Social immunity behaviour among ants infected by specialist and generalist fungi

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Social insects are distinguished by their lifestyle of living in groups with division of labour, cooperative brood care, and reproduction limited to a few colony members. Social insects often build large colonies with remarkable densities of highly related individuals and this can lead to an increased pathogen pressure. Our review focuses on interactions of ants with two important taxonomic groups of fungi infecting ants: Hypocreales (Ascomycota) and Entomophthorales (Entomophthoromycotina), and their different infection strategies, including host manipulation for optimal spore dispersal in the specialised ant pathogens. In social insects such as ants, resistance to pathogens is present at the colony level, with social immunity in addition to the individual resistance. We describe how ants use both organizational and behavioural defence strategies to combat fungal pathogens, with emphasis on highly specialised fungi from the genera *Ophiocordyceps* and *Pandora*.

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Introduction

Social insects are distinguished by their lifestyle of living in groups with division of labour, cooperative brood care, and reproduction limited to a few colony members. Individual members rely for their survival on the survival of the whole colony, and therefore they nurture and protect the colony. The ecological and evolutionary benefits of social living come at the cost of a greater risk of pathogen infection. A colony of highly related individuals that share physical space and frequently interact creates favourable conditions for pathogens to spread, which ultimately can lead to colony collapse. This pressure is believed to heavily influence the evolution of social organization in general, shaping the structure of the colony and leading to emergence of the so-called 'social immunity'. This term was first used by Cremer *et al.* in their 2007 review [1] and, since then, the concept has been adopted in other studies of social insect defence [2–5]. Many of these studies are based on laboratory set-ups with colonies or cohorts of insects kept in conditions with varying degrees of similarity to their natural habitat. However, it has been suggested that studies in natural settings are needed to give us a fuller picture of social immunity responses [6••].

In this review, we will highlight research on fungal pathogens and social immunity in ants, as studied in their natural habitats, or in laboratory conditions mimicking natural settings. First, we will briefly present the fungal pathogens. Then, we will review recent findings on different aspects of ants' social immunity, from laboratory and field-based studies, with a special section on specialist ant pathogenic fungi, and will end with a brief discussion of a specialised ectoparasitic group of fungi for which impact on the ant hosts is still rather unresolved.

Fungal pathogens of ants

In nature, ants are attacked by parasites of various taxonomic origins, including insects, helminths, protozoans and pathogenic viruses, bacteria, and fungi [7,8•]. Table 1 summarises some main lifestyle features of ant-parasitic fungal groups (pathogens and ectoparasites).

In the context of social immunity responses in ants fungal pathogens are by far the most studied, and a recent review of 114 years of ant-fungi studies showed that the majority of research papers focused on two genera of generalist fungal pathogens, *Metarhizium* and *Beauveria*, which are the anamorphic (asexual) stages of fungi from the Order Hypocreales, Phylum Ascomycota [6••]. These fungi are common in the soil and have complex life cycles with long lasting spores that typically survive for prolonged periods in the soil [9]. Some species in these genera are known as generalists and some isolates can infect several or many insect host species from different taxa [10••]. In addition, some of the hypocrealean entomopathogenic fungi are plant endophytes; they can colonise plant tissues and promote plant growth [11]. This relationship resembles mycorrhizal symbiosis with nitrogen–carbon exchange

Table 1

Summary of typical lifestyle features of selected fungal pathogens and ectoparasites of ants

	Asexual stages of Hypocreales	Sexual stages of Hypocreales	Entomophthorales	Laboulbeniales
Host specificity	Low	High	High	High
Lifestyle	Facultative pathogens	Obligate pathogens	Obligate pathogens	Obligate ectoparasites
Host manipulation	No	Yes	Yes	No
Virulence	Low	High	High	Unknown
Prevalence on ants in nature	Rare and at low prevalence	Common and occasionally at high prevalence	Common and occasionally at high prevalence	Common and occasionally at high prevalence
Notable genera/species	*Metarhizium* spp., *Beauveria* spp.	*Ophiocordyceps* spp.	*Pandora formicae*, *P. myrmecophaga*	*Rickia wassmanii*, *Laboulbenia formicarum*

between the fungus and the plant [12,13]. Species in these genera are, however, rarely found to kill ants in nature and laboratory studies have shown that high numbers of spores are needed for them to infect and kill ants [10••].

In contrast, specialist pathogenic fungi present a significant challenge to affected colonies. Examples can be found among *Ophiocordyceps* spp., ascomycete fungi killing ants in tropical regions. This genus contains the sexual stages of hypocrealean insect pathogenic fungi. Fungi from the entomophthoralean genus *Pandora* are also specialists and these are often prevalent in temperate regions [14–16]. Although distantly related, these two specialist fungal genera have evolved striking similarities in the ways that they manipulate the behaviour of infected ant hosts. Just before death of an infected ant, the fungi cause symptoms of summit disease (ants seeking elevated positions before death), and a pre-death bite that attaches the ant where it dies [8•]. These pathogens have often been studied in natural settings, due to their high natural prevalence in their host populations and their striking external features.

Specialist fungal pathogens are very interesting to study in the social immunity context. They generally rely on a small number of spores to infect the host, provided a certain spore threshold is reached [10••]. They serve as a prime example of the co-evolutionary arms race where the pathogen is specialised to kill a specific host and the host find new ways of defence; these host/pathogen interactions can act as a test of the concept stating that social immunity gives social insects an advantage in this battle.

Social defences: organization

One obvious aspect of colony resistance to pathogens in their natural habitat is the physical and temporal organization of the space the colonies occupy. Many species of ants live in perennial colonies with complex nest structures, with the reproductive queen (or queens) and brood typically occupying the most central and protected location [17]. Controlled humidity and temperature inside the

nest may act as disease-preventive measures, eliminating the development of fungi which require high levels of moisture to grow [16,18]. Division of labour, age-related task allocation (polyethism), heterogeneity in space that different task groups occupy, and limited between-group interaction all contribute to limiting transmission of pathogens [19,20]. Outside-nest foragers, a task group with the highest risks of acquiring pathogen propagules from the environment, have limited access to the brood area with the most valuable groups, and are generally recruited from older individuals, which are less valuable from the colony-survival perspective [21,22]. This is thought to both limit the possibility for pathogen entrance and transmission in the nest and to limit the colony-level cost of individual deaths.

Nest material itself can harbour substances having antimicrobial properties. Wood ants are known to seek out and collect conifer resin to incorporate it in the nest substrate as a type of prophylaxis against infection [23]. Moreover, ants use collected resin preferentially to protect brood [24], and are able to enhance antimicrobial properties of this 'medication' with their own venom acid secretions [25].

Social defences: behaviours

Social withdrawal is a phenomenon characteristic for social immunity. Individuals allegedly remove themselves from the colony when close to death, supposedly in an altruistic act to save nestmates, as shown in a study of ants poisoned with CO_2 [26]. Upon challenge and infection with a pathogenic fungus (*Metarhizium brunneum*), *Myrmica rubra* ants showed less attraction towards nestmates and colony cues, whereas their phototropism slightly increased, which has been proposed as the behavioural mechanism for pre-death social isolation [27•]. A recent study exposing ants to lower doses of CO_2 also reported decreases in altruistic rescue behaviour I *Formica cinerea*, which could also be a result of lower social commitment for exposed ants [28]. Generally, it seems that ants facing disease become less responsive to social cues [29]. However, known examples of social withdrawal observed in nature (and not experimentally induced)

seem to be a product of pathogen manipulation of the host rather than a direct host response to infection [16,30].

As prophylactic measures, ants display nest hygiene behaviours constitutively as a first line of defence against pathogens, and these include management of waste and corpses, grooming (removing spores) from themselves and other individuals (allogrooming), and use of poison. Survival benefits of corpse removal have been shown experimentally [31]. Also, the effects of selfgrooming and allogrooming have in numerous laboratory studies been shown to mitigate the effects of high host density on pathogen transmission [32–34]. Recently, there is an increasing evidence of poison use in disease control, including using poison to kill infected pupae in early stages of infection, when ants unpack contaminated pupae and spray them with poison from the gaster [35–37]. It seems that the behavioural repertoire of ants challenged with a pathogen threat, for example, in the form of high doses of spores of generalist fungal insect pathogens like *Metarhizium* spp., is sufficient to contain the risks they might encounter.

Indeed, based on our knowledge, no published evidence exists for colony-level epizootics caused by generalist fungi in ants in nature, even though the presence of entomopathogenic fungi seems common in the environment [38–40]. Surprisingly, ants may even seem to prefer nesting sites contaminated with fungal conidia [41–43]. A possible explanation for this phenomenon could be that the fungal odour provides a cue indicating a humid and humus-rich environment, while the fungus in itself is not much of a threat [43]. This could indicate that ants can rely on the effect of prophylactic social defences against frequently encountered generalist pathogens. It seems that in more natural settings, the real challenge comes during stressful conditions, for example during colony founding, that would render the colony unusually susceptible to enemies. In a laboratory experiment, *Camponotus castaneus* ant nests were divided into small subcolonies resembling colony founding stages. Most of these colonies collapsed when a single sporulating cadaver infected with *Beauveria bassiana* was introduced, both when ants were prevented from and allowed to remove the corpses [44•].

Specialist pathogens
The major fungal challenge for ants in nature comes from specialised pathogens, effective against healthy colonies and with biological properties supporting epizootic development. Interestingly, these pathogens seem to infect only workers outside of the nest, mostly the foragers [45]. This is important for our general understanding of social immunity, because laboratory studies of collective behaviours are mostly based on pathogens being introduced into the nest. In natural conditions, it seems that more complex perennial nests are fortresses that are very difficult for pathogens to penetrate, while at the same time, colonies provide susceptible hosts in abundance in the areas surrounding the nests [45].

The epidemiology of *Ophiocordyceps camponoti-rufipedis* infection of *Camponotus rufipes* ants in a rain forest in Brazil resembles a chronic infection [45] and similar long term chronic infection has been documented in *Pandora formicae* infections of *Formica polyctena* ants in a Danish forest floor [16]. The *Ophiocordyceps* fungus infects foragers, and manipulates these ants to leave the area they normally occupy, climb vegetation, and bite onto the margin or vein of a leaf to become fixed and die in this position. This is a developmental necessity for the fungus, because it requires sufficient time and humidity to produce fruiting bodies with new infective spores approximately 7–10 days post mortem. The authors have not observed any specific nestmate behaviours towards infected *O. camponoti-rufipedis* infected *Camponotus rufipes* ant cadavers in the field [45] and in a lab colony observation of *C. castaneus* ants did not alter their behaviour towards nestmates injected with the fungus *Ophiocordyceps kimflemingiae* (=*unilateralis*) [46]. The infected ants did not provoke aggressive behaviour from the uninfected nestmates and they were not secluded from the colony resources [46]. The authors speculate that this particular fungus, which exploits only the least valuable fraction of the colony population, has not induced the evolution of special detection mechanisms in the host. However, other host/pathogen systems involving *Ophiocordyceps* may differ; it has been observed in ants in the genus *Cephalotes* that ants remove infected and killed nestmates from tree trunks [14,47].

Wood ants [genus *Formica*] infected with *Pandora formicae*, unlike the *Ophiocordyceps*-infected ants, do not disperse far from the nest shortly before death, and cadavers can be found attached to grass and small twigs in the busiest colony surroundings, close to the nest or at the nest margin and close to the busiest foraging trails [16,48]. If the humidity is favourable, the fungus develops a massive layer of mature spore-shooting conidiophores on the ant's body within 24 hours after death. In this case, healthy nestmates are very committed to removing these deadly cadavers (see Figure 1) and as much as 80% can be removed each day [J Małagocka, PhD thesis, University of Copenhagen, 2016]. Ants remove diseased cadavers 'most painstakingly', as Marikovsky [48] states, with a repertoire of biting, pulling, detachment of body parts, and teamwork. *Pandora* spores are actively discharged and they are most likely highly virulent. Spore production from numerous cadavers around the nest indeed resembles a fungal siege on the ant fortress. It is understandable that social immunity is applied in full force here to protect the nest, with workers engaged in removing cadavers acquiring a considerable load of spores and putting themselves at high risk of infection.

Figure 1

Current Opinion in Insect Science

A red wood ant worker, *Formica polyctena*, striving to remove a nestmate cadaver killed by *Pandora formicae*, firmly attached to grass and covered in fungal conidiophores, photographed in Bidstrup forest in Middle Zealand, Denmark.

The *Pandora/Formica* system has probably co-evolved for a long time [10••], and therefore it is plausible to hypothesize that specialised detection mechanisms in ants have evolved to face this threat, especially because highly infective spores are produced shortly after host death. Cuticular hydrocarbon (CHC) analysis of fungal-killed cadavers sampled from three colonies in the forest showed that indeed these cadavers have a specific CHC composition different from uninfected ants. Field experiments, with control freeze-killed versus fungus-killed cadavers glued to Y-shaped bamboo sticks placed above a busy trail near an *F. polyctena* nest, supported the hypothesis that this potential odour cue could be detected by nestmates [J Małagocka, PhD thesis, University of Copenhagen, 2016]. Workers removed the infected cadavers preferentially, which indicates inclusion of a specific *Pandora*-induced behavioural response in the wood ant social immunity repertoire.

Interactions with fungal ectoparasites

The interactions between other types of microorganisms present in or on ants are but have so far not received much attention. Non-lethal ectoparasites, the Laboulbeniales

fungi infesting ants, are highly host-specific, common [49] and often considered rather neutral because they do not penetrate the host cuticle and only attach to the surface of the ant [50]. However, it seems that their presence can have a variety of context-dependent effects on host populations. Konrad *et al*. [51] suggested that superficial infestation of *Lasius* ants with a host-specific fungus, *Laboulbenia formicarum*, can be beneficial upon pathogenic fungus challenge. This is possibly thanks to increased immune stimulation and sanitary behaviour of infected *Lasius* ants. The same fungus can however be a burden on stressed hosts. A different ectoparasite in the Laboulbeniales, *Rickia wessmanii* infesting *Myrmica scabrinodis* ants, was shown to reduce the recognition ability of an infested host population which resulted in inability to discriminate kin; this was possibly due to increasing variation of CHC, which diluted the kin signal and led to higher acceptance of strangers by the affected colony [52]. Also, evidence exists for reduced survival of *M. scabrinodis* upon *R. wessmanii* infestation [53]. These complex interactions remain to be studied in more detail to improve our understanding of the impact infestations by species of Laboulbeniales have on host ants and the

interactions between these fungi and, for example, the specialised fungal pathogens from the genera *Ophiocordyceps* and *Pandora*.

Conclusions

We have presented the major features of social immunity in ants towards specialist and generalist fungal pathogens. Available literature demonstrates that the repertoire of prophylactic behaviours provides protection against infection with generalist fungal pathogens in most tested conditions, while the relation between social immunity and infection success is more complex in the case of specialist pathogens. We highlight the value and need for studies in the field or including more ecologically probable set-ups mimicking natural situations. With the inclusion of complementary sets of methods in studies, our view of social immunity will rapidly expand. General assumptions will be challenged, and can inspire a deeper appreciation for the evolutionary interplay between social hosts and their pathogens.

Conflict of interest statement

Nothing declared.

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