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# Symbioses among ants and microbes

Corrie S Moreau

Ants have been shown to engage in symbiosis across the tree of life, although our knowledge is far from complete. These interactions range from mutualistic to parasitic with several instances of manipulation of host behavior. Nutrient contributions in these symbioses include both farming for food and nitrogen recycling by gut-associated microbes. Interestingly, the ants that are mostly likely to host diverse and likely functional gut microbial communities are those that feed on extreme diets. Although we do see many instances of symbiosis between ants and microbes, there are also examples of species without a functional gut microbiome.

Symbiosis among microbes and eukaryotic hosts is common and often considered a hallmark of multicellular evolution [1]. This is true among many of the over 13 000 species of ants, although symbiosis between ants and microbes are not ubiquitous. These microbial-ant symbiotic interactions span the tree of life and include microbial eukaryotes, fungi, viruses, and bacteria. These interactions range from pathogenic to mutualistic, with many relationships still not well understood. Although our knowledge of the diversity of these microbes in ants is growing rapidly, and in some cases we know the function and interaction with the host, we still have much to learn about — the little things that run the little things that run the world!

## Address

Cornell University, Departments of Entomology and Ecology & Evolutionary Biology, 129 Garden Avenue, Ithaca, NY, 14850, USA

Corresponding author: Moreau, Corrie S ([corrie.moreau@cornell.edu](mailto:corrie.moreau@cornell.edu))

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## Microbial eukaryotes

A number of microbial eukaryotes have been documented in ants, including some with very interesting transmission modes. One such example is the tetradonematid nematode, *Myrmecconema neotropicum* that infects the giant turtle ant, *Cephalotes atratus* [2]. As the ant is the intermediate host, the parasitic nematode needs to ensure

transmission to the definitive avian host, which it does by morphological and behavioral manipulation of the ant host. Once the eggs of the nematode reach maturity they turn the abdomen of the ant red to mimic fruits that are attractive to birds. In addition, the nematode reduces ant aggression and alters behavior such that the ant walks with its abdomen raised high, increasing the chances a bird will eat the ant's abdomen, which easily detaches in infected hosts.

Another microbial eukaryote that is known to infect ants and alter their behavior is the trematode, *Dicrocoelium dendriticum*, called the lancet liver fluke. The lancet liver fluke has two intermediate hosts. The first is a terrestrial snail and the second is an ant, which ingests a cyst left in the snail's trail that ants lick for moisture. Once the flukes mature in the ant, the infected individual leaves the nest in the evening, climbs a blade of grass, bites onto the blade and stays there all night. It will repeat this behavior each evening until it is finally consumed by the ultimate host, a cow or other grazing mammal [3].

Some microbial eukaryotes strikingly alter the behavior of an ant host, but in many cases, we know little about either the diversity of these microbes or the impacts on the host. We will begin to get a better understanding of this diversity by using high-throughput sequencing methods with eukaryotic primers. In one such study, we recovered Botryosphaerales fungi, *Adelina* (Coccidia) and Colpodea ciliates from samples of the ant *Daceton armigerum* (Ramalho *et al.* in review). This suggests there are likely a diversity of microbial eukaryotes present in many species and I am hopeful using these amplicon-based methods we may begin to understand the diversity of these microbes across the tree of life.

## Fungi

Likely the most well-known and best understood interaction between ants and fungi involve the fungus-farming ants. In this interaction the over 200 species of fungus-farming ants provide a suitable substrate for their associated fungus to grow within their nests. For the fungus this ensures a habitable and well-protected location to grow. In return, the ants harvest the gongylidia of their monocultural fungal garden as their sole food source. This mutualistic interaction has been occurring across the attine ants for roughly 55 million years [4] and although this mutualism occurs in one clade of ants (attines), the fungal partners have been acquired and switched several times, coming from two distantly related groups of fungi (gilled and coral fungi). Within this mutualism there are also fungal parasites (*Escovopsis*) that

can infect the cultivated fungus. To defend their fungal garden, the ants have a second mutualism with bacteria (*Pseudonocardia*), which produce antibiotics that inhibit the growth of *Escovopsis* [5].

Another group of fungi that have been associated with ants are the black yeasts (*Chaetothyriales*, Ascomycota) [6–9,10\*]. These fungi are associated with ants that live in domatia or build carton nests. Interestingly phylogenetic analysis of these fungi shows that unrelated ants with similar nesting behavior may have closely related black yeasts in their nests regardless of geographic location. In addition, this association with ants has evolved multiple times across this fungal group [6,8]. For the carton nesting ants, the black yeasts appear to stabilize the nest material and possibly to keep other fungi out of the carton [6]. The role for the domatia nesting species is less clear, although the black yeasts that are found in the nests of some species have also been found in the guts of adult and larval ants, suggesting they may be farmed for food [7,9]. Black yeasts from another fungal genus (*Phialophora*, Ascomycota) are also commonly found associated with the cuticle of fungus-farming ants.

Not all intimate interactions between fungi and ants are beneficial. Probably the most charismatic example of this are the zombie ant fungi (*Ophiocordyceps*), which alter the behavior of their ant hosts [11]. In this parasitism the fungal spore enters an ant worker's body through physical contact with the spore. Once the fungus is mature within the body of the ant host it triggers a series of complex behavioral changes that ensure the fungi will release its spores outside of the ant nest. The mechanism of behavioral manipulation is not completely understood, but manipulation of the ant brain is induced by an array of compounds produced by the fungus [12]. Not only are the manipulative behaviors species specific for each host, but these behaviors have evolved, sometimes convergently, to match the environmental conditions of the of the host's geographic range [13\*].

### Viruses

As in many other groups of organisms, we know almost nothing about the diversity and impact of viruses in ants. The few viruses that have been documented mainly come from two invasive ant species (*Solenopsis invicta* and *Linepithema humile*) where the viruses were targeted as potential control agents [14,15,16\*]. There are studies underway to document the diversity and potential factors shaping viral communities across ants. Although not directly looking at viruses infecting ants, one study investigated the number and diversity of endogenous viral elements (EVEs) found across 19 ant genomes [17\*]. A diverse group of EVEs were discovered in all screened ant host genomes and in many cases are similar to previously identified exogenous viruses. In addition, these EVEs may hint at viruses that are more likely to be chronic infections in ant hosts.

### Bacteria

The richest literature regarding microbes associated with ants is from the bacterial world. Bacteria have been surveyed from the cuticle, mouth, reproductive organs, and every digestive compartment of the gut. Interestingly, ants that feed at extremes have more gut associated bacteria, especially herbivores (Figure 1). Generalists species have low to no gut bacterial communities, which is thought to be due to their diverse and general diets. This adds to the growing body of literature that suggests not all species have a functional or necessary gut-bacterial community [10\*,18\*\*,19\*].

### Wolbachia

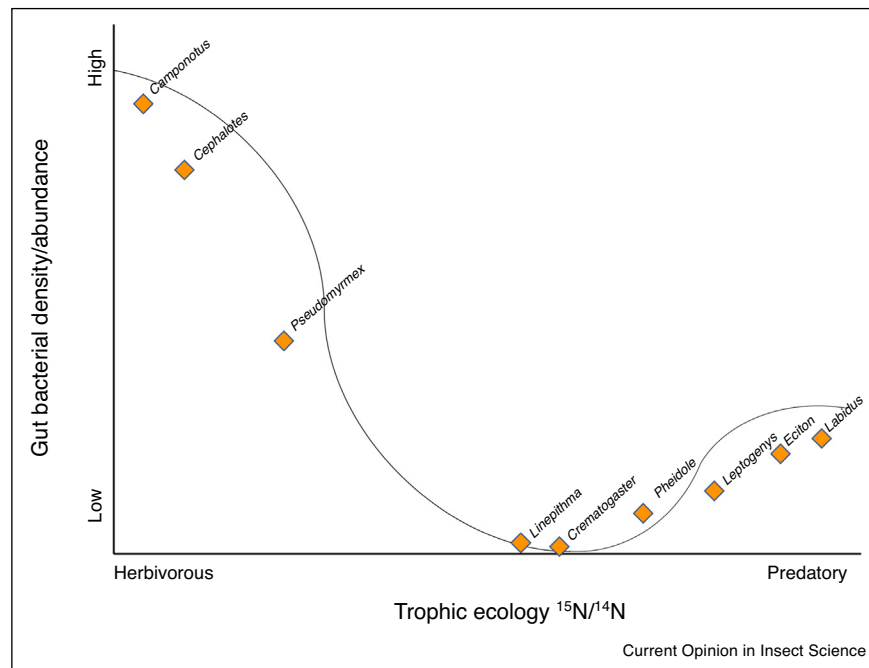
Despite the fact that there have been more studies on *Wolbachia* in ants than any other bacterial group we still know almost nothing about its function within ants. In ants there have been numerous studies that documented the presence and absence of *Wolbachia* (~35% of all ants are infected with *Wolbachia*), investigated host-associated factors that may structure *Wolbachia* diversity, and detailed their transmission mode (for a recent review see Ref. [20]). Interestingly, part of the difficulty in understanding if *Wolbachia* has any impact on ant hosts is due to two factors: 1) most ants in a colony are non-breeding workers, which poses an evolutionary dead end if *Wolbachia* is a reproductive manipulator and 2) it has been documented that older workers may lose their infection or at least have *Wolbachia* in low enough titers to not be detectable. There is some evidence to suggest that *Wolbachia* may influence sex ratios in ants, but this has only been documented in one species, *Monomorium pharaonis* [21\*].

### Nutritional provisioning

Bacteria associated with the gut have been demonstrated to upregulate the diets of several ant groups. In the carpenter ants and their relatives (Camponotini), these intracellular bacteria from the genus *Blochmannia* are housed in midgut-associated bacteriocytes. This relationship between camponotine ants and *Blochmannia* has been maintained for over 40 million years [22] and there appears to be co-diversification between these bacteria and their ant hosts [23]. In this symbiosis these host-specific bacteria provide essential amino acids to their host [24], which are thought to have nitrogen poor diets.

In the case of the herbivorous turtle ants (*Cephalotes*), a core community of gut-associated bacteria has been demonstrated to upregulate the host's diet through recycling of nitrogenous waste across 46 million years of evolution [25\*\*]. These bacteria transform urea into essential amino acids which are used by the host. Interestingly several other herbivorous ant groups have been found to host bacteria closely related to those in *Cephalotes*, suggesting that these bacteria may serve a similar role across herbivores [26].

Figure 1



Trophic ecology versus gut bacterial abundance in several genera of ants. Each diamond represents an ant genus with trophic ecology measured as the ratio of  $^{15}\text{N}/^{14}\text{N}$  and the bacterial abundance measured as the number of 16S rRNA read counts as assessed using qPCR. Data were derived from the following publications: [26,28\*,31, and Pringle *et al.* in press]. The curve was included to show the U-shaped pattern of gut-associated communities related to host diet.

Although functionality has only been demonstrated in herbivorous ant species, there are hints that gut-associated bacteria may also be important in predatory species. Entomoplasmatales bacteria have been found associated with the gut tissues of several predatory groups, including the army ants [27,28\*], although they are not exclusive to this group of hosts. Although it is still unknown if these bacteria benefit their hosts, it does appear that if you feed at the extremes of the trophic scale (herbivores and carnivores), as measured as the ratio of heavy-nitrogen to light-nitrogen through stable isotopic enrichment, you are likely to have gut-associated bacteria (Figure 1). Although the reason for this U-shaped curve in Figure 1 is unknown it is likely due to missing nutrients and vitamins in extreme diets resulting in reliance on gut-associated microbes to supplement the hosts' diet.

### Symbiont transmission

Across the ants, microbes are transmitted through a diversity of mechanisms. These include environmentally acquired (horizontally), through social interactions (horizontally), behavioral vertical transmission, and reproductive vertical transmission. Horizontal transmission to the host is usually not intentional and these microbes are often acquired from the environment or through interactions with other

infected individuals. Vertical transmission can occur through behavioral or maternal routes. In the case of behavioral transmission this is often through ritualized behaviors such as oral-oral or oral-anal tropholaxis [29]. Reproductive transmission usually occurs with microbes being transmitted by the mother through the egg [23,30].

Most pathogens are horizontally transmitted from the environment or through species interactions, which limits the chances the microbes will co-diversify with their hosts. For microbes that are vertically transmitted, either behaviorally or maternally through the egg, there are higher chances of co-diversification [22].

### Conclusions

Host-associated microbes have had profound impacts on their ant hosts. These interactions range from pathogenic to mutualistic and span almost all known microbial groups. In some cases, the costs or benefits are known for the host, but in most systems the majority of the interactions are not well understood. We are only now beginning to move beyond documenting the diversity to experimentally understanding what these microbes do within a host. This is an exciting time to study the microbiome.

## Conflicts of interest statement

Nothing declared.

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