

Review

Interplay between gut symbionts and behavioral variation in social insects

Joanito Liberti^{1,2,#}, Philipp Engel¹ and Amélie Cabirol¹

Social insects exhibit a high degree of intraspecific behavioral variation. Moreover, they often harbor specialized microbial communities in their gut. Recent studies suggest that these two characteristics of social insects are interlinked: insect behavioral phenotypes affect their gut microbiota composition, partly through exposure to different environments and diet, and in return, the gut microbiota has been shown to influence insect behavior. Here, we discuss the bidirectional relationship existing between intraspecific variation in gut microbiota composition and behavioral phenotypes in social insects.

Addresses

¹ Department of Fundamental Microbiology, University of Lausanne, Switzerland

² Department of Ecology and Evolution, University of Lausanne, Switzerland

Corresponding authors: Liberti, Joanito (joanito.liberti@unil.ch), Cabirol, Amélie (amelie.cabirol@unil.ch)

Present address: Department of Genetics and Evolution, University of Geneva, Switzerland.

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Introduction

Colonies of social insects are self-organized entities whose ecological success relies on a structured yet flexible allocation of behavioral tasks among individuals [1–4]. Such behavioral variation can be organized into three categories: (1) developmentally determined and irreversible behavioral specialization, which results from developmental programs producing distinct reproductive and nonreproductive castes, as well as morphologically distinct subcastes of workers undertaking nonreproductive tasks such as foraging, brood nursing, and nest defense; (2) plastic behavioral specialization of monomorphic workers undergoing an age-related

transition between nonreproductive behavioral tasks, which can be accelerated or reversed depending on colony needs; and (3) general behavioral variation, which refers to behavioral traits that are expressed by all castes and subcastes, yet having different optima in distinct (sub)castes, for example, social interactions, exploratory behavior, and cognitive abilities, such as learning and memory [5,6].

Which factors drive behavioral variation between and within castes is a key question in social insect research [7]. Recent evidence suggests that there is an interplay between gut microbiota composition and behavioral variation across social insects. While the homeostatic colony environment ensures the stability of microbial communities in the gut of many social insects [8,9], the abundance and prevalence of bacterial species can differ between individuals belonging to different castes and subcastes [10–12]. But what is the causal direction in these associations? This review first presents known consequences of intraspecific behavioral specialization and variation on gut microbiota composition. Then, it discusses recent evidence and new perspectives as to how variation in gut microbiota composition may support behavioral specialization and variation between and within castes of both monomorphic and polymorphic social insect species.

Gut microbiota composition as a consequence of host behavioral phenotypes

Social insects harbor gut microbiota of varying degrees of diversity and stability [13]. Such variation occurs both across and within different social insect lineages. For example, termites host some of the most diverse gut communities, mainly composed of protozoa or bacteria [14–16]. Both ants and wasps often associate with simpler communities, showing large variation between species, colonies, and individuals [17–22], with some exceptions where either primary symbionts dominate or microbial communities are relatively stable across colonies and individuals [21,23,24]. Eusocial corbiculate bees, that is, honeybees, bumblebees, and stingless bees, in contrast to most solitary bees [25–28], harbor specialized gut microbial communities, which are composed of the same core genera. However, there seem to be differences in the amount of inter- and intra-specific variation in the gut microbiota across these bees [27,28].

Castes and subcastes of social insects vary in genes [7,29,30], physiology [3,7,31], dietary preferences [32–35], and social interactions [36,37]. Given demonstrated effects

of such variables on bacterial community assembly and maintenance in the gut [10,21,38], it may not be surprising that many studies found (sub)caste-specific variation in gut microbiota composition across social insects [12]. Developmentally determined behavioral specialization has been associated with differences in the gut microbiota composition of ants [39–42], termites [33,43], bees [11,44], and wasps [17]. While most research has revealed differences between queens, males, and nonreproductive workers [17,33,39–44], two studies showed differences in the gut bacterial communities of morphologically distinct worker subcastes in fungus-growing termites of the genera *Macrotermes* and *Odontotermes* [33,45]. This should encourage future investigation of gut microbiota variation between polymorphic workers.

In monomorphic species, evidence that plastic behavioral specialization led to different gut microbial communities between subcastes of workers has accumulated this past couple of years. In the honeybee *Apis mellifera*, the nurse-to-forager transition is accompanied by a shift in gut bacterial communities and a decrease in bacterial diversity [35,46,47]. The gut microbiota composition of both behavioral subcastes also differs from that of winter bees that remain in the hive during the cold winter season and live much longer [35]. Given that young nurse honeybees, but not old foragers, feed on pollen, such a shift in diet may support the gut microbiota differences reported between these subcastes [35,46,47]. Indeed, while dietary pollen restriction reduced the abundance of all core gut microbiota members, supplementation with nectar and pollen phytochemicals increased gut bacterial diversity and abundance in this species [48,49]. An age-related change in diet was also suggested to affect the gut microbiota composition of the fungus-growing termite *Macrotermes gilvus*, especially after the final molt [4,45]. Consistently, hornets hunting honeybees to feed their brood harbored honeybee gut microbiota members in their guts [18]. Additionally, feeding often involves the unintentional consumption of xenobiotics, such as pesticides or herbicides, shown to induce dysbiosis in the gut of several insect species [50,51]. Differential exposure to such chemicals may trigger differences in the gut microbiota composition of indoor and outdoor workers. Finally, foraging increased the presence of opportunistic bacteria in the gut of honeybees and ants, therefore showing that the environment plays an important role in gut microbiota structure [35,36]. Environmental effects were also reported in the invasive wasp *Vespula vulgaris* whose gut microbial communities differed according to their native or introduced range, with some range-specific gut symbionts [19]. However, no subcaste-associated differences in gut microbiota composition were observed in the ant *Temnothorax nylanderii*, suggesting that differences in host physiology or behavior are not always linked to differences in gut microbiota composition [39].

Compared with differences between (sub)castes, relatively little is known about the link between behavioral variation among individuals of the same (sub)caste and gut microbiota composition. Variation in the frequency of social interactions and in the structure of the social network might affect the propensity of mutualistic or commensal bacteria to colonize the gut after adult emergence [11,36]. In honeybees, the presence or absence of trophallaxis, that is, the oral exchange of fluids, during early adulthood modulated bacterial community structure in the gut [38,52]. The effects of other behavioral traits on the gut microbiota composition have not been reported but might indirectly be mediated by a change in the environment, diet, or physiology of the individuals [10]. Overall, the impact of behavioral specialization and variation on gut microbiota composition remains to be unraveled for most social insect species to better understand the mechanisms by which insect behavioral ecology drives variation in gut bacterial communities.

Gut microbiota composition as a cause of host behavioral phenotypes

Given the known effects of gut microbiota on animal behavior, in particular via the gut-brain axis [53,54], it is apparent to ask whether interindividual differences in gut microbiota composition contribute to behavioral variation in social insects. Specialized microbe–host associations may be under stronger selection for effects on behavior to evolve. However, testing the role of microbes in modulating behavioral phenotypes requires controlled experimental approaches that precisely manipulate the composition and/or load of the gut microbiota and has therefore proved challenging. For most social insects, the gut microbiota is not yet culturable, and insect hosts have not been rendered microbiota free if not using experimentally confounding antibiotic treatments. Thus, only a handful of studies have so far attempted to test the causal role of the gut microbiota on behavioral specialization and variation.

No evidence for a role of gut microbes in developmentally determined behavioral specialization

Whether gut microbes play a causal role in developmentally determined behavioral specialization (e.g. queens, drones, workers or in polymorphic worker subcastes) has yet to be demonstrated. We can speculate that microbial strains associated with specific castes may be under selection to bias developmental trajectories to favor those castes. Because adult behavioral specialization is determined early in ontogenesis, such effects should only occur at the early stages in life (i.e. larval development) and when at least part of the microbiota is vertically transmitted. These effects would be more likely to evolve if they would not reduce the overall fitness of the insect society (e.g. by skewing sex ratios or the proportion of individuals in each caste to ratios that

are suboptimal at the colony level), as this may compromise microbial survival. However, the intrinsic dispersal abilities of microbes may also play a role. Selfish microbial strains might bias host development toward foraging castes, giving them opportunities to invade other colonies, especially if they can persist in the environment. In several insect species, manipulative bacteria that can reside in the gut (but are not exclusively gut associated), such as *Wolbachia*, *Spiroplasma*, *Rickettsia*, *Cardinium*, and *Arsenophonus*, were shown to skew host sex ratios in favor of females [55]. However, there are only a few studies investigating similar processes in social insects [56]. One study found no effect of *Wolbachia* on the sex ratio of the ant *Formica exsecta* [57], while a second study found that *Wolbachia* infection was associated with a 13% average increase in females in *Monomorium pharaonis* ants [58].

Evidence for gut microbes influencing worker behavioral specialization

Within the worker caste, there may be opportunities for microbes to bias individual task specialization and associated physiological states [12,43]. In this case, vertical transmission and microbiota maintenance throughout development would not be required, and such effects could be induced by bacteria that are acquired horizontally at the adult stage. Bacterial strains that are segregated between individuals specialized in different tasks could additionally play roles in maintaining behavioral specialization, for example, by supporting alternative nutritional requirements for the host to express specialized behaviors [12]. However, there is only limited evidence that the gut microbiota can alter or support task allocation.

Recent studies investigated the link between the gut microbiota and the development of cuticular hydrocarbons (CHCs), which allow social insects to discriminate nestmates from non-nestmates and to signal the functional specialization of behavioral subcastes [59,60]. It was proposed that gut microbes may alter the composition of CHCs with repercussions on social insect communication and division of labor [61–63]. Feeding *Acromyrmex* leaf-cutter ants with antibiotics concomitantly led to reducing bacterial loads and the abundance of a few CHCs and increasing aggression, compared to ants that were either fed a sucrose solution or their typical fungus garden diet [62]. However, the authors noted that the shifts in CHC profiles probably resulted from a direct effect of the antibiotic on the host metabolism rather than an indirect effect through the gut microbiota. Another study found similar effects on the honeybee CHC profile when continuously feeding gut bacterial suspensions [63]. However, a more recent study comparing microbiota-free and microbiota-colonized bees could not replicate these findings and suggested that the association between the gut microbiota and CHC profiles may have been spurious because of uncontrolled co-housing effects in laboratory

experiments [64]. Additionally, the latter study did not see any effect of gut microbes on a suite of physiological traits that vary between nurses and foragers (e.g. body and gut weight, hypopharyngeal gland size and gene expression). While gut microbiota inoculation accelerated the timing at which honeybees performed explorations into a foraging arena in the laboratory, no effect was observed on the total number of trips they performed. In contrast, another recent study, which assessed the effects of inoculations with specific core members, found no effect on the onset of foraging trips but significant and opposing effects (depending on the inoculated microbe) on host foraging rates, suggesting that the microbiota has the potential to modulate the behavioral specialization of worker subcastes [65]. Because of the differences in microbiome inoculations and experimental setups, these two studies [48,49] are not necessarily in disagreement. However, more research is needed to validate these findings to assess whether these microbial effects are simply a product of generally improved cognition [66,67] or underlie adaptations to diverging host specialization and to quantify the relative contribution of gut microbes compared with other cues regulating host division of labor.

Gut microbes can affect general behavioral phenotypes

Although research exploring the impact of the gut microbiota on developmentally determined behavioral specialization is lacking, and studies on plastic behavioral specialization are limited to a few, there is increasing evidence suggesting that gut microbes can influence cognitive and behavioral traits that are expressed by all colony members. Recent studies in bumblebees and honeybees showed that gut microbes can generally improve learning and memory [66–68], an effect that was either induced by specific bacterial lineages [66,68] or resulted from the combined effects of core gut microbiota members [67]. Two studies also reported differences in sucrose responsiveness between microbiota-free and microbiota-colonized honeybees [69,70], although these results could not be replicated by two independent studies [67,71]. Variation in host genetics, developmental conditions, or inoculated microbial strains may be the reason for these discrepant results. The honeybee gut microbiota was also shown to promote host social behavior and to contribute to increasing the specialization of social interactions between nestmates [37]. Bees inoculated with the native gut microbiota indeed formed stronger and more specific social bonds than microbiota-free bees. However, the mechanisms through which the microbiota and the social insect brain interact to produce these behavioral phenotypes are yet to be fully elucidated. Research on the gut microbiota — brain axis in honeybees and bumblebees has identified candidate metabolites produced by specific bacterial strains, which may underlie the reported effects on learning, memory, and social interactions [37,66,68,72]. Bacterial production of LPA glycerophospholipid in bumblebees and indole derivatives in honeybees was shown to

promote learning and memory performances [66,68]. Elevated levels of amino acids were also found in the brain of gut microbiota-colonized honeybees compared with microbiota-free bees, some of which positively correlated with the number of social interactions of individual bees [37]. How precisely these metabolites act on the brain functions supporting cognitive performance and social behavior remains unclear, but differential gene expression and proteomic analyses revealed effects of the gut microbiota on brain chromatin regulation, alternative splicing, synaptic neurotransmission, and amino acid biosynthesis [72]. In mammals, four main routes of communication between the gut microbiota and the brain have been identified: (1) via the systemic circulation and direct action of metabolites on the brain or indirect action of metabolites on (2) the enteric nervous system, (3) the immune system, and (4) the enteroendocrine cells or enterocytes in the gut [73]. These routes have not yet been identified in social insects, but the latter was reported in *Drosophila melanogaster* [74]. In honey bees, the binding of bacterially produced indole derivatives to a receptor located on enterocytes was shown to be required for memory improvements [66], but the link between this receptor and memory formation remains unknown. Research in that direction will help identify conserved mechanisms of gut microbiota–brain communication and characterize the selection pressures that shaped bacteria metabolic functions.

Conclusions

Research on the interplay between the gut microbiota and the behavior of social insects is in its early stages. Mounting evidence suggests that individuals that perform different behaviors are often colonized by distinct microbes, or host the same microbes at different abundances, as a consequence of host physiology, dietary preferences, and social interactions. Pioneering experimental research suggests that the gut microbiota supports host cognitive abilities and sociality but seems unlikely to influence ontogenetic programs that underlie caste-specific behaviors. However, a few honeybee studies suggest that the microbiota may be among the factors inducing or supporting worker division of labor.

Future research should prioritize the development of additional social insect models where the composition of the microbiota and the host genetics can be experimentally determined. This will enable broadening our understanding of the causal effects of gut microbes on social insect behavior, as opposed to the correlative approaches and natural surveys that have provided a large body of knowledge on the host factors determining microbiome composition. Social insects include many closely related lineages where (often complex) behavioral traits have evolved independently multiple times [75,76]. This provides unique opportunities to explore how host behavioral traits influence gut microbiota assembly, diversity, and stability and

how gut micro-organisms have contributed to the evolution of behavioral specialization and variation. With recent advances in behavioral quantification, omics approaches and gnotobiotic techniques, the time seems ripe to establish new mechanistic models across multiple social insect lineages. This work will allow for comparative studies to determine the generality/specificity of proximate mechanisms and to explore the evolutionary history of interactions between the gut microbiota and animal behavior.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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