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Trade-offs in group living: transmission and disease resistance in leaf-cutting ants

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Sociality can be associated with significant costs due to the increased risk of disease transmission. However, in some organisms the costs may be offset by benefits due to improvements in defences against parasites. To examine this possible trade-off between infection risk and disease resistance, we used *Acromyrmex* leaf-cutting ants and the entomopathogenic fungus *Metarhizium anisopliae* as the model system. Ants exposed to the parasite were found to have substantially improved survival when they were kept with nest-mates, while the cost of being in a group in terms of increased disease transmission was very low. The efficiency of transmission is described by the transmission parameter, which decreased with increasing host density showing that transmission rates are inversely density dependent. Both grooming and antibiotic secretions appeared to be important in resistance against the parasite, with the defences of small workers being particularly effective. The results indicate that leaf-cutting ant colonies may have much greater resistance to disease than would be predicted from the high densities of host individuals within them. Unlike most organisms, group living in these ants may actually be associated with a net benefit in terms of disease dynamics.

Keywords: disease; resistance; transmission; leaf-cutting ants; entomopathogen

1. INTRODUCTION

Parasitic organisms are an important driving force in evolution, having significant effects on the life history and reproductive success of their hosts (Tomkins & Begon 1999). The outcome of the host–parasite interaction depends upon the dynamics of the relationship, with transmission being a key process (Ewald 1994; McCallum *et al.* 2001). In the simplest models of horizontal transmission, the number of new infections per unit time is vSP, where S is the density of susceptible hosts, P is the density of infectious parasites and v is the transmission parameter that describes the efficiency of parasite transmission to the host (Anderson & May 1981). In most such models, v is assumed to be a constant, and so the rate of new infections will be density dependent, increasing linearly with the density of both hosts and parasites (Anderson & May 1981; Dwyer & Elkington 1993). This density-dependent rate of transmission, known as the mass-action assumption (McCallum *et al.* 2001), has profound implications for the evolution of social behaviour. Adopting a group-living lifestyle carries with it an inevitable increase in the density of individuals and the frequency of interactions between them. Disease transmission within the group will be further facilitated where groups consist of closely related individuals (Hamilton 1987; Schmid-Hempel 1998). Consequently, sociality has been predicted to be associated with greater rates of disease transmission (Alexander 1974; Freeland 1976). Such a positive relationship has been observed in many intraspecific studies (e.g. Freeland 1976; Davies *et al.* 1991; Côté & Poulin 1995; Møller *et al.* 2001; Tella 2002). In some species, individuals reared in groups have been shown to divert more resources to immune system development than when reared in isolation, in order to cope with this effect (Reeson *et al.* 1998; Wilson *et al.* 2002).

In addition to the obvious costs of disease transmission, group living may also be associated with benefits to the host in its relationship with parasites. These have been largely ignored in studies of disease dynamics. For example, being in a social group in which food is shared amongst individuals may enable the survival of diseased individuals that are unable to forage for themselves (Loehle 1995), and many primates in particular show high rates of allogrooming that are effective at removing ectoparasites (Barton 1985). With regard to disease dynamics, there may therefore be a trade-off involved in group living, between the cost of increased transmission and the benefit of improved disease resistance. However, it is a trade-off that is thought, in general, to resolve to a net cost, with group size and disease impact being positively correlated in spite of any resistance benefits that may also occur (Loehle 1995).

Possible exceptions to this general rule are the social insects, which provide the most extreme examples of sociality. Their colonies are characterized by all of the features of social behaviour that can increase disease transmission: high densities of host individuals, enhanced frequencies of interactions between individuals and close relatedness of individuals within the group (Schmid-Hempel 1998). They can also exhibit complex defence mechanisms that may result in individuals having a greater resistance to disease when they are in a group (Rosengaus *et al.* 1998; Traniello *et al.* 2002). Many insect pathogens,

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such as the fungus *Metarhizium*, are able to either evade or degrade the immune response of a host insect. Defence mechanisms, such as grooming and antibiotic secretions, that prevent penetration of the cuticular barrier are therefore the primary deterrents to infection (Boucias & Pendland 1998). However, with the exception of some well-studied social insects, such as honeybees (Bailey & Ball 1991) and bumble-bees (Schmid-Hempel 1998), very little is known about the dynamics of host–parasite relationships in the vast majority of species.

We investigated the disease dynamics trade-off in group living using the leaf-cutting ant *Acromyrmex echinatior* Forel (Hymenoptera: Formicidae: Attini) as the host and a generalist entomopathogenic fungus, *Metarhizium anisopliae* (Metschnikoff) (Deuteromycotina: Hyphomycetes) as the parasite. *Acromyrmex* ants have large, long-lived colonies with a complex alloethic division of labour in the worker population that is based upon an essentially bimodal size distribution of workers (Wetterer 1999). With the possible exception of phorid flies (Schmid-Hempel 1998), the ants themselves are not known to suffer from any specialist parasites. However, there are a number of records of leaf-cutting ants being infected with generalist parasites, such as the entomopathogenic fungi *Beauveria* and *Metarhizium* (Alves & Sosa Gómez 1983; Diehl-Fleig *et al.* 1992; C. W. Jackson and W. O. H. Hughes, unpublished data; W. O. H. Hughes, L. Thomsen, J. Eilenberg and J. J. Boomsma, unpublished data). Leaf-cutting ants are thought to protect themselves against parasites such as these by intensive self-grooming and allogrooming (Kermarrec & Decharme 1982; Kermarrec *et al.* 1986; Jaccoud *et al.* 1999). In addition, the ants excrete compounds with antibiotic properties from the mandibular (Knapp *et al.* 1994) and metapleural glands (Hölldobler & Wilson 1990; Nascimento *et al.* 1996; Bot *et al.* 2002). In a crucial experiment by Poulsen *et al.* (2002*b*), *Acromyrmex* ants with non-functioning metapleural glands were found to have substantially reduced resistance to *M. anisopliae*, thus demonstrating the importance of these glands in disease defence.

Using this *Acromyrmex–Metarhizium* model system, we quantify both the benefit of improved disease defences and the cost of greater disease transmission. To establish the effect of group size on disease transmission, we then use these data to calculate the transmission parameters (v) of the host–parasite interaction. We also investigate the impact of the three main proposed defence mechanisms (self-grooming, allogrooming and the production of antibiotic compounds) on the host–parasite relationship to elucidate the mechanisms underlying the trade-off. It has been suggested that the small worker caste may have a key role in disease defence (Jaccoud *et al.* 1999; Poulsen *et al.* 2002*a*), and the fact that they have relatively large metapleural glands, compared with their body size, supports this (Bot & Boomsma 1996). We therefore compare both the effectiveness of the defence mechanisms and the overall resistance of small and large workers.

2. MATERIAL AND METHODS

(a) *General methodology*

Colonies of *Acromyrmex echinatior* were collected from Gamboa, Panama, during April 1996, 1999 and 2000. They were maintained in the laboratory on a diet of bramble leaves and rice grains. For the experimental replicates, ants were removed from the colony and kept in plastic pots (diameter: 2.5 cm; height: 4 cm) at 24 °C with an *ad libitum* supply of water and 10% sucrose water. We defined small workers (SW) as having a head width of 1.0–1.4 mm and large workers (LW) as having a head width of 2.1–2.3 mm. To reduce variation in age between experimental ants, all ants were taken from the surface of the fungus garden and callow individuals were not used. No effects of the colony of origin were found in any of the experiments and so these data are not considered further.

The strain of *M. anisopliae* var. *anisopliae* used in these experiments, KVL 01-106, was isolated from soil samples collected at the same site in Gamboa, Panama, as that from where the ant colonies were collected (W. O. H. Hughes, L. Thomsen, J. Eilenberg and J. J. Boomsma, unpublished data). The host and parasite used therefore represented a natural system. Cultures were passaged through selective media (Lacey & Brooks 1997) three times before use. For the experiments, spore (conidia) suspensions were created from recently sporulating culture plates in a 0.05% solution of Triton-X surfactant. The suspensions were quantified using a haemocytometer and diluted to the required concentrations. Quantities of 0.5 μl were applied to the thoraxes of ants using a micropipette. Control ants had 0.5 μl of a 0.05% Triton-X solution applied in the same way. All treated ants had been previously marked with nail varnish to allow their later identification. The viability of spores in all the suspensions was checked (Lacey & Brooks 1997) and was greater than 95% in all cases. After application, ant mortality was assessed daily. Dead individuals were also removed from the experimental pots on a daily basis, thus preventing any secondary cycling of the pathogen. The cadavers were surface sterilized (Lacey & Brooks 1997), placed in individual vials with wet cotton wool and monitored for the appearance of external features (conidiophores and conidia) that are diagnostic of a *Metarhizium* infection. Confirmation rates for *Metarhizium* infection were generally high (greater than 90%) for treated ants. None of the control ants exhibited any symptoms of *Metarhizium* infection.

(b) *The benefit of group living*

To examine whether being in a group bestows a benefit on the resistance of ants to disease, individual ants were treated with *M. anisopliae* as described above and then maintained either in isolation or together with two or five nest-mates. These nest-mates were either all similarly treated or were all uninfected. All the ants used were SWs. The treated ants had suspensions of 1×10^5, 1×10^6 or 1×10^7 spores ml^{-1} or the control solution, applied to their thoraxes. Each group size was replicated 10 times at each spore concentration. The ants used came from five colonies (Ae47, 48, 109, 124 and 152) with two replicates in each treatment coming from each colony. We recorded the mortality of the treated individuals over a 10 day period. Ant survival was examined using a Cox proportional-hazards regression model, with group type (number of ants and whether only a single ant in the group or all the ants were treated) and dose as variables. Individual analyses were also carried out for each dose. The survival distributions were examined at each dose in an overall and pairwise manner using Kaplan–Meier survival tests and the Breslow statistic.

(c) *The cost of group living*

A second similar experiment focused on the mortality of the untreated individuals in the group in order to quantify the cost due to disease transmission of being in a group. Focal ants were treated with either a 1×10^7 spores ml^{-1} suspension or the control solution and placed with one, two or five untreated nest-mates. The treatment involving single untreated individuals was repeated using focal ants treated with spore concentrations of 1×10^5 and 1×10^6 spores ml^{-1} in order to examine the relationship between dose and transmission. This treatment was also repeated using the sporulating cadavers of LW nest-mates as the focal ants ($1.2 \times 10^7 \pm 1.6 \times 10^6$ *Metarhizium* spores per cadaver, mean ± s.e., $n = 5$). In all cases, the focal ants were removed after 48 h and the mortality of the remaining untreated ants was monitored for 16 days. Each treatment was replicated 20 times, with equal numbers of replicates involving ants from each of four colonies (Ae47, 48, 109 and 124). All the ants used were SWs.

The mortality of the non-treated individuals was examined using Cox proportional-hazards analyses. A first test included only the data for ants exposed to the 1×10^7 spores ml^{-1} suspension or the control solution, and examined the effect of group size on survival. A second test then examined all the data for ants maintained in isolation, and examined the effect of dose on survival. Survival distributions were compared both overall and pairwise in a similar manner with the Breslow statistic (Kaplan–Meier survival test). The transmission parameters (v) were also calculated for each group size at the 1×10^7 dose, following the method derived from Anderson & May (1981) and adapted by Dwyer & Elkington (1993):

$$v = (-1/P) \times \ln[1 - (I/S)],$$

where P is the pathogen density, I is the number of individuals infected and S is the number of susceptible individuals. As the ants were only exposed to the treated ants for a 48 h period, and dead ants were removed to prevent secondary infections, we ignored any effects of time. The number of individuals infected was therefore calculated as the mortality at the end of the experiment adjusted for control mortality using Abbott's correction formula (Abbott 1925), in order to distinguish parasite-induced mortality from that due to other causes. In order to avoid problems due to 100% infection rates, replicates for each colony were pooled before the calculations, giving four replicates per group size.

(d) *The efficacy of the defence mechanisms*

The secretion of antibiotic compounds may reduce the viability of the spores, while both self-grooming and allogrooming may reduce the number, and possibly the viability, of spores on the cuticle. To quantify the effects of these mechanisms, we examined the number and viability of spores on the cuticles of ants exposed to *M. anisopliae*. Focal ants were treated with 0.5 µl of a 1×10^8 spores ml^{-1} suspension of the fungus. As controls, ants were freshly killed with CO_2 before treatment. Ants were kept either in isolation or with two SW nest-mates to investigate the importance of individual-level and group-level defences. The number and viability of spores on the cuticles of the treated ants were quantified immediately after application, and 1 h and 24 h later. The treated ant was placed into an Eppendorf vial containing 1 ml of 0.05% Triton-X solution and immediately vortexed for 1 min to remove any spores from the cuticle of the ant. The spore concentration was then immediately counted with a haemocytometer and 100 µl of the spore suspension was plated onto a selective medium plate. After 24 h, the viability of the spores was quantified. Each treatment was replicated six times with SWs and six times with LWs as the focal individuals to examine whether the defence mechanisms differed between the castes. Equal numbers of ants were used from each of two colonies of *A. echinatior* (Ae47 and 109).

Univariate analyses of variance (ANOVAs) were used to examine the way that time, caste or treatment affected the number or viability of spores remaining on the cuticles of ants. For each of the two dependent variables measured (number of spores and viability of spores), two separate three-way ANOVAs were performed in order that they each had balanced designs. The first included only the data for ants unable to groom (those at 0 h and the dead ants at 1 h and 24 h after application), to examine whether the number or viability of spores on the cuticles changed over time and whether any change in number or viability differed between the two castes. The second ANOVA was carried out for each variable using all the data at 1 h and 24 h to examine if spore number or viability was affected by treatment or caste or whether there was a difference between 1 h and 24 h after application.

(e) *The effect of worker caste on disease resistance*

Small workers and LWs were treated with either a 1×10^5 spores ml^{-1} suspension or with a control solution of 0.05% Triton-X. The ants were then maintained in isolation for a 10 day period and their survival was recorded daily. Each caste was replicated 20 times with each treatment, with equal numbers of replicates using ants from each of four colonies (Ae33, 47, 48 and 109). The effects of treatment and caste on ant mortality were examined using a Cox proportional-hazards model, and the survival distributions were compared using a Kaplan–Meier survival test.

3. RESULTS

(a) *The benefit of group living*

Both the dosage of *M. anisopliae* applied and the number of nest-mates the treated ants were maintained with affected the survival of the ants, with there being a significant interaction between the two factors (Wald statistic = 21.6, d.f. = 12, $p = 0.043$) (figure 1). The survival distributions of the groups differed significantly at all three doses of *Metarhizium* (1×10^5: Breslow statistic = 29.3, d.f. = 4, $p < 0.0001$; 1×10^6: Breslow statistic = 48.2, d.f. = 4, $p < 0.0001$; 1×10^7: Breslow statistic = 39.7, d.f. = 4, $p < 0.0001$), but not in the controls (Breslow statistic = 3.52, d.f. = 4, $p = 0.48$). The mortality of treated ants maintained in isolation was high at all doses of *M. anisopliae*, while treated ants maintained with nest-mates in groups of any size had a hazard ratio of death 3.5–7.5 times lower when exposed to the lowest dose (Wald statistic = 13.1, d.f. = 4, $p = 0.001$) (figure 1*c*). This was regardless of whether one or all of the group had been treated with the fungus. At the highest dose, the mortality of treated ants maintained with untreated nest-mates was also high, but the hazard ratio was reduced when all the ants in the group were treated with *M. anisopliae* (Wald statistic = 34.3, d.f. = 4, $p = 0.001$) (figure 1*a*). This difference was clearer at the 1×10^6 dosage, where the hazard ratio of death was 4.3–5.3 times lower when the ants were in a group of six individuals that had all been treated with *M. anisopliae* (Wald statistic = 24.1, d.f. = 4,

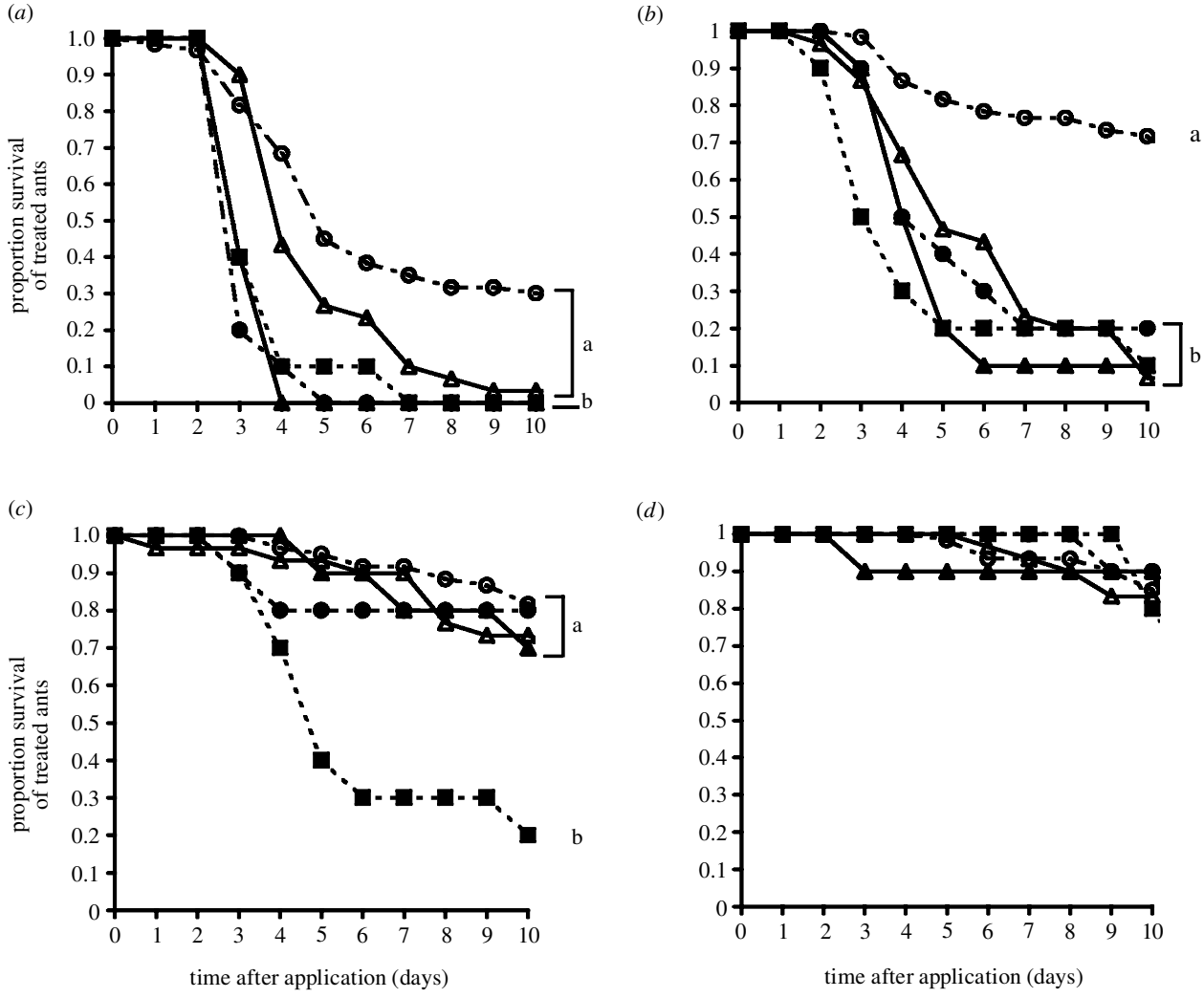

Figure 1. Survival curves for ants treated with *Metarhizium* suspensions of (*a*) 1×10^7, (*b*) 1×10^6, (*c*) 1×10^5 spores ml^{-1} or (*d*) a control solution and maintained after infection either alone (filled squares), in groups of three (open triangles) or six treated (open circles) individuals or in groups of one treated and two (filled triangles) or five (filled circles) untreated individuals. Different letters indicate treatments the survival curves of which differed from one another at $p < 0.01$ in pairwise comparisons with the Kaplan–Meier survival test.

$p < 0.001$) (figure 1*b*). The mortality of ants treated with the control solution was low and the hazard ratios did not differ between the group types (Wald statistic = 1.45, d.f. = 4, $p = 0.228$) (figure 1*d*).

(b) *The cost of group living*

At the 1×10^7 dose, mortality of ants exposed to treated nest-mates was found to be significantly higher than those exposed to untreated nest-mates (Wald statistic = 8.23, d.f. = 1, $p = 0.004$), but the difference was relatively low with very little disease transmission occurring (figure 2*a*,*b*). There was no effect of group size on ant mortality (Wald statistic = 1.03, d.f. = 2, $p = 0.597$), in spite of many more ants in the group treatments being exposed, although the survival of treated ants maintained in groups of two was lower than when they were maintained in groups of five (figure 2*a*). For the ants maintained in isolation after the initial 48 h exposure period, the survival distributions differed significantly between the doses (Breslow statistic = 35.9, d.f. = 4, $p < 0.0001$). Although mortality was also slightly higher in the *M. anisopliae* applications than in the controls, this treatment effect was entirely due to the very high mortality of ants exposed to sporulating cadavers (figure 2*b*). These ants had a hazard ratio of death at least 4.2 times higher than those in any of the other treatments (Wald statistic = 27.4, d.f. = 4, $p < 0.001$).

Very few (0–30%) of the dead ants in most of the treatments exhibited features diagnostic of a *Metarhizium* infection. The exception was in the cadaver treatment where 88% of the ants that died following exposure to a sporulating cadaver then themselves produced *Metarhizium* conidia and conidiophores. The transmission parameter (v) decreased significantly as group size increased ($F_{2,9} = 4.68$, $p = 0.04$). There was very little difference in the efficiency of transmission when ants were either maintained in isolation or in groups of two individuals, but the transmission efficiency was far lower when ants were maintained in groups of five individuals (figure 3).

(c) *The efficacy of the defence mechanisms*

There was a significant reduction over time in the numbers of spores remaining on the ant cuticles even when the ants were dead ($F_{2,30} = 6.27$, $p = 0.005$) (figure 4*a*).

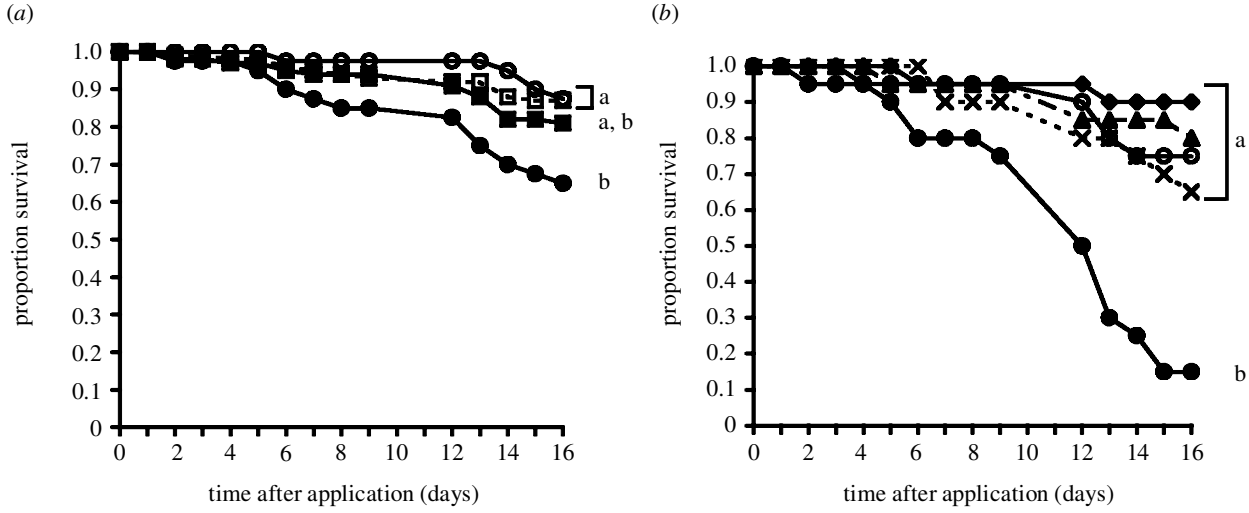

Figure 2. Survival curves for ants exposed to an infected nest-mate for 48 h in (*a*) groups of two or five, or (*b*) alone. The infected nest-mates were treated with *Metarhizium* spore suspensions of 1×10^7(high treatment), 1×10^6 (medium treatment), 1×10^5 (low treatment) spores ml^{-1}, with control solutions or were sporulating cadavers. Different letters indicate treatments the survival curves of which differed from one another at $p < 0.01$ in pairwise comparisons with the Kaplan–Meier survival test. (*a*) Filled circles, two ants, high treatment; open circles, two ants, control; filled squares, five ants, high treatment; open squares, five ants, control. (*b*) Filled diamonds, control; crosses, high treatment; open circles, medium treatment; filled triangles, low treatment; filled circles, cadaver.

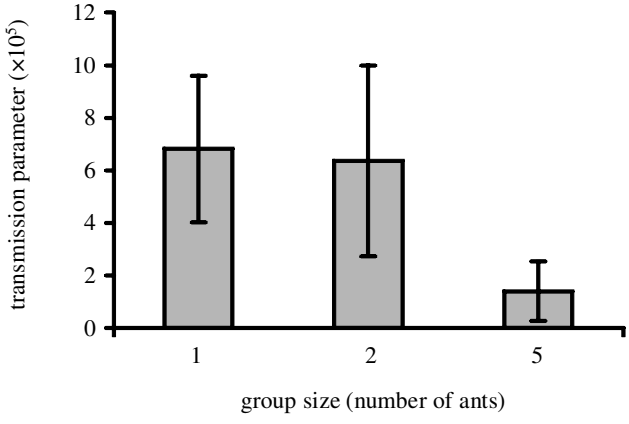

Figure 3. Transmission parameters (v) for ants exposed for 48 h to an infected nest-mate, either alone or in groups of two or five ants. The infected nest-mates were treated with a *Metarhizium* spore suspension of 1×10^7 spores ml^{-1} (means ± 95% confidence intervals).

This was the case for both LWs and SWs (caste-time interaction: $F_{2,30} = 0.025$, $p = 0.976$) with there being no overall difference between the castes ($F_{1,30} = 1.25$, $p = 0.273$). At 1 h and 24 h after application there was a significant interaction between the effects of treatment and the caste of the focal ant on the numbers of spores remaining ($F_{2,60} = 3.51$, $p = 0.036$). The numbers of spores on live ants, even in isolation, were much lower than on dead ants, with most of the reduction occurring during the first hour after application. Small workers were more efficient at spore removal than LWs when kept in isolation. The numbers of spores on ants maintained with nest-mates were generally lower than when they were kept in isolation, with LWs particularly benefiting from the presence of nest-mates.

The viability of the spores remaining on the ant cuticles decreased over time from the initial level of *ca.* 90% immediately after application, but this was not statistically significant ($F_{2,30} = 2.75$, $p = 0.08$) and did not differ between the castes ($F_{2,30} = 1.86$, $p = 0.174$) (figure 4*b*). At both 1 h and 24 h after application, the viability of spores applied to SWs was lower than those applied to LWs ($F_{1,60} = 4.64$, $p = 0.035$). There was neither a significant effect of treatment on spore viability ($F_{2,60} = 2.22$, $p = 0.118$), nor interaction between treatment and caste ($F_{2,60} = 2.43$, $p = 0.097$). However, the viability of spores on LWs maintained with nest-mates did appear to be somewhat lower than for spores on LWs that were either dead, or alive but maintained in isolation. It should be noted that the power of these tests to avoid a type II error was low (less than 50% in all cases) and so non-significant results must be treated with caution.

(d) *The effect of worker caste on disease resistance*

Treatment with *M. anisopliae* was found to significantly affect the survival of ants; mortality was high in treated ants in both castes and very low in the control ants (Wald statistic = 16.6, d.f. = 1, $p < 0.001$) (figure 5). Although LWs had 1.9 times the hazard ratio of death of SWs, no significant difference was found between the castes (Wald statistic = 0.825, d.f. = 1, $p = 0.364$), and the interaction between treatment and caste was also non-significant (Wald statistic = 0.004, d.f. = 1, $p = 0.947$). The survival distributions of the castes were not found to differ significantly in either the control (Breslow statistic = 0.22, d.f. = 1, $p = 0.64$) or *Metarhizium* (Breslow statistic = 0.56, d.f. = 1, $p = 0.45$) treatments, although the mortality of LWs treated with *M. anisopliae* was substantially higher than that of treated SWs (figure 5).

4. DISCUSSION

There were found to be clear beneficial effects to being in a group for *Acromyrmex* leaf-cutting ants exposed to the

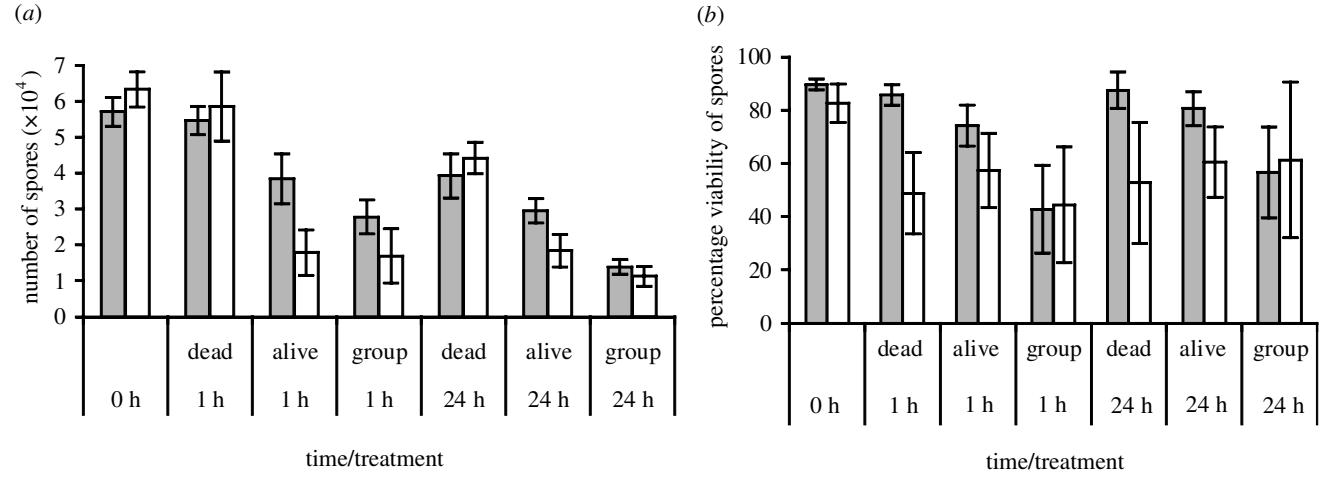

Figure 4. Mean ± s.e. (*a*) number and (*b*) viability of *Metarhizium* spores on ant cuticles at 0, 1 or 24 h after application to large (shaded bars) or small (open bars) workers that were either dead, alive and alone, or alive and with two uninfected nest-mates.

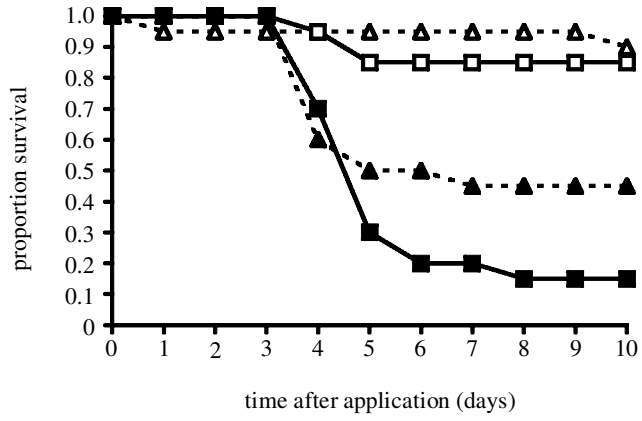

Figure 5. Survival curves for large workers (squares) or small workers (triangles) treated with either a *Metarhizium* suspension of 1×10^5 spores ml^{-1} (filled symbols) or a control solution (open symbols).

parasitic fungus used in these experiments. Individuals exposed to a dose of 1×10^5 *M. anisopliae* spores ml^{-1} had a much greater chance of surviving the infection when they were maintained with nest-mates than when they were kept in isolation. That this was due to some physiological deficiency in the isolated ants seems unlikely, given the lack of effect of group size on the mortality of the control ants, the identical maintenance conditions and the short time-span of the experiment. The presence of nest-mates therefore appears to heighten the resistance of ants to disease. However, at higher doses, benefits from being in a group were only seen if all the individuals in the group had been directly exposed to the parasite. This suggests that a direct challenge to an individual stimulates its defence mechanisms to a greater degree than does the indirect challenge presented by an infected nest-mate. Exposure to pathogens can increase grooming behaviour in leaf-cutting ants (Kermarrec & Decharme 1982; Kermarrec *et al.* 1986; Jaccoud *et al.* 1999; Currie & Stuart 2001). It may also be possible for ants to increase the flow of the secretion into the reservoirs of the metapleural gland by contracting their thoracic muscles (Bot *et al.* 2001). This greater response then appears to benefit other infected members of the group, resulting in an improvement in the group-level defence that is greater than the sum of the parts.

In addition, there was found to be very little cost due to disease transmission associated with being in a group. Exposure to individuals dosed with as high as 5000 spores resulted in only low levels of transmission to the untreated individuals. Concordantly, the transmission parameter (v) decreased as the group size of uninfected ants increased, and the mass action assumption was not upheld. Such a decrease of transmission parameter with host density means that the regulatory effect of the parasite on the host will be reduced (Hochberg 1991). It indicates that the high-density host populations that occur in colonies of social insects, such as leaf-cutting ants, may be much more resistant to disease than would be expected based on a linear density–transmission relationship.

Although some studies have found considerable transmission of parasites between social insect individuals (Kramm *et al.* 1982; Rosengaus & Traniello 1997; Schmid-Hempel 1998; Rosengaus *et al.* 2000), this does not appear to be the case here. In addition to the effective defences of the ants, this may also be related to the likely relative genetic heterogeneity of the groups (due to the high mating frequencies of *Acromyrmex* queens; Boomsma *et al.* 1999). Transmission is lower between less related individuals (Shykoff & Schmid-Hempel 1991) with the result that more genetically heterogeneous groups suffer less from parasites (Baer & Schmid-Hempel 1999). The results of the present study suggest that the often-proposed cost to sociality of increased disease risk may not apply to many social insects. In fact, increased group size may actually bestow a net benefit in terms of disease dynamics, by improving the survival of any infected individuals with little risk of the disease spreading. Similar beneficial effects of group living have been found in termites (Rosengaus *et al.* 1998; Rosengaus & Traniello 2001), and it seems likely that they may also occur in many other social insects. In addition, the group sizes used here were extremely small relative to the natural size of a colony, and it has been shown in termites that individuals can gain immunity to disease through exposure to infected

individuals (Traniello *et al.* 2002). For both these reasons, the trade-off in group living between the risk of transmission and improved disease resistance may be biased towards a net benefit to an even greater degree than is suggested by the current results. This may explain why so few diseases are known from leaf-cutting ant colonies, and why attempts at biological control have proved so problematic (Kermarrec & Decharme 1982; Kermarrec *et al.* 1986). It also generates the prediction that unlike most other animals (Davies *et al.* 1991; Côté & Poulin 1995; Møller *et al.* 2001; Tella 2002), in many social insects there may not be an intraspecific, positive relationship between group size and the impact of disease.

The exception to the low rate of transmission was the treatment in which individual ants were presented with sporulating cadavers. These were associated with very high rates of transmission and mortality of previously uninfected ants. This is unsurprising given the large number of spores present on a leaf-cutting ant cadaver and their possibly greater virulence due to their recent passage through the host insect (Lacey & Brooks 1997). It illustrates the substantial danger posed to ants by infectious cadavers. Leaf-cutting ants and many other social insects are extremely fastidious about removing hazardous waste, including corpses or diseased individuals, from the nest (Kermarrec *et al.* 1986; Schmid-Hempel 1998; Hart *et al.* 2002). Given the risk posed to the colony by individuals producing parasite propagules, this behaviour is likely to be extremely important in preventing the colony being overcome by disease.

Evidence was found to support the roles of all three proposed defence mechanisms (self-grooming, allogrooming and the secretion of antibiotic compounds) in resistance to *M. anisopliae*. The numbers of spores on ant cuticles decreased more over time when the ants were alive than when they were dead, presumably due to self-grooming. In the case of LWs, the decrease in the number of spores appeared greater when the infected ant was with nestmates, suggesting that allogrooming also occurred. Although not quantified, allogrooming of infected individuals was regularly observed during these experiments. In addition, the viability of spores on ant cuticles decreased over time, and was lower on the cuticles of SWs than LWs. The presence of antibiotic secretions on the cuticles is the most probable explanation. That this was the case even when the ants were dead, suggests it was related to compounds already present on the cuticle rather than any produced in response to exposure to the parasite. Unlike other sources of antibiotic compounds, such as the mandibular glands (Knapp *et al.* 1994), the metapleural glands continuously excrete their contents onto the cuticles of ants (Bot *et al.* 2001). In addition, certain cuticular compounds have been found to have antibiotic properties in other insects (Boucias & Pendland 1998), and possibly also in leaf-cutting ants. However, the importance of the metapleural gland in disease resistance has been demonstrated by bioassay (Poulsen *et al.* 2002*b*) and antibiotic compounds produced by the metapleural glands are the most likely reason for the reduction in spore viability on the ant cuticles.

The impact of these mechanisms on disease resistance was demonstrated by the survival distributions of LWs and SWs exposed to *M. anisopliae*. Given that the dose of spores was the same, the smaller size of SWs, and therefore greater density of spores, would have been predicted to result in them suffering a greater or faster mortality than LWs (Ebert *et al.* 2000). In fact, no significant difference was found between the castes, and there was a strong tendency towards the opposite pattern. Small workers would therefore appear to be more resistant to *Metarhizium* than LWs. In another study of parasitism of *Acromyrmex* ants, Kermarrec *et al.* (1990) found the opposite pattern, however this may have been due to a within-host density-dependent effect of the parasite population. The results of the present study support previous suggestions that SWs may be the caste most involved in protecting the colony against diseases (Kermarrec *et al.* 1986; Jaccoud *et al.* 1999; Poulsen *et al.* 2002*a*). Small workers of *Acromyrmex* have large metapleural glands relative to their body size (Bot & Boomsma 1996), and the composition of the glands also differs from that of LWs (D. Ortius-Lechner and J. J. Boomsma, unpublished data). The greater reduction in spore viability on the cuticles of SWs than LWs may be a result of these differences. Small workers also appear to be better at grooming, which may be due to their smaller mouthparts, antibiotic salivary secretions and greater efficiency at filtering out small particles in their infra-buccal pocket (Kermarrec *et al.* 1986).

In conclusion, we found that group living has a net benefit in terms of disease dynamics in leaf-cutting ants. Infected individuals benefit from an improved disease resistance associated with being with nest-mates, while the cost of increased disease transmission is very low. The efficiency of this is exemplified by the transmission parameter (v), which decreases as group size increases. The results support the probable role of both grooming and the production of antibiotic compounds in the defence reaction, and SWs appeared more resistant to disease than LWs. The results help explain why so few parasites are known for leaf-cutting ants and indicate that social insect colonies may be far more resistant to disease than would be indicated by the densities of host individuals within them. It appears that the effective defences against disease of leaf-cutting ants have allowed them to not only overcome a fundamental feature of host–parasite relationships, but also actually turn what is normally a cost of group living into a benefit.

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