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Disease resistance in a weaver ant, *Polyrhachis dives*, and the role of antibiotic-producing glands

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Abstract Parasites represent one of the main threats to all organisms and are likely to be particularly significant for social animals because of the increased potential for intragroup transmission. Social animals must therefore have effective resistance mechanisms against parasites and one of the most important components of disease resistance in ants is thought to be the antibiotic-producing metapleural gland. This gland is ancestral in ants, but has been lost secondarily in a small number of species. It is unknown whether these evolutionary losses are due to a reduction in parasite pressure or the replacement of the gland's function with other resistance mechanisms. Here we used the generalist entomopathogenic fungus *Metarhizium* to compare the disease resistance of a species of a weaver ant, *Polyrhachis dives*, which has lost the metapleural gland, with that of the well-studied leaf-cutting ant *Acromyrmex echinatior* and two other ant species, *Myrmica ruginodis* and *Formica fusca*, all of which have metapleural glands. The *P. dives* weaver ants had intermediate resistance when kept individually, and similar resistance to *A. echinatior* leaf-cutting ants when kept in groups, suggesting that the loss of the metapleural gland has not resulted in weaver ants having reduced disease resistance. *P. dives* weaver ants self-groomed at a significantly higher rate than the other ants examined and apparently use their venom for resistance, as they had reduced resistance when their venom gland was blocked and the venom was shown in vitro to prevent the germination of fungal spores. Unexpectedly, the leaf-cutting ant *A. echinatior* also had reduced resistance to *Metarhizium* when its venom gland was blocked. It therefore appears that the evolutionary loss of the metapleural gland does not result in reduced disease resistance in *P. dives* weaver ants, and that this at least in part may be due to the ants having antimicrobial venom and high self-grooming rates. The results therefore emphasise the importance of multiple, complementary mechanisms in the disease resistance of ant societies.

Keywords Parasite · Grooming · Metapleural gland · Social insect · Venom gland · Weaver ant

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Introduction

The social lifestyle of group-living animals results in many benefits but is also associated with significant costs, notably from parasites (Kraus and Ruxton 2002). Sociality may result in greater intragroup transmission of parasites due to the high density and interaction rate of individuals within a group (Alexander 1974; Wilson et al. 2003). The pressure from parasites may be particularly significant in social insects because individuals within their groups are highly related, making it more likely that a parasite that has entered the colony will be able to transmit successfully to further individuals and adapt to the particular genetic make-up of the colony (Hamilton 1987; Sherman et al. 1988; Hughes and Boomsma 2006). Social insects cope with parasites using a suite of sophisticated defence mechanisms (Schmid-Hempel 1998; Boomsma et al. 2005). Individuals have the first-line defences of behavioural avoidance, self-grooming and antimicrobial secretions to prevent infection occurring (Rosengaus et al. 1999a, 2000; Hughes et al.

2002; Poulsen et al. 2002; Yanagawa et al. 2008), and the second-line defence of the physiological immune response to minimise the impact of infections (Rosengaus et al. 1999b; Baer et al. 2005; Bocher et al. 2007; Schlüns and Crozier 2009). Social insects also have group-level defences such as division of labour, waste management and social immunity, which in the broad sense involves allogrooming and the social upregulation of defence mechanisms (Rosengaus et al. 1998; Hart and Ratnieks 2001; Hughes et al. 2002; Traniello et al. 2002; Ugelvig and Cremer 2007; Castella et al. 2008; Walker and Hughes 2009; Ugelvig et al. 2010; Waddington and Hughes 2010).

One of the most important components of disease resistance in ants has long been considered to be the metapleural gland (Hölldobler and Wilson 1990; Boomsma et al. 2005). This gland is ancestral in ants and is thought to have the exclusive function of producing an antimicrobial secretion in almost all species (Hölldobler and Engel-Siegel 1984; Bolton 1995; Schlüns and Crozier 2009). The morphology is very similar across ants, with the glands occurring as a pair at the posterolaterial corners of the thorax and the gland reservoirs often being visible externally as a swelling (bulla) in the cuticle. The secretion from the metapleural gland spreads passively out over the cuticle (Bot et al. 2001), but is also actively groomed over the cuticle in at least some species (Fernandez-Marin et al. 2006). The secretions of the metapleural gland from a broad range of ant species have been shown to have antimicrobial properties in vitro (Beattie et al. 1986; Veal et al. 1992; Mackintosh et al. 1995; Bot et al. 2002). The importance of the gland in disease resistance was conclusively demonstrated by Poulsen et al. (2002), who showed that *Acromyrmex* leaf-cutting ants with non-functional glands were far more susceptible to a parasite than ants with functioning glands. The gland carries a significant energetic cost to *Acromyrmex* workers, further indicating its importance (Poulsen et al. 2002). Accordingly, the relative size of the gland differs between the various castes of ants within a colony according to their need or role in disease resistance (Angus et al. 1993; Bot and Boomsma 1996; De Souza et al. 2006; Poulsen et al. 2006; Hughes et al. 2010), and male ants in most ant species have no metapleural gland at all because they are short-lived and protected by workers (Hölldobler and Wilson 1990; Mackintosh et al. 1999). Further comparative evidence of the importance of the gland comes from the leaf-cutting ants which have evolved larger metapleural glands than their closest relatives due to a change in parasite pressure (Hughes et al. 2008), and ant species which have a socially parasitic lifestyle having reduced metapleural glands due to being protected by the workers of their host ant species (Brown 1968; Sumner et al. 2003; Yek and Mueller 2011).

However, a small number of ant species that are not social parasites have also lost the metapleural gland. All of these are within the subfamily Formicinae, with the metapleural gland having been lost on one occasion in *Oecophylla* (tribe Oecophyllini), and on one or more occasions in *Camponotus* and *Polyrhachis* (tribe Camponotini; Johnson et al. 2003). Given the generally accepted importance of the metapleural gland in ant disease resistance, the evolutionary loss of the gland in these species is intriguing. Several hypotheses suggest themselves. Firstly, many of the species without a metapleural gland are arboreal and so have a reduced exposure to the parasites (particularly fungi and nematodes) which abound in soil (Hölldobler and Engel-Siegel 1984; Boomsma et al. 2005). Secondly, some of the species without a metapleural gland (*Oecophylla*, some *Polyrhachis* and some *Camponotus*) are weaver ants, making their nest to a varying extent out of larval silk (Hölldobler and Wilson 1990). It seems logical that a silken nest may be more hygienic than one built in the soil for example, and it is also possible that silk itself may have antimicrobial properties. Species adopting an arboreal lifestyle or weaving their nests out of silk may therefore be less exposed to parasites, have less need for the metapleural gland and accordingly have lost it to save on the energetic cost (Johnson et al. 2003). However, many species of *Camponotus* and *Polyrhachis* which lack a metapleural gland do not weave their nests out of silk and are not arboreal (Robson and Kohout 2007), and the vast majority of arboreal ant species have retained the metapleural gland. In addition, silk of the weaver ant *Polyrhachis dives* is in fact heavily contaminated with a facultative fungal parasite (Fountain and Hughes 2011). Finally, it may be that the species without a metapleural gland have replaced its function with alternative defences. The Formicinae subfamily, including all of the species without a metapleural gland, are characterised by having an enlarged venom gland which excretes through an acidopore venom made up predominantly (up to 60%) of formic acid (Blum 1992). It is not known if this venom is effective against the parasites that commonly attack ants, but the high acidity of formic acid (3.77 pK_a) makes it plausible.

Here we investigate the disease resistance of a silk-nesting weaver ant, *P. dives*, which is one of the species which has lost the metapleural gland and which would therefore be hypothesised to have reduced resistance. *P. dives* is an arboreal nesting species, which constructs its nests out of sheets of silk and can have polydomous colonies with little intercolony aggression (Robson and Kohout 2005, 2007). We use the generalist entomopathogenic fungus *Metarhizium anisopliae* as a model parasite because this is a virulent, natural parasite of many ant species (Schmid-Hempel 1998; Hughes et al. 2004a,b). We first compare the individual resistance of *P. dives* with the

well-studied leaf-cutting ant *Acromyrmex echinatior* and two other ant species, *Myrmcia ruginodis* and *Formica fusca*, that have a metapleural gland to place the relative resistance of *P. dives* in context and test whether *P. dives* show relatively frequent self-grooming. We then focus on the comparison of *P. dives* to *A. echinatior*, examining the resistance of ants in groups to test whether the species differ in the frequency of allogrooming, and the resistance of ants with and without functioning metapleural and venom glands to test whether *P. dives* derives chemical resistance from its venom. Finally we determine in vitro the antimycotic activity of the venom from *P. dives*.

Methods

The experiments used four colonies of a Southeast Asian weaver ant *P. dives* (subfamily Formicinae) and four colonies of the Panamanian leaf-cutting ant *Acromyrmex echinatior* (subfamily Myrmicinae). In addition, four colonies each of the European ants *F. fusca* (subfamily Formicinae) and *Myrmica ruginodis* (subfamily Myrmicinae) were used for comparison in Experiment 1 as representatives from the same subfamilies as the two main species, and of the same cuticular melanisation (black in the case of *P. dives* and *F. fusca*, medium-brown in the case of *A. echinatior* and *M. ruginodis*) as this can affect disease resistance in other insects (Wilson et al. 2001). All colonies had been maintained in a laboratory under controlled conditions for at least 6 months at the time of use and were apparently healthy, with normal brood production and worker mortality rates. Differences in size between ant species were controlled for by standardising the parasite dose applied as conidia/mm^2 of body surface. Ants were maintained during experiments at 25°C and 80% RH, with ad libitum water and 10% sucrose solution throughout. We used a strain of *Metarhizium anisopliae* that is exotic to all the ant species examined (isolate 144467, CABI; isolated from the soil of a maize field in Canada). In addition, in Experiment 4 we used an isolate of *Aspergillus flavus* (cultured from a sporulating *F. fusca* cadaver), a fungus that is known to be a common facultative parasite of ants (Schmid-Hempel 1998; Hughes and Boomsma 2004; Hughes et al. 2004b). The viability of the fungal conidia suspensions used was checked and confirmed to be >90% in all cases.

Experiment 1: Comparative disease resistance of individual ants

To determine the resistance of *P. dives*, as compared with other ants, 60 ants (15 per colony) from each of the four ant species were treated with a suspension of *Metarhizium* conidia in 0.05% Triton-X surfactant at a dose of 370 conidia/mm^2 body area. Sixty ants per species were also treated with a control solution of 0.05% Triton-X. For each ant, 0.5 μl of either *Metarhizium* suspension or control solution was applied to the dorsal surface of the thorax with a micropipette. Following treatment, ants were placed in individual containers (diameter 5 cm, height 8 cm). The frequency of self-grooming (rubbing antennae or body with legs, licking legs or body with mouthparts) by the treated ants was then observed for 20-s periods at 1, 2, 3, 4, 5, 10, 15, 30 and 60 min after treatment, with each separate occurrence of self-grooming being recorded. The survival of the treated ants was monitored for 14 days, with dead ants removed daily, surface sterilised (Lacey 1997), and then monitored for a further 7 days for the appearance of conidia and conidiophores diagnostic of *Metarhizium* infection.

Experiment 2: The importance of group defences in disease resistance

A *Metarhizium* conidia suspension was applied at 370 conidia/mm^2 to 90 *P. dives* and 90 *A. echinatior* workers (30 from each of three colonies, for each species) following the same procedure as before, but with the ants placed in groups of three (n=30) per container (7 cm diameter, 15 cm height). The same numbers of ants were treated with the control solution of 0.05% Triton-X and these were also placed in groups of three. Self-grooming and allogrooming were recorded for 20-s periods at 1, 2, 3, 4, 5, 10, 15, 30 and 60 min after treatment, and survival monitored for 14 days.

Experiment 3: The importance of gland secretions in disease resistance

Following Poulsen et al. (2002), gland openings of ants were blocked using nail varnish to establish the importance of the gland in disease resistance. The acidopore through which venom is released was blocked in *P. dives*, while the openings of either the metapleural glands or the venom gland were blocked in *A. echinatior*. As controls, a similar quantity of nail varnish was applied to the pronotum in both species. Ants were left for 24 h and any individuals which had lost the nail varnish during this time or which appeared to be behaving abnormally were removed. The ants were then treated with either a suspension of *Metarhizium* conidia or a control solution. The dose of *Metarhizium* used was selected based on preliminary trials to give approximately 50% mortality and differed between the two ant species. A concentration of 5×10^5 conidia/ml was used

for *A. echinatior*, equating to 20 conidia/mm^2 body surface, while 1×10^7 conidia/ml was used for *P. dives*, equating to 370 conidia/mm^2 body surface. For each of the five species/gland closure combinations (*P. dives* with acidopore open or closed; *A. echinatior* with metapleural gland closed, venom gland closed, or both glands open), 60 ants were treated with *Metarhizium* and 60 with the control solution (15 in each case from each of four colonies).

Experiment 4: Antimycotic activity of weaver ant venom in vitro

The poison gland and reservoir were dissected from 120 *P. dives* workers, pooled and centrifuged. Petri dishes (90 mm diameter) of selective media (Saboraud dextrose agar with 0.1 g/l dodin, 0.05 g/l streptomycin sulphate and 0.1 g/l chloramphenicol) were seeded with 250 μl of a 1×10^6 conidia/ml *Metarhizium* suspension. Doses of 3 μl of either poison gland secretion, 0.05% Triton-X or a dilution series of formic acid (>98%, Fisher) were applied to the *Metarhizium* plates. Each 3 μl dose was applied in a 4-mm-diameter circle, which has approximately the same area as the body surface of a *P. dives* worker (ca. 13.5 mm^2). After a period of 36 h at 22°C the proportion of spores germinating was determined using a light microscope at 400× magnification. The experiment was repeated using media plates (without dodin) that had been seeded with 250 μl of a suspension of 1×10^6 *Aspergillus flavus* conidia/ml. Each treatment was repeated five times.

Statistical analyses

The effects of species, treatment and colony on the survival of individual ants in Experiments 1 and 3 were analysed using a Cox proportional-hazards regression model. Pair-wise comparisons were conducted using the Breslow statistic in a Kaplan–Meier analysis. Where ants were kept in groups in Experiment 2, survival was instead analysed using a repeated measures General Linear Model to identify significant survival differences over time, between species and treatments. The numbers of ants self-grooming over the course of the 20-s observation periods in Experiment 1 was analysed using a Generalized Estimating Equation (GEE) with binomial distribution and logit link function to test for differences in grooming frequency between species and treatments, with the effect of colony nested within species. A GEE was also used with the data from Experiment 2 to analyse the numbers of ant groups from each colony in which self-grooming or allogrooming took place in each 20-s observation period, with a Poisson distribution and log link function. All analyses were conducted in SPSS 19.0 with non-significant terms removed in a stepwise manner to arrive at the minimum adequate models.

Results

Experiment 1: comparative resistance of individual ants

There was a significant interaction between the effects of treatment and species on the survival of ants (Wald=20.9, df=3, P<0.001). Ants treated with the control solution had low mortality in all four species, and this was also true for *F. fusca* ants treated with the *Metarhizium* parasite (Fig. 1a). Mortality was significantly greater in both *P. dives* and *M. ruginodis* ants treated with *Metarhizium*, with there being approximately 65% mortality in both species, while there was 100% mortality of *A. echinatior* workers exposed to *Metarhizium* (Fig. 1a). *Metarhizium* sporulated from 50% to 95% of the cadavers of ants treated with *Metarhizium*, and from none of those treated with the control solution. Species differed significantly in their frequencies of self-grooming (Wald χ^2=184.9, df=3, P<0.001), but there was no effect of treatment on this, with ants increasing their grooming to a similar extent in response to the control solution as to the parasite solution (Wald χ^2=0.057, df=1, P=0.811). *P. dives* exhibited the most self-grooming, engaging in this at least 50% more than any other species and almost six times more often than *A. echinatior* (Fig. 1b).

Experiment 2a: the importance of group defences

Ants had lower survival when treated with *Metarhizium* ($F_{1,116}$=24.5, P<0.001), and survival did not differ between the species ($F_{1,116}$=0.315, P=0.576; Fig. 2a). There was a significant interaction between the effects of species and treatment on the frequencies of self-grooming (Wald χ^2= 5.82, df=1, P=0.016). *P. dives* self-groomed far more frequently than *A. echinatior*, with the frequency in *P. dives* being high immediately after treatment and then declining whereas self-grooming in *A. echinatior* remained at a lower level throughout the observation period (Fig. 2b). The rates of self-grooming were similar in ants treated with control solution and parasite solution in both species. The frequencies of allogrooming increased immediately after treatment in both species, but was approximately twice as frequent throughout in *A. echinatior* as in *P. dives* (Wald χ^2= 30.2, df=1, P<0.001; Fig. 2c). Allogrooming frequency did not differ between treatments, with the rate increasing in response to the control solution as well as to the parasite solution (Wald χ^2=0.191, df=1, P=0.662).

Experiment 3: the importance of gland secretions for parasite defence

Survival in both *A. echinatior* and *P. dives* was significantly lower when ants were exposed to *Metarhizium* (Wald χ^2=

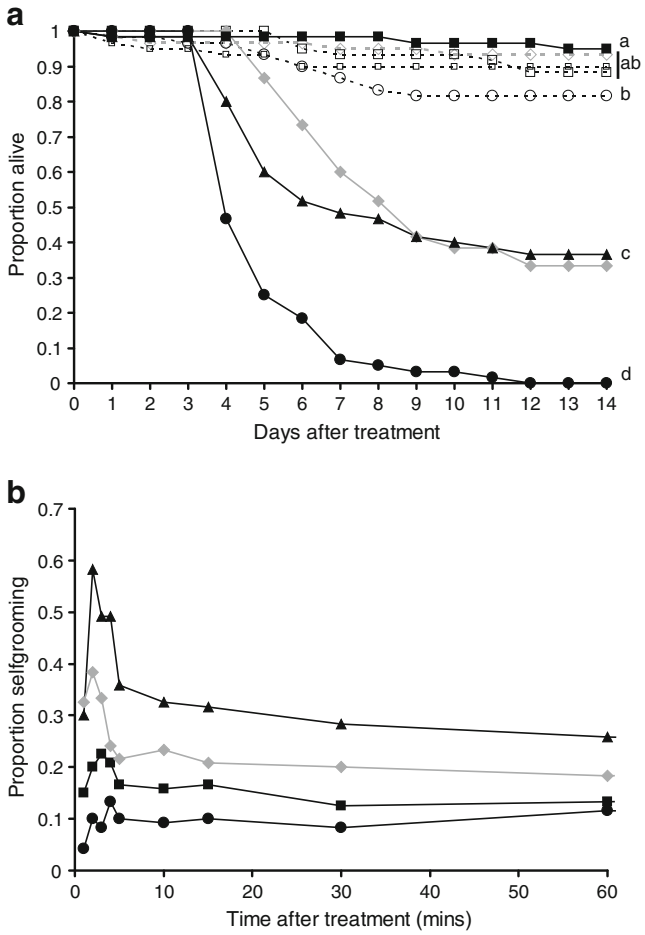

Fig. 1 Individual survival and self-grooming in Experiment 1. **a** Survival and **b** mean self-grooming frequencies of *Polyrhachis dives* weaver ants (triangles), *Acromyrmex echinatior* leaf-cutting ants (*circles*), *Formica fusca* (*squares*) and *Myrmica ruginodis* (*grey diamonds*) ants that were treated with either the *Metarhizium anisopliae* fungal parasite (*solid lines*, *filled symbols*) or 0.05% Triton-X control solution (*dashed lines* and *open symbols*) and then kept individually. Different letters in (a) indicate treatments which differed significantly from one another in Kaplan–Meier pairwise comparisons. Error bars and control data excluded from (b) for clarity

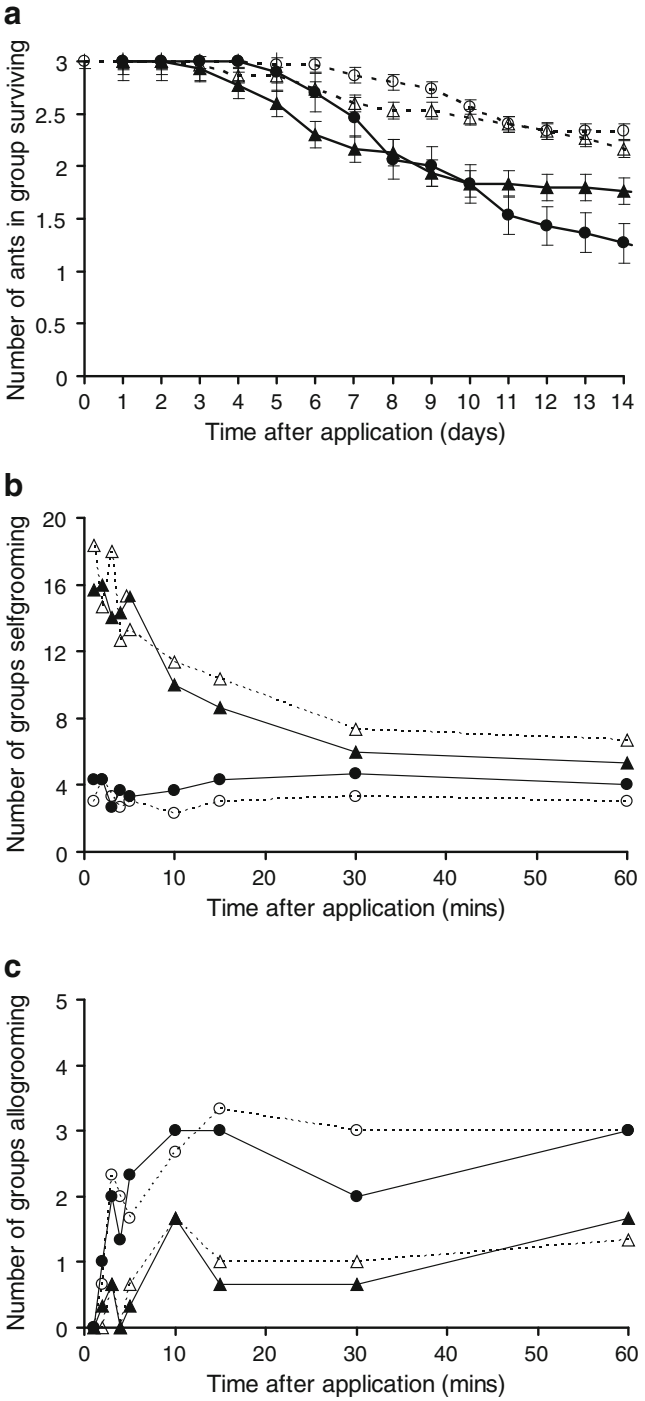

Fig. 2 Group survival and grooming in Experiment 2. **a** Mean ± SE number of ants in group surviving, **b** number of ant groups with self-grooming and **c** number of ant groups with allogrooming for *Polyrhachis dives* weaver ants (*triangles*) and *Acromyrmex echinatior* leaf-cutting ants (*circles*), that were treated with either the *Metarhizium anisopliae* fungal parasite (*solid lines*, *filled symbols*) or 0.05% Triton-X control solution (*dashed lines*, *open symbols*). Error bars excluded from (b) and (c) for clarity

54.3, df=1, P<0.001 and Wald χ^2=52.4, df=1, P<0.001, respectively) and when their glands were blocked (Wald= 24.5, df=1, P<0.001 and Wald=11.2, df=1, P<0.001, respectively; Fig. 3). Survival was not significantly reduced by gland blockage when ants were exposed to the control solution, whereas gland blockage resulted in significantly lower survival when ants were exposed to the *Metarhizium* parasite (Fig. 3).

Experiment 4 results — antimycotic activity of formic acid and venom in vitro

Aspergillus was less susceptible to the effects of formic acid, with a concentration of at least 10% formic acid being required to completely inhibit germination whereas a concentration of 2% formic acid was sufficient to completely inhibit germination of *Metarhizium* (Fig. 4). The

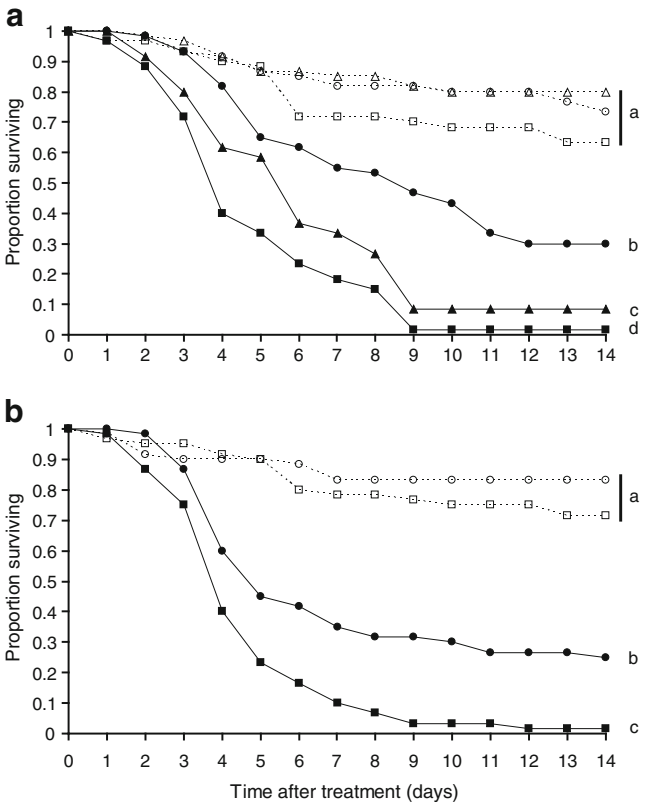

Fig. 3 Survival of **a** *Acromyrmex echinatior* leaf-cutting ants and **b** *Polyrhachis dives* weaver ants that had either their venom gland (*squares*) or metapleural gland (*triangles*; *A. echinatior* only as *P. dives* lacks a metapleural gland) blocked with nail varnish, or had nail varnish applied to the pronotum as a control (*circles*), and which were then treated with either the *Metarhizium anisopliae* fungal parasite (*solid lines*, *filled symbols*) or with 0.05% Triton-X control solution (*dashed lines*, *open symbols*). For each species, different letters indicate treatments which differed significantly from one another in Kaplan–Meier pairwise comparisons

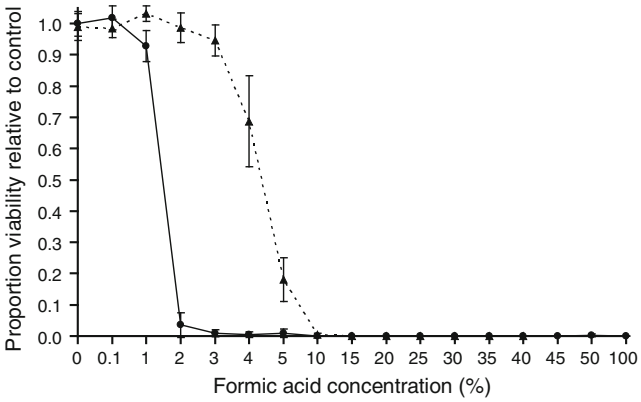

Fig. 4 Proportion of *Metarhizium anisopliae* (*circles*, *solid line*) and *Aspergillus flavus* (*triangles*, *dashed line*) fungal conidia germinating on media treated with different concentrations of formic acid. The venom of *Polyrhachis dives* weaver ants in comparison resulted in 0% germination of both fungi

venom gland reservoir of *P. dives* workers contained approximately 0.47 μl of secretion. The venom of *P. dives* completely inhibited the germination of both *Aspergillus* and *Metarhizium*, and thus had similar antimycotic activity to at least 10% formic acid.

Discussion

The antibiotic-producing metapleural gland has been shown experimentally to have a major role in the resistance of leaf-cutting ants to fungal parasites (Poulsen et al. 2002) and is generally thought to be important to the disease resistance of ants in general (Boomsma et al. 2005; Schlüns and Crozier 2009). However, we found that the lack of a metapleural gland did not mean that the weaver ant *P. dives* is more susceptible to parasites. In fact, *P. dives* workers were considerably more resistant to the *Metarhizium* fungal parasite than *A. echinatior* leaf-cutting ants. As the parasite used is a generalist entomopathogen that all the ant species tested will encounter, and, as the strain used was exotic to all species, the species differences found cannot be due to host–parasite coevolution. The results therefore do not support the hypothesis that weaver ants have lost the metapleural gland as part of an evolution of reduced investment in disease resistance in response to a lower parasite threat associated with an arboreal or weaving lifestyle (Johnson et al. 2003; Boomsma et al. 2005).

An alternative explanation for the loss of the metapleural gland in weaver ants is that they have evolved to rely on alternative resistance mechanisms. The self-grooming results provide support for this. In all species, self-grooming and allogrooming occurred to a similar extent after application of Triton-X control solution and *Metarhizium* suspension, indicating that the response was to the presence of a foreign substance on the cuticle rather than recognition of the parasite. Workers of *P. dives* self-groomed substantially more than any of the other ant species when kept individually in Experiment 1 and also self-groomed significantly more than *A. echinatior* workers when kept in groups in Experiment 2. Self-grooming has been shown to be effective at removing fungal spores from the cuticles of ants and other social insects (Hughes et al. 2002; Yanagawa et al. 2008). It is possible that the ant species may differ in the efficiency of conidia removal by grooming, but the higher survival of *P. dives* suggests that its more frequent self-grooming does improve its disease resistance. It is notable that of the four ant species examined, *A. echinatior* had the lowest frequency of self-grooming and also had the lowest resistance to *Metarhizium* when ants were kept individually. There was much less difference in resistance between *P. dives* weaver ants and *A. echinatior* leaf-cutting ants when ants were kept in groups. In contrast to the self-grooming frequencies, *A. echinatior* had higher rates of

allogrooming than *P. dives*. It therefore appears that disease resistance in *A. echinatior* places a stronger emphasis on group-level defence than *P. dives* which places more emphasis on individual defence.

The gland blockage experiment provides evidence that the secretion from the venom gland is a further mechanism by which weaver ants resist parasites. Blocking the metapleural gland of leaf-cutting ants with nail varnish has previously been shown to substantially increase their susceptibility to *Metarhizium* (Poulsen et al. 2002), and we also found this to be the case in our study. Blocking the acidopore of weaver ants had a similar effect by reducing their survival when they were exposed to *Metarhizium*, but not under control conditions. In both species, gland blockage did not increase the mortality of ants exposed to the control solution, so did not appear to result in any general physiological stress. Weaver ants with a functioning venom gland therefore are more resistant to *Metarhizium*, suggesting that the secretion has antimycotic properties. This was confirmed in vitro with the venom inhibiting the germination of 100% of both *Metarhizium* and *Aspergillus* spores to the same extent as >10% formic acid. The secretion of formic acid by the venom gland is characteristic of the Formicinae ant subfamily (Hölldobler and Wilson 1990; Blum 1992), so the antimicrobial action of venom would seem likely to be a common feature of formicine ants that may make the metapleural gland of less importance in this subfamily. It is notable in this regard that the species in Experiment 1 with the greatest resistance to *Metarhizium* was *Formica fusca*, a formicine ant which both produces formic acid in its venom and which has a metapleural gland. However, blocking the venom gland of *A. echinatior* workers also reduced their resistance to *Metarhizium* and, surprisingly, did so to a similar extent to blockage of the metapleural gland. Venom of ants from other subfamilies has been shown to have antimicrobial properties (Storey et al. 1991; Orivel et al. 2001; Zelezetsky et al. 2005), and it may therefore be the case that the venom of ants in general is important in disease resistance. The silken nests of *P. dives* are contaminated with many viable spores of fungi (Fountain and Hughes 2011), so while the venom may be used in directly defending the ants, it would not appear to be used in sterilising the nest.

The results suggest that the while the metapleural gland may be important in the disease resistance of some ant species, it is not necessarily essential, at least for the resistance of mature, adult ants. Weaver ants which lack the metapleural gland are individually more resistant than some ants which have the gland and this appears to be at least in part due to higher frequencies of self-grooming behaviour. Furthermore, the formic acid-containing venom of weaver ants, and also the venom of leaf-cutting ants, appears to have antimicrobial properties making it too important in resistance to parasites. The results therefore emphasise the multiple, complementary behavioural and chemical defence mechanisms that ants use to resist disease. It will be very interesting to see whether the disease resistance of other species of weaver ants is similar to that of *P. dives*, and how the evolutionary regaining of the metapleural gland by some species of *Camponotus* has impacted their disease resistance.

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