

Arboreality and the evolution of disease resistance in ants

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Abstract. 1. Parasites are an important selective force for almost all organisms and drive the evolution by hosts of defence mechanisms that are energetically costly. The strength of parasitism will vary between host species according to their specific ecology and life history, and so the optimal investment in costly resistance mechanisms is also likely to vary between host species with differing ecologies.

2. Parasites are particularly important for social species such as ants, but very little is known about the strength of selection in different species. It has been suggested that, because arboreality reduces exposure to soil-borne fungal pathogens, arboreal ant species may invest less in disease resistance. However, testing hypotheses such as this requires data on disease resistance in multiple species, and such studies have not previously been attempted.

3. Here we examine the arboreality hypothesis by comparing the disease resistance of seven Neotropical ant species with different degrees of arboreality. We exposed ants to controlled doses of the generalist, virulent fungal parasite, *Metarhizium anisopliae* (Metchnikoff) Sorokin. We then monitored survival, parasite sporulation, and the anti-fungal grooming response of the ants.

4. Contrary to the hypothesis, we found that arboreal species were not less resistant to *M. anisopliae* than species that were ground-dwelling, and that the species that inhabited both arboreal and ground habitats had the greatest resistance. Surprisingly, the most resistant species was one that lacked the antibiotic-producing metapleural gland, previously considered the lynchpin of disease resistance in ants.

5. The results suggest that it may be the diversity of parasites encountered that is the greatest selection pressure. Further experimental studies with other parasites are needed to confirm the generality of the results, and similar comparative studies of other taxa are needed to understand the relationship between host ecology and the evolution of disease resistance.

Key words. Grooming, life history, metapleural gland, parasite, social insect.

Introduction

Parasitism is of great evolutionary significance because of the profound effects that it has on host life history, sexual selection, and population dynamics (Tomkins & Begon, 1999; Hudson *et al.*, 2001). Hosts have thus evolved a diverse array of adaptations to prevent infection and, when necessary, minimise the damage that it causes. These mechanisms vary

between taxa, from the sophisticated physiological immunity of vertebrates, to behaviours that reduce the likelihood of parasite infection and proliferation (Sheldon & Verhulst, 1996; Boomsma *et al.*, 2005; Schmid-Hempel, 2005). However, all of these mechanisms are costly and thus traded-off against other traits. Consequently, species are likely to express different levels of resistance because of differences in their ecologies, and thus the amount of parasite exposure that they receive.

Parasite transmission is often density-dependent (McCallum *et al.*, 2001), and group living has therefore been considered to be associated with elevated pathogenic risks (Alexander, 1974). This is exacerbated in social insects because their colonies consist of large numbers of highly related individuals

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interacting in close proximity. Social insects such as ants have accordingly evolved an array of sophisticated defence mechanisms to counter the costs associated with group living (Boomsma *et al.*, 2005; Schlüns & Crozier, 2009), and these may in fact bestow a net benefit in resistance to ant colonies (Rosengaus *et al.*, 1998; Hughes *et al.*, 2002; Cremer *et al.*, 2007). These mechanisms consist of individual- and colony-level responses, which act to prevent infection and, if this fails, restrict the damage it causes and minimise its subsequent transmission to other members of the group. The metapleural gland, which is unique to ants, is considered to be of particular significance in ant disease resistance (Hölldobler & Wilson, 1990). It produces a secretion that contains effective antimicrobial compounds (Ortius-Lechner *et al.*, 2000; Bot *et al.*, 2002), the production of which incurs a significant metabolic cost (Poulsen *et al.*, 2002). Ants also prevent infections by using self- or allogrooming to remove parasites from body surfaces or actively spread antibiotic secretions (Hughes *et al.*, 2002; Fernandez-Marin *et al.*, 2006). In addition, the cuticle itself acts as an important barrier, the effectiveness of which can depend on the level of melanisation (Reeson *et al.*, 1998; Barnes & Siva-Jothy, 2000). When these measures fail to prevent infection, the insect immune system works to minimise its effect (Rosengaus *et al.*, 1999, 2007; Rolff & Siva-Jothy, 2003; Baer *et al.*, 2005; Bocher *et al.*, 2007). Colony-level responses, or 'social immunity' in the broad sense (Cremer *et al.*, 2007), usually consist of behavioural interactions between individuals. Allogrooming between nestmates is a particularly effective mechanism at preventing the introduction and spread of parasites in a colony (Rosengaus *et al.*, 1998; Hughes *et al.*, 2002; Traniello *et al.*, 2002; Yanagawa *et al.*, 2008; Morelos-Juárez *et al.*, 2010), and may be adaptive, with groups developing a more rapid response following repeated exposure to the same pathogen (Walker & Hughes, 2009).

All defence mechanisms are, to varying extents, energetically costly and it therefore seems likely that if selection pressures from parasites are low it will be adaptive for species to invest less in disease resistance. In particular, arboreality has been suggested to allow such a reduced investment in resistance (Johnson *et al.*, 2003; Boomsma *et al.*, 2005). Soil commonly contains high numbers of entomopathogenic fungal spores whereas the load on vegetation is orders of magnitude lower (Rath *et al.*, 1992; Keller *et al.*, 2003; Hughes *et al.*, 2004b; Meyling & Eilenberg, 2006; Pilz *et al.*, 2008; Reay *et al.*, 2008; Tuininga *et al.*, 2009; Griffiths & Hughes, 2010). Arboreality will thus involve reduced exposure to fungal parasites and arboreal species may as a result evolve to invest less in costly resistance to them. Indeed, the secondary loss of metapleural glands in some species has been attributed to this (Johnson *et al.*, 2003).

In this study, we compare the disease resistance of seven Neotropical ant species with varying nesting strategies and degrees of arboreality. We do this at a study site in Panama for which parasite pressure from fungi appears to be substantially lower in the arboreal than ground habitats (Hughes *et al.*, 2004b; Griffiths & Hughes, 2010). The spectrum of natural parasites of these ant species is, as with almost all social insect species, unknown. We therefore used the cosmopolitan

Metarhizium anisopliae (Metschnikoff Sorokin) (Deuteromycotina: Hyphomycetes) as the experimental parasite. This is a virulent, generalist entomopathogenic fungus that has been reported to infect at least 18 ant species from the Myrmicinae and Formicinae subfamilies, including ants close to the field site of this study, is capable of causing fatal epizootics within colonies, but can also be resisted by ants, depending on their species and group composition amongst other things (Boucias & Pendland, 1998; Schmid-Hempel, 1998; Jaccoud *et al.*, 1999; Hughes *et al.*, 2002, 2004a; Poulsen *et al.*, 2002, 2006; Lopez & Orduz, 2003; Sumner *et al.*, 2003; Hughes & Boomsma, 2004; Chapuisat *et al.*, 2007; Ugelvig & Cremer, 2007; Reber *et al.*, 2008, 2011; Castilho *et al.*, 2010; Ugelvig *et al.*, 2010). It thus represents a natural threat to the ant species studied, but one which, because of its very wide range of insect hosts, will not have coevolved to exploit a particular species. *Metarhizium* is the asexual anamorph form of the teleomorph ascomycete *Metacordyceps*, which is also a parasite of ants and other arthropods (Stensrud *et al.*, 2005; Sung *et al.*, 2007). *Metarhizium* infects through the host's cuticle, with death typically occurring 2–10 days after infection due to the utilisation of host nutritional resources, direct damage to organs from hyphal proliferation and the effects of toxic secondary metabolites (Boucias & Pendland, 1998). The fungus then produces characteristic conidia and conidiophores on the external surface of the host insect, making it an easily recognisable, semelparous, obligate killer. The biology of infection through the cuticle, hyphal proliferation within the host insect and semelparous spore production after host death, is therefore similar to some other fungal entomopathogens of ants, such as *Beauveria*, *Aspergillus*, and *Ophiocordyceps* (Boucias & Pendland, 1998; Meyling & Eilenberg, 2007; Hughes *et al.*, 2009; Evans *et al.*, 2011), although *Metarhizium* is by no means a proxy for them. *Metarhizium* is most common in soil, being particularly abundant in the rhizosphere where it can utilise root exudates, but is also found on vegetation (Sosa-Gómez *et al.*, 2001; Meyling & Eilenberg, 2007; St Leger, 2008; Vega *et al.*, 2009; Bruck, 2010). It will therefore be encountered by ants during scouting, foraging, waste management and particularly during nest excavation. Here we test the hypothesis that arboreal ant species have a lower ability to resist *M. anisopliae* than ground-dwelling species. Furthermore, we determine whether *Camponotus sericeiventris* Guérin-Méneville, an arboreal species that does not possess metapleural glands (Hölldobler & Engel-Siegel, 1984), has particularly low resistance or compensates for the lack of a metapleural gland with alternative disease resistance mechanisms.

Materials and methods

All fieldwork was carried out on Barro Colorado Island, Panama (79°51'W, 9°09'N), between May and July 2009. The density of *Metarhizium* alone in soil near this location is 1×10^3 to 5×10^4 CFU g⁻¹ (Hughes *et al.*, 2004b). In contrast, the density of all microbes on canopy vegetation on Barro Colorado Island is only 12–560 CFU cm⁻² (Griffiths & Hughes, 2010), while sporefall in the understorey is 15 CFU cm⁻² h⁻¹

(Arnold & Herre, 2003), similar to other tropical forests (Gilbert & Reynolds, 2005). The site's arboreal habitat is therefore associated with substantially less exposure to microbes than the ground. We examined seven ant species which ranged from species that both nested and foraged exclusively in trees, to those that nested in the soil and foraged predominantly on the ground (in order of decreasing arboreality: *Azteca velox* Forel, *Cephalotes atratus* Linnaeus, *Camponotus sericeiventris*, *Ectatomma tuberculatum* Olivier, *Atta colombica* Guérin-Méneville, *Eciton burchellii* Westwood, and *Ectatomma ruidum* Roger). All seven species foraged to a greater or lesser extent on understorey vegetation, but differed in whether they foraged predominantly, occasionally or only very rarely on the ground. All seven species foraged during the day. Individuals were collected from the nest entrances to reduce their age variation and were experimented with on the day of sampling. For experimental trials, ants were kept in plastic pots (diameter: 2 cm; height: 10 cm) at room temperature ($\sim 28^\circ\text{C}$) with an *ad libitum* supply of water and 10% sucrose solution.

Metarhizium anisopliae conidia suspensions were generated from freshly sporulating plates of *M. anisopliae* strain KVL 02-73 (isolated from soil collected in nearby Gamboa, Panama; Hughes *et al.*, 2004b) in a 0.05% Triton-X solution. Focal individuals, marked with a paint spot for ease of identification, were treated with a size-specific dose (Table 1) of either a 0.05% Triton-X control solution or one of two batches of a *M. anisopliae* conidia suspension (batch 1: 1.56×10^6 conidia ml^{-1} ; batch 2: 1.64×10^6 conidia ml^{-1}). No significant differences were found between the batches in survival or behaviour ($P > 0.05$ in all cases), so the data were pooled. Size-specific doses were calculated using the mean body lengths of five ants from each species, which generated dose volumes of between 0.2 and 0.5 μl and between ~ 320 and ~ 800 *Metarhizium* conidia (Table 1). Our aim was to expose the ants to a significant, but potentially resistible, parasite challenge, rather than to necessarily simulate natural exposure levels, although the 1×10^3 to 5×10^4 density of *Metarhizium* conidia per g of soil means the dose may not be unrealistic (Hughes *et al.*, 2004b). Body length was measured to the nearest 0.5 mm using dead ants placed flat on their ventral side and was defined as the length of an ant from the anterior of the head to the tip of the gaster. For treatment, ants were restrained using sterilised forceps and doses were applied to the dorsal surface of the thorax using a micropipette.

Table 1. Doses for the seven experimental ant species controlled for body size (body length).

Ant species	Mean ant size (mm)	Treatment dose
<i>Azteca velox</i>	4.67	0.2 μl ; ~ 320 conidia
<i>Cephalotes atratus</i>	12.00	0.5 μl ; ~ 800 conidia
<i>Camponotus sericeiventris</i>	12.60	0.5 μl ; ~ 800 conidia
<i>Ectatomma tuberculatum</i>	9.92	0.4 μl ; ~ 640 conidia
<i>Atta colombica</i>	7.42	0.3 μl ; ~ 480 conidia
<i>Eciton burchellii</i>	9.92	0.4 μl ; ~ 640 conidia
<i>Ectatomma ruidum</i>	8.17	0.3 μl ; ~ 480 conidia

Experiment 1: individual survival following parasite exposure

To compare the effects of *M. anisopliae* infection on the individual survival of ants of different species, individuals were treated as described above and placed in isolation. Four ants were used from each of four colonies of each species for each treatment, resulting in 16 ants per treatment per species (see below for details of how this was handled statistically). Mortality was recorded daily for 10 days and dead ants were removed from the experimental pots on the day of death. Cadavers were surface-sterilised (Lacey, 1997), and kept in a Petri dish on damp filter paper for 14 days to monitor for the emergence of *M. anisopliae* conidiophores and conidia. The number of ants producing *M. anisopliae* conidia and the time taken for this to occur was recorded. In addition, the number of *M. anisopliae* conidia present on fully sporulating cadavers (i.e. dead ants on which *M. anisopliae* conidia had been present for 10 days or longer; Boucias & Pendland, 1998) was quantified. Three cadavers from each species, selected randomly, were placed into individual vials containing 2 ml of 0.05% Triton-X solution and vortexed for 1 min to remove conidia from the cuticle (Hughes *et al.*, 2004a). The concentrations of conidia present were then estimated for each species using a haemocytometer.

Experiment 2: behavioural responses to parasite exposure

The extent to which behavioural resistance mechanisms occur in different species in response to treatment with *M. anisopliae* was examined. Focal individuals were treated with an *M. anisopliae* conidia suspension or control solution as described above, placed either in isolation or in a group with three untreated nest-mates and observed for 30 s periods at 0, 1, 2, 4, and 8 min after treatment, as most grooming occurs during this time period (Walker & Hughes, 2009; Graystock & Hughes, in press). The frequencies of antennal self-grooming, tibial self-grooming, and abdominal self-grooming were recorded during the observation periods. The frequencies of these behaviours were combined to derive the total self-grooming frequency. Rubbing of the metapleural glands (Fernandez-Marin *et al.*, 2006) was not observed, possibly because the conidia were applied in solution. The numbers of interactions between untreated ants and focal individuals, specifically antennal inspection of the focal ant, reciprocal antennation between the focal ant and nest-mate, allogrooming of the focal ant and aggression towards the focal ant, were also recorded for individuals in groups. Behaviours that occurred for more than a 5 s period were split into multiple events, the number of which depended on the total length of the behaviour (e.g. 20 s of allogrooming was recorded as four allogrooming events). Two trials were performed per colony of each species for each treatment, resulting in eight replicates per species for each treatment and group type.

Statistical analysis

The effects of species, treatment, and colony on survival and the time taken for sporulation (the first appearance of conidia)

were analysed using Cox proportional-hazards regression models. The frequencies of self-grooming and allogrooming across all the observation periods were totalled and compared between species and treatments using a Generalized Linear Mixed Model (GLMM), with colony included as a random factor. A GLMM was similarly used to examine the numbers of conidia present on sporulating cadavers 14 days after death. All analyses were conducted in SPSS 19.0 with non-significant ($P > 0.05$) terms removed in a stepwise manner until the minimum adequate model was obtained. Each species was categorised according to its nest location (arboreal or ground), foraging location (arboreal, intermediate or ground-foraging), and cuticular melanisation (black, medium-brown, or light-brown). The relationships across species between these traits, survival, and grooming were examined using Spearman's rank correlations.

Results

Experiment 1: individual survival following parasite exposure

The species differed significantly in their mortality rates (Wald = 61.1, d.f. = 6, $P < 0.001$) and ants treated with *M. anisopliae* had significantly lower survival than those treated with the control solution (Wald = 48.8, d.f. = 1, $P < 0.001$; Fig. 1). Most species had a high survival when treated with the control solution, although *Az. velox* and *Eci. burchellii* survived less well, losing 44% and 63% of ants over the 10-day period respectively (Fig. 1a). Both of these species, as well as *A. colombica* and *Ect. ruidum* (which had high control survival rates), were highly susceptible to *M. anisopliae* with 80–100% of ants having died after the 10 day experimental period (Fig. 1b). The survival of *Ca. sericeiventris* and *Ect. tuberculatum*, in contrast, was not greatly affected by exposure to *M. anisopliae* with most ants resisting the parasite. The survival of *Ce. atratus* was also relatively high, with more than 63% of ants surviving exposure.

No cadavers of ants treated with control solution sporulated with *Metarhizium*, or any other parasite, whereas many of the cadavers of ants treated with *Metarhizium* sporulated with the characteristic conidia and conidiophores of *Metarhizium*. The time taken for infected cadavers to produce *M. anisopliae* conidia varied significantly between species (Wald = 13.5, d.f. = 6, $P = 0.036$). Most species produced a similar number of sporulating cadavers over a 14-day period with the exception of *Ca. sericeiventris*, which produced no sporulating cadavers, and *Ce. atratus*, for which only 50% of dead ants were sporulating after 14 days (Fig. 2a). *Azteca velox* cadavers sporulated at a slower rate than those of other species, reaching 90% of dead ants after 8 days compared to between 80% and 100% after 4 days for other species (Fig. 2a). Only *Ect. tuberculatum* and *Ect. ruidum* had 100% of cadavers sporulating within the 14-day period, and this occurred more rapidly for *Ect. tuberculatum*, which reached 100% after 2 days compared to 7 days for *Ect. ruidum* (Fig. 2a). The number of conidia produced by sporulating cadavers differed significantly between species ($F_{6,14} = 15.2$, $P < 0.001$; Fig. 2b). *Ectatomma tuberculatum* cadavers produced a larger number of *M. anisopliae* conidia than other species (Fig. 2b). Furthermore, *At.*

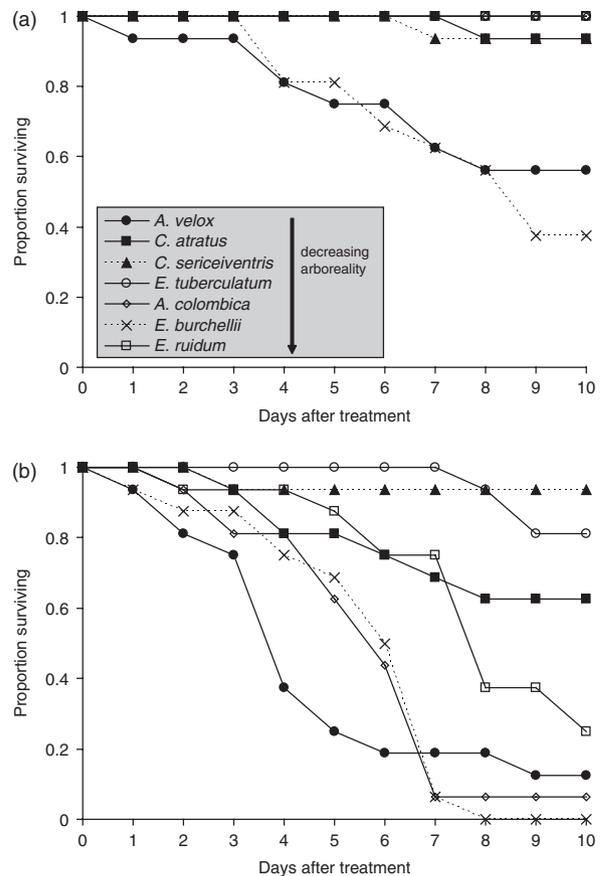


Fig. 1. Survival of ants that were treated with (a) a control solution or (b) a *Metarhizium anisopliae* conidia suspension and placed in isolation for 10 days ($n = 16$). Ants were from seven species that varied in their extent of arboreality (from top to bottom in key). Note that survival of five of the species was very high in (a) and lines thus overlap.

colombica, *Eci. burchellii*, and *Ect. ruidum* cadavers appeared to produce more conidia than *Az. velox*, *Ce. atratus*, and *Ca. sericeiventris* cadavers (Fig. 2b).

Experiment 2: behavioural responses to parasite exposure

The total frequency of self-grooming differed significantly between species ($F_{6,210} = 8.38$, $P < 0.001$), but not between ants exposed to *Metarhizium* and the control solution ($F_{1,210} = 0.118$, $P = 0.731$), with the interaction between species and treatment being marginally non-significant ($F_{6,210} = 1.96$, $P = 0.073$). *Camponotus sericeiventris* consistently self-groomed more than individuals of other species (Fig. 3). *Cephalotes atratus* appeared to self-groom more after treatment with *M. anisopliae* conidia, while *Ect. ruidum* treated with *M. anisopliae* self-groomed twice as frequently in isolation than in a group (Fig. 3). The number of allogrooming events performed by untreated nest-mates on treated individuals did not differ significantly between species ($F_{6,216} = 0.414$, $P = 0.87$) or treatments ($F_{1,216} = 0.135$, $P =$

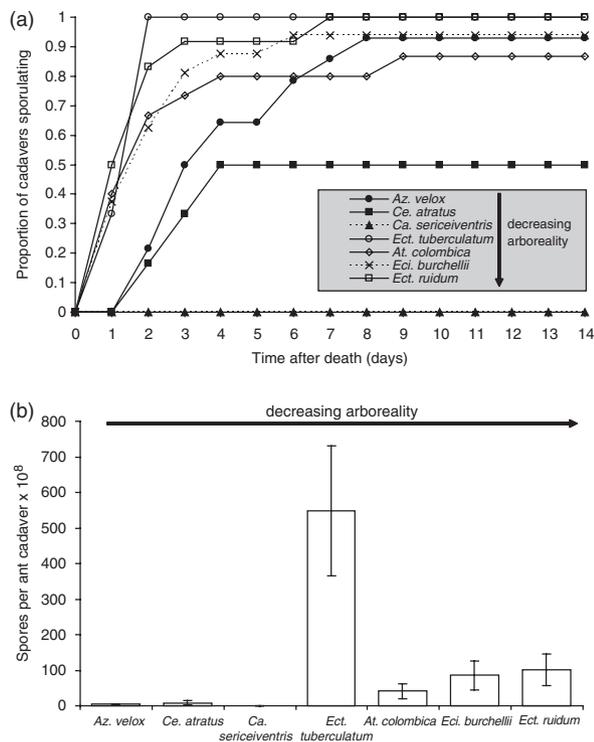


Fig. 2. Production of *Metarhizium anisopliae* conidia from ant cadavers. (a) Proportion of ant cadavers producing conidia over a 14-day period following death. (b) Mean (\pm SE) number of conidia present per sporulating cadaver. Ants were from seven species that varied in their extent of arboreality [from top to bottom in key for (a) and from left to right in (b)].

0.714). *Ectatomma tuberculatum* and *At. colombica* ants were allogroomed at a lower frequency than other species (Fig. 3c). *Camponotus sericeiventris* individuals treated with *M. anisopliae* were allogroomed more frequently than ants treated with the control solution; the reverse is true, however, for *Az. velox* and *Ce. atratus* ants, which were allogroomed less frequently when treated with *M. anisopliae* (Fig. 3c).

Across species relationships

Although species therefore varied significantly in survival, there were no significant correlations between this and nest location ($\rho = 0.15$, $n = 7$, $P = 0.749$), foraging location ($\rho = 0.216$, $n = 7$, $P = 0.642$) or cuticular melanisation ($\rho = 0.392$, $n = 7$, $P = 0.384$).

Discussion

Survival after *M. anisopliae* exposure varied significantly between species, demonstrating that they differed in their ability to resist the parasite (see Table 2 for a summary of the results). Although our sample size in each species was limited, the experiment therefore had sufficient statistical power to detect differences between species, but these differences were

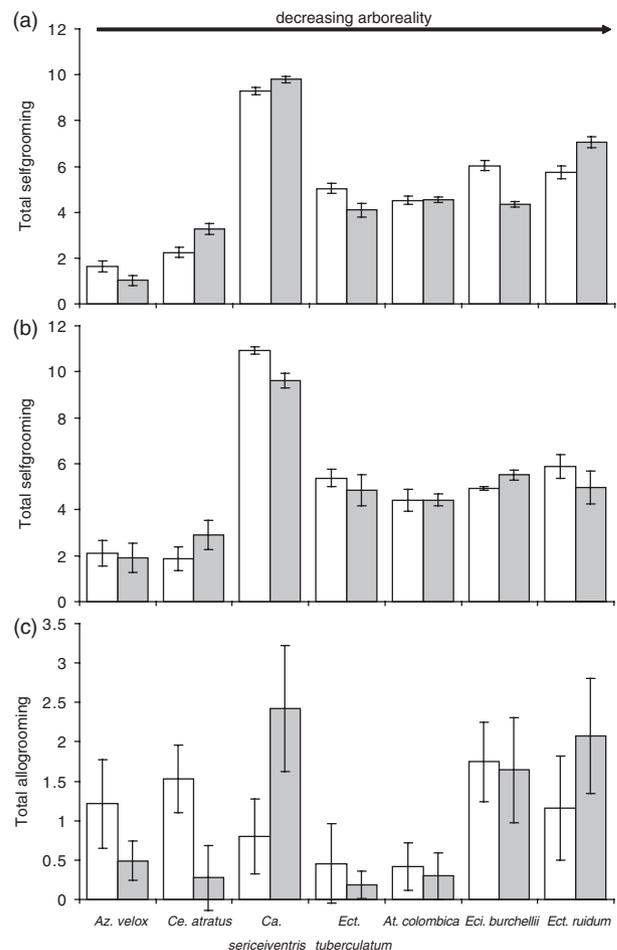


Fig. 3. Mean (\pm SE) number of grooming behaviours of focal ants following treatment with either a control solution (open bars) or a *Metarhizium anisopliae* suspension (shaded bars), for (a) self-grooming in isolation, (b) self-grooming with three untreated nest-mates, and (c) allogrooming with three untreated nest-mates. Species are presented in order of decreasing arboreality, as indicated by arrow.

not in the directions predicted. *Camponotus sericeiventris*, an arboreal nesting, and arboreal and ground foraging species, was the most successful at resisting *M. anisopliae* infection, with 90% of ants still surviving at the end of the 10 day experimental period and none of its cadavers sporulating with the parasite. *Atta colombica*, *Ect. ruidum*, *Az. velox*, and *Eci. burchellii* were most susceptible to the parasite. These species had very low survival and, while control mortality was also high in the latter two of these species, the high proportions of cadavers sporulating with *Metarhizium* indicate it was the primary cause of death. It is notable that *Ca. sericeiventris* showed much higher rates of self-grooming and allogrooming than the other species studied, while *Az. velox* performed self-grooming behaviour at very low frequencies. This trend is similar for the remaining species, *At. colombica*, *Ce. atratus*, *Eci. burchellii*, *Ect. ruidum*, and *Ect. tuberculatum*, all of which performed self-grooming behaviours and nest-mate interactions at lower frequencies than *Ca. sericeiventris* and were less

Table 2. Summary of the nesting and foraging biology of the seven ant species studied (varying in degree of arboreality from top to bottom), together with the results of the disease resistance experiments [median survival time (days) after exposure to *M. anisopliae*, with the relative hazard ratio in parentheses]. Levels of self-grooming (SG) and allogrooming (AG) observed during the trials, presence of the metapleural gland (MG), and cuticular melanisation are also indicated.

Ant species	Nesting strategy	Foraging strategy	MG	Cuticle	Resistance to		
					SG	AG	<i>M. anisopliae</i>
<i>Azteca velox</i>	Arboreal (tree cavities/carton; Longino, 1991)	Arboreal (Longino, 1991)	Yes	Brown	1	0.5	4 (1.64)
<i>Cephalotes atratus</i>	Arboreal (tree cavities; Corn, 1980)	Arboreal and ground (Corn, 1980)	Yes	Black	3	0.3	10 (1.2)
<i>Camponotus sericeiventris</i>	Arboreal (tree cavities; Yamamoto & Del-Claro, 2008)	Arboreal and ground (Yamamoto & Del-Claro, 2008)	No	Black	10	2.4	10 (1.02)
<i>Ectatomma tuberculatum</i>	Ground (Wheeler, 1986)	Arboreal (Wheeler, 1986)	Yes	Light brown	4	0.2	10 (1.09)
<i>Atta colombica</i>	Ground (Hölldobler & Wilson, 1990)	Ground and arboreal (Hölldobler & Wilson, 1990)	Yes	Light brown	5	0.3	6 (1.72)
<i>Eciton burchellii</i>	Ground (bivouacs of clustered ants) (Kronauer <i>et al.</i> , 2007)	Ground (Couzin & Franks, 2003)	Yes	Brown	4	1.6	6.5 (1.38)
<i>Ectatomma ruidum</i>	Ground (Lachaud, 1985)	Ground (Lachaud, 1985)	Yes	Brown	7	2.1	8 (1.26)

successful at resisting *M. anisopliae* infection (this is most obviously the case for *At. colombica*, *Eci. burchellii*, and *Ect. ruidum*). Such self-grooming and allogrooming has previously been shown to be effective at removing fungal spores and reducing the effects of exposure (Rosengaus *et al.*, 1998; Hughes *et al.*, 2002; Yanagawa *et al.*, 2008). The grooming occurred at similar frequencies for ants treated with the control solution as those treated with *Metarhizium*, indicating that it is not stimulated by specific recognition of the parasite but is rather a general response to contamination of the cuticle with a foreign substance. Specific responses, such as metapleural gland grooming, may be more likely to be stimulated by dry conidia of fungal parasites.

One of the most interesting points that emerge from this is that *Ca. sericeiventris*, the only species studied that does not possess metapleural glands, was the most successful at resisting *M. anisopliae* infection. This gland has, until now, been considered as the lynchpin of ant disease resistance, and was thought to be the main mechanism by which ants offset the cost of group living on disease resistance (Hölldobler & Wilson, 1990; Boomsma *et al.*, 2005). The high survival of *Ca. sericeiventris* without metapleural glands shows that this is not necessarily the case, and that this species may compensate for the absence of the gland by using alternative, but at least equally effective mechanisms, of which grooming appears likely to be a major component.

The results presented here demonstrate that species with varying ecologies differ in their ability to resist *M. anisopliae* infection (Table 2), but that there is no clear correlation with arboreality. Although the only entirely arboreal species, *Az. velox*, was particularly susceptible to *M. anisopliae* infection, it appears that this was little to do with arboreality *per se*. Most notably, the three species that live both arboreally and terrestrially (*Ca. sericeiventris*, *Ce. atratus*, and *Ect. tuberculatum*) were more successful at resisting *M. anisopliae* infection than the other species studied, suggesting that they have evolved greater defences against disease than entirely arboreal or entirely terrestrial species. These heightened defences may be in the form of grooming behaviour, or also the

physiological immune response or the composition of antibiotic secretions. It seems likely that the pathogen species will vary between arboreal and terrestrial habitats, in which case the heightened resistance of the species that move between the canopy and the forest floor may be an adaptation to resist a more diverse pathogenic risk. In addition, all the species studied foraged in the understorey, which has a higher microbial load than vegetation in the canopy, although this load is still much less than on the ground (Arnold & Herre, 2003; Hughes *et al.*, 2004b; Gilbert & Reynolds, 2005; Griffiths & Hughes, 2010). The study was limited to only seven ant species and similar data are needed from many more to allow a rigorous analysis including phylogenetic constraints. The study was also limited to a single, generalist parasite and experiments with other natural parasites of the relevant ant species are needed to confirm the generality of the results. Investigations of the *Ophiocordyceps* species which appear to be specialised pathogens of many arboreal *Camponotus* species would be particularly interesting (Hughes *et al.*, 2009; Pontopidan *et al.*, 2009; Evans *et al.*, 2011). However, the results do suggest that arboreality is not associated with the clear-cut effect that might have been predicted (Johnson *et al.*, 2003; Boomsma *et al.*, 2005).

In conclusion, the metapleural gland, which until now has been considered to be the defining feature of disease resistance in ants (Boomsma *et al.*, 2005; Fernandez-Marin *et al.*, 2006), has been shown here to be effectively replaced in at least one ant species, possibly by behavioural resistance mechanisms. Since disease resistance mechanisms are thought to be a major factor in the ecological success of ants and are energetically costly, a number of alternative mechanisms may be employed by species according to their particular threats and life histories. The results do not support arboreal species having reduced investment in disease resistance and it may instead be the diversity of parasites to which a species is exposed that provides the greatest selection pressure. Similar comparative studies of other taxa are needed to improve our understanding of how variation in ecology affects the selection pressure exerted by parasites on their hosts.

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