Microbial associates and social behavior in ants

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Abstract: Current research in life sciences provides advances on how animal-associated microbes affect behavior and its underlying neurophysiology. However, studies in this field are often limited to individuals outside of their social context, and neglect social dynamics. Contrarily, animals and humans develop and live in complex societies where they constantly adjust physiology and behavior to social interactions. To improve our understanding of how microbes and hosts interact and produce phenotypes at social and group levels, we need to broaden our experimental approaches to a group-level dimension. Here we point out that eusocial insects, and ants in particular, are ideal models for this purpose. We first examine the most common types of microorganismal associations that ants engage in, and then briefly summarize what is known about the role of symbiotic microbes in ant social behavior. Finally, we propose future directions in the field in the light of recent technical advances in behavior measuring techniques.

Keywords: Social Evolution; Commensal Microbes; Primary Endosymbionts; Ants; Group-Level Behavior

1. INTRODUCTION

In the last two decades, the microbes associated with animals, including humans, have been at the center of a multidisciplinary scientific revolution. In life sciences, correlations and causal links have been established between microbiota and a plethora of aspects relevant to animal biology and human health. A key concept emerging from this conspicuous body of scientific work is the existence of an intimate relationship between gut microbes, gut and brain, which is usually defined as the “microbiota-gut-brain axis” [1–3]. According to this model, the communities of bacteria living in the animal gut communicate with the gut itself and the brain through multiple routes. In humans, these include the vagus nerve and the hypothalamic-pituitary axis, as well as host- and bacteria-produced neurotransmitters, cytokines and other bacterial metabolites [4]. This network of exchanges affects individual neurophysiology and the resulting behavioral output (Fig. 1a). Although gut microbes attract most of the interest in the scientific community, relevant connections between brains and animal-associated microbes can be broadened to include bacteria dwelling in all locations of the host body [5–8].

Effects of microbiota on behavior are frequently described in the current literature, but little is known about the relationships between the microbiota-gut-brain axis and the social life of animals. This is surprising because dialogues between animal-associated bacteria and nervous systems, similar to any other organismal trait, are expected to coevolve with the social systems of their animal hosts. The microbiota is therefore likely to influence brains and behaviors across levels of biological organization and in multiple contexts. For example, in animals with parental cares, parents sometimes drive developmental trajectories of brood (e.g. eusocial insect larvae developing in workers vs reproductive individuals), playing a role in determining their adult phenotypes [9–11] (Fig. 1b). If associated microbes consistently affect behaviors of parents and developing offspring, these are also likely to affect their interactions, with consequences on individual and social phenotypes.

Microbial effects on behavior are not limited to developmental dynamics. Contrarily, when the development of a group-living animal is accomplished, its life still largely depends on iterative adjustments of behavior and underlying physiology to interactions with its social partners, where all involved parties are affected by their associated microbiota (Fig. 1b). Finally, group-level properties emerge from microbiota-affected individual

traits and social interactions, and the resulting group performance contributes to the pool of genes passing to the next generations (Fig. 1c). Is it possible to determine to what extent symbiotic microbes affect lives of group-living animals across levels of biological organization?

2. MODEL SYSTEMS

Conducting experimental work on animals seems a reasonable way to explore the roles of microbiota in social dynamics. However, the models to be used for this purpose need to have three essential features: first, they must live in relatively stable groups; second, they must consistently establish symbioses with microorganisms; third, they must be suitable for experimental manipulations in the laboratory or in natural conditions. The classic laboratory-adapted animals of contemporary behavioral neuroscience [12] not always fulfill these conditions. For example, *Drosophila* fruit flies are easy to rear and very well characterized, and gut microbes affect their physiological and behavioral traits. These include specific appetites for certain foods [13], reproductive choices and mating [14–17], “social recognition” of related individuals [18], and even locomotion [19]. However, fruit flies have limited interactions with conspecifics, only forming relatively ephemeral gatherings in the proximity of food sources where individuals interact to compete for territories or mate [20]. Contrarily, rodents such as mice and rats, commonly used in life sciences to model human systems, live in familial groups relatively similar to those of humans, and are employed in a variety of behavior-relevant microbiome studies [21, 22]. However, laboratory-adapted rodents often host microbial communities that differ from those of natural populations [23] and have relatively complex brains, with relatively high cognitive abilities and important effects of experience on behavior. Such traits complicate experimental approaches.

The ideal models to investigate group-level behavior effects of associated microbes are group-living animals with relatively simple brains and behaviors. From this perspective, eusocial insect societies can be considered as systems of elementary cooperative modules acting altruistically to maximize the reproductive output of their groups. In their colonies, cohesion and stable division of reproductive labor are maintained because of genetic homogeneity and group-level benefits [24], while simple and stereotypical behavioral patterns of individuals generate complex group-level phenotypes. Such emerging dynamics characterize key features of insect eusocial life, including for example the ability of finding and exploiting food sources or to build nests with sophisticated architectures.

From a more practical point of view, eusocial insect individuals perform working tasks that are not always directly correlated with their appetitive needs (i.e., a non-starving worker would altruistically engage in foraging if its colony needs food [25]), and communicate extensively with each other across a variety of contexts. These traits make them suitable for laboratory-based behavioral experiments in which individuals and groups are challenged with operational tasks. In addition, eusocial insects host relatively simple communities of symbiotic bacteria compared to other animals and including humans [26, 27], which is ideal when roles of specific microbial strains need to be investigated.

Honeybees are by far the best characterized species among eusocial insects due to their economic importance as pollinators and producers of honey. Therefore, their microbiome has already attracted a significant interest from the scientific community. However, ants offer different experimental advantages. For example, compared to honeybees, they are relatively easy to work with in laboratory conditions, as they do not fly and can be hosted in limited space. Contrarily, honeybees form very large colonies and usually need to forage in natural settings, which in temperate climates limits experimental work to around half of the year. Finally, ants are a large and diverse group of insects comprising >12000 described species. Research questions about the role of microbes in ant behavior can thus be tailored based on a variety of species-specific features.

3. ANTS: MICROBIAL ASSOCIATIONS AND SOCIAL BEHAVIOR

3.1 Types of symbiosis

Insect associations with microbes are distributed along a broad spectrum, with bacterial parasitism and obligate mutualism at the two extremes. Along the non-parasitic side, bacteria are classified based on the degree of intimacy of their association with insects. For example, “commensals” live on the insect cuticle, in exocrine glands or in the gut lumen [28], and their activity sometimes provides advantages to their hosts [29, 30]. Insect symbionts are different from commensals in that their presence is usually necessary to the survival of the host, on which they may even depend completely. Some of these microbes, called primary endosymbionts, live in specialized host cells (bacteriocytes), from where they provide nutritional benefits [31]. Primary endosymbionts are strictly vertically transmitted, i.e., from mother to offspring, and have simplified small genomes resulting from a long process of erosion throughout evolutionary time [32]. This extreme

101 genomic simplification prevents them from living anywhere else than the bacteriocytes of their hosts, and for
102 this reason they are considered as intermediate stages between free-living bacteria and endosymbiotic cellular
103 organelles [33]. To some extent similar to primary endosymbionts, secondary endosymbionts can be found in
104 the insect hemolymph or organs, and are sometimes vertically transmitted from mother to offspring. However,
105 these are less intimately linked to their insect hosts and their transition from commensals to symbionts is
106 generally more recent than that of primary endosymbionts [34].

107

108

3.2 Ant commensals and behavior

Across eusocial insects, individuals belonging to different castes within the same society generally harbor distinct microbiomes (reviewed in [35]). In ants, differences in the communities of associated microbes are found between workers and queens [36, 37] and among groups of workers [38] (but see [39]). Based on such differences, it has been hypothesized that the composition of gut microbe communities within insect societies varies based on functional roles of colony members, ultimately resulting in increased fitness benefits [35]. Effects of insect-associated microbes are usually investigated via comparing control and microbiota remodeled individuals, with the latter most often treated with antibiotics. Commensal microbes of eusocial insects have been shown to serve nutritional upgrading roles for their hosts [30, 40] and are thought to be also involved in socially relevant dynamics.

In humans, commensals largely contribute to the biosynthesis of neuroactive molecules [41–46]. Although in principle this may also occur in ants, at present the only evidence of microorganismal effects on neurotransmitter biosynthesis comes from parasitic fungi such as *Ophiocordyceps unilateralis*, which manipulates ant behavior to reach the fruiting stage and efficiently propagate [47]. Similarly, bacteria-produced semiochemicals (chemicals conveying signals to other organisms, affecting their behavior) are widely distributed across insects (reviewed in [48]), but mechanisms underlying the putative roles of ant-associated microbes in the production of such chemicals are largely unknown. There is some evidence for a direct role of cuticle-dwelling microbes on cuticular hydrocarbons, with a single study on *Pogonomyrmex* harvester ants (Fig. 2a) in which topical administration of antibiotics was found to alter cuticular hydrocarbon (CHC) profiles of workers [49]. Such findings are backed by work on fruit flies suggesting that CHC may be used by cuticular microbes as sources of carbon [16]. Another related study on ant semiochemicals that is unique in its scope shows that pyrazines serving as trail pheromones in leaf-cutting *Atta sexdens rubropilosa* (Fig. 2b) are produced by the ant-associated microbe *Serratia marcescens* [50].

Recent work on *Acromyrmex* leaf-cutting ants (Fig. 2c) proposes that indirect effects of microbe metabolism on the production of semiochemicals may also be possible [51]. For example, insect cuticular hydrocarbons are produced in the oenocytes, which are also the center of the insect intermediate metabolism [52]. The availability of compounds involved in the biosynthesis of cuticular hydrocarbons may therefore be affected by

136 gut microbes, which would in principle result in effects on the composition of ant CHC blends [51].

137

138 **3.3 Ant primary endosymbionts and their putative roles on social behavior**

139 Only few ant taxa are known to harbor primary endosymbionts. Among those, *Cardiocondyla obscurior*
140 (Fig. 2d) (subfamily Myrmicinae), which hosts *Westeberhardia cardiocondylae* [53, 54], and genera within the
141 tribe Camponotini (subfamily Formicinae, Fig. 2e), which live in symbiosis with *Blochmannia* [55]. Closely
142 related to one another, *Westeberhardia* and *Blochmannia* are both γ -proteobacteria belonging to the family
143 Enterobacteriaceae, upgrading the nutrition of their ant hosts by contributing to the biosynthesis of amino acids.
144 In particular, the 530kb genome of *Westeberhardia* encodes for enzymes of the shikimate pathway. These
145 ultimately lead to 4-hydroxyphenylpyruvate, which is likely converted into tyrosine by the ant host metabolic
146 machinery [54]. The 700 kb genome of *Blochmannia* [28] includes genes involved in nitrogen recycling
147 (urease and glutamine synthetase) and the biosynthesis of aromatic and branched amino acids (tyrosine and
148 tryptophan and leucine, isoleucine and valine) [56, 57]. For *Westeberhardia* and *Blochmannia*, the contribution
149 to the biosynthesis of tyrosine, involved in the establishment of cuticle in ants [54, 58] and in insect in general,
150 is thought to be a key feature of the symbiosis. The role of endosymbionts in cuticle formation is also supported
151 by the fact that both *Westeberhardia* and *Blochmannia* peak across the end of metamorphosis and the beginning
152 of adult life, when the ant cuticle is established. Accordingly, long-term antibiotic treatment on developing
153 *Camponotus* workers results in individuals with a thinner cuticle [58].

154 The metabolic activity of primary endosymbionts provides chemicals required for the formation of a
155 functional cuticle. This protects the ant from dehydration but is also coated with a blend of long-chain
156 hydrocarbons mediating social interactions such as nestmate recognition [59], the process by which individuals
157 discriminate colony mates from strangers. If cuticle thickness and structure affect cuticular hydrocarbon blends
158 qualitatively and/or quantitatively, the activity of *Westeberhardia* and *Blochmannia* might be indirectly
159 involved in social interactions within ant colonies. In addition, the metabolic activity of these endosymbionts
160 might further affect social interactions because compounds deriving from aromatic amino acids (i.e., tyrosine
161 and tryptophan) are precursors of neurotransmitters such as dopamine and serotonin, which affect animal
162 neurophysiology and behavior. In *Blochmannia* and *Westeberhardia* this would be especially relevant because

163 they achieve their peaks during the late pupation and early adulthood of their ant hosts, when ants establish
164 their colony-specific blend of cuticular hydrocarbons, learn to discriminate nestmates from non-nestmates
165 based on such blends [34] and begin developing their behavioral profile through early life experiences [60].
166 Nevertheless, it must be stressed that at present there is hardly any evidence for primary endosymbionts to
167 affect ant social behavior, and all the stated hypotheses have not yet been experimentally investigated.

168 The scarcity of studies explicitly linking ant primary endosymbionts and behavior is partly due to technical
169 limitations. Experimental manipulations of microbial communities (microbiota remodeling) can be conducted
170 via rearing individuals in sterile conditions, or administering specific microbial strains via the food. However,
171 experiments involving ants often rely on the use of antibiotics. While these provide invaluable help in selecting
172 and controlling culturable symbiotic microbes of insects in in vitro assays [61], they tend to result in undesired
173 effects when used to suppress microbes within live insects. For example, antibiotics usually do not target a
174 single bacterial strain, potentially resulting in the suppression of the targeted endosymbionts but also of
175 commensal species, making impossible to disentangle the role of specific bacteria. In addition, the
176 experimental suppression of primary endosymbionts should target the phases in which these are most active,
177 such as metamorphosis and early adult life. However, ants do not feed in these phases, which precludes
178 antibiotics administration via the food. Finally, some antibiotics are known to interfere with animal host
179 physiology, for example by affecting mitochondrial function [62, 63]. In order to limit such side effects that
180 potentially impair physiology and behavior, a reasonable alternative approach would be silencing genes of
181 primary endosymbionts via RNAi.

182

183 **3.4 Incidental effects of microbial partners on social dynamics**

184 Microbial partners do not necessarily need to affect the function of nervous systems in order to influence
185 behavioral dynamics of animals, including ants. For example, microbe-suppressed *Acromyrmex* leaf-cutting
186 ants were recently found to have low levels of some microbe-produced antibiotic chemicals [64], and were
187 aggressed in social recognition assays with their nestmates [51]. In that case, microbe suppression disrupted
188 colony-level cohesion and triggered an effect on social behavior. Such an increase of aggression towards
189 individual lacking chemical defenses against bacteria probably occurs because immunocompromised

190 individuals would increase the risk of disease occurrence in the colony, and are kept at bay via the aggressive
191 behavior of their nestmates.

192 In principle, incidental effects of microbes on social life can be extended to any ant trait that is potentially
193 affected by microbial partners. For example, if ant-associated gut commensals affect host appetites for specific
194 foods as it is found in fruit flies [13, 29, 65], the food consumption of an ant will depend on its microbiota. As
195 colony-level nutrient homeostasis depends on individual feeding [66], the colony-level investment in foraging
196 activity is also likely to depend on the microbiota. Similarly, maternally inherited primary endosymbionts
197 support larval growth and upgrade nutrition during metamorphosis [54, 67]. As the metabolic activity of
198 primary endosymbionts contributes to the ant adult phenotypes, this might in principle contribute to individuals'
199 behavior in the long term, ultimately affecting social interactions.

200

201 **4. EXPERIMENTAL APPROACHES TO MICROBIAL EFFECTS ON SOCIAL BEHAVIOR**

202 Since the 1980's, researchers in biology have been implementing techniques to automatically measure the
203 activity of animals in experimental settings [68]. Such methods rely on computer algorithms extracting
204 quantitative measures of behavior via pattern analysis applied to recorded images. The rise of these systems
205 has improved experimental reliability because computer algorithms are not subject to variations typical of
206 human performance [68]. Initially, automatic behavior observation was found to be particularly suited for
207 quantifying animal locomotion [69, 70], behaviors occurring unpredictably [71] or over long periods [72, 73].
208 With progress in camera resolution and integration of code identification, tracking technology became suitable
209 to study the behavior of animal groups. Automatic tracking is usually conducted on groups of insects moving
210 on 2D arenas [74]. In recent systems, individuals are tagged with unique markers (matrix codes such as QR or
211 ArUco [75–78], Fig 3a; or combinations of color dots painted on ants [79–82], Fig 3b), and images are taken
212 by video cameras placed on top of the arenas. Systems based on automatic identification of individual codes
213 allow determining the positions of all insects within a given colony at pre-set time intervals, which are used to
214 determine basic attributes of individual behavior such as speed, distance traveled or activity levels. Interaction
215 networks within insect colonies can reconstructed by measuring inter-individual distances over time.

216 In the last few years, the integration of behavioral pattern recognition based on machine learning and

217 computer vision has allowed to automatically classify not only individual identities, but also different
218 behaviors and interactions among individuals [83, 84]. These next generation systems do not rely anymore on
219 the positions of individual tags, but perform analyses of videos based on the recognition of insect shapes and
220 movements. These innovations are ideal to investigate the behavior of ants at individual and group levels,
221 which is in turn the best approach to evaluate effects of symbiotic microbes on behavior. From an individual
222 perspective, it is in principle possible to monitor microbiota-remodeled individuals over long periods of time,
223 which allows detecting even slight modifications on their movement patterns. Concerning social behaviors, it
224 is possible to quantify different types of interactions (e.g., antennal contacts or trophallaxis [85]) and for
225 example correlate those with the spread of horizontally transmitted commensal microbes or pathogens. Finally,
226 the effects of symbiotic microbes on group-level phenotypes can be explored by tracking groups of ants
227 engaging in cooperative behaviors. This can be done, for example, by measuring the performance of colonies
228 including known proportions of microbiota-remodeled individuals, or including/excluding specific bacterial
229 strains from the microbial communities of all individuals within colonies. Testable group-level behaviors
230 include structure construction, cooperative transport of large prey items, nest choice etc.

231 To conclude, it is important to emphasize that most studies still rely on the offline processing of the data.
232 While this allows to compensate for errors and to employ more advanced and expensive computational
233 techniques, or even apply future knowledge to previous experiments by reprocessing previously collected data,
234 it does not allow for rapid insights into the experiment itself. Recent progresses in technology, such as the use
235 of Graphics Processing Units to accelerate computation, allow to complement the traditional way of conducting
236 these studies with real-time data. In this way, while individuals are tracked, data is recorded for further
237 processing and at the same time analyzed, and preliminary results are made available to scientists. By
238 combining real-time processing with traditional experimental methods, it will be possible to identify individual
239 behavioral attributes and group emerging properties before the end of an experiment, therefore increasing the
240 rate of discovery.

241

242 **5. CONCLUSION**

243 Microorganisms associated with group-living animals potentially impact social interactions of their hosts,

but knowledge in this field is still limited. On the other hand, determining whether and how microbial partners affects social dynamics is key to contextualize the evolution of the interactions between microbiota and brains. Future research effort in the field should be conducted in multiple directions. First, by addressing the direct roles of microbes in semiochemical production in eusocial Hymenoptera; second, by disentangling the indirect effects of bacterial partners, such as gut commensals, on the production of semiochemicals. This should be especially focused on the relationship between chemicals produced by microbes and the metabolism in the insect oenocytes; third, by exploring effects of microbes that do not directly impact socially relevant traits but incidentally affect social dynamics. Next generation video tracking systems based on artificial intelligence and real-time data analysis will be instrumental to delineate how such microbial effects on individuals are mirrored at social and group levels.

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REFERENCES

1. Bienenstock J, Kunze W, Forsythe P (2015) Microbiota and the gut-brain axis. *Nutr Rev* 73 Suppl 1:28–31. <https://doi.org/10.1093/nutrit/nuv019>
2. Carabotti M, Scirocco A, Maselli MA, Severi C (2015) The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol* 28:203–209
3. Tognini P (2017) Gut Microbiota: A Potential Regulator of Neurodevelopment. *Front Cell Neurosci* 11: <https://doi.org/10.3389/fncel.2017.00025>
4. Mayer EA, Knight R, Mazmanian SK, et al (2014) Gut Microbes and the Brain: Paradigm Shift in Neuroscience. *J Neurosci* 34:15490–15496. <https://doi.org/10.1523/JNEUROSCI.3299-14.2014>
5. Mändar R (2013) Microbiota of male genital tract: Impact on the health of man and his partner. *Pharmacological Research* 69:32–41. <https://doi.org/10.1016/j.phrs.2012.10.019>
6. Wijkert JHHM van de, Borgdorff H, Verhelst R, et al (2014) The Vaginal Microbiota: What Have We Learned after a Decade of Molecular Characterization? *PLOS ONE* 9:e105998. <https://doi.org/10.1371/journal.pone.0105998>
7. Urbaniak C, Cummins J, Brackstone M, et al (2014) Microbiota of Human Breast Tissue. *Appl Environ Microbiol* 80:3007–3014. <https://doi.org/10.1128/AEM.00242-14>
8. Chen YE, Fischbach MA, Belkaid Y (2018) Skin microbiota–host interactions. *Nature* 553:427–436.

- 276 <https://doi.org/10.1038/nature25177>
- 277 9. Linksvayer TA (2006) Direct, Maternal, and Sibsocal Genetic Effects on Individual and Colony Traits
278 in an Ant. *Evolution* 60:2552–2561. <https://doi.org/10.1111/j.0014-3820.2006.tb01889.x>
- 279 10. Teseo S, Châline N, Jaisson P, Kronauer DJC (2014) Epistasis between adults and larvae underlies caste
280 fate and fitness in a clonal ant. *Nat Commun* 5:3363. <https://doi.org/10.1038/ncomms4363>
- 281 11. Wu M, Walser J-C, Sun L, Kölliker M (2020) The genetic mechanism of selfishness and altruism in
282 parent-offspring coadaptation. *Science Advances* 6:eaaw0070. <https://doi.org/10.1126/sciadv.aaw0070>
- 283 12. Mathuru AS, Libersat F, Vyas A, Teseo S (2020) Why behavioral neuroscience still needs diversity?: A
284 curious case of a persistent need. *Neuroscience & Biobehavioral Reviews* 116:130–141.
285 <https://doi.org/10.1016/j.neubiorev.2020.06.021>
- 286 13. Leitão-Gonçalves R, Carvalho-Santos Z, Francisco AP, et al (2017) Commensal bacteria and essential
287 amino acids control food choice behavior and reproduction. *PLOS Biology* 15:e2000862.
288 <https://doi.org/10.1371/journal.pbio.2000862>
- 289 14. Arbuthnott D, Levin TC, Promislow DEL (2016) The impacts of Wolbachia and the microbiome on mate
290 choice in *Drosophila melanogaster*. *J Evol Biol* 29:461–468. <https://doi.org/10.1111/jeb.12788>
- 291 15. Morimoto J, J. Simpson S, Ponton F (2017) Direct and trans-generational effects of male and female gut
292 microbiota in *Drosophila melanogaster*. *Biology Letters* 13:20160966.
293 <https://doi.org/10.1098/rsbl.2016.0966>
- 294 16. Ringo J, Sharon G, Segal D (2011) Bacteria-induced sexual isolation in *Drosophila*. *Fly (Austin)* 5:310–
295 315. <https://doi.org/10.4161/fly.5.4.15835>
- 296 17. Sharon G, Segal D, Ringo JM, et al (2010) Commensal bacteria play a role in mating preference of
297 *Drosophila melanogaster*. *Proc Natl Acad Sci U S A* 107:20051–20056.
298 <https://doi.org/10.1073/pnas.1009906107>
- 299 18. Lizé A, McKay R, Lewis Z (2014) Kin recognition in *Drosophila*: the importance of ecology and gut
300 microbiota. *ISME J* 8:469–477. <https://doi.org/10.1038/ismej.2013.157>
- 301 19. Schretter CE, Vielmetter J, Bartos I, et al (2018) A gut microbial factor modulates locomotor behaviour
302 in *Drosophila*. *Nature* 563:402–406. <https://doi.org/10.1038/s41586-018-0634-9>
- 303 20. Teseo S, Veerus L, Mery F (2016) Fighting experience affects fruit fly behavior in a mating context.
304 *Naturwissenschaften* 103:38. <https://doi.org/10.1007/s00114-016-1368-x>
- 305 21. Kennedy EA, King KY, Baldrige MT (2018) Mouse Microbiota Models: Comparing Germ-Free Mice
306 and Antibiotics Treatment as Tools for Modifying Gut Bacteria. *Front Physiol* 9:
307 <https://doi.org/10.3389/fphys.2018.01534>
- 308 22. Cheung SG, Goldenthal AR, Uhlemann A-C, et al (2019) Systematic Review of Gut Microbiota and
309 Major Depression. *Front Psychiatry* 10:. <https://doi.org/10.3389/fpsy.2019.00034>
- 310 23. Viney M (2018) The gut microbiota of wild rodents: Challenges and opportunities: Laboratory Animals.
311 <https://doi.org/10.1177/0023677218787538>
- 312 24. Teseo S, Kronauer DJC, Jaisson P, Châline N (2013) Enforcement of reproductive synchrony via policing
313 in a clonal ant. *Curr Biol* 23:328–332. <https://doi.org/10.1016/j.cub.2013.01.011>
- 314 25. Greenwald EE, Baltiansky L, Feinerman O (2018) Individual crop loads provide local control for

collective food intake in ant colonies. *eLife* 7:e31730. <https://doi.org/10.7554/eLife.31730>

26. Sapountzis P, Zhukova M, Hansen LH, et al (2015) Acromyrmex Leaf-Cutting Ants Have Simple Gut Microbiota with Nitrogen-Fixing Potential. *Appl Environ Microbiol* 81:5527–5537. <https://doi.org/10.1128/AEM.00961-15>

27. Chua K-O, Song S-L, Yong H-S, et al (2018) Microbial Community Composition Reveals Spatial Variation and Distinctive Core Microbiome of the Weaver Ant *Oecophylla smaragdina* in Malaysia. *Scientific Reports* 8:10777. <https://doi.org/10.1038/s41598-018-29159-2>

28. Douglas AE (2015) Multiorganismal Insects: Diversity and Function of Resident Microorganisms. *Annu Rev Entomol* 60:17–34. <https://doi.org/10.1146/annurev-ento-010814-020822>

29. Sannino DR, Dobson AJ, Edwards K, et al (2018) The *Drosophila melanogaster* Gut Microbiota Provisions Thiamine to Its Host. *mBio* 9:. <https://doi.org/10.1128/mBio.00155-18>

30. Sapountzis P, Zhukova M, Shik JZ, et al (2018) Reconstructing the functions of endosymbiotic Mollicutes in fungus-growing ants. *eLife* 7:e39209. <https://doi.org/10.7554/eLife.39209>

31. Baumann P, Moran NA, Baumann L (2006) Bacteriocyte-Associated Endosymbionts of Insects. In: Dworkin M, Falkow S, Rosenberg E, et al (eds) *The Prokaryotes: Volume 1: Symbiotic associations, Biotechnology, Applied Microbiology*. Springer New York, New York, NY, pp 403–438

32. Wernegreen JJ (2015) Endosymbiont evolution: Predictions from theory and surprises from genomes. *Ann N Y Acad Sci* 1360:16–35. <https://doi.org/10.1111/nyas.12740>

33. Reyes-Prieto M, Latorre A, Moya A (2014) Scanty microbes, the “symbionelle” concept. *Environ Microbiol* 16:335–338. <https://doi.org/10.1111/1462-2920.12220>

34. Wernegreen JJ (2012) Strategies of genomic integration within insect-bacterial mutualisms. *Biol Bull* 223:112–122

35. Sinotte VM, Renelies-Hamilton J, Taylor BA, et al (2020) Synergies Between Division of Labor and Gut Microbiomes of Social Insects. *Frontiers in Ecology and Evolution* 7:

36. Johansson H, Dhaygude K, Lindström S, et al (2013) A Metatranscriptomic Approach to the Identification of Microbiota Associated with the Ant *Formica exsecta*. *PLOS ONE* 8:e79777. <https://doi.org/10.1371/journal.pone.0079777>

37. Brown BP, Wernegreen JJ (2016) Deep divergence and rapid evolutionary rates in gut-associated Acetobacteraceae of ants. *BMC Microbiology* 16:140. <https://doi.org/10.1186/s12866-016-0721-8>

38. Koto A, Nobu MK, Miyazaki R (2020) Deep Sequencing Uncovers Caste-Associated Diversity of Symbionts in the Social Ant *Camponotus japonicus*. *mBio* 11:. <https://doi.org/10.1128/mBio.00408-20>

39. Ramalho MO, Duplais C, Orivel J, et al (2020) Development but not diet alters microbial communities in the Neotropical arboreal trap jaw ant *Daceton armigerum*: an exploratory study. *Sci Rep* 10:1–12. <https://doi.org/10.1038/s41598-020-64393-7>

40. Hu Y, Sanders JG, Łukasik P, et al (2018) Herbivorous turtle ants obtain essential nutrients from a conserved nitrogen-recycling gut microbiome. *Nat Commun* 9:1–14. <https://doi.org/10.1038/s41467-018-03357-y>

41. Clarke G, Stilling RM, Kennedy PJ, et al (2014) Minireview: Gut Microbiota: The Neglected Endocrine Organ. *Mol Endocrinol* 28:1221–1238. <https://doi.org/10.1210/me.2014-1108>

- 354 42. Yano JM, Yu K, Donaldson GP, et al (2015) Indigenous bacteria from the gut microbiota regulate host
355 serotonin biosynthesis. *Cell* 161:264–276. <https://doi.org/10.1016/j.cell.2015.02.047>
- 356 43. Mazzoli R, Pessione E (2016) The Neuro-endocrinological Role of Microbial Glutamate and GABA
357 Signaling. *Front Microbiol* 7:. <https://doi.org/10.3389/fmicb.2016.01934>
- 358 44. Ríos-Covián D, Ruas-Madiedo P, Margolles A, et al (2016) Intestinal Short Chain Fatty Acids and their
359 Link with Diet and Human Health. *Front Microbiol* 7:. <https://doi.org/10.3389/fmicb.2016.00185>
- 360 45. Byrne CS, Chambers ES, Alhabeeb H, et al (2016) Increased colonic propionate reduces anticipatory
361 reward responses in the human striatum to high-energy foods. *Am J Clin Nutr* 104:5–14.
362 <https://doi.org/10.3945/ajcn.115.126706>
- 363 46. Strandwitz P (2018) Neurotransmitter modulation by the gut microbiota. *Brain Res* 1693:128–133.
364 <https://doi.org/10.1016/j.brainres.2018.03.015>
- 365 47. de Bekker C, Ohm RA, Loreto RG, et al (2015) Gene expression during zombie ant biting behavior
366 reflects the complexity underlying fungal parasitic behavioral manipulation. *BMC Genomics* 16:620.
367 <https://doi.org/10.1186/s12864-015-1812-x>
- 368 48. Calcagnile M, Tredici SM, Talà A, Alifano P (2019) Bacterial Semiochemicals and Transkingdom
369 Interactions with Insects and Plants. *Insects* 10:. <https://doi.org/10.3390/insects10120441>
- 370 49. Dosmann A, Bahet N, Gordon DM (2016) Experimental modulation of external microbiome affects
371 nestmate recognition in harvester ants (*Pogonomyrmex barbatus*). *PeerJ* 4:e1566.
372 <https://doi.org/10.7717/peerj.1566>
- 373 50. Silva-Junior EA, Ruzzini AC, Paludo CR, et al (2018) Pyrazines from bacteria and ants: convergent
374 chemistry within an ecological niche. *Sci Rep* 8:1–7. <https://doi.org/10.1038/s41598-018-20953-6>
- 375 51. Teseo S, van Zweden JS, Pontieri L, et al (2019) The scent of symbiosis: gut bacteria may affect social
376 interactions in leaf-cutting ants. *Animal Behaviour* 150:239–254.
377 <https://doi.org/10.1016/j.anbehav.2018.12.017>
- 378 52. Arrese EL, Soulages JL (2010) Insect Fat Body: Energy, Metabolism, and Regulation. *Annual Review*
379 *of Entomology* 55:207–225. <https://doi.org/10.1146/annurev-ento-112408-085356>
- 380 53. Russell JA, Moreau CS, Goldman-Huertas B, et al (2009) Bacterial gut symbionts are tightly linked with
381 the evolution of herbivory in ants. *PNAS* 106:21236–21241. <https://doi.org/10.1073/pnas.0907926106>
- 382 54. Klein A, Schrader L, Gil R, et al (2016) A novel intracellular mutualistic bacterium in the invasive ant
383 *Cardiocondyla obscurior*. *ISME J* 10:376–388. <https://doi.org/10.1038/ismej.2015.119>
- 384 55. Williams LE, Wernegreen JJ (2015) Genome evolution in an ancient bacteria-ant symbiosis: parallel gene
385 loss among *Blochmannia* spanning the origin of the ant tribe Camponotini. *PeerJ* 3:e881.
386 <https://doi.org/10.7717/peerj.881>
- 387 56. Stoll S, Feldhaar H, Gross R (2009) Transcriptional profiling of the endosymbiont *Blochmannia*
388 *floridanus* during different developmental stages of its holometabolous ant host. *Environ Microbiol*
389 11:877–888. <https://doi.org/10.1111/j.1462-2920.2008.01808.x>
- 390 57. Fan Y, Thompson JW, Dubois LG, et al (2013) Proteomic analysis of an unculturable bacterial
391 endosymbiont (*Blochmannia*) reveals high abundance of chaperonins and biosynthetic enzymes. *J*
392 *Proteome Res* 12:704–718. <https://doi.org/10.1021/pr3007842>
- 393 58. Sinotte VM, Freedman SN, Ugelvig LV, Seid MA (2018) *Camponotus floridanus* Ants Incur a Trade-Off

394 [between Phenotypic Development and Pathogen Susceptibility from Their Mutualistic Endosymbiont](#)
395 [Blochmannia](#). *insects* 9:e58. <https://doi.org/10.3390/insects9020058>

396 59. (2010) *Insect Hydrocarbons: Biology, Biochemistry, and Chemical Ecology*. In: Cambridge Core.
397 [/core/books/insect-hydrocarbons/DCD6067FC5A92251705BAF499FB4B432](#). Accessed 18 May 2020

398 60. Bernadou Abel, Schrader Lukas, Pable Julia, et al (2018) Stress and early experience underlie dominance
399 status and division of labour in a clonal insect. *Proceedings of the Royal Society B: Biological Sciences*
400 285:20181468. <https://doi.org/10.1098/rspb.2018.1468>

401 61. Itoh H, Jang S, Takeshita K, et al (2019) Host–symbiont specificity determined by microbe–microbe
402 competition in an insect gut. *PNAS* 116:22673–22682. <https://doi.org/10.1073/pnas.1912397116>

403 62. Chatzispyrou IA, Held NM, Mouchiroud L, et al (2015) Tetracycline antibiotics impair mitochondrial
404 function and its experimental use confounds research. *Cancer Res* 75:4446–4449.
405 <https://doi.org/10.1158/0008-5472.CAN-15-1626>

406 63. Moullan N, Mouchiroud L, Wang X, et al (2015) Tetracyclines disturb mitochondrial function across
407 eukaryotic models: a call for caution in biomedical research. *Cell Rep.*
408 <https://doi.org/10.1016/j.celrep.2015.02.034>

409 64. Poulsen M, Bot ANM, Boomsma JJ (2003) The effect of metapleural gland secretion on the growth of a
410 mutualistic bacterium on the cuticle of leaf-cutting ants. *Naturwissenschaften* 90:406–409.
411 <https://doi.org/10.1007/s00114-003-0450-3>

412 65. Consuegra J, Grenier T, Baa-Puyoulet P, et al (2020) *Drosophila*-associated bacteria differentially shape
413 the nutritional requirements of their host during juvenile growth. *PLOS Biology* 18:e3000681.
414 <https://doi.org/10.1371/journal.pbio.3000681>

415 66. Csata E, Gautrais J, Bach A, et al (2020) Ant Foragers Compensate for the Nutritional Deficiencies in
416 the Colony. *Current Biology* 30:135-142.e4. <https://doi.org/10.1016/j.cub.2019.11.019>

417 67. Feldhaar H, Straka J, Krischke M, et al (2007) Nutritional upgrading for omnivorous carpenter ants by
418 the endosymbiont *Blochmannia*. *BMC Biol* 5:48. <https://doi.org/10.1186/1741-7007-5-48>

419 68. Noldus LPJJ, Spink AJ, Tegelenbosch RAJ (2001) EthoVision: A versatile video tracking system for
420 automation of behavioral experiments. *Behavior Research Methods, Instruments, & Computers* 33:398–
421 414. <https://doi.org/10.3758/BF03195394>

422 69. Buresová O, Bolhuis JJ, Bures J (1986) Differential effects of cholinergic blockade on performance of
423 rats in the water tank navigation task and in a radial water maze. *Behav Neurosci* 100:476–482.
424 <https://doi.org/10.1037//0735-7044.100.4.476>

425 70. Spruijt B, Pitsikas N, Algeri S, Gispen WH (1990) Org2766 improves performance of rats with unilateral
426 lesions in the fimbria fornix in a spatial learning task. *Brain Research* 527:192–197.
427 [https://doi.org/10.1016/0006-8993\(90\)91137-6](https://doi.org/10.1016/0006-8993(90)91137-6)

428 71. Martin BR, Prescott WR, Zhu M (1992) Quantitation of rodent catalepsy by a computer-imaging
429 technique. *Pharmacology Biochemistry and Behavior* 43:381–386. [https://doi.org/10.1016/0091-3057\(92\)90166-D](https://doi.org/10.1016/0091-3057(92)90166-D)

430

431 72. Spruijt BM, Gispen WH (1983) Prolonged animal observation by use of digitized video displays.
432 *Pharmacology, biochemistry and behavior* 19:765–769

433 73. Olivo RF, Thompson MC (1988) Monitoring animals' movements using digitized video images.
434 *Behavior Research Methods, Instruments, & Computers* 20:485–490.

<https://doi.org/10.3758/BF03202701>

74. Noldus L, Spink AJ, Tegelenbosch R a. J (2002) Computerised video tracking, movement analysis and behaviour recognition in insects. *Comput Electron Agric* 35:201–227. [https://doi.org/10.1016/S0168-1699\(02\)00019-4](https://doi.org/10.1016/S0168-1699(02)00019-4)
75. Mersch DP, Crespi A, Keller L (2013) Tracking Individuals Shows Spatial Fidelity Is a Key Regulator of Ant Social Organization. *Science* 340:1090–1093. <https://doi.org/10.1126/science.1234316>
76. Heyman Y, Shental N, Brandis A, et al (2017) Ants regulate colony spatial organization using multiple chemical road-signs. *Nature Communications* 8:ncomms15414. <https://doi.org/10.1038/ncomms15414>
77. Greenwald E, Eckmann J-P, Feinerman O (2019) Colony entropy—Allocation of goods in ant colonies. *PLOS Computational Biology* 15:e1006925. <https://doi.org/10.1371/journal.pcbi.1006925>
78. Quque M, Bles O, Bénard A, et al (2020) Hierarchical networks of food exchange in the black garden ant *Lasius niger*. *Insect Sci*. <https://doi.org/10.1111/1744-7917.12792>
79. Ulrich Y, Burns D, Libbrecht R, Kronauer DJC (2015) Ant larvae regulate worker foraging behavior and ovarian activity in a dose-dependent manner. *Behav Ecol Sociobiol* 1–8. <https://doi.org/10.1007/s00265-015-2046-2>
80. Ulrich Y, Saragosti J, Tokita CK, et al (2018) Fitness benefits and emergent division of labour at the onset of group living. *Nature* 560:635–+. <https://doi.org/10.1038/s41586-018-0422-6>
81. Ulrich Y, Kawakatsu M, Tokita CK, et al (2020) Emergent behavioral organization in heterogeneous groups of a social insect. *bioRxiv* 2020.03.05.963207. <https://doi.org/10.1101/2020.03.05.963207>
82. Gal A, Saragosti J, Kronauer DJC (2020) anTraX: high throughput video tracking of color-tagged insects. *bioRxiv* 2020.04.29.068478. <https://doi.org/10.1101/2020.04.29.068478>
83. Kabra M, Robie AA, Rivera-Alba M, et al (2013) JAABA: interactive machine learning for automatic annotation of animal behavior. *Nat Methods* 10:64–67. <https://doi.org/10.1038/nmeth.2281>
84. Mathis A, Mamidanna P, Cury KM, et al (2018) DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat Neurosci* 21:1281–1289. <https://doi.org/10.1038/s41593-018-0209-y>
85. Gernat T, Rao VD, Middendorf M, et al (2018) Automated monitoring of behavior reveals bursty interaction patterns and rapid spreading dynamics in honeybee social networks. *PNAS* 115:1433–1438. <https://doi.org/10.1073/pnas.1713568115>

466
467 **Figure 1. Potential effects of ant-associated microbes on behaviour across levels of biological**
468 **organization.** Investigable effects appear in magenta boxes, whereas tests and techniques to investigate such
469 effects are shown in cyan boxes.

470

Figure 2. Ant species used in microbiota studies. a. *Pogonomyrmex barbatus*. Photo by April Nobile /

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©AntWeb 2002 - 2020. Licensing: Creative Commons Attribution License. **c.** *Acromyrmex echinator*. Photo

courtesy of Panagiotis Sapountzis. **d.** *Cardiocondyla obscurior*. Photo by Eli M. Sarnat / URL:

<https://www.antweb.org/bigPicture.do?name=casent0171038&shot=p&number=1> Image Copyright

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by April Nobile / URL: <https://www.antweb.org/bigPicture.do?name=casent0103673&shot=p&number=1>

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Figure 3. Examples of individual identifiers for ants. a. *Camponotus* workers marked with ArUco codes in a video tracking experimental set up. **b.** Color-marked worker of *Dinoponera gigantea*. Photo courtesy of Nicolas Châline.