging activity of honeybee colonies. *Behav. Ecol. Sociobiol.* **73**, 34. doi:10.1007/s00265-019-2644-5
- Belcaid, M., Casaburi, G., McNulty, S. J., Schmidbaur, H., Suria, A. M., Moriano-Gutierrez, S., Pankey, M. S., Oakley, T. H., Kremer, N., Koch, E. J. et al. (2019). Symbiotic organs shaped by distinct modes of genome evolution in cephalopods. *Proc. Natl. Acad. Sci. USA* **116**, 3030–3035. doi:10.1073/pnas.1817322116
- Berdahl, A., Torney, C. J., Ioannou, C. C., Faria, J. J. and Couzin, I. D. (2013). Emergent sensing of complex environments by mobile animal groups. *Science* **339**, 574–576. doi:10.1126/science.1225883
- Beshers, S. N. and Fewell, J. H. (2001). Models of division of labor in social insects. *Annu. Rev. Entomol.* **46**, 413–440. doi:10.1146/annurev.ento.46.1.413
- Beshers, S. N., Huang, Z. Y., Oono, Y. and Robinson, G. E. (2001). Social inhibition and the regulation of temporal polyethism in honey bees. *J. Theor. Biol.* **213**, 461–479. doi:10.1006/jtbi.2001.2427
- Bicca-Marques, J. C. and Garber, P. A. (2005). Use of social and ecological information in tamarin foraging decisions. *Int. J. Primatol.* **26**, 1321–1344. doi:10.1007/s10764-005-8855-9
- Boes, K. E. (2010). Honeybee colony drone production and maintenance in accordance with environmental factors: an interplay of queen and worker decisions. *Insect. Soc.* **57**, 1–9. doi:10.1007/s00040-009-0046-9
- Bonabeau, E., Theraulaz, G., Deneubourg, J.-L., Aron, S. and Camazine, S. (1997). Self-organization in social insects. *Trends Ecol. Evol.* **12**, 188–193. doi:10.1016/S0169-5347(97)01048-3
- Bongers, K. S., Chanderraj, R., Woods, R. J., McDonald, R. A., Adame, M. D., Falkowski, N. R., Brown, C. A., Baker, J. M., Winner, K. M., Fergle, D. J. et al. (2023). The gut microbiome modulates body temperature both in sepsis and health. *Am. J. Respir. Crit. Care. Med.* **207**, 1030–1041. doi:10.1164/rccm.202201-0161OC
- Bonilla-Rosso, G. and Engel, P. (2018). Functional roles and metabolic niches in the honey bee gut microbiota. *Curr. Opin. Microbiol.* **43**, 69–76. doi:10.1016/j.mib.2017.12.009
- Bordenstein, S. R. and Theis, K. R. (2015). Host biology in light of the microbiome: ten principles of holobionts and hologenomes. *PLoS Biol.* **13**, e1002226. doi:10.1371/journal.pbio.1002226
- Breed, M. D., Guzmán-Novoa, E. and Hunt, G. J. (2004). Defensive behavior of honey bees: organization, genetics, and comparisons with other bees. *Annu. Rev. Entomol.* **49**, 271–298. doi:10.1146/annurev.ento.49.061802.123155
- Brian, M. V. (2012). *Social Insects: Ecology and Behavioural Biology*. Springer Science & Business Media.
- Brochet, S., Quinn, A., Mars, R. A., Neuschwander, N., Sauer, U. and Engel, P. (2021). Niche partitioning facilitates coexistence of closely related honey bee gut bacteria. *eLife* **10**, e68583. doi:10.7554/eLife.68583
- Buffington, S. A., Di Prisco, G. V., Auchtung, T. A., Ajami, N. J., Petrosino, J. F. and Costa-Mattioli, M. (2016). Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* **165**, 1762–1775. doi:10.1016/j.cell.2016.06.001
- Conly, J. M. and Stein, K. (1992). The production of menaquinones (vitamin K2) by intestinal bacteria and their role in maintaining coagulation homeostasis. *Prog. Food Nutr. Sci.* **16**, 307–343.
- Cook, C. N. and Breed, M. D. (2013). Social context influences the initiation and threshold of thermoregulatory behaviour in honeybees. *Anim. Behav.* **86**, 323–329. doi:10.1016/j.anbehav.2013.05.021
- Cook, C. N., Lemanski, N. J., Mosquero, T., Ozturk, C., Gadau, J., Pinter-Wollman, N. and Smith, B. H. (2020). Individual learning phenotypes drive collective behavior. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 17949. doi:10.1073/pnas.1920554117
- Copeland, D. C., Anderson, K. E. and Mott, B. M. (2022a). Early queen development in honey bees: social context and queen breeder source affect gut microbiota and associated metabolism. *Microbiol. Spectr.* **10**, e00383–e00322. doi:10.1128/spectrum.00383-22
- Copeland, D. C., Maes, P. W., Mott, B. M. and Anderson, K. E. (2022b). Changes in gut microbiota and metabolism associated with phenotypic plasticity in the honey bee *Apis mellifera*. *Front. Microbiol.* **13**, 1059001. doi:10.3389/fmicb.2022.1059001
- Corby-Harris, V., Maes, P. and Anderson, K. E. (2014). The bacterial communities associated with honey bee (*Apis mellifera*) foragers. *PLoS ONE* **9**, e95056. doi:10.1371/journal.pone.0095056
- Corona, M., Hughes, K. A., Weaver, D. B. and Robinson, G. E. (2005). Gene expression patterns associated with queen honey bee longevity. *Mech. Ageing Dev.* **126**, 1230–1238. doi:10.1016/j.mad.2005.07.004
- Corona, M., Velarde, R. A., Remolina, S., Moran-Lauter, A., Wang, Y., Hughes, K. A. and Robinson, G. E. (2007). Vitellogenin, juvenile hormone, insulin signaling, and queen honey bee longevity. *Proc. Natl. Acad. Sci. USA* **104**, 7128–7133. doi:10.1073/pnas.0701909104
- Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D. M. et al. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **318**, 283–287. doi:10.1126/science.1146498
- Crailsheim, K., Schneider, L. H. W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R. and Schöffmann, B. (1992). Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): dependence on individual age and function. *J. Insect Physiol.* **38**, 409–419. doi:10.1016/0022-1910(92)90117-V
- Cryan, J. F. and Dinan, T. G. (2012). Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* **13**, 701–712. doi:10.1038/nrn3346

- Ding, N., Zhang, X., Zhang, X. D., Jing, J., Liu, S. S., Mu, Y. P., Peng, L. L., Yan, Y. J., Xiao, G. M., Bi, X. Y. et al. (2020). Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. *Gut* **69**, 1608–1619. doi:10.1136/gutjnl-2019-319127
- Dou, W., Sun, B., Miao, Y., Huang, D. and Xiao, J. (2023). Single-cell transcriptome sequencing reveals *Wolbachia*-mediated modification in early stages of *Drosophila* spermatogenesis. *Proc. R. Soc. B* **290**, 20221963. doi:10.1098/rspb.2022.1963
- Douglas, A. E. (2019). Simple animal models for microbiome research. *Nat. Rev. Microbiol.* **17**, 764–775. doi:10.1038/s41579-019-0242-1
- Elgart, M., Stern, S., Salton, O., Gnainsky, Y., Heifetz, Y. and Soen, Y. (2016). Impact of gut microbiota on the fly's germ line. *Nat. Commun.* **7**, 11280. doi:10.1038/ncomms11280
- Engel, P., Kwong, W. K., McFrederick, Q., Anderson, K. E., Barribeau, S. M., Chandler, J. A., Cornman, R. S., Dainat, J., de Miranda, J. R., Doublet, V. et al. (2016). The bee microbiome: impact on bee health and model for evolution and ecology of host–microbe interactions. *mBio* **7**, e02164–15.
- Feng, Y., Peng, Y., Song, X., Wen, H., An, Y., Tang, H. and Wang, J. (2022). Anopheline mosquitoes are protected against parasite infection by tryptophan catabolism in gut microbiota. *Nat. Microbiol.* **7**, 707–715. doi:10.1038/s41564-022-01099-8
- Fluri, P., Lüscher, M., Wille, H. and Gerig, L. (1982). Changes in weight of the pharyngeal gland and haemolymph titres of juvenile hormone, protein and vitellogenin in worker honey bees. *J. Insect Physiol.* **28**, 61–68. doi:10.1016/0022-1910(82)90023-3
- Gesù, C. M. D., Matz, L. M., Bolding, I. J., Fultz, R., Hoffman, K. L., Gammazza, A. M., Petrosino, J. F. and Buffington, S. A. (2022). Maternal gut microbiota mediate intergenerational effects of high-fat diet on descendant social behavior. *Cell Rep.* **41**, 111461. doi:10.1016/j.celrep.2022.111461
- Gilley, D. C., Tarpay, D. R. and Land, B. B. (2003). Effect of queen quality on interactions between workers and dueling queens in honeybee (*Apis mellifera* L.) colonies. *Behav. Ecol. Sociobiol.* **55**, 190–196. doi:10.1007/s00265-003-0708-y
- Gmeinbauer, R. and Crailsheim, K. (1993). Glucose utilization during flight of honeybee (*Apis mellifera*) workers, drones and queens. *J. Insect Physiol.* **39**, 959–967. doi:10.1016/0022-1910(93)90005-C
- Gnainsky, Y., Zfanya, N., Elgart, M., Omri, E., Brandis, A., Mehlman, T., Itkin, M., Malitsky, S., Adamski, J. and Soen, Y. (2021). Systemic regulation of host energy and oogenesis by microbiome-derived mitochondrial coenzymes. *Cell Rep.* **34**, 108583. doi:10.1016/j.celrep.2020.108583
- Grueter, C. C., Qi, X., Zinner, D., Bergman, T., Li, M., Xiang, Z., Zhu, P., Migliano, A. B., Miller, A., Krützen, M. et al. (2020). Multilevel organisation of animal sociality. *Trends Ecol. Evol.* **35**, 834–847. doi:10.1016/j.tree.2020.05.003
- Guidugli, K. R., Nascimento, A. M., Amdam, G. V., Barchuk, A. R., Omholt, S., Simões, Z. L. P. and Hartfelder, K. (2005). Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* **579**, 4961–4965. doi:10.1016/j.febslet.2005.07.085
- Haydak, M. H. (1970). Honey bee nutrition. *Annu. Rev. Entomol.* **15**, 143–156. doi:10.1146/annurev.en.15.010170.001043
- Henry, Y. and Colinet, H. (2018). Microbiota disruption leads to reduced cold tolerance in *Drosophila* flies. *Sci. Nat.* **105**, 59. doi:10.1007/s00114-018-1584-7
- Hill, J. H., Massaquoi, M. S., Sweeney, E. G., Wall, E. S., Jahl, P., Bell, R., Kallio, K., Derrick, D., Murtaugh, L. C., Parthasarathy, R. et al. (2022). Befa, a microbiota-secreted membrane disrupter, disseminates to the pancreas and increases β cell mass. *Cell Metab.* **34**, 1779–1791.e9. doi:10.1016/j.cmet.2022.09.001
- Hoover, S. E. R., Keeling, C. I., Winston, M. L. and Slessor, K. N. (2003). The effect of queen pheromones on worker honey bee ovary development. *Naturwissenschaften* **90**, 477–480. doi:10.1007/s00114-003-0462-z
- Huang, Z. Y. and Robinson, G. E. (1992). Honeybee colony integration: worker–worker interactions mediate hormonally regulated plasticity in division of labor. *Proc. Natl Acad. Sci. USA* **89**, 11726–11729. doi:10.1073/pnas.89.24.11726
- Huang, Z.-Y. and Robinson, G. E. (1995). Seasonal changes in juvenile hormone titers and rates of biosynthesis in honey bees. *J. Comp. Physiol. B* **165**, 18–28. doi:10.1007/BF00264682
- Jemielity, S., Chapuisat, M., Parker, J. D. and Keller, L. (2005). Long live the queen: studying aging in social insects. *AGE* **27**, 241–248. doi:10.1007/s11357-005-2916-z
- Jia, Y., Jin, S., Hu, K., Geng, L., Han, C., Kang, R., Pang, Y., Ling, E., Tan, E. K., Pan, Y. et al. (2021). Gut microbiome modulates *Drosophila* aggression through octopamine signaling. *Nat. Commun.* **12**, 2698. doi:10.1038/s41467-021-23041-y
- Jones, J. C., Fruciano, C., Marchant, J., Hildebrand, F., Forslund, S., Bork, P., Engel, P. and Hughes, W. O. H. (2018). The gut microbiome is associated with behavioural task in honey bees. *Insect. Soc.* **65**, 419–429. doi:10.1007/s00040-018-0624-9
- Kapheim, K. M., Rao, V. D., Yeoman, C. J., Wilson, B. A., White, B. A., Goldenfeld, N. and Robinson, G. E. (2015). Caste-specific differences in hindgut microbial communities of honey bees (*Apis mellifera*). *PLoS ONE* **10**, e0123911. doi:10.1371/journal.pone.0123911
- Keller, L. and Nonacs, P. (1993). The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav.* **45**, 787–794. doi:10.1006/anbe.1993.1092
- Kešnerová, L., Mars, R. A. T., Ellegaard, K. M., Troilo, M., Sauer, U. and Engel, P. (2017). Disentangling metabolic functions of bacteria in the honey bee gut. *PLoS Biol.* **15**, e2003467. doi:10.1371/journal.pbio.2003467
- Kešnerová, L., Emery, O., Troilo, M., Liberti, J., Erkosar, B. and Engel, P. (2020). Gut microbiota structure differs between honeybees in winter and summer. *The ISME Journal* **14**, 801–814.
- King, A. J. and Cowlshaw, G. (2007). When to use social information: the advantage of large group size in individual decision making. *Biol. Lett.* **3**, 137–139. doi:10.1098/rsbl.2007.0017
- Knoll, S., Pinna, W., Varcasia, A., Scala, A. and Cappai, M. G. (2020). The honey bee (*Apis mellifera* L., 1758) and the seasonal adaptation of productions. Highlights on summer to winter transition and back to summer metabolic activity. A review. *Livest. Sci.* **235**, 104011. doi:10.1016/j.livsci.2020.104011
- Krause, J., Krause, P. of F. B. and E. J., Ruxton, G. D., Ruxton, G. and Ruxton, I. G. (2002). *Living in Groups*. OUP Oxford.
- Kuszevska, K. and Woyciechowski, M. (2014). Risky robbing is a job for short-lived and infected worker honeybees. *Apidologie* **45**, 537–544. doi:10.1007/s13592-014-0267-4
- Kwong, W. K. and Moran, N. A. (2016). Gut microbial communities of social bees. *Nat. Rev. Microbiol.* **14**, 374–384. doi:10.1038/nrmicro.2016.43
- Kwong, W. K., Mancenido, A. L. and Moran, N. A. (2017). Immune system stimulation by the native gut microbiota of honey bees. *R. Soc. Open Sci.* **4**, 170003. doi:10.1098/rsos.170003
- LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D. and Ventura, M. (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Curr. Opin. Biotechnol.* **24**, 160–168. doi:10.1016/j.copbio.2012.08.005
- Lee, F. J., Rusch, D. B., Stewart, F. J., Mattila, H. R. and Newton, I. L. G. (2015). Saccharide breakdown and fermentation by the honey bee gut microbiome. *Environ. Microbiol.* **17**, 796–815. doi:10.1111/1462-2920.12526
- Leonhardt, S. D., Menzel, F., Nehring, V. and Schmitt, T. (2016). Ecology and evolution of communication in social insects. *Cell* **164**, 1277–1287. doi:10.1016/j.cell.2016.01.035
- Li, L., Solvi, C., Zhang, F., Qi, Z., Chittka, L. and Zhao, W. (2021). Gut microbiome drives individual memory variation in bumblebees. *Nat. Commun.* **12**, 6588. doi:10.1038/s41467-021-26833-4
- Liberti, J., Kay, T., Quinn, A., Kesner, L., Frank, E. T., Cabirol, A., Richardson, T. O., Engel, P. and Keller, L. (2022). The gut microbiota affects the social network of honeybees. *Nat. Ecol. Evol.* **6**, 1471–1479. doi:10.1038/s41559-022-01840-w
- Lucchetti, M. A., Kilchenmann, V., Glauser, G., Praz, C. and Kast, C. (2018). Nursing protects honeybee larvae from secondary metabolites of pollen. *Proc. R. Soc. B* **285**, 20172849. doi:10.1098/rspb.2017.2849
- Lyte, M. (2011). Probiotics function mechanistically as delivery vehicles for neuroactive compounds: microbial endocrinology in the design and use of probiotics. *BioEssays* **33**, 574–581. doi:10.1002/bies.201100024
- Ma, X., Xiao, W., Li, H., Pang, P., Xue, F., Wan, L., Pei, L. and Yan, H. (2021). Metformin restores hippocampal neurogenesis and learning and memory via regulating gut microbiota in the obese mouse model. *Brain Behav. Immun.* **95**, 68–83. doi:10.1016/j.bbi.2021.02.011
- Manthey, C., Johnston, P. R., Nakagawa, S. and Rolff, J. (2022). Complete metamorphosis and microbiota turnover in insects. *Mol. Ecol.* **32**, 6543–6551. doi:10.1111/mec.16673
- Martinson, V. G., Danforth, B. N., Minckley, R. L., Rueppell, O., Tingek, S. and Moran, N. A. (2011). A simple and distinctive microbiota associated with honey bees and bumble bees. *Mol. Ecol.* **20**, 619–628. doi:10.1111/j.1365-294X.2010.04959.x
- Martinson, V. G., Moy, J. and Moran, N. A. (2012). Establishment of characteristic gut bacteria during development of the honeybee worker. *Appl. Environ. Microbiol.* **78**, 2830–2840. doi:10.1128/AEM.07810-11
- McAnulty, S. J., Kerwin, A. H., Koch, E., Nuttall, B., Suria, A. M., Collins, A. J., Schleicher, T. R., Rader, B. A. and Nyholm, S. V. (2023). 'Failure to launch': development of a reproductive organ linked to symbiotic bacteria. *mBio* **14**, e0213122. doi:10.1128/mbio.02131-22
- Miller, A. K., Westlake, C. S., Cross, K. L., Leigh, B. A. and Bordenstein, S. R. (2021). The microbiome impacts host hybridization and speciation. *PLoS Biol.* **19**, e3001417. doi:10.1371/journal.pbio.3001417
- Moeller, A. H., Ivey, K., Cornwall, M. B., Herr, K., Rede, J., Taylor, E. N. and Gunderson, A. R. (2020). The lizard gut microbiome changes with temperature and is associated with heat tolerance. *Appl. Environ. Microbiol.* **86**, e01181–e01120. doi:10.1128/AEM.01181-20
- Moran, N. A. (2015). Genomics of the honey bee microbiome. *Curr. Opin. Insect Sci.* **10**, 22–28. doi:10.1016/j.cois.2015.04.003
- Mott, C. M. and Breed, M. D. (2012). Insulin modifies honeybee worker behavior. *Insects* **3**, 1084–1092. doi:10.3390/insects3041084

- Motta, E. V. S., Raymann, K. and Moran, N. A. (2018). Glyphosate perturbs the gut microbiota of honey bees. *Proc. Natl. Acad. Sci. USA* **115**, 10305–10310. doi:10.1073/pnas.1803880115
- Naggal, J. and Cryan, J. F. (2021). Host genetics, the microbiome & behaviour—a ‘holobiont’ perspective. *Cell Res.* **31**, 832–833. doi:10.1038/s41422-021-00512-x
- Nelson, C. M., Ihle, K. E., Fondrk, M. K., Jr, Page, R. E. and Amdam, G. V. (2007). The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol.* **5**, e62. doi:10.1371/journal.pbio.0050062
- Nieuwdorp, M., Gilijamse, P. W., Pai, N. and Kaplan, L. M. (2014). Role of the microbiome in energy regulation and metabolism. *Gastroenterology* **146**, 1525–1533. doi:10.1053/j.gastro.2014.02.008
- O'Donnell, M. P., Fox, B. W., Chao, P.-H., Schroeder, F. C. and Sengupta, P. (2020). A neurotransmitter produced by gut bacteria modulates host sensory behaviour. *Nature* **583**, 415–420. doi:10.1038/s41586-020-2395-5
- Oi, C. A., van Zweden, J. S., Oliveira, R. C., Van Oystaeyen, A., Nascimento, F. S. and Wenseleers, T. (2015). The origin and evolution of social insect queen pheromones: novel hypotheses and outstanding problems. *BioEssays* **37**, 808–821. doi:10.1002/bies.201400180
- Ortiz-Alvarado, Y., Clark, D. R., Vega-Melendez, C. J., Flores-Cruz, Z., Domingez-Bello, M. G. and Giray, T. (2020). Antibiotics in hives and their effects on honey bee physiology and behavioral development. *Biol. Open* **9**, bio053884.
- Oster, G. F. and Wilson, E. O. (1978). *Caste and Ecology in the Social Insects*. Princeton University Press.
- Page, R. E., Jr, Erber, J. and Fondrk, M. K. (1998). The effect of genotype on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. A* **182**, 489–500. doi:10.1007/s003590050196
- Pearce, A. N., Huang, Z. Y. and Breed, M. D. (2001). Juvenile hormone and aggression in honey bees. *J. Insect Physiol.* **47**, 1243–1247. doi:10.1016/S0022-1910(01)00109-3
- Poutahidis, T., Kearney, S. M., Levkovich, T., Qi, P., Varian, B. J., Lakritz, J. R., Ibrahim, Y. M., Chatzigiagkos, A., Alm, E. J. and Erdman, S. E. (2013). Microbial symbionts accelerate wound healing via the neuropeptide hormone oxytocin. *PLoS ONE* **8**, e78898. doi:10.1371/journal.pone.0078898
- Powell, J. E., Martinson, V. G., Urban-Mead, K. and Moran, N. A. (2014). Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. *Appl. Environ. Microbiol.* **80**, 7378–7387. doi:10.1128/AEM.01861-14
- Powell, J. E., Eiri, D., Moran, N. A. and Rangel, J. (2018). Modulation of the honey bee queen microbiota: effects of early social contact. *PLoS ONE* **13**, e0200527.
- Qiao, H., Keesey, I. W., Hansson, B. S. and Knaden, M. (2019). Gut microbiota affects development and olfactory behavior in *Drosophila melanogaster*. *J. Exp. Biol.* **222**, jeb192500. doi:10.1242/jeb.192500
- Raymann, K. and Moran, N. A. (2018). The role of the gut microbiome in health and disease of adult honey bee workers. *Curr. Opin. Insect Sci.* **26**, 97–104. doi:10.1016/j.cois.2018.02.012
- Raymann, K., Shaffer, Z. and Moran, N. A. (2017). Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol.* **15**, e2001861. doi:10.1371/journal.pbio.2001861
- Ricke, S. C., Dittoe, D. K. and Olson, E. G. (2022). Microbiome applications for laying hen performance and egg production. *Poult. Sci.* **101**, 101784. doi:10.1016/j.psj.2022.101784
- Roager, H. M. and Licht, T. R. (2018). Microbial tryptophan catabolites in health and disease. *Nat. Commun.* **9**, 3294. doi:10.1038/s41467-018-05470-4
- Robinson, G. E. (1987). Regulation of honey bee age polyethism by juvenile hormone. *Behav. Ecol. Sociobiol.* **20**, 329–338. doi:10.1007/BF00300679
- Robinson, G. E. (1992). Regulation of division of labor in insect societies. *Annu. Rev. Entomol.* **37**, 637–665. doi:10.1146/annurev.en.37.010192.003225
- Rohrscheib, C. E., Bondy, E., Josh, P., Riegler, M., Eyles, D., van Swinderen, B., Weible, M. W. and Brownlie, J. C. (2015). Wolbachia influences the production of octopamine and affects drosophila male aggression. *Appl. Environ. Microbiol.* **81**, 4573–4580. doi:10.1128/AEM.00573-15
- Rowland, C. M. and McLellan, A. R. (1987). Seasonal changes of drone numbers in a colony of the honeybee, *Apis mellifera*. *Ecol. Model.* **37**, 155–166. doi:10.1016/0304-3800(87)90023-8
- Scheiner, R., Page, R. and Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (*Apis mellifera*). *Apidologie* **35**, 133–142. doi:10.1051/apido:2004001
- Schwarz, R. S., Moran, N. A. and Evans, J. D. (2016). Early gut colonizers shape parasite susceptibility and microbiota composition in honey bee workers. *Proc. Natl. Acad. Sci. USA* **113**, 9345–9350. doi:10.1073/pnas.1606631113
- Seeley, T. D. (1982). Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav. Ecol. Sociobiol.* **11**, 287–293. doi:10.1007/BF00299306
- Seeley, T. D. and Kolmes, S. A. (1991). Age polyethism for hive duties in honey bees – illusion or reality? *Ethology* **87**, 284–297. doi:10.1111/j.1439-0310.1991.tb00253.x
- Sgritta, M., Dooling, S. W., Buffington, S. A., Momin, E. N., Francis, M. B., Britton, R. A. and Costa-Mattioli, M. (2019). Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron* **101**, 246–259.e6. doi:10.1016/j.neuron.2018.11.018
- Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I. and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **107**, 20051–20056. doi:10.1073/pnas.1009906107
- Shreiner, A. B., Kao, J. Y. and Young, V. B. (2015). The gut microbiome in health and in disease. *Curr. Opin. Gastroenterol.* **31**, 69–75. doi:10.1097/MOG.0000000000000139
- Shropshire, J. D. and Bordenstein, S. R. (2016). Speciation by symbiosis: the microbiome and behavior. *mBio* **7**, e01785–e01715. doi:10.1128/mBio.01785-15
- Shu, R., Hahn, D. A., Jurkevitch, E., Liburd, O. E., Yuval, B. and Wong, A. C.-N. (2021). Sex-dependent effects of the microbiome on foraging and locomotion in *Drosophila suzukii*. *Front. Microbiol.* **12**, 656406. doi:10.3389/fmicb.2021.656406
- Silva, Y. P., Bernardi, A. and Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front. Endocrinol.* **11**, 25. doi:10.3389/fendo.2020.00025
- Silva, V., Palacios-Muñoz, A., Okray, Z., Adair, K. L., Waddell, S., Douglas, A. E. and Ewer, J. (2021). The impact of the gut microbiome on memory and sleep in *Drosophila*. *J. Exp. Biol.* **224**, jeb23619.
- Simcock, N. K., Gray, H., Bouchebti, S. and Wright, G. A. (2018). Appetitive olfactory learning and memory in the honeybee depend on sugar reward identity. *J. Insect Physiol.* **106**, 71–77. doi:10.1016/j.jinsphys.2017.08.009
- Slankster, E., Lee, C., Hess, K. M., Odell, S. and Mathew, D. (2019). Effect of gut microbes on olfactory behavior of *Drosophila melanogaster* larva. *Bios* **90**, 227–238. doi:10.1893/0005-3155-90.4.227
- Stabentheiner, A., Kovac, H. and Brodschneider, R. (2010). Honeybee colony thermoregulation – regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS ONE* **5**, e8967. doi:10.1371/journal.pone.0008967
- Strandwitz, P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res.* **1693**, 128–133. doi:10.1016/j.brainres.2018.03.015
- Suito, T., Nagao, K., Juni, N., Hara, Y., Sokabe, T., Atomi, H. and Umeda, M. (2022). Regulation of thermoregulatory behavior by commensal bacteria in *Drosophila*. *Biosci. Biotechnol. Biochem.* **86**, 1060–1070. doi:10.1093/bbb/zbac087
- Tarpy, D. R., Mattila, H. R. and Newton, I. L. G. (2015). Development of the honey bee gut microbiome throughout the queen-rearing process. *Appl. Environ. Microbiol.* **81**, 3182–3191. doi:10.1128/AEM.00307-15
- Teseo, S., van Zweden, J. S., Pontieri, L., Koopij, P. W., Sørensen, S. J., Wenseleers, T., Poulsen, M., Boomsma, J. J. and Sapountzis, P. (2019). The scent of symbiosis: gut bacteria may affect social interactions in leaf-cutting ants. *Anim. Behav.* **150**, 239–254. doi:10.1016/j.anbehav.2018.12.017
- Trevelline, B. K. and Kohl, K. D. (2022). The gut microbiome influences host diet selection behavior. *Proc. Natl. Acad. Sci. USA* **119**, e2117537119. doi:10.1073/pnas.2117537119
- van Zweden, J. S. and d'Ettorre, P. (2010). *Nestmate Recognition in Social Insects and the Role of Hydrocarbons*. Cambridge University Press.
- Varian, B. J., Poutahidis, T., DiBenedictis, B. T., Levkovich, T., Ibrahim, Y., Didyk, E., Shikhman, L., Cheung, H. K., Hardas, A., Ricciardi, C. E. et al. (2017). Microbial lysate upregulates host oxytocin. *Brain Behav. Immun.* **61**, 36–49. doi:10.1016/j.bbi.2016.11.002
- Vernier, C. L., Chin, I. M., Adu-Oppong, B., Krupp, J. J., Levine, J., Dantas, G. and Ben-Shahar, Y. (2020). The gut microbiome defines social group membership in honey bee colonies. *Sci. Adv.* **6**, eabd3431. doi:10.1126/sciadv.abd3431
- Wang, Y. and Xie, Z. (2022). Exploring the role of gut microbiome in male reproduction. *Andrology* **10**, 441–450. doi:10.1111/andr.13143
- Ward, A. and Webster, M. (2016). *Sociality: The Behaviour of Group-Living Animals*. Cham: Springer International Publishing.
- Wilson, E. O. and Hölldobler, B. (2005). Eusociality: origin and consequences. *Proc. Natl. Acad. Sci. USA* **102**, 13367–13371. doi:10.1073/pnas.0505858102
- Winston, M. L. (1991). *The Biology of the Honey Bee*. Cambridge, MA: Harvard University Press.
- Wong, A. C.-N., Wang, Q.-P., Morimoto, J., Senior, A. M., Lihoreau, M., Neely, G. G., Simpson, S. J. and Ponton, F. (2017). Gut microbiota modifies olfactory-guided microbial preferences and foraging decisions in *Drosophila*. *Curr. Biol.* **27**, 2397–2404.e4. doi:10.1016/j.cub.2017.07.022
- Wu, W.-L., Adame, M. D., Liou, C.-W., Barlow, J. T., Lai, T.-T., Sharon, G., Schretter, C. E., Needham, B. D., Wang, M. I., Tang, W. et al. (2021). Microbiota regulate social behaviour via stress response neurons in the brain. *Nature* **595**, 409–414. doi:10.1038/s41586-021-03669-y
- Xie, F., Anderson, C. L., Timme, K. R., Kurz, S. G., Fernando, S. C. and Wood, J. R. (2016). Obesity-dependent increases in oocyte mRNAs are associated with increases in proinflammatory signaling and gut microbial abundance of lachnospiraceae in female mice. *Endocrinology* **157**, 1630–1643. doi:10.1210/en.2015-1851
- Zayed, A., Naeger, N. L., Rodríguez-Zas, S. L. and Robinson, G. E. (2012). Common and novel transcriptional routes to behavioral maturation in worker and male honey bees. *Genes Brain Behav.* **11**, 253–261.

- Zhang, Z., Mu, X., Cao, Q., Shi, Y., Hu, X. and Zheng, H. (2022a). Honeybee gut *Lactobacillus* modulates host learning and memory behaviors via regulating tryptophan metabolism. *Nat. Commun.* **13**, 2037. doi:10.1038/s41467-022-29760-0
- Zhang, Z., Mu, X., Shi, Y. and Zheng, H. (2022b). Distinct roles of honeybee gut bacteria on host metabolism and neurological processes. *Microbiol Spectr* **10**, e02438-e02421.
- Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C. and Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via bacterial metabolism and hormonal signaling. *Proc. Natl. Acad. Sci. USA* **114**, 4775–4780. doi:10.1073/pnas.1701819114
- Zheng, H., Steele, M. I., Leonard, S. P., Motta, E. V. S. and Moran, N. A. (2018). Honey bees as models for gut microbiota research. *Lab. Anim. (NY)* **47**, 317–325. doi:10.1038/s41684-018-0173-x
- Zheng, P., Wu, J., Zhang, H., Perry, S. W., Yin, B., Tan, X., Chai, T., Liang, W., Huang, Y., Li, Y. et al. (2021). The gut microbiome modulates gut–brain axis glycerophospholipid metabolism in a region-specific manner in a nonhuman primate model of depression. *Mol. Psychiatry* **26**, 2380–2392. doi:10.1038/s41380-020-0744-2
- Zhu, L., Liao, R., Wu, N., Zhu, G. and Yang, C. (2019). Heat stress mediates changes in fecal microbiome and functional pathways of laying hens. *Appl. Microbiol. Biotechnol.* **103**, 461–472. doi:10.1007/s00253-018-9465-8