

Rapid anti-pathogen response in ant societies relies on high genetic diversity

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Social organisms are constantly exposed to infectious agents via physical contact with conspecifics. While previous work has shown that disease susceptibility at the individual and group level is influenced by genetic diversity within and between group members, it remains poorly understood how group-level resistance to pathogens relates directly to individual physiology, defence behaviour and social interactions. We investigated the effects of high versus low genetic diversity on both the individual and collective disease defences in the ant *Cardiocondyla obscurior*. We compared the antiseptic behaviours (grooming and hygienic behaviour) of workers from genetically homogeneous and diverse colonies after exposure of their brood to the entomopathogenic fungus *Metarhizium anisopliae*. While workers from diverse colonies performed intensive allogrooming and quickly removed larvae covered with live fungal spores from the nest, workers from homogeneous colonies only removed sick larvae late after infection. This difference was not caused by a reduced repertoire of antiseptic behaviours or a generally decreased brood care activity in ants from homogeneous colonies. Our data instead suggest that reduced genetic diversity compromises the ability of *Cardiocondyla* colonies to quickly detect or react to the presence of pathogenic fungal spores before an infection is established, thereby affecting the dynamics of social immunity in the colony.

Keywords: inbreeding; social immunity; hygienic behaviour; grooming; *Cardiocondyla* ants; *Metarhizium* fungi

1. INTRODUCTION

The susceptibility of individuals to disease is strongly affected by genetic effects. These include the presence of particular alleles at resistance loci (Carius *et al.* 2001; Lambrechts *et al.* 2005) and the level of homozygosity within host organisms, i.e. the inbreeding effect (O'Brien & Evermann 1988; Arkush *et al.* 2002; Whiteman *et al.* 2006; Luong *et al.* 2007; but see cases where particular resistant genotypes become fixed (Ouborg *et al.* 2000; Haag *et al.* 2003; Spielman *et al.* 2004)). The underlying genetics can affect two lines of host disease defence: its anti-parasite behaviour and physiological immune system (Arkush *et al.* 2002; Luong *et al.* 2007).

(a) Disease susceptibility and levels of defence in social insects

In societies, disease dynamics are not only affected by selection at the individual level, but also the group level. Among others, this is true for insect societies (social bees and wasps, ants and termites) that consist of reproducing queens and multiple generations of their worker offspring. Because social insects live together in close

proximity and interact intimately, disease spread between colony members is expected to be facilitated in comparison to solitary individuals (Shykoff & Schmid-Hempel 1991; Schmid-Hempel 1998). To counteract the high risk of disease transmission within colonies, insect societies have evolved cooperative social defences that complement the immune response of individual group members (social immunity; Cremer *et al.* 2007). Similar to individual defence mechanisms, which include both behavioural (e.g. parasite avoidance and self-grooming) and physiological responses (e.g. the innate immune system and antimicrobial gland secretions), social immunity comprises behavioural and physiological defences that are based on interactions between two or more individuals (Cremer *et al.* 2007). Examples are allogrooming of exposed nest-mates or brood (Schmid-Hempel 1998), 'hygienic behaviour', where workers detect and remove diseased and parasitized brood from the nest (Rothenbuhler & Thompson 1956; Wilson-Rich *et al.* 2009), and enrichment of the nest material with antimicrobially active substances from the environment (Christe *et al.* 2003). It has also been shown that social interactions within colonies directly affect the physiological susceptibility of individual group members (Traniello *et al.* 2002; Ugelvig & Cremer 2007).

(b) Genetic effects on disease resistance in social insects

Similar to individual organisms, colony-level disease susceptibility is influenced by the presence of specific alleles

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at loci affecting disease resistance. Furthermore, it is affected by the overall genetic diversity of group members. The genetic diversity in colonies has two components: a within-individual (e.g. homozygosity level) and a between-individual (e.g. nest-mate relatedness) component.

In ants and bees, individuals from distinct matri- and patriline in a single colony can differ in their physiological disease susceptibility (Baer & Schmid-Hempel 2003; Palmer & Oldroyd 2003; Hughes & Boomsma 2004; Reber *et al.* 2008; Hughes *et al.* 2010). Individuals may also have different thresholds to perform antiseptic behaviours (Beshers & Fewell 2001; Gramacho & Spivak 2003). A genetic basis of these traits is further corroborated by breeding experiments that allowed creating susceptible versus resistant colonies in honeybees (Rothenbuhler & Thompson 1956; Spivak & Reuter 2001; Swanson *et al.* 2009). Furthermore, molecular studies combined with quantitative trait analyses have revealed a number of suggestive loci that influence hygienic behaviour (Lapidge *et al.* 2002; Oxley *et al.* 2010).

The presence of multiple queens and multiple mating by queens not only creates a larger set of potential resistance alleles being present in the colony, but it also decreases the relatedness between nest-mates. Thereby, between-individual genetic diversity is increased, which in turn reduces disease transmission efficiency. Multiple mating has therefore been suggested as an evolutionary response to parasite pressure (Hamilton 1987; Sherman *et al.* 1988). Numerous studies on ants and bees have confirmed that colonies with multiple mated queens (Baer & Schmid-Hempel 1999, 2001; Tarpay 2003; Hughes & Boomsma 2004; Tarpay & Seeley 2006; Seeley & Tarpay 2007), as well as multiple as opposed to single queens (Liersch & Schmid-Hempel 1998; Reber *et al.* 2008), suffer from lower parasite loads.

While multiple mating and multiple queens only affect the between-individual component of genetic diversity, inbreeding simultaneously lowers both within-individual and between-individual diversity. This leads to colonies with high between-nest-mate relatedness and individuals with high levels of homozygosity. Studies of inbreeding in social insects have focused mostly on the within-individual component, and found no negative effect of inbreeding on either the physiological or the behavioural defences of individuals (Gerloff *et al.* 2003; Calleri *et al.* 2006). To our knowledge, only a single study on termites has investigated the colony-level effects of inbreeding that result from the interplay of within- and between-individual genetic diversity. Colonies had greater mortality and higher microbial loads after only a single generation of inbreeding, for which the authors suggest potentially lower allogrooming between workers in inbred colonies as a likely mechanism (Calleri *et al.* 2006).

(c) *Anti-pathogen defence in Cardiocondyla ants*

To investigate the effects of genetic diversity on individual and social immunity in social Hymenoptera, we compared the disease resistance and antiseptic behaviours of genetically homogeneous and diverse colonies of the ant *Cardiocondyla obscurior* after exposing brood to the entomopathogenic fungus *Metarhizium anisopliae*. We used an experimental set-up that allowed us to test for

differences between genetically diverse and homogeneous ant colonies with respect to (i) their susceptibility to the disease (measured as worker survival), (ii) their expression of self- and allogrooming as well as hygienic behaviour (removal of diseased brood from the nest), and (iii) their discrimination ability between pathogenic and non-pathogenic brood treatments.

2. MATERIAL AND METHODS

(a) *Experimental colonies*

Cardiocondyla obscurior colonies were collected from Una, Bahia, Brazil, and reared in the laboratory as described previously (Cremer & Heinze 2003). The social structure of *C. obscurior* colonies in the field is characterized by highly variable queen numbers, intra-nest mating and the exchange of individuals between nests (Heinze & Delabie 2005; Heinze *et al.* 2006). We used 16 field colonies to rear two different colony types (eight colonies each) over 4 years, i.e. 8–10 generations. *Genetically diverse colonies* contained 3–20 queens, the offspring of which were allowed to mate freely with each other and with incoming individuals from other colonies. *Genetically homogeneous colonies* were obtained by strict brother–sister mating, a single queen per colony and no migration into the nest (Schrempf *et al.* 2006). We therefore created eight independent inbred lines and eight independent diverse colonies of *C. obscurior* from our 16 original field colonies.

(b) *Simulations of within- and between-individual genetic diversity*

To characterize the difference in genetic diversity between the two colony types, we performed simulations revealing that strict inbreeding decreased both the within- and the between-individual genetic diversity of colony members as compared with the diverse colonies (inbreeding coefficient: homogeneous colonies, $F_{IT} = 0.87$; diverse colonies, $F_{IT} = 0.06–0.28$; worker–worker relatedness: homogeneous colonies, $r = 0.98$; diverse colonies, $r = 0.16–0.60$; for details, see the electronic supplementary material, tables S1, S2 and figure S1).

(c) *Experimental set-up and fungal infection of larvae*

From each of the 16 colonies, we set up two replicates containing five workers and nine larvae, which were presented in groups of three outside the brood chamber. Before the start of the experiment, larvae were colour-marked by ingestion of different food colours (Schwartau). Larvae in each group were coded with the same colour and received one of three larval treatments. In the *live spore treatment*, larvae were exposed to 0.3 μl of live conidiospores of the entomopathogenic fungus *M. anisopliae* var. *anisopliae* (strain KVL 03–143/Ma 275 dissolved in a 0.05% Triton X-100 solution (Sigma) at a concentration of 10^9 spores ml^{-1}). In the *dead spore treatment*, larvae received the same amount and concentration of spores, which, however, had been UV-killed and thus not able to germinate (Ugelvig & Cremer 2007). Larvae in the *sham control* received a spore-free solution of Triton X-100. Different combinations of colour code and treatment were used in different replicates and colonies, so that the observations occurred blindly with respect to larval treatment (for details, see the electronic supplementary material).

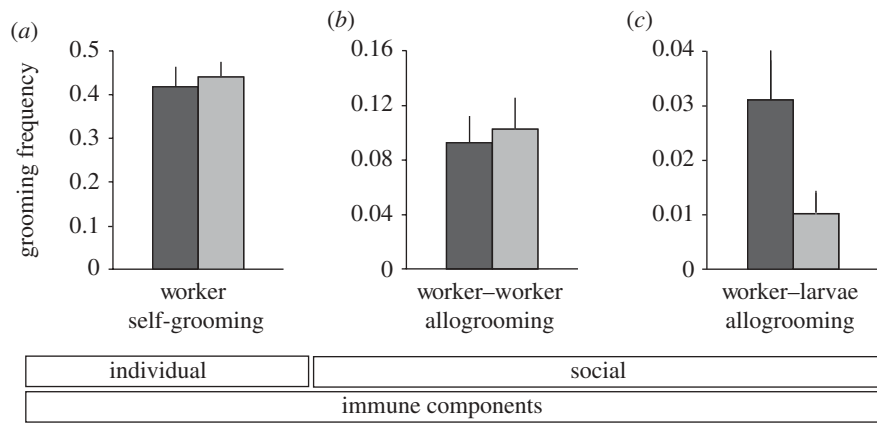


Figure 1. Individual and social grooming behaviour. Total grooming frequencies of workers from genetically diverse (dark grey bars) and homogeneous (light grey bars) ant colonies. (a) Worker self-grooming, (b) worker-worker allogrooming, and (c) larval allogrooming performed by adult workers (eight diverse and eight homogeneous colonies, two replicates per colony, mean + s.e.m.). Only worker-larvae allogrooming frequency was significantly lower in homogeneous than in diverse ant colonies.

(d) Behavioural observations and larval location

Immediately after the start of the experiment and for the following 4 days, we performed 10 scan samplings per day (each 1–2 s) to record ant worker behaviour. We observed the anti-septic behaviours, *worker self-grooming*, *worker-worker allogrooming* and *worker-larvae allogrooming*, as well as the non-disease-related behaviours, *worker running*, *worker-worker antennation* and *general brood care* (i.e. larval handling and feeding) (for details, see the electronic supplementary material). The frequencies of the observed behaviours were averaged across the two replicates per colony and arcsin square-root-transformed to obtain normal distributions prior to statistical analysis. As the same behaviours were recorded in a time series, we used two-way ANOVAs with repeated measures and post hoc contrasts (JMP 8; for detailed statistical analyses and MANOVA summary tables, see the electronic supplementary material, tables S3–S9).

The location of the larvae was observed every 15 min on day 1 and daily for the following 8 days. Both types of colonies carried most larvae into the brood chamber immediately after the start of the experiment, independent of larval treatment (for details, see the electronic supplementary material). Some of the larvae that had originally been carried into the brood chamber were later removed again and left outside to die. Larval removal data were analysed by Cox proportional regression analysis (SPSS 13; for details on the statistical analyses, see the electronic supplementary material).

(e) Histological analysis of the time course of larval infection

We determined the infection status of the live-spore-treated larvae over the course of the experiment by repeating the experimental set-up and taking out the larvae for histological analysis either 2, 24, 96 or 168 h after spore application (each in eight replicates; for details, see the electronic supplementary material).

(f) Survival and infection status of workers

We recorded the death of workers by *Metarhizium* infections for 12 days after the start of the experiment and analysed the survival data by the Cox proportional regression analysis (SPSS 13; see the electronic supplementary material for details).

3. RESULTS

(a) Survival and infection status of workers

Of 160 worker ants in the experiment, only a single individual (from a homogeneous colony) died from *Metarhizium* infection. The risk of workers dying from infection was thus similarly low for workers in the homogeneous and diverse colonies (Cox proportional regression; colony-type effect: Wald statistics = 0.27, d.f. = 1, $p = 0.61$).

(b) Non-disease-related behaviours

Ants from the diverse colonies spent more time running than workers in the homogeneous colonies (repeated-measures ANOVA, between-subject effect of colony type: $F_{1,14} = 9.14$, $p = 0.009$). The two colony types did not show significant differences in either antennation frequency ($F_{1,14} = 2.821$, $p = 0.12$) or general brood care activity ($F_{5,42} = 0.96$, $p = 0.45$; for details, see the electronic supplementary material, tables S3, S4 and S7).

(c) Grooming behaviour

There was no significant difference between the two colony types in worker self-grooming (figure 1a; repeated-measures ANOVA: between-subject effect of colony type: $F_{1,14} = 0.09$, $p = 0.77$) or worker-worker allogrooming (figure 1b; $F_{1,14} = 0.04$, $p = 0.84$).

By contrast, worker-larvae allogrooming frequency was significantly higher in the diverse than in the homogeneous colonies (figure 1c; $F_{1,46} = 5.24$, $p = 0.03$). Furthermore, the two colony types differed in their time pattern of larval grooming (as revealed by a significant interaction between colony type and time: $F_{4,43} = 2.70$, $p = 0.04$). In the homogeneous colonies, larval grooming frequency was consistently low throughout the experiment (figure 2a; repeated-measures ANOVA for homogeneous colonies only, within-subject time effect: $F_{4,20} = 0.16$, $p = 0.96$), whereas larval grooming frequency was significantly higher in the first 29 h after larval treatment in the diverse colonies (figure 2a; repeated-measures ANOVA for diverse colonies only: within-subject time effect: $F_{4,20} = 4.23$, $p = 0.01$; post hoc contrasts of starting point to late (greater than 29 h) time points: always $p < 0.01$; for details of

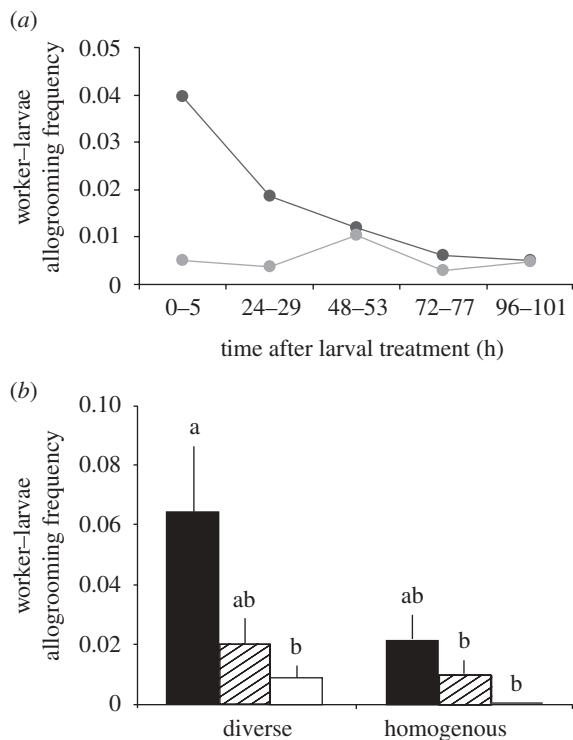


Figure 2. Larval grooming by adult workers over time and in response to larval treatment. (a) Daily worker-larvae allogrooming frequency of workers from genetically diverse (dark grey line) and homogeneous (light grey line) colonies. (b) Total worker-larvae allogrooming frequency by workers from the genetically diverse and homogeneous colonies towards the three larval treatments: live-spore-treated larvae (black bars), dead-spore-treated larvae (hatched bars) and sham-treated larvae (white bars; mean + s.e.m.; significance groups (letters) obtained by all pairwise comparisons of ANOVA post hoc Tukey tests). Workers from diverse colonies intensively groomed live-spore-treated larvae within the first 29 h of the experiment, while larval grooming was consistently low and did not differ between larval treatments in the homogeneous colonies.

pairwise comparisons, see the electronic supplementary material, table S8).

Worker-larvae allogrooming frequencies did not only differ across time but also between the three larval treatments for the two colony types (i.e. the six treatment groups; figure 2b; repeated-measures ANOVA, between-subject treatment group effect: $F_{5,42} = 4.00$, $p = 0.005$). Workers from the diverse colonies groomed the live-spore-treated larvae more intensively than the sham-control-treated larvae and the dead-spore-treated larvae at intermediate frequencies. The workers from the genetically homogeneous colonies, however, did not groom the live-spore-treated larvae significantly more often than the control larvae, which may be owing to overall low levels of grooming (figure 2b; post hoc all pairwise Tukey test; $p < 0.05$ for diverse colonies \times live spores versus diverse colonies \times sham control/homogeneous colonies \times dead spores/homogeneous colonies \times sham control; all other pairwise comparisons non-significant). Furthermore, the preferential grooming of live-spore-treated larvae by the workers in the diverse colonies was caused mainly by an intensive early grooming of these larvae (same repeated-measures ANOVA: within-subject

time effect: $F_{4,39} = 2.74$, $p = 0.04$), post hoc contrasts of starting point to 48–53 h $p = 0.06$, and to greater than 53 h always $p < 0.01$).

(d) Hygienic behaviour

Both colony types initially carried larvae into the brood chamber and subsequently removed some of them (figure 3; for details, see the electronic supplementary material). The larvae that were removed were almost exclusively live-spore-treated (figure 3a,b; Cox proportional regression; pathogenic–non-pathogenic treatment; diverse colonies: Wald statistics = 7.03, d.f. = 1, $p = 0.008$; homogeneous colonies: Wald statistics = 7.98, d.f. = 1, $p = 0.005$). Larvae treated with either dead spores or sham control were removed at low rates, not significantly different from zero for both colony types at any time (Fisher exact test: $p > 0.4$; for details, see the electronic supplementary material) and not significantly different from each other (Cox proportional regression; dead spores–sham control: Wald statistics = 1.45, d.f. = 1, $p = 0.23$).

Cumulative removal frequencies of pathogen-treated larvae did not differ significantly between the genetically diverse and homogeneous colonies towards the end of the experiment (Fisher's exact test: d.f. = 1; 72 h, $p = 0.22$; 96 h and following, $p = 0.39$). Importantly, however, the timing of when colonies started to remove larvae differed between colony types. Only the diverse colonies removed larvae from the brood chamber within the first 24 h after spore application. This early removal of larvae was nearly significantly higher in the diverse than in homogeneous colonies (Fisher's exact test at 24 h, $p = 0.07$) and was significantly different from zero only in the diverse colonies ($p = 0.05$). By the time the homogeneous colonies removed the first larvae, the diverse colonies had already taken out 50 per cent of the overall removed larvae (with the remaining 50% to be removed within 96 h; figure 3a,b).

(e) Time pattern of larval infection

Histological analyses revealed that fungal spores had not yet penetrated and infected larvae at either 2 h or 24 h after spore application (figure 3c(i)), while mycelial growth occurred inside the bodies of larvae analysed at 96 h and 168 h after spore treatment (figure 3c(ii)).

4. DISCUSSION

Our study on collective antiseptic and hygienic behaviours in the ant *C. obscurior* revealed higher allogrooming frequencies and earlier nest removal of pathogen-treated larvae in genetically diverse than in homogeneous colonies. We can thus conclude that inbreeding compromises efficient early anti-pathogen defence in ant societies.

(a) Worker activity and disease susceptibility

Ants from genetically diverse colonies showed higher individual running activity than workers from homogeneous colonies, similar to a higher foraging activity reported in genetically more diverse honeybee colonies (Mattila & Seeley 2007). Apart from that, the frequencies of other non-disease-related behaviours, such as antennation and

general brood care behaviour as well as most antiseptic behaviours (see below), did not differ between the ants from the two colony types, indicating that inbreeding did not lower all activities equally.

Both colony types also showed a similarly low risk of workers contracting the disease from the exposed brood, revealing that inbreeding did not make the ants more susceptible to *Metarhizium* infection. Overall low cross-infection has also been found among nest-mate termite nymphs and leaf-cutter ant workers (Rosengaus *et al.* 1998; Hughes *et al.* 2002).

(b) Grooming behaviour

Ants from both colony types performed worker self-grooming and worker-worker allogrooming at the same frequencies (figure 1*a,b*). Previously, data on termites also revealed no negative effect of inbreeding on self-grooming rates (Calleri *et al.* 2006). Likewise, allogrooming frequencies were not found to be affected by genetic diversity in leaf-cutting ants (Hughes & Boomsma 2004). Together, these studies show that individual and social antiseptic behaviour does not seem to be compromised in genetically homogeneous insect societies. Given the similar disease resistance levels (survival) and individual and social grooming activities, we can also conclude that the physiological immunity of individual ants was not likely to have been affected by the 8–10 generations of brother–sister matings. The same has been found after one generation of inbreeding in termites and bumble-bees (Gerloff *et al.* 2003; Calleri *et al.* 2006).

In contrast to these similarities, workers from homogeneous colonies were compromised in worker–larvae allogrooming, an important component of social immunity (figure 1*c*). Only ants from diverse colonies showed intensive grooming of live-spore-treated larvae in the first 29 h after the start of the experiment (figure 2*a,b*). A similar immediate but temporary upregulation in grooming behaviour has been reported in diverse colonies of termites and other ant species after colony exposure to pathogens (Kermarrec *et al.* 1986; Rosengaus *et al.* 1998; Jaccoud *et al.* 1999; Currie & Stuart 2001), but its absence in inbred colonies is an entirely novel finding of this study.

What causes this deficit in expressing early antiseptic behaviour towards the pathogen-treated larvae in ants from genetically homogeneous colonies? At the individual level, an overall higher homozygosity of workers may (i) result in a lower general physical performance, including antiseptic behaviours (comparable to Luong *et al.* 2007); or (ii) affect the ants' ability to detect and/or trigger a response to the pathogen. Our data indicate that the first explanation is highly unlikely, as workers from genetically homogeneous colonies did not show a lower rate of grooming themselves (figure 1*a*) or other adult workers (figure 1*b*). Neither did they show less general brood care.

Instead, it seems likely that homozygosity affects the ability of individual ants to detect or respond to live fungal spores or that differences in between-individual genetic diversity affect colony-level performance. All physiological abilities of individuals being equal, the high relatedness between nest-mates in inbred colonies

may reduce the likelihood that the threshold to react to the stimulus 'spores' with the action 'grooming behaviour' is reached in any group member. Our data are thus also in line with response threshold models for division of labour (Beshers & Fewell 2001).

(c) Hygienic behaviour

To the best of our knowledge, this study is the first to describe hygienic behaviour in ants (i.e. the detection and removal of diseased brood). So far, the behaviour has only been known for honeybees (Rothenbuhler & Thompson 1956; Wilson-Rich *et al.* 2009), which raise their brood in individual brood cells. Ant brood, on the other hand, is clumped together in brood piles that can facilitate larval cross-infection (L. V. Ugelvig & S. Cremer 2006, unpublished data). This suggests that hygienic behaviour may be at least equally important in ants as in honeybees. The ants in our experiment excluded larvae from the brood chamber over the duration of a week (figure 3), which covers the time course of a typical *Metarhizium* infection (Ugelvig & Cremer 2007). The expression of this hygienic behaviour is a direct consequence of our treatment of larvae with fungal spores, as it did not occur in an additional control set-up where colonies had received only fungus-free control larvae (electronic supplementary material).

All colonies only removed the live-spore-treated larvae at frequencies significantly different from zero, but the timing of larval removal differed between the two colony types. Only ants from the diverse colonies started removal of live-spore-treated larvae within the first 24 h after spore application, and histological analyses revealed that fungal spores had not yet penetrated, i.e. infected, these 'early-removed' larvae (figure 3*c*(i)). 'Late-removed' larvae, on the other hand, contained mycelial growth inside their bodies (figure 3*c*(ii)). This confirms the time pattern of *Metarhizium* infection in other arthropods, where attachment of spores to the host cuticle and its penetration was found to occur after approximately 6 h and 24–48 h, respectively (Vestergaard *et al.* 1999; Arruda *et al.* 2005). Thus, ants from both colony types reacted to signs of larval sickness by late larval removal, but only ants from diverse colonies had the additional capacity to perceive and/or react to the presence of the live, pathogenic fungal spores prior to infection. The fact that larvae exposed to dead fungal spores and spore-free controls were treated similarly indicates that the ants detect and respond to cues of the live spores themselves or cues released throughout the germination process, and not the sheer physical presence of non-germinating spores.

These cues are likely to be of a chemical nature, as termites react to volatile compounds released from spores of *M. anisopliae* in an olfactometer (Mburu *et al.* 2009), and volatile compounds of another fungal pathogen (*Ascospaera apis*; chalkbrood disease) have been shown to elicit hygienic behaviour in honeybees (Swanson *et al.* 2009). Furthermore, honeybee workers that uncap infected brood cells in their colonies apparently have better chemosensory capacities than bees not performing this task (Masterman *et al.* 2001; Gramacho & Spivak 2003; Wilson-Rich *et al.* 2009).

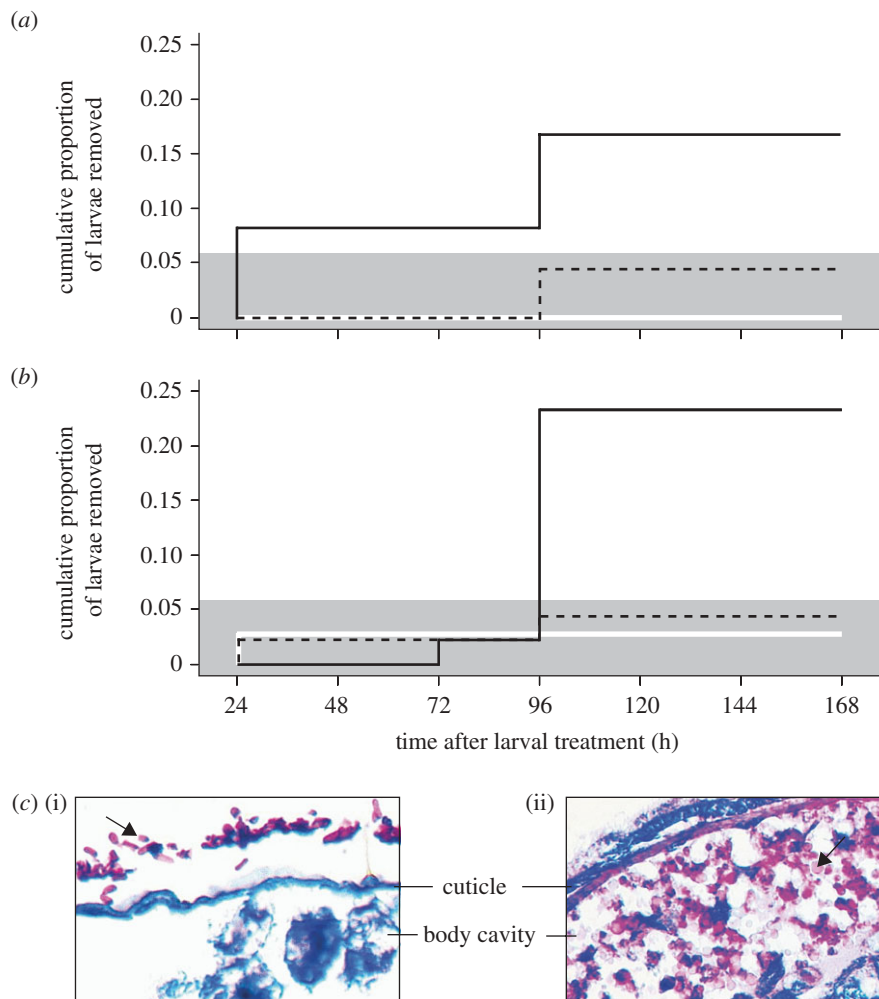


Figure 3. Larval removal from the brood chamber depending on infection status. Workers from (a) genetically diverse and (b) homogeneous colonies (means of eight colonies each, two replicates per colony) removed live-spore-treated larvae (black solid line) significantly more often than larvae treated with the non-pathogenic dead spores (black dashed line) or sham-treated (white solid line). Removal frequencies of non-pathogenic larvae did not significantly differ from zero in either colony type (as indicated by the grey shading showing the zone of non-significant difference from zero). Only workers from diverse colonies started removal of pathogen-treated larvae within the first 24 h after spore application. (c) Histology of *Cardiocondyla* larvae (i) within the first 24 h after live spore application ($n = 16$) and (ii) from 96 h onwards ($n = 16$; fungus stained pink, ant tissue blue). Fungal spores (i, arrow) did not enter the larval bodies within the first 24 h. From 96 h onwards, substantial hyphal growth (ii, arrow) was detectable inside the body of removed larvae.

(d) Potential benefits of early antiseptic behaviours

We suggest a twofold prophylactic effect of the intensive early grooming of live-spore-treated larvae (figure 2a) and the early removal of those larvae from the brood chamber (figure 3a). First, quick grooming of fungal spores, before they attach firmly or enter the host cuticle, is most efficient to decrease spore number on the exposed individuals. This reduces the likelihood of individual infection (Rosengaus *et al.* 1998; Hughes *et al.* 2002), especially if spore dosage drops below a critical threshold value (this was not likely to be the case in our experiment owing to the high initial exposure dosage). Second, early grooming and in particular early removal will lower the risk of cross-infection of hitherto unexposed larvae in the brood pile. The early anti-pathogen defences observed in the genetically diverse colonies therefore probably affect both individual and colony-level disease dynamics. The failure of workers from homogeneous colonies to perform those early social defences may thus

explain the high parasite load observed in inbred field colonies of social insects (Calleri *et al.* 2006).

(e) Potential basis of impaired antiseptic behaviours

As the reduction of antiseptic behaviours was observed in all eight inbred lines of *Cardiocondyla*, each originating from a different field colony, we conclude that the described phenomenon is not a result of a specific genotype with low chemical detection ability, but rather is an effect of decreased genetic diversity within colonies or individuals. Currently, we can only speculate that a possible underlying mechanism may originate from either a reduced chemosensory capacity of the inbred adult workers to detect the pathogen, or a weaker 'groom me' or 'remove me' signal elicited by the inbred larvae. This may be caused by a different interaction of the fungal spores with the cuticle of inbred versus non-inbred

larvae. However, studies on genetically diverse termites and ants (Jaccoud *et al.* 1999; Mburu *et al.* 2009) suggest that workers already change their behaviour in the presence of pure dried spores of *M. anisopliae* without any interaction with the host cuticle, making an impaired pathogen detection capacity of the adult *Cardiocondyla* ants a more likely explanation.

Alternatively, the discrimination capacities of individual workers from the genetically homogeneous colonies are unaltered but fail to trigger the same response as observed in workers from the genetically diverse colonies. We consider this unlikely as the ants from homogeneous colonies also ultimately remove sick larvae from the nest. Lower pathogen perception abilities of individual ants in response to their homozygosity coupled with a low between-individual diversity in task thresholds (Beshers & Fewell 2001) would be a sufficient mechanism to explain the observed colony-level differences.

Our behavioural approach thus allowed us to bring forward novel and testable hypotheses on the mechanisms underlying the observed delay in anti-pathogen behaviours in genetically uniform ant societies. To disentangle whether the observed phenomena are mainly based on differences in individual homozygosity or on differences in genetic relatedness between group members, future studies should compare colonies differing only in homozygosity or relatedness levels. Given the heritable differences in chemosensory capacities of honeybees (Gramacho & Spivak 2003; Spivak *et al.* 2003; Swanson *et al.* 2009), it would be highly valuable to put such future studies into a neuro-immunological framework.

Performed experiments comply with the laws of Germany.

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