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Ant microbial endosymbionts and the emergent properties of social groups

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Abstract: In the last fifteen years, research on animal models has provided advances on how gut symbiotic microbes affect behavior and its underlying neurophysiology. However, most studies on the gut microbiota only take into exam individual behavior without considering social dynamics. Contrarily, animals and humans 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 functional individual, social and collective phenotypes, we need to broaden our experimental approaches to a group-level dimension. The ideal models for this purpose are social animals living in stable symbioses with microbes, such as eusocial insects. In our research, we investigate *Camponotus* carpenter ants and their obligate bacterial symbiont *Blochmannia* from a behavioral ecology perspective. We aim to create ant colonies including differential proportions of bacteria-free individuals by suppressing *Blochmannia* with antibiotics. Then, using a machine learning-based video tracking system, we will study network features and group-level behavior of such experimental colonies.

Keywords: Social Evolution; Microbiota-Gut-Brain Axis; Ants; Group-Level Behavior; Machine Learning; Real Time Data Analysis

1. INTRODUCTION

In the last 15 years, the gut microbiota has been at the center of a scientific revolution. Correlations, but also causal links have been established between gut microbe activity and a plethora of aspects relevant to human biology and health. A key concept emerging from this large body of scientific work is the existence of an intimate relationship between gut microbes, the gut and the brain, the microbiota-gut-brain axis [1]–[3]. According to this model, the complex communities of bacteria living in the animal gut are in communication with the gut itself and the brain through multiple channels. These include the vagus nerve, the hypothalamic-pituitary axis, neurotransmitters produced by the animal body and the bacteria themselves, cytokines and other bacterial metabolites. These connections constitute a dynamic three-party system affecting animal neurophysiology and the resulting behavioral output.

Little is known about how the relationship between the microbiota-gut-brain axis of individual animals and their social environment. This is surprising because, from a behavioral ecology perspective, the world of many group-living animals, including humans, is made of multiple complex social relationships. Social animals need to iteratively adapt their behavior and underlying physiology to their social environment. Inversely, each individual contributes to the social environment of its partners, affecting their physiology and behavior through social interactions. As gut microbes play a role in determining individual behavior, they are also likely to affect interindividual relationships within social groups. Finally, through this influence on social interactions, microbes are predicted to ultimately affect the emergent properties of social groups (Figure 1). Therefore, can we establish mechanistic links between symbiotic microbes and behavior at social and group levels?

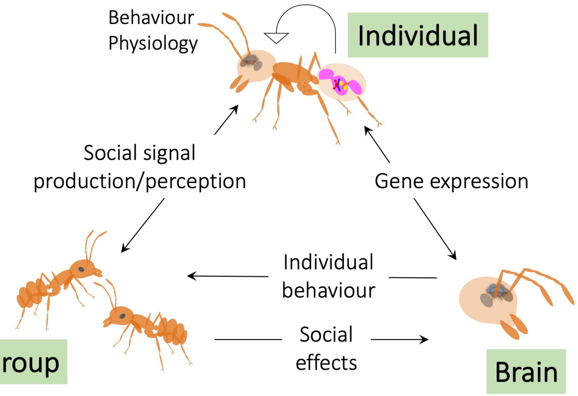

Figure 1. Predicted effects of gut microbes at different levels of biological organization.

One way to explore this question is conducting experimental research on animal models. However, these model animals need to have three essential features: first, they need to live in stable social groups; second, they must establish permanent symbioses with gut microorganisms; finally, they must be suitable for experimental work on social and group-level behavior in laboratory settings. The classic animal models of biology do not always fulfill these conditions. For example, *Drosophila* fruit flies are easy and inexpensive to rear, and very well characterized. *Drosophila* studies show that commensal gut microbes affect physiological and behavioral aspects, such as specific appetites for certain foods [4], reproductive choices and mating [5]–[8], as well as the recognition of related individuals [9]. However, fruit flies do not live in stable social groups and engage in little social interactions besides mating and fighting. Contrarily, mice and rats are relatively similar

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† Serafino Teseo is the presenter of this paper.

to humans from a social perspective, and have already been employed for a variety of behavior-relevant gut microbiome studies [10], [11]. However, they have complex gut microbe communities, complex brains and relatively high cognitive abilities, which complicates experimental approaches. Finally, to our knowledge there is limited evidence for group-level cooperative behaviors in mice and rats.

Eusocial insects like ants are instead good models to investigate how gut microbes are involved in social and group-level behavior. Their societies can be considered as aggregations of simple cooperative modules (individual workers) acting for the benefit of the colony. Interestingly, simple behavioral patterns of individual ants produce extremely complex group-level phenotypes, such as the nests of *Atta* leaf-cutter ants, or the living bridges of *Eciton* army ants and *Oecophylla* weaver ants. From a more practical perspective, and contrary for example to fruit flies, ants perform working tasks and communicate with each other, which makes them suitable for behavioral experiments in laboratory conditions. Contrary to mice, ants have relatively simple brains, as well as simple communities of symbiotic bacteria [12], [13].

2. BACKGROUND AND WORKING HYPOTHESIS

Ant-associated microbes appear to be involved in socially relevant physiological dynamics. In particular, several recent studies have investigated the relationship between microbes and the colony-specific cuticular hydrocarbons of ants [14]–[18]. In our research, we aim to explore this field further, with a special focus on microbial influences during ant behavioral ontogeny.

The model system of our research is the association of carpenter ants of the genus *Camponotus* (family Formicidae, subfamily Formicinae) and their endosymbiont *Blochmannia*. *Camponotus* ants are among the most successful ant genera in terms of species number and global distribution, and most carpenter ant species are easy to keep in laboratory conditions. *Blochmannia* (γ-Proteobacteria) is a primary obligate intracellular endosymbiont not only of *Camponotus* but of the whole ant tribe Camponotini [19], [20], which also includes the genera *Calomyrmex*, *Colobopsis*, *Dinomyrmex*, *Echinopla*, *Opisthopsis*, *Overbeckia* and *Polyrhachis*. It is related to free-living Enterobacteriaceae such as *Escherichia coli* and to several obligate insect intracellular endosymbionts such as *Baumannia*, *Sodalis* and *Wigglesworthia* [21]–[23], [20]. *Blochmannia*'s most closely related bacteria appear to be some facultative endosymbionts of mealybugs (order Hemiptera, family Pseudococcidae). This suggests that the ant-*Blochmannia* association may have emerged through the nutritional symbiosis of a common ancestor of Camponotini with sap-feeding insects [24]. *Blochmannia* is hosted in specialized cells (bacteriocytes) of the ant host internal midgut epithelium [25]–[27].

Interestingly, the 700 kb genome of *Blochmannia* [28] includes genes involved in nitrogen recycling and the synthesis of amino acids such as tyrosine, tryptophan, leucine, isoleucine and valine [29], [30]. These amino acids have potential behavior-relevant effects. For example, tyrosine and tryptophan are precursors of neurotransmitters such as dopamine and serotonin, which directly affect individual neurophysiology. In addition, dopamine also acts as a hormonal regulator in ants [31], which may as well affect individual and group-level behavior. Finally, branched amino acids (leucine, isoleucine and valine), appear to be involved in the biosynthesis of cuticular hydrocarbons [32], on which eusocial insect nestmate recognition is based.

The abundance of *Blochmannia* varies throughout the ant host life stages, and studies on *Camponotus floridanus* show that *Blochmannia* titers are low in eggs and larvae and increase significantly during metamorphosis, peaking in late pupation and early adult stages [29], [33]. Importantly, the life cycle phases in which *Blochmannia* peaks are also highly relevant for the ant behavioral ontogeny. For example, in these phases, ants develop their colony-specific blend of cuticular hydrocarbons and learn to discriminate nestmates from non-nestmates based on such blends [34].

Given that *Blochmannia* provides behavior-relevant molecules in a sensitive time window for the ant host behavior, our working hypothesis is that behavioral ontogeny in carpenter ants depends at least partially on the action of its endosymbiont. We predict that long-lasting effects on behavior (e.g., individual social impairment) could be produced via suppressing *Blochmannia* in such critical phases.

3. METHODS

Ant workers are treated with antibiotics (Rifampicin) either via feeding or via microinjections (Rifampicin dissolved in 1x phosphate buffer saline (PBS)) using a pressure injector connected to a pulled glass capillary (Figure 2). Treated and control workers are then mixed in varying proportions (0%, 33%, 66%, 100%) in 50-individual experimental colonies. Each ant is marked with a QR code glued on the thorax as an individual identifier during experiments. The video tracking device is constituted of ant-hosting units installed on an anti-vibration table, each with a single queenless experimental ant colony. Each unit comprises a foraging area and a nest, both isolated from external light and with 850 nm infrared light-sensitive webcams placed on top. Nests are exposed to infrared light to collect images in darkness conditions; foraging areas are exposed to visible and UV light or infrared light in a 12h:12h LD alternation. Webcams are connected to a computer where all processing takes place. Video streams are saved while at the same time processed in real-time by the system. To perform these operations, the system uses two high-performance Graphics Processing Units (GPUs).

4. PERSPECTIVES: INTEGRATING MACHINE LEARNING AND REAL TIME DATA ANALYSIS IN ANT VIDEO TRACKING

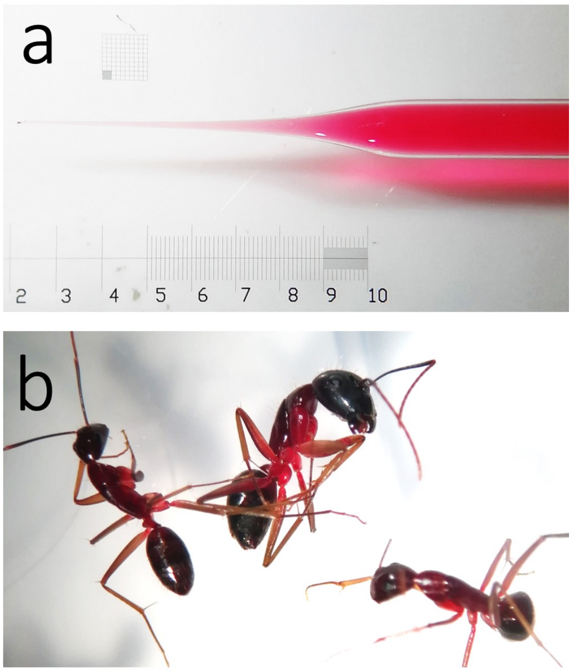

Figure 2. a. A pulled glass capillary loaded with red food dye used here to assess how injected liquid diffuses into the ant hemolymph. b. Ice-anesthetized injected ants.

The processing software will consist of two subsystems, one for tracking and the other for analysis. Instead of relying exclusively on standard QR code recognition, the tracking will be based on machine learning. The use of machine learning will provide resilience to partially invisible or damaged QR codes, and enrichment of the QR-based positional tracking with information on behavioral patterns. The analysis part will process the tracker output to produce real-time models of the behavior of single individuals, as well as interaction models of ant subgroups and of the colony as a whole.

We will first proceed by creating training and test sets for the machine learning-based tracker. The training will be constituted of videos of ants with QR codes, labeled with the ID corresponding to the QR code itself; and of videos of the same individuals during social or individual behaviors, qualitatively labeled according to the type. The training set will then be employed to teach the tracking system to recognize individual identity and behavior, and the test set will be used to measure the accuracy of the learned system.

5. CONCLUSIONS

Disentangling the crosstalk of gut microbes, guts, individual brains and societies is essential for understanding animal and human behavior, and may ultimately contribute to create opportunities to treat disorders such as depression and autism. Our research aims to move steps in these directions by determining how individual gut microbes influence and are influenced by social dynamics.

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