



# Disease Dynamics in Ants: A Critical Review of the Ecological Relevance of Using Generalist Fungi to Study Infections in Insect Societies

R.G. Loreto<sup>\*,§,1</sup> and D.P. Hughes<sup>\*,1</sup>

<sup>\*</sup>Pennsylvania State University, University Park, PA, United States

<sup>§</sup>CAPES Foundation, Ministry of Education of Brazil, Brasília, DF, Brazil

<sup>1</sup>Corresponding authors: E-mail: raquelgloreto@gmail.com; dhughes@psu.edu

## Contents

1. Introduction	2
2. Origin and Trends of Using Generalist Fungal Parasites to Study Ant–Fungal Parasite Interactions	5
3. The Ecological Relevance of Laboratory Experimentation With <i>Beauveria</i> and <i>Metarhizium</i> in Ants	6
4. Natural Occurrence of <i>Beauveria</i> and <i>Metarhizium</i> in Ants: Opportunistic Parasites?	11
5. Future Perspectives	14
Acknowledgments	15
Supplementary Data	16
References	16

## Abstract

It is assumed that social life can lead to the rapid spread of infectious diseases and outbreaks. In ants, disease outbreaks are rare and the expression of collective behaviors is invoked to explain the absence of epidemics in natural populations. Here, we address the ecological approach employed by many studies that have notably focused (89% of the studies) on two genera of generalist fungal parasites (*Beauveria* and *Metarhizium*). We ask whether these are the most representative models to study the evolutionary ecology of ant–fungal parasite interactions. To assess this, we critically examine the literature on ants and their interactions with fungal parasites from the past 114 years (1900–2014). We discuss how current evolutionary ecology approaches emerged from studies focused on the biological control of pest ants. We also analyzed the ecological relevance of the laboratory protocols used in evolutionary ecology studies employing generalist parasites, as well as the rare natural occurrence of these parasites

on ants. After a detailed consideration of all the publications, we suggest that using generalist pathogens such as *Beauveria* and *Metarhizium* is not an optimal approach if the goal is to study the evolutionary ecology of disease in ants. We conclude by advocating for approaches that incorporate greater realism.



## 1. INTRODUCTION

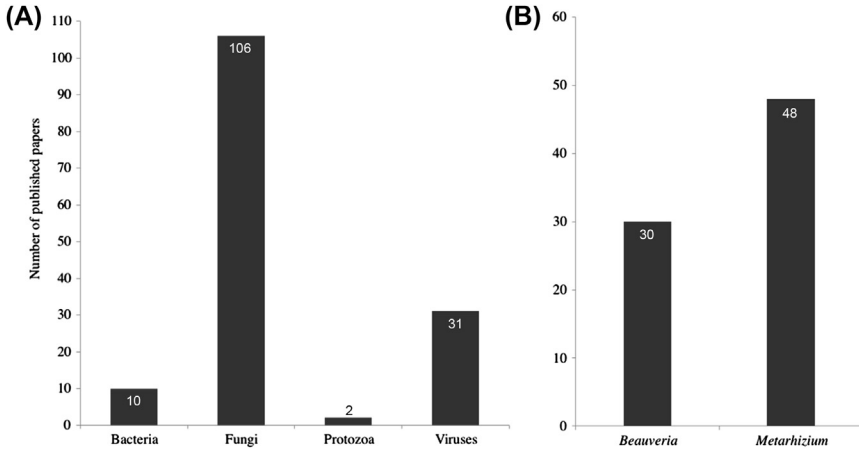
The emergence of the insect societies is one of the great evolutionary transitions in organic life (Maynard Smith & Szathmáry, 1995), and the notable dominance of ants, and social insects in general, implies advantages of group living over solitary life. One advantage is the extended life span of reproductive castes as a result of the protective nature of a colony (Keller & Genoud, 1997). As a counterpoint, major costs may exist for social life. Notably, group living presents conditions that are assumed to be ideal for parasite development and transmission. The organization and dynamics of the colony depend on the exchange of information and resources among nestmates, resulting in high rates of contact within the confined environment that is the nest. This close contact is compounded by elevated relatedness, an overlap of generations with reproducing and sterile adults, pupae, larvae, and eggs in the same space, and the controlled microclimate within the nest. Thus, it is assumed that social life can lead to the rapid spread of infectious diseases and outbreaks (Cremer, Armitage, & Schmid-Hempel, 2007).

Social insects, like all insects, are host to a diverse array of macroparasites and microparasites. Macroparasites include parasitic insects (eg, flies, wasps, and strepsipterans), worms (nematodes, trematodes, and cestodes) as well as mites, beetles, lepidopterans, and other arthropods that enter the nest to sequester resources (Schmid-Hempel, 1998). However, we know from standard epidemiological theory that macroparasitic infections tend not to lead to major disease outbreaks that strongly affect the host population (Anderson & May, 1979). On the other hand, microparasites (bacteria, fungi, protozoa and viruses) can cause epidemics that in turn slow down population growth. In ants, the abundance of entomopathogenic fungi in tropical forests has been suggested to act as an important control of ant populations (Evans, 1974). The potential for these microparasites to cause infectious diseases has been hypothesized as a major break on the evolution of sociality (Alexander, 1974; Arneberg, Skorping, Grenfell, & Read, 1998; Côté & Poulin, 1995).

A second hypothesis emerged from studies motivated by the use of infectious disease agents as biological control tools of ants and termites (Chouvenc, Su, & Grace, 2011), as well as the efforts aimed at reducing infectious disease spread in bees (Bailey & Ball, 1991). Overall, these studies indicate that those same conditions that could facilitate disease spread would also result in complex collective behaviors that could mitigate the parasite threat, a phenomena described as “social immunity” (Cremer et al., 2007). This second hypothesis (the social immunity hypothesis) predicts that the emergent properties of sociality, such as collective behavior, in fact lead to disease control and not necessarily an increased rate of transmission, as predicted by the previous hypothesis (ie, that social living promotes epidemics). This socially mediated dampening down of disease inside the nest may be particularly true for ants (discussed in this chapter) and termites (Chouvenc et al., 2011), where a summary of the literature would conclude that they are very efficient at collectively avoiding and controlling disease spread. For these two important groups of insects, social immunity is often used to explain the absence of disease outbreaks in natural populations, despite the apparently ideal ecological conditions for the rapid spread of infectious diseases within the colony.

Recently in the literature on disease in ant societies, we have encountered many studies supporting the social immunity hypothesis. The majority of them use fungal parasites as a source of disease (Fig. 1A and Boomsma, Schmid-Hempel, and Hughes (2005)). Overall, these studies show changes in the behavior of the colony and conclude that social immunity in ant societies is very efficient. To examine this in more detail, we analyzed 114 years of literature (1900–2014) on ant–fungal parasite interactions. Most studies used the generalist entomopathogenic fungi *Beauveria* and *Metarhizium* (89% of studies on ant–fungal parasite interactions, Fig. 1B). This is likely due to the enthusiasm and then subsequent failure of the attempt to use these two genera of fungi to control pest ants (this chapter), which mirrors a review of 50 years of similar studies in termites (Chouvenc et al., 2011). Although these fungi did not result in successful biological control in the field, they were found to kill ant workers, thus elevating these fungi to important tools for laboratory-based assays of infection. From there the approach has shifted to a framework that draws general conclusions about the evolutionary ecology of ant–parasite interactions.

It appears to us that our current knowledge on the ecology and evolution of ant–fungal parasite interactions is heavily drawn from studies using



**Figure 1** Number of publications exploring the different group of microparasites of ants over last 14 years (2001–14). (A) Number of published papers exploring each of the four categories of microparasites: bacteria (10), fungi (106), protozoa (2), and viruses (31). When more than one parasite was used in the same publication, the publication was listed once for each parasite. (B) Number of publications on ant–parasite interactions using *Beauveria* or *Metarhizium* as infectious disease agents.

simplistic conditions, using far higher doses of parasite propagules than those experienced in nature, and often presented to ants in unrealistic routes (Table S1). We suggest that employing a methodology that lacks ecological relevance may lead to incorrect conclusions. This, in turn, possibly prevents general insights into the evolution and ecology of ant–fungal parasite interactions. The equivocal assumption that such studies illuminate our understanding of ant–fungal coevolution is now being compounded by the adoption of formal mathematical models (Novak & Cremer, 2015; Theis, Ugelvig, Marr, & Cremer, 2015). However, if the biology is not realistic to begin with, then such approaches have limited utility. In addition, we also observed few records of natural infections of ants by generalist fungi. In line with that, it has been suggested that *Beauveria* and *Metarhizium* are not obligate insect parasites, but rather facultative/opportunistic associates that also interact with plants (Gao et al., 2011; Vega, 2008). We also raise the issue for how the sublethal effect of these infections has been considered in ant–generalist parasite studies. Taken together, we argue that the use of *Beauveria* and *Metarhizium* as a tool to understand infectious diseases in ants will better serve us when implemented in conditions that reflect natural conditions. Additionally, studies on parasites known to be specialized on ants are encouraged, as they would significantly complement our current knowledge.



## 2. ORIGIN AND TRENDS OF USING GENERALIST FUNGAL PARASITES TO STUDY ANT–FUNGAL PARASITE INTERACTIONS

During the second half of 1980s, the economic importance of leaf-cutting ants (*Atta* and *Acromyrmex*) and fire ants (*Solenopsis*) led to efforts to develop entomopathogenic fungi as biological control agents of these ants. Special attention was devoted to fungi from the genera *Beauveria* and *Metarhizium* due to their successful application on other groups of insects (Goettel, Eilenberg, & Glare, 2010). The initial tests for pathogenicity and host susceptibility to different strains of *Beauveria* and *Metarhizium* were promising, with high mortality and sporulation rates on individual workers (Alves, Stimac, & Camargo, 1988; Sánchez-penã & Thorvilson, 1992; Silva & Diehl-Fleig, 1988; Stimac, Alves, & Camargo, 1987). However, the application of these fungi at the colony level both in laboratory and field conditions had inconsistent results that were not sufficiently robust for effective pest control (Diehl-Fleig, Silva, Specht, & Valim-Labres, 1993; Kermarrec, Febvay, & Decharme, 1986; Pereira & Stimac, 1992; Stimac, Alves, & Camargo, 1989). For example, Stimac et al. (1989) reported an average of 80% of control in *Solenopsis* spp. nests by *Beauveria bassiana*, while Oi, Pereira, Stimac, and Wood (1994) reported 24% of nest mortality using a similar technique of fungal application.

In the biological control of pest insects, the social life of ants can add difficulties for controlling these organisms compared to solitary insects. In general, only a small portion of the colony explores the outside environment at one time, limiting the exposure of the colony to the fungi. Compounding the problem of using such fungi as biological control tools is that the queen, the reproductive unit of the colony, tends to be well protected, especially in mature colonies, making it unlikely that an application of fungi would eliminate her. Thus, the application of the fungus may decrease the number of ants temporarily, but it does not necessarily lead to control. Additionally, the behavioral ecology of the ants could be a barrier for the establishment of the onward infection chain needed for successful biological control. From the earliest reports of field applications of entomopathogenic fungi in ant colonies, researchers found that the ants would abandon areas of the nest where the inoculation with the fungi occurred (Machado, Diehl-Fleig, Silva, & Lucchese, 1988), as well as removing the inoculated baits (Diehl-Fleig & Lucchese, 1991) and infected cadavers (Pereira & Stimac, 1992) away from their nest.

In an attempt to link the inconsistency around ant control following the application of entomopathogenic fungi with general insights into colony defenses, [Jaccoud, Hughes, and Jackson \(1999\)](#) studied the epizootiology of *Metarhizium* infections in leaf-cutting ants. Although they seemed to be motivated by biological control, these authors stepped away from the traditional framework, focusing more on the ecology of the interaction. A few years later, [Hughes, Eilenberg, and Boomsma \(2002\)](#) published the first paper on ant–fungal parasite interactions that explicitly considered the ability of ants to survive fungal infections, making clear that the framework of the paper was not biological control. Before this, biological control focused studies used ant mortality, never survival, as the most relevant measurement ([Table S1](#)). It was the beginning of a new trend, which would shift the focus of the ant–fungal parasite studies from biological control to the ecology and evolution of infectious diseases in ants.



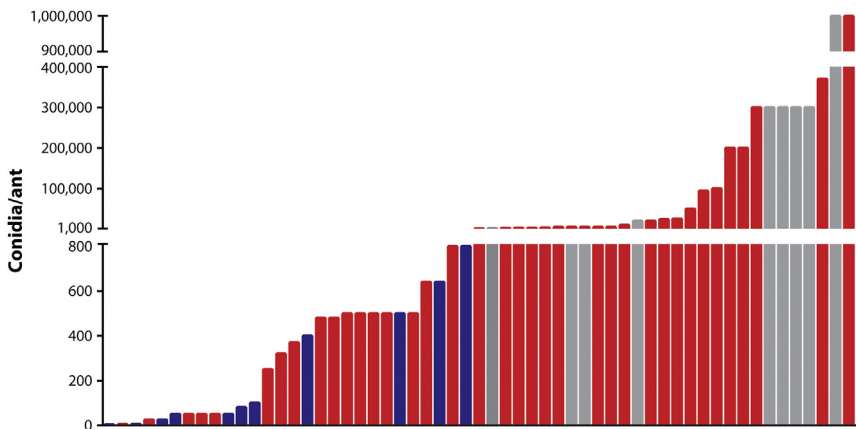
### **3. THE ECOLOGICAL RELEVANCE OF LABORATORY EXPERIMENTATION WITH *BEAUVERIA* AND *METARHIZIUM* IN ANTS**

The difficulty of working with ant colonies in the wild and the convenience of generalist parasites likely explain why the majority of ant–fungal parasite studies have been performed in the laboratory. These studies have shaped our understanding of ant–fungal parasite interactions and, more broadly, the ecology and evolution of disease threats in ant societies. The controlled conditions of laboratory experiments, achieved by simplifying setups, do provide valuable information on how general defenses are organized. However, basing our view only on studies performed in such contexts can be problematic because they may not translate to defenses implemented in more complex environments. Environmental complexity, such as daily temperature fluctuation ([Murdock, Paaijmans, Cox-Foster, Read, & Thomas, 2012](#)), spatial heterogeneity ([Brockhurst & Koskella, 2013](#)), and community diversity ([Orlofske, Jadin, Preston, & Johnson, 2012](#); [Thrall, Hochberg, Burdon, & Bever, 2007](#)), is known to play an important role in host–parasite interactions. We could reasonably assume that is also true where ants are the host.

One major issue with laboratory setups is how the conidia (ie, infective propagule of the fungal pathogen) encounter ants. This is the first step of the infection, and is, therefore, ecologically relevant for the interaction. As in other insects, ants can get infected when they encounter fungal conidia

(Hajek & St. Leger, 1994; Kermarrec et al., 1986). These conidia would be scattered on the soil and other surfaces where the ants nest and forage for food. Therefore, a realistic protocol for infection would be mixing fungi into the soil and exposing the ants to it. This was often done in biological control research (Pereira & Stimac, 1992; Pereira, Stimac, & Alves, 1993), where it was established that applying spores in soil considerably decreased the infection rate (Fuxa & Richter, 2004; Pereira & Stimac, 1992; Pereira et al., 1993; Stimac, Pereira, Alves, & Wood, 1993). In studies on behavioral immunity of ants, the experimental arenas are often sterile petri dishes (Konrad et al., 2012; Tragust, Mitteregger, et al., 2013).

Despite the appeal of naturalistic conditions such as placing spores in soil or having ants walk over conidia applied on filter paper (Castella, Chapuisat, & Christe, 2008; Chapuisat, Oppliger, Magliano, & Christe, 2007; Mattoso, Moreira, & Samuels, 2012; Reber, Castella, Christe, & Chapuisat, 2008; Schmidt, Linksvayer, Boomsma, & Pedersen, 2011), most researchers have adopted a peculiar approach that is not natural. Out of the 57 papers published in the last 12 years on ant ecological immunology, 31 used a topical application of conidia in a suspension applied directly onto the ant cuticle (Fig. 2 and Table S1). These conidia suspensions are often very concentrated, with the number of spores ranging from  $10^4$  to  $10^9$  conidia



**Figure 2** Number of conidia applied per ant in experiments using topical application of conidial suspension as exposure method to *Beauveria* or *Metarhizium*. The blue (black in print versions) bars represent the experiments where survival was not decreased by the parasite. The red (dark gray in print versions) bars represent the experiments where survival was decreased by the parasite. Gray bars are studies where survival was not measured. The doses in the first five studies were below or equal to those found in nature (see Table S2).

per mL (Fig. 2). Such concentrations are unlikely to be found in nature. The highest reported concentration of conidia in the soil for either *Beauveria* or *Metarhizium* is  $6.8 \times 10^3$  and  $5 \times 10^4$  colony-forming units (CFU) per gram, respectively (Hughes, Thomsen, Eilenberg, & Boomsma, 2004; Keller, Kessler, & Schweizer, 2003). It is difficult to estimate how many spores an ant would come in contact with based on the number of CFU found in 1 g of soil. It would also be challenging to translate the number of CFU per gram into a solution of conidia (per mL). However, for the majority of studies, the experimental dosages are orders of magnitude higher than what can be found in natural situations (Fig. 2). In those studies, justifications for the applied dosages are rare. When provided, they are often related to experimental need to ensure the infection process (Reber, Purcell, Buechel, Buri, & Chapuisat, 2011) or to elicit a behavioral response (Pull, Hughes, & Brown, 2013). Moreover, the topical application of conidia solution does not reflect the natural route of infection, which is via ants brushing against a source of spores or walking over them. The application method in the laboratory setup eliminates ecological features of what is an important step of the ant–fungal parasite interactions.

Fungal conidia could also potentially be transmitted inside the nest by contacting a nestmate that had direct contact with the fungi. As discussed above, the sibling–sibling transmission is assumed to be an important constraint of social living. Konrad et al. (2012) showed that ants infected with a high dosage of conidia ( $10^9$  mL<sup>-1</sup>) subsequently transmitted conidia to 17 ants out of 45 (37%) they had contact with. However, in the same study, the mortality of these secondarily exposed nestmates due to the fungus was only 3 out of 150 worker ants (2%). A study by Reber et al. (2011) had shown that in two different experiments, using a total of 1555 ants, only one was infected and died by secondary contact with conidia via nestmates. Therefore, if realistic spore loads were applied, it is unlikely that secondary infections and subsequent deaths would have occurred.

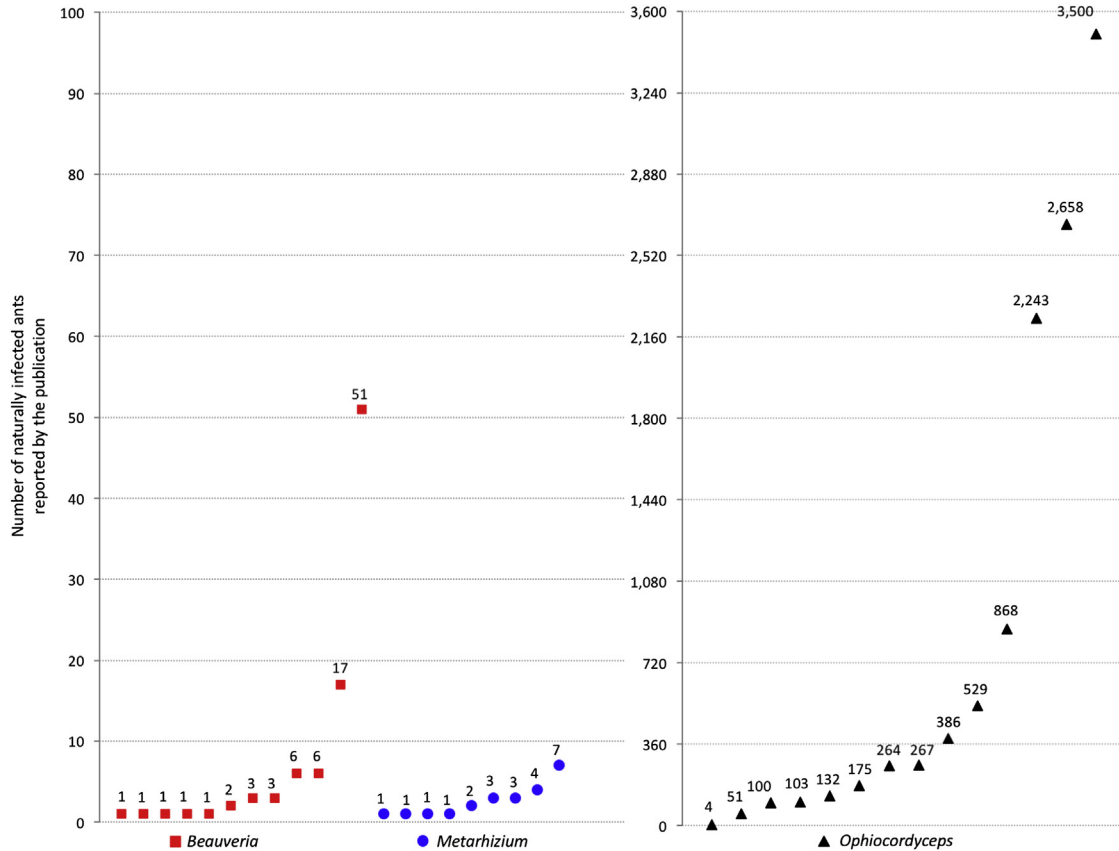
We suggest, based on our review of the literature, that ants might not naturally encounter the high amounts of conidia typically used for experimental infections and certainly not as highly concentrated solutions of conidia directly applied to their bodies. However, it could be possible that worker ants come in contact with a large amount of conidia if they are handling infectious cadavers (either of an ant or some other insect). The encounter with an infectious cadaver may happen when the ants are digging the nest galleries or foraging directly on the forest floor. The amount of conidia produced by a cadaver varies according to the ant species (Walker &



Hughes, 2011), but it was estimated in the leaf-cutter ant *Acromyrmex echinator* to be  $1.2 \times 10^7$  ( $\pm 1.6 \times 10^6$ ) conidia per cadaver (Hughes et al., 2002). Studies on biological control have investigated the mortality of workers following the introduction of cadavers in the nest, showing that it correlates with the number of cadavers (Pereira & Stimac, 1992). However, it remains to be investigated how ants react to situations where they encounter infectious cadavers.

Since the initial infection is a key point in any host–parasite interaction, we looked at 31 studies that topically applied conidia of *Beauveria* or *Metarhizium*. For those that compared infected and control ants, we found, not surprisingly, that ant survival is less where researchers expose the ants to higher loads of conidia (Fig. 2). The lower doses of fungi ( $10^4$  mL<sup>-1</sup>) used in the studies also did not affect grooming levels (Reber et al., 2011). Grooming is the most common behavior used to measure social immunity. In some cases, the higher doses of conidia ( $10^7$  mL<sup>-1</sup>) resulted in decreased grooming levels (Okuno, Tsuji, Sato, & Fujisaki, 2012). In some cases, the approach taken is perhaps not as objective as it should be. For example, in a study of founding *Lasius niger* queens, Pull et al. (2013) state that queens were “exposed to a higher dosage of conidia (2  $\mu$ L of conidia solution at a concentration of  $5 \times 10^8$  conidia mL<sup>-1</sup>) in order to elicit a greater anti-septic grooming response from the queens.” In Fig. 3 of the same study (Pull et al., 2013), the researchers report an increase in self-grooming behavior as a response to the parasite. These are possible examples of how our ecologically unrealistic setups could produce misleading conclusions into the evolutionary ecology of diseases in ant societies.

During our literature research we came across very few negative results. This implies that most of the publications discovered an effect of fungal application on the tested subject. This could be due to the robustness of our current knowledge or, after evaluating the implemented protocols, a bias toward positive results, likely driven by the nonrealistic applications of conidia. Only one publication was entirely based on a negative result (Reber & Chapuisat, 2012b), while the other ones had the negative results buried among the highlighted, positive, results (Okuno et al., 2012; Tragust, Ugelvig, Chapuisat, Heinze, & Cremer, 2013). As such, the negative results, when present, did not receive their due attention. In studies of disease dynamics, reporting the failure of the disease agent to transmit (ie, a negative result) is of fundamental interest because it reveals aspects of context-dependent virulence, which is a property of both host and parasite (Ebert, 1994; Read, 1994).



**Figure 3** Number of natural infections of ants by generalist and specialist fungi reported in publications. All the publications that reported the number of isolates or cadavers for *Beauveria* (red (light gray in print versions) squares), *Metarhizium* (blue (gray in print versions) circle) or *Ophiocordyceps* (black triangles) were included. Note that it was necessary to include a second x-axis for reporting the number of ants naturally infected by *Ophiocordyceps* sp. The publications used for this figure are also reported in [Table S3](#).

Very recently, researchers started adopting mathematical models to explore disease transmission within ant societies (Novak & Cremer, 2015; Theis et al., 2015). These models are based on assumptions from empirical data, collected following laboratory studies, such as those that elicited grooming (Theis et al., 2015) or those which observed cross infections among nestmates (Novak & Cremer, 2015). We have already discussed that such results might be due to the nature of the laboratory setups, and likely do not represent the real ecology of ant–fungal interactions. Consequently, the theoretical models based on these results may also be only applicable to these nonnatural conditions. Mathematical explorations are certainly illuminating, but we encourage them to be parameterized based on a realistic ecological basis (Andersen et al., 2012).

Although we highlight the limitation of the laboratory studies that do not reflect natural conditions, we do not suggest that laboratory studies themselves are the problem. The simplification of the system is necessary to establish cause and effect. We suggest that it would be fairly easy to use more realistic conidia loads and/or exposure methods, and to change some other aspects to incorporate more biological relevance into laboratory setups. Despite the limitations of the protocols in the published studies, the generally held view that an ant nest is a “fortress” (Schmid-Hempel, 1998), well defended against diseases, seems to be corroborated by the absence of outbreaks of *Beauveria* or *Metarhizium* in their societies. However, as other fungi are commonly found infecting ants (Araujo, Evans, Geiser, Mackay, & Hughes, 2015; Csata et al., 2013; Evans, Elliot, & Hughes, 2011), we question if the absence of *Beauveria* or *Metarhizium* is in fact due to the efficient social defenses.



#### **4. NATURAL OCCURRENCE OF *BEAUVERIA* AND *METARHIZIUM* IN ANTS: OPPORTUNISTIC PARASITES?**

The use of *Beauveria* and *Metarhizium* to study ant diseases is often justified by the assumption that they are common parasites of ants (Reber et al., 2011; Ribeiro et al., 2012; Tragust, Mitteregger, et al., 2013; Tranter, LeFevre, Evison, & Hughes, 2015; Walker & Hughes, 2011). The reasons for this assumption are (1) these fungi are soil-borne parasites, commonly associated with the soil of ant nests, and (2) a few records of natural infections of these parasites in ants do exist. Although *Beauveria* and *Metarhizium* do cause epizootics in other insects, such as coleopterans (Townsend, Glare, & Willoughby, 1995), hemipterans (Shimazu, 1989), and lepidopterans (Tefera & Pringle, 2003; Townsend et al., 1995), there is no record of a

natural epizootic events in ant colonies and natural infections are rather isolated and very rare (Fig. 3 and Table S3).

*Beauveria* and *Metarhizium* incidence is relatively higher on ground nesting founding queens—up to 10% (Cardoso, 2010) (Fig. 3 and Table S3). Following the nuptial flight, the reproductive female attempts to found a colony. This is a critical and vulnerable stage for the future queen, where she has limited resources until the first batch of workers emerge and start foraging. At this stage of the colony, generalist and opportunistic parasites may represent a bottleneck for the next generation of colonies, since the infected, nonresistant queens will die. Consequently, the workers may be potentially preselected (via their mother) for resistance, since only the queen lays eggs in the colony. Because the parasites are not equally distributed on soil (Hughes, Thomsen, et al., 2004; Keller et al., 2003), the variation in the level of exposure of the queens may be an important source of selection, removing from the population queens that are more susceptible to generalist parasites or priming the immune system of the queen. It might be the reason for the strong “colony effect,” where workers from different colonies have different responses under laboratory conditions (Baer, Krug, Boomsma, & Hughes, 2005; Fountain & Hughes, 2011; Hughes, Petersen, et al., 2004; Ribeiro et al., 2012). Nonetheless, the overall success of the founder queens is low and more than 95% of them die before laying the first batch of eggs (Hölldobler & Wilson, 1990).

The bottleneck the queens go through in the founding stage of the colony could, in part, explain why there are few records of worker cadavers infected by these generalist fungi in nature. Hughes, Thomsen, et al. (2004) actively searched for ant cadavers around the nests of leaf-cutting ants and found zero cadavers of *Beauveria* or *Metarhizium* infected ants. They also collected 3300 live ants over a year to verify infections from which only 3 (0.09%) were infected by *Metarhizium anisopliae* (Fig. 3 and Table S3). The fungus *Beauveria* was not detected. Reber and Chapuisat (2012a) collected 4050 ants from 81 colonies of *Formica selysi*. They found that *Beauveria* prevalence at the colony level was 17% (14 colonies out of 81), but at individual level, it was only 0.42% (17 ants out of 4050). In that study, zero workers were infected by *Metarhizium*. A few other publications have reported that live ants collected in field and kept in the laboratory until death showed fungal growth following few days of postmortem incubation of the cadaver (Baird, Woolfolk, & Watson, 2007; Hughes, Thomsen, et al., 2004; Sánchez-penã & Thorvilson, 1992) (Fig. 3 and Table S3).

These sampling methodologies, which collect ants and establish infection status following their death and the subsequent growth of the fungus

following incubation do not account for sublethal infections. Sublethal effects of parasites, such as reduced fertility and competitiveness, are considered important ecological and evolutionary impacts of diseases (Boots et al., 2003; Boots & Norman, 2000). This important aspect of host–parasite ecology has not been considered in the ant studies we examined. Interestingly, in our opinion, this would be one of the aspects to be explored and better characterized in laboratory setups. Considering that ant workers are sterile, a reduction in fertility or competitiveness might not be the most appropriate parameter for studying sublethal effect. On the other hand, their social nature can open new avenues to study sublethal effects of parasite. For example, do worker ants infected by either *Metarhizium* or *Beauveria* forage less or generally alter their expression of altruism to nestmates?

Other fungi in the genus *Aspergillus* are also used (Table S1) because they are considered opportunistic parasites, while species in the genera *Beauveria* and *Metarhizium*, are considered to be obligate pathogens in ant studies (Novak & Cremer, 2015; Tranter et al., 2015). Ironically, the fungus *Aspergillus*, claimed as an opportunistic parasite, is often present in ants collected both in the field and those reared in the laboratory (Lacerda et al., 2014; Lacerda, Della Lucia, Pereira, Peternelli, & Totola, 2010; Rodrigues, Silva, Bacci, Forti, & Pagnocca, 2010; Tranter, Graystock, Shaw, Lopes, & Hughes, 2014). If *Beauveria* and *Metarhizium* were common parasites in ants, as claimed, we would expect to find them as frequently or more often than the opportunistic parasite, *Aspergillus*. It is worth noting that *Beauveria* and *Metarhizium* play a role in other ecological interactions, such as their role as endophytes, or as associates of roots in the rhizosphere and as mycoparasites (Vega et al., 2009). For example, *Metarhizium* spp. are known to colonize roots and promote plant growth (St. Leger, 2008). Genomes of *Metarhizium* species reveal many genes involved in the colonization of plant tissue (Gao et al., 2011). In fact, an emerging view among mycologists is that they are facultative parasites of insects (Bidochka, Clark, Lewis, & Keyhani, 2010; Oulevey, Widmer, Koelliker, & Enkerli, 2009; Padilla-Guerrero, Barelli, Gonzalez-Hernandez, Torres-Guzman, & Bidochka, 2011). Additionally, these two fungi have been treated as opportunistic parasites in the biological control arena (Brodeur, 2012).

The notion that the fungi *Beauveria* and *Metarhizium* are a common parasite of ants is widespread, but most likely not true. For *Metarhizium*, there are some extreme cases in the ant literature where it is even described as a “specialist parasite” (Tranter et al., 2014, 2015). The rare natural infection of ants by *Beauveria* and *Metarhizium* (discussed in this chapter), their alternative lifestyles in plants (Vega et al., 2009), and their opportunistic nature

recognized by both mycology and biological control disciplines (Brodeur, 2012) indicate that these fungi are not necessarily a potent threat for ants. Therefore, we suggest that there is no evidence for assuming that *Beauveria* and *Metarhizium* are common parasites of ants in natural populations, and the designation of any of them as “specialized parasites” is wrong.



## 5. FUTURE PERSPECTIVES

*Beauveria* and *Metarhizium* have been and continue to be important tools to understand how ants organize their defenses against opportunistic threats. However, we consider the unjustified methodologies implemented by researchers as a barrier to knowledge collection. It is important to reconsider the unrealistic doses of conidia and exposure routes, a reality also discussed in other host–parasite systems (Poulin, 2010a, 2010b). High concentrations of microbial parasites will always affect ant mortality or behavior, but they do not allow us to objectively understand the link between social behavior and evolved behaviors that function to mitigate diseases. We are not implying that high doses cannot be used, when, for example, the goal is simply to kill, study within host growth or better understand the terminal stages of the infection (ie, prior to death) (Heinze & Walter, 2010). However, where the goal is to understand hygienic behaviors, ant-to-ant cross infection and social network disruption following exposure, we strongly recommend future research efforts incorporate ecologically relevant conditions. Recently, a few studies have used more realistic setups to investigate ants and diseases (Loreto, Elliot, Freitas, Pereira, & Hughes, 2014; Quevillon, Hanks, Bansal, & Hughes, 2015), which prove that it is feasible and fairly simple to implement. We hope to see more ant–parasite studies continue in this direction.

Although we highlight that the two focal parasites on ant studies are rather rare and likely opportunistic in natural populations, we understand the importance of these models. However, we were surprised by the lack of information on sublethal doses and its effects, both at individual and colony levels. Perhaps, it is due to the experimental ease by which high doses of conidia can be used to produce high mortality rates (Fig. 3). Additionally, there were no studies on the detection and response to infectious cadavers, a likely real scenario. *Beauveria* and *Metarhizium* may be useful models to investigate these unknown aspects of ant–parasites interactions, but we would also argue that our view could be biased by the limitation of these models.

While the current studies on ant–parasite interactions support the view that they possess a robust social immunity, there are many other parasites, including other fungi, which are specialized in infecting ant societies (Csata et al., 2013; Loreto et al., 2014; Marikovsky, 1962). It is worth noting that studies investigating immune defenses in honeybees and bumblebees predominantly focus on specialized parasites (Bailey & Ball, 1991), which can contribute to the loss of these beneficial insects. These fungi have been overlooked in studies focused on the ecology and evolution of ant–parasite interactions. They are very common in the natural habitat of ants (Table S3), have a prevalence as high as 100% at the colony level (Konrad, Grasse, Tragust, & Cremer, 2015; Loreto et al., 2014), and allow for both field and laboratory experimentation (de Bekker et al., 2014). We see a lot of potential in exploring these specialized interactions: they could lead us to a broader understanding of social immunity and how parasites can overcome collective defenses.

Finally, models are often chosen for particular reasons. Due to ethical and logistical reasons, most of what we know about the molecular mechanisms of diseases in humans comes from artificial models, such as *Drosophila melanogaster* (Keebaugh & Schlenke, 2014; Lemaitre & Hoffmann, 2007), *Caenorhabditis elegans* (Markaki & Tavernarakis, 2010), and the mouse (Rosenthal & Brown, 2007). However, these models often lack the social component of disease spread, which is an important factor in human societies. In addition, a disease model that reflects the low genetic diversity and high density of our agricultural and livestock groups would be beneficial. Social insects have the social properties that reflect high-density living of closely related individuals. The effort to prevent colony collapse disorder in bees has advanced our knowledge on diseases in insect societies. However, we suggest that ants may be a better model to study the ecology and evolution of group living disease dynamics due to the larger sizes of their colonies of the nonwinged workers. If we study these social models in the proper way, many valuable lessons could be learned from them.

## ACKNOWLEDGMENTS

We are thankful to Dr. Rebeca Rosengaus, Dr. Klaus Reinhardt, and Dr. Andrew Read for helpful discussions and to three anonymous reviewers for their constructive comments. We are also thankful to Djoshkun Shengjuler for assisting us in the making of the figures for this manuscript. RGL is funded by CAPES-Brazil (grant 6203-10-8). DPH is funded by Pennsylvania State University. Part of this work was funded by NSF (grant 1414296) as part of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program.

## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/bs.adgen.2015.12.005>.

## REFERENCES

- Alexander, R. D. (1974). The evolution of social behavior. *Annual Review of Ecology and Systematics*, 5, 325–383.
- Alves, S. B., Stimac, J. L., & Camargo, M. T. V. (1988). Susceptibility of *Solenopsis invicta* Buren and *Solenopsis saevissima* fr. Smith to isolates of *Beauveria bassiana* Bals. vuill. *Anais da Sociedade Entomologica do Brasil*, 17, 379–388.
- Andersen, S. B., Ferrari, M., Evans, H. C., Elliot, S. L., Boomsma, J. J., & Hughes, D. P. (2012). Disease dynamics in a specialized parasite of ant societies. *PLoS One*, 7, e36352.
- Anderson, R. M., & May, R. M. (1979). Population biology of infectious-diseases: part I. *Nature*, 280, 361–367.
- Araujo, J. P. M., Evans, H. C., Geiser, D. M., Mackay, W. P., & Hughes, D. P. (2015). Unravelling the diversity behind the *Ophiocordyceps unilateralis* (Ophiocordycipitaceae) complex: three new species of zombie-ant fungi from the Brazilian Amazon. *Phytotaxa*, 220, 224–238.
- Arneberg, P., Skorping, A., Grenfell, B., & Read, A. F. (1998). Host densities as determinants of abundance in parasite communities. *Proceedings of the Royal Society B*, 265, 1283–1289.
- Baer, B., Krug, A., Boomsma, J. J., & Hughes, W. O. H. (2005). Examination of the immune responses of males and workers of the leaf-cutting ant *Acromyrmex echinator* and the effect of infection. *Insectes Sociaux*, 52, 298–303.
- Bailey, L., & Ball, B. (1991). Honey bee pathology. *Honey Bee Pathology*, 1–200.
- Baird, R., Woolfolk, S., & Watson, C. E. (2007). Survey of bacterial and fungal associates of black/hybrid imported fire ants from mounds in Mississippi. *Southeastern Naturalist*, 6, 615–632.
- de Bekker, C., Quevillon, L. E., Smith, P. B., Fleming, K. R., Ghosh, D., Patterson, A. D., & Hughes, D. P. (2014). Species-specific ant brain manipulation by a specialized fungal parasite. *BMC Evolutionary Biology*, 14.
- Bidochka, M. J., Clark, D. C., Lewis, M. W., & Keyhani, N. O. (2010). Could insect phagocytic avoidance by entomogenous fungi have evolved via selection against soil amoeboid predators? *Microbiology-Sgm*, 156, 2164–2171.
- Boomsma, J. J., Schmid-Hempel, P., & Hughes, W. O. H. (2005). Life histories and parasite pressure across the major groups of social insects. Insect evolutionary ecology. In *Proceedings of the Royal Entomological Society's 22nd symposium* (pp. 139–175).
- Boots, M., Greenman, J., Ross, D., Norman, R., Hails, R., & Sait, S. (2003). The population dynamical implications of covert infections in host-microparasite interactions. *Journal of Animal Ecology*, 72, 1064–1072.
- Boots, M., & Norman, R. (2000). Sublethal infection and the population dynamics of host-microparasite interactions. *Journal of Animal Ecology*, 69, 517–524.
- Brockhurst, M. A., & Koskella, B. (2013). Experimental coevolution of species interactions. *Trends in Ecology & Evolution*, 28, 367–375.
- Brodeur, J. (2012). Host specificity in biological control: insights from opportunistic pathogens. *Evolutionary Applications*, 5, 470–480.
- Cardoso, S. R. S. (2010). *Morfogênese de ninhos iniciais de Atta spp. (Hymenoptera: Formicidae), mortalidade em condições naturais e avaliação da ação de fungos entomopatogênicos* (Ph.D. UNESP, Botucatu).
- Castella, G., Chapuisat, M., & Christe, P. (2008). Prophylaxis with resin in wood ants. *Animal Behaviour*, 75, 1591–1596.



- Chapuisat, M., Oppliger, A., Magliano, P., & Christe, P. (2007). Wood ants use resin to protect themselves against pathogens. *Proceedings of the Royal Society B*, *274*, 2013–2017.
- Chouvenc, T., Su, N.-Y., & Grace, J. K. (2011). Fifty years of attempted biological control of termites – analysis of a failure. *Biological Control*, *59*, 69–82.
- Côté, I. M., & Poulin, R. B. (1995). Parasitism and group-size in social animals: a meta-analysis. *Behavioral Ecology*, *6*, 159–165.
- Cremer, S., Armitage, S. A. O., & Schmid-Hempel, P. (2007). Social immunity. *Current Biology*, *17*, R693–R702.
- Csata, E., Czekes, Z., Eros, K., Nemet, E., Hughes, M., Csoz, S., & Marko, B. (2013). Comprehensive survey of Romanian myrmecoparasitic fungi: new species, biology and distribution. *North-Western Journal of Zoology*, *9*, 23–29.
- Diehl-Fleig, E., & Lucchese, M. E. D. P. (1991). Behavioral responses of *Acromyrmex striatus* workers (Hymenoptera: Formicidae) in the presence of entomopathogenic fungi. *Revista Brasileira de Entomologia*, *35*, 101–108.
- Diehl-Fleig, E., Silva, M. E. D., Specht, A., & Valim-Labres, M. E. (1993). Efficiency of *Beauveria bassiana* for *Acromyrmex* spp. control (Hymenoptera: Formicidae). *Anais da Sociedade Entomologica do Brasil*, *22*, 281–285.
- Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science*, *265*, 1084–1086.
- Evans, H. C. (1974). Natural control of arthropods, with special reference to ants (Formicidae), by fungi in the tropical high forest of Ghana. *Journal of Applied Ecology*, *11*, 37–49.
- Evans, H. C., Elliot, S. L., & Hughes, D. P. (2011). Hidden diversity behind the zombie-ant fungus *Ophiocordyceps unilateralis*: four new species described from carpenter ants in Minas Gerais, Brazil. *PLoS One*, *6*.
- Fountain, T., & Hughes, W. O. H. (2011). Weaving resistance: silk and disease resistance in the weaver ant *Polyrhachis dives*. *Insectes Sociaux*, *58*, 453–458.
- Fuxa, J. R., & Richter, A. R. (2004). Effects of soil moisture and composition and fungal isolate on prevalence of *Beauveria bassiana* in laboratory colonies of the red imported fire ant (Hymenoptera: Formicidae). *Environmental Entomology*, *33*, 975–981.
- Gao, Q. A., Jin, K., Ying, S. H., Zhang, Y. J., Xiao, G. H., Shang, Y. F. ... Wang, C. S. (2011). Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. *PLoS Genetics*, *7*.
- Goettel, M. S., Eilenberg, J., & Glare, T. (2010). Entomopathogenic fungi and their role in regulation of insect populations. In L. I. Gilbert, & S. S. Gill (Eds.), *Insect control: Biological and synthetic agents*. London: Academic Press.
- Hajek, A. E., & St. Leger, R. J. (1994). Interactions between fungal pathogens and insect hosts. *Annual Review of Entomology*, *39*, 293–322.
- Heinze, J., & Walter, B. (2010). Moribund ants leave their nests to die in social isolation. *Current Biology*, *20*, 249–252.
- Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Cambridge: Harvard University Press.
- Hughes, W. O. H., Eilenberg, J., & Boomsma, J. J. (2002). Trade-offs in group living: transmission and disease resistance in leaf-cutting ants. *Proceedings of the Royal Society B*, *269*, 1811–1819.
- Hughes, W. O. H., Petersen, K. S., Ugelvig, L. V., Pedersen, D., Thomsen, L., Poulsen, M., & Boomsma, J. J. (2004). Density-dependence and within-host competition in a semelparous parasite of leaf-cutting ants. *BMC Evolutionary Biology*, *4*.
- Hughes, W. O. H., Thomsen, L., Eilenberg, J., & Boomsma, J. J. (2004). Diversity of entomopathogenic fungi near leaf-cutting ant nests in a neotropical forest, with particular reference to *Metarhizium anisopliae* var. *anisopliae*. *Journal of Invertebrate Pathology*, *85*, 46–53. e1001300.
- Jaccoud, B. D., Hughes, W. O. H., & Jackson, C. W. (1999). The epizootiology of a *Metarhizium* infection in mini-nests of the leaf-cutting ant *Atta sexdens rubropilosa*. *Entomologia Experimentalis et Applicata*, *93*, 51–61.

- Keebaugh, E. S., & Schlenke, T. A. (2014). Insights from natural host–parasite interactions: the *Drosophila* model. *Developmental and Comparative Immunology*, *42*, 111–123.
- Keller, L., & Genoud, M. (1997). Extraordinary lifespans in ants: a test of evolutionary theories of ageing. *Nature*, *389*, 958–960.
- Keller, S., Kessler, P., & Schweizer, C. (2003). Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae*. *BioControl*, *48*, 307–319.
- Kermarrec, A., Febvay, G., & Decharme, M. (1986). Protection of leaf-cutting ants from bio-hazards: is there a future for microbiological control? In C. S. Lofgren, & R. K. Meer (Eds.), *Fire ants and leaf-cutting ants: Biology and management* (p. 433). Boulder and London: Westview Press.
- Konrad, M., Grasse, A. V., Tragust, S., & Cremer, S. (2015). Anti-pathogen protection versus survival costs mediated by an ectosymbiont in an ant host. *Proceedings of the Royal Society B*, *282*, 20141976.
- Konrad, M., Vyleta, M. L., Theis, F. J., Stock, M., Tragust, S., Klatt, M. ... Cremer, S. (2012). Social transfer of pathogenic fungus promotes active immunisation in ant colonies. *PLoS Biology*, *10*.
- Lacerda, F. G., Della Lucia, T. M. C., Desouza, O., Pereira, O. L., Kasuya, M. C. M., De Souza, L. M. ... De Souza, D. J. (2014). Social interactions between fungus garden and external workers of *Atta sexdens* (Linnaeus) (Hymenoptera: Formicidae). *Italian Journal of Zoology*, *81*, 298–303.
- Lacerda, F. G., Della Lucia, T. M. C., Pereira, O. L., Peternelli, L. A., & Totola, M. R. (2010). Mortality of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae) workers in contact with colony waste from different plant sources. *Bulletin of Entomological Research*, *100*, 99–103.
- Lemaitre, B., & Hoffmann, J. (2007). The host defense of *Drosophila melanogaster*. *Annual Review of Immunology*, *25*, 697–743.
- Loreto, R. G., Elliot, S. L., Freitas, M. L. R., Pereira, T. M., & Hughes, D. P. (2014). Long-term disease dynamics for a specialized parasite of ant societies: a field study. *PLoS One*, *9*, e103516.
- Machado, V., Diehl-Fleig, E., Silva, M. E. D., & Lucchese, M. E. D. P. (1988). Observed reactions in colonies of some species of *Acromyrmex* (Hymenoptera: Formicidae) when inoculated with entomopathogenic fungi. *Ciencia e Cultura (Sao Paulo)*, *40*, 1106–1108.
- Marikovskiy, P. I. (1962). On some features of behaviour of the ant *Formica rufa* L. infected with fungous disease. *Insectes Sociaux*, *9*, 173–179.
- Markaki, M., & Tavernarakis, N. (2010). Modeling human diseases in *Caenorhabditis elegans*. *Biotechnology Journal*, *5*, 1261–1276.
- Mattoso, T. C., Moreira, D. D. O., & Samuels, R. I. (2012). Symbiotic bacteria on the cuticle of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* protect workers from attack by entomopathogenic fungi. *Biology Letters*, *8*, 461–464.
- Maynard Smith, J., & Szathmáry, E. (1995). *The major transitions in evolution* (p. 346). Oxford: Oxford University Press.
- Murdock, C. C., Paaijmans, K. P., Cox-Foster, D., Read, A. F., & Thomas, M. B. (2012). Rethinking vector immunology: the role of environmental temperature in shaping resistance. *Nature Reviews Microbiology*, *10*, 869–876.
- Novak, S., & Cremer, S. (2015). Fungal disease dynamics in insect societies: optimal killing rates and the ambivalent effect of high social interaction rates. *Journal of Theoretical Biology*, *372*, 54–64.
- Oi, D. H., Pereira, R. M., Stimac, J. L., & Wood, L. A. (1994). Field applications of *Beauveria bassiana* for control of the red imported fire ant (Hymenoptera: Formicidae). *Journal of Economic Entomology*, *87*, 623–630.

- Okuno, M., Tsuji, K., Sato, H., & Fujisaki, K. (2012). Plasticity of grooming behavior against entomopathogenic fungus *Metarhizium anisopliae* in the ant *Lasius japonicus*. *Journal of Ethology*, *30*, 23–27.
- Orlofske, S. A., Jadin, R. C., Preston, D. L., & Johnson, P. T. J. (2012). Parasite transmission in complex communities: predators and alternative hosts alter pathogenic infections in amphibians. *Ecology*, *93*, 1247–1253.
- Oulevey, C., Widmer, F., Koelliker, R., & Enkerli, J. (2009). An optimized microsatellite marker set for detection of *Metarhizium anisopliae* genotype diversity on field and regional scales. *Mycological Research*, *113*, 1016–1024.
- Padilla-Guerrero, E. I., Barelli, L., Gonzalez-Hernandez, G. A., Torres-Guzman, J. C., & Bidochka, M. J. (2011). Flexible metabolism in *Metarhizium anisopliae* and *Beauveria bassiana*: role of the glyoxylate cycle during insect pathogenesis. *Microbiology-Sgm*, *157*, 199–208.
- Pereira, R. M., & Stımac, J. L. (1992). Transmission of *Beauveria bassiana* within nests of *Solenopsis invicta* (Hymenoptera: Formicidae) in the laboratory. *Environmental Entomology*, *21*, 1427–1432.
- Pereira, R. M., Stımac, J. L., & Alves, S. B. (1993). Soil antagonism affecting the dose-response of workers of the red imported fire ant, *Solenopsis invicta*, to *Beauveria bassiana* conidia. *Journal of Invertebrate Pathology*, *61*, 156–161.
- Poulin, R. (2010a). The scaling of dose with host body mass and the determinants of success in experimental cercarial infections. *International Journal for Parasitology*, *40*, 371–377.
- Poulin, R. (2010b). The selection of experimental doses and their importance for parasite success in metacercarial infection studies. *Parasitology*, *137*, 889–898.
- Pull, C. D., Hughes, W. O. H., & Brown, M. J. F. (2013). Tolerating an infection: an indirect benefit of co-founding queen associations in the ant *Lasius niger*. *Naturwissenschaften*, *100*, 1125–1136.
- Quevillon, L. E., Hanks, E. M., Bansal, S., & Hughes, D. P. (2015). Social, spatial, and temporal organization in a complex insect society. *Scientific Reports*, *5*.
- Read, A. F. (1994). The evolution of virulence. *Trends in Microbiology*, *2*, 73–76.
- Reber, A., Castella, G., Christe, P., & Chapuisat, M. (2008). Experimentally increased group diversity improves disease resistance in an ant species. *Ecology Letters*, *11*, 682–689.
- Reber, A., & Chapuisat, M. (2012a). Diversity, prevalence and virulence of fungal entomopathogens in colonies of the ant *Formica selysi*. *Insectes Sociaux*, *59*, 231–239.
- Reber, A., & Chapuisat, M. (2012b). No evidence for immune priming in ants exposed to a fungal pathogen. *PLoS One*, *7*.
- Reber, A., Purcell, J., Buechel, S. D., Buri, P., & Chapuisat, M. (2011). The expression and impact of antifungal grooming in ants. *Journal of Evolutionary Biology*, *24*, 954–964.
- Ribeiro, M. M. R., Amaral, K. D., Seide, V. E., Souza, B. M. R., Lucia, T. M. C. D., Kasuya, M. C. M., & de Souza, D. J. (2012). Diversity of fungi associated with *Atta bisphaerica* (Hymenoptera: Formicidae): the activity of *Aspergillus ochraceus* and *Beauveria bassiana*. *Psyche*, *2012*, 1–6.
- Rodrigues, A., Silva, A., Bacci, M., Jr., Forti, L. C., & Pagnocca, F. C. (2010). Filamentous fungi found on foundress queens of leaf-cutting ants (Hymenoptera: Formicidae). *Journal of Applied Entomology*, *134*, 342–345.
- Rosenthal, N., & Brown, S. (2007). The mouse ascending: perspectives for human-disease models. *Nature Cell Biology*, *9*, 993–999.
- Sánchez-penã, S. R., & Thorvilson, H. G. (1992). 2 Fungi infecting red imported fire ant (Hymenoptera: Formicidae) founding queens from Texas. *Southwestern Entomologist*, *17*, 181–182.
- Schmid-Hempel, P. (1998). *Parasites in social insects*. Princeton: Princeton University Press.
- Schmidt, A. M., Linksvayer, T. A., Boomsma, J. J., & Pedersen, J. S. (2011). No benefit in diversity? The effect of genetic variation on survival and disease resistance in a polygynous social insect. *Ecological Entomology*, *36*, 751–759.

- Shimazu, M. (1989). *Metarhizium cylindrospora* chen et guo (Deuteromycotina, Hyphomycetes), the causative agent of an epizootic on *Graptosaltria nigrofusca* Motchulski (Homoptera, Cicadidae). *Applied Entomology and Zoology*, 24, 430–434.
- Silva, M. E. D., & Diehl-Fleig, E. (1988). Evaluation of different strains of entomopathogenic fungi to control the ant *Atta sexdens piriventris* Santschi 1919 (Hymenoptera: Formicidae). *Anais da Sociedade Entomologica do Brasil*, 17, 263–270.
- St. Leger, R. J. S. (2008). Studies on adaptations of *Metarhizium anisopliae* to life in the soil. *Journal of Invertebrate Pathology*, 98, 271–276.
- Stimac, J. L., Alves, S. B., & Camargo, M. T. V. (1987). Susceptibility of *Solenopsis* spp. to different species of entomopathogenic fungi. *Anais da Sociedade Entomologica do Brasil*, 16, 377–388.
- Stimac, J. L., Alves, S. B., & Camargo, M. T. V. (1989). Control of *Solenopsis* spp. (Hymenoptera: Formicidae) with *Beauveria bassiana* Vuill. in laboratory and field conditions. *Anais da Sociedade Entomologica do Brasil*, 18, 95–104.
- Stimac, J. L., Pereira, R. M., Alves, S. B., & Wood, L. A. (1993). Mortality in laboratory colonies of *Solenopsis invicta* (Hymenoptera: Formicidae) treated with *Beauveria bassiana* (Deuteromycetes). *Journal of Economic Entomology*, 86, 1083–1087.
- Tefera, T., & Pringle, K. L. (2003). Effect of exposure method to *Beauveria bassiana* and conidia concentration on mortality, mycosis, and sporulation in cadavers of *Chilo partellus* (Lepidoptera: Pyralidae). *Journal of Invertebrate Pathology*, 84, 90–95.
- Theis, F. J., Ugelvig, L. V., Marr, C., & Cremer, S. (2015). Opposing effects of allogrooming on disease transmission in ant societies. *Philosophical Transactions of the Royal Society B-Biological Sciences*, 370.
- Thrall, P. H., Hochberg, M. E., Burdon, J. J., & Bever, J. D. (2007). Coevolution of symbiotic mutualists and parasites in a community context. *Trends in Ecology & Evolution*, 22, 120–126.
- Townsend, R. J., Glare, T. R., & Willoughby, B. E. (1995). The fungi *Beauveria* spp. cause epizootics in grass grub populations in Waikato. In *48th New Zealand plant protection conference* (Vol. 48, pp. 237–241) (Hastings, New Zealand).
- Tragust, S., Mitteregger, B., Barone, V., Konrad, M., Ugelvig, L. V., & Cremer, S. (2013). Ants disinfect fungus-exposed brood by oral uptake and spread of their poison. *Current Biology*, 23, 76–82.
- Tragust, S., Ugelvig, L. V., Chapuisat, M., Heinze, J., & Cremer, S. (2013). Pupal cocoons affect sanitary brood care and limit fungal infections in ant colonies. *BMC Evolutionary Biology*, 13.
- Tranter, C., Graystock, P., Shaw, C., Lopes, J. F. S., & Hughes, W. O. H. (2014). Sanitizing the fortress: protection of ant brood and nest material by worker antibiotics. *Behavioral Ecology and Sociobiology*, 68, 499–507.
- Tranter, C., LeFevre, L., Evison, S. E. F., & Hughes, W. O. H. (2015). Threat detection: contextual recognition and response to parasites by ants. *Behavioral Ecology*, 26, 396–405.
- Vega, F. E. (2008). Insect pathology and fungal endophytes. *Journal of Invertebrate Pathology*, 98, 277–279.
- Vega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S. ... Roy, H. E. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, 2, 149–159.
- Walker, T. N., & Hughes, W. O. H. (2011). Arboreality and the evolution of disease resistance in ants. *Ecological Entomology*, 36, 588–595.