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No benefit in diversity? The effect of genetic variation on survival and disease resistance in a polygynous social insect

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Abstract. 1. Multiple mating by queens has been shown to enhance disease resistance in insect societies, because higher genetic diversity among nestmates improves collective immune defences or offers a certain level of herd immunity. However, it has remained ambiguous whether polygynous societies with large numbers of queens also benefit from increased genetic diversity.

2. We used one of the very few ant species that can be reared across generations, the pharaoh ant, *Monomorium pharaonis* Linnaeus, to create experimental colonies with two types of enhanced genetic diversity: (i) mixed workers from three divergent inbred lineages representing the 'polygyny-equivalent' of multiple mating by queens (i.e. increased between-worker variation); and (ii) uniform workers whose overall heterozygosity was increased by two subsequent generations of crossing between the same divergent inbred lineages (i.e. increased within-worker variation).

3. We found significant differences in worker survival among the three inbred lineages, with exposure to conidiospores of the fungal pathogen *Beauveria bassiana* causing significant mortality to the workers independently of their diversity type. Increased diversity did not improve the resistance to *Beauveria*.

4. Enhanced heterozygosity colonies had worker survival rates similar to the most resistant inbred lineage, whereas colonies with mixed workers from the three inbred lineages had lower worker and larval survival. Workers did not show any infection-avoidance behaviour.

5. Average larval survival appeared unaffected by the presence of conidiospores. It benefitted from increased heterozygosity but was reduced in mixed colonies independent of infection. This suggests that negative, but cryptic social interactions in mixed colonies may affect overall survival.

6. The present results do not provide evidence for or against a link between increased genetic variation and increased disease resistance in pharaoh ants, but show that colonies differ considerably in general survival. Thus, increasing the genetic diversity of pharaoh ant colonies may not provide survival advantages in the face of pathogen exposure, and polygyny and polyandry may not be directly comparable mechanisms for creating adaptive resistance towards pathogens.

Key words. Ants, *Beauveria bassiana*, breeding experiment, entomopathogenic fungi, *Monomorium pharaonis.*

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Introduction

Living in groups provides benefits of cooperation, but increases the risk of disease transmission (Anderson & May, 1985). Large societies of ants, bees, wasps, and termites are expected to experience considerable pressure from parasites and pathogens, because dense groups of related individuals living in stable environments are attractive hosts to exploit (Schmid-Hempel, 1998; Boomsma *et al*., 2005). Many insect societies possess social mechanisms of disease defence in addition to the individual-level defences that are common to solitary ancestors. This combination of individual and social defences may help to offset the increased disease pressures of social living (e.g. Rosengaus *et al*., 1998; Hughes *et al*., 2002; Boomsma *et al*., 2005; Cremer *et al*., 2007; Evans & Spivak, 2010). Social defences include grooming behaviours and the division of labour with some individuals specializing in hygienic behaviours (Evans & Spivak, 2010), as well as the use of antimicrobial compounds obtained during foraging (Chapuisat *et al*., 2007; Bulmer *et al*., 2009) and domesticated symbionts (Little *et al*., 2006). Individual defences include encapsulation responses (Baer *et al*., 2005) and secretions from specialised glands (e.g. Hughes *et al*., 2008a). These multiple lines of defence make social insect colonies difficult to invade, because they function as dual-palisade fortresses from the perspective of potential parasites (e.g. Wilson, 1971; Hughes *et al*., 2008b).

Genetic diversity among nestmates may enhance a colony's ability to combat infection (Hamilton, 1987) by affecting both individual- and social lines of defence. For example, increased genetic diversity is likely to increase the efficiency of the division of labour and to facilitate colony-level homeostasis (Crozier & Page, 1985; Oldroyd & Fewell, 2007). The 'pure stands breed diseases' paradigm has been particularly influential in stimulating work to explain the evolution and maintenance of multiple mating by queens (polyandry) in outbreeding hymenopteran societies headed by a single queen, as this is a costly, but powerful, mechanism for increasing intracolonial genetic variation (reviewed in Boomsma & Ratnieks, 1996; Crozier & Fjerdingstad, 2001; Strassmann, 2001). Several studies with polyandrous or polygynous social insects have shown that increased inter-individual genetic diversity in colonies is indeed associated with increased disease resistance (e.g. Baer & Schmid-Hempel, 1999; Schmid-Hempel & Crozier, 1999; Tarpy, 2003; Hughes & Boomsma, 2004; Tarpy & Seeley, 2006; Reber *et al*., 2008). Examples of potential mechanisms through which genetic diversity may influence disease resistance range from genetically determined hygienic behaviours (Rothenbuhler, 1964) and heritable variation for antimicrobial peptides (Decanini *et al*., 2007), to heritable size variation in worker metapleural glands (Hughes *et al*., 2010).

It is seldom made explicit that genetic diversity in eusocial colonies both has an inter-individual and intra-individual (heterozygosity) component, which may affect defences to a variable extent. While a fair amount of studies have been devoted to the inter-individual component as found in species with multiple patrilines, only three studies have addressed the intra-individual component. Calleri *et al*. (2006) showed that groups of inbred termites suffered higher mortality when exposed to a fungal pathogen, and Ugelvig *et al*. (2010) showed that groups of inbred *Cardiocondyla* ants are less good in detecting infections before they spread. However, Gerloff *et al*. (2003) did not find any negative effects of one generation of inbreeding in a bumblebee when measuring the immune response of single individuals. The explicit study of individual heterozygosity as a variable for disease resistance is particularly relevant when genetic diversity is generated by varying numbers of queens breeding in the same colony, as this type of colony structure often involves substantial degrees of inbreeding (Haag-Liautard *et al*., 2009), limited dispersal across occupied patches, and thus considerable genetic population structuring (Sundström *et al*., 2005).

We used the pharaoh ant, *Monomorium pharaonis* Linnaeus, as an experimental model system, as this global pest species has extremely high worldwide genetic differentiation (measured as $F_{ST} = 0.751 \pm 0.006$ SE) among its global populations (Schmidt *et al*., 2010). This differentiation means that crossing and mixing of lineages can create substantial differences in intra- and inter-individual genetic diversity, enabling the production of colonies of increased intra- and inter-individual diversity, which can subsequently be exposed to experimentally controlled infection regimes. In addition to shedding light on the relevance of two distinct types of genetic variation for colony level disease resistance, the present study addresses the effect of genetic variation on survival in groups of pharaoh ants and the extent to which polyandry and polygyny are equivalent mechanisms for enhancing adaptive genetic diversity in insect societies, an issue that has remained somewhat controversial (Keller & Reeve, 1994; Boomsma & Ratnieks, 1996; Schmid-Hempel & Crozier, 1999; Hughes *et al*., 2008c).

Materials and methods

Study species

Pharaoh ants form large polygynous colonies and are commonly found as pests in buildings. New queens and males mate within their natal nest (all queens are singly inseminated) and pharaoh ants can thus potentially be kept in the laboratory indefinitely (Peacock & Baxter, 1949; Berndt & Eichler, 1987). They only spread to new areas by walking or by being inadvertently transported via human-mediated jump dispersal (Edwards & Baker, 1981; Schmidt *et al*., 2010), and gene flow between colonies may therefore be rare, at least in the semi-natural habitats associated with human settlement. Microsatellite markers have previously been employed to show that *M. pharaonis* colonies, both from laboratories and from the field, have very low levels of genetic diversity and are genetically highly differentiated between sites but without any correlation between genetic and geographical distance (Schmidt *et al*., 2010). For the present study, experimental groups of workers and brood using three different source colonies were created (U1, U3, and U5; Schmidt *et al*., 2010), each of them an inbred lineage originally collected in the USA

(U1 and U3) or UK (U5), and subsequently kept in laboratories for more than 10 years.

Controlled crosses to create a hybrid stock colony with enhanced heterozygosity

We created ants with high intra-individual diversity through controlled crosses between the three inbred laboratory lineages U1, U3, and U5. Controlled breeding is not possible with most ants (see e.g. Cremer & Heinze, 2003; Schrempf *et al*., 2006), so the approach reported here is novel, and has only been used in one other ant species (Ugelvig *et al*., 2010). The crosses were made over two generations (Fig. 1), first crossing colonies U5 (females) and U3 (males) and subsequently crossing the hybrid females with males from colony U1. This created a colony with high heterozygosity of the individual ants, while hardly affecting the inter-individual genetic diversity (Fig. 1, see also below) as the diversity in the source colonies (U1, U3, and U5) was very low (Schmidt *et al*., 2010). The production of sexuals (virgin queens and males) for the crosses was induced by removing the mother queens from large colony fragments, waiting *c*. 40 days for the workers to rear a new sexual brood, and removing this sexual brood at the pupal stage, keeping males and females separate. Groups of 15–20 females and 10–15 males were then combined in 10-cm-diameter Petri plates and mating often occurred immediately. Approximately 1 week later, the newly mated queens were supplemented with brood and workers from other colonies to boost growth rates of these incipient colonies, as the queens are practically incapable of founding new colonies by themselves (Peacock *et al*., 1955; A.M. Schmidt, pers. obs.). The colonies were regularly checked for the first 2 months and any sexuals reared from the supplemental brood were removed before eclosing to ensure only next generation offspring were obtained. The generation time was approximately 5 months.

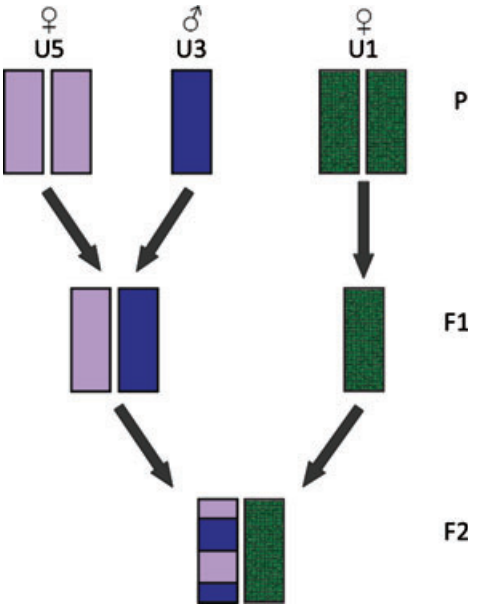

Fig. 1. The crossing scheme applied to obtain individuals of high genetic diversity in *Monomorium pharaonis*. The bars represent putative alleles at any marker locus; as males are haploid these are represented by one bar only. After two crossings (generations) the individuals of the F2 generation had contributions from all three parental lineages.

Experimental groups

Groups of ants were chosen as the survival of single *M. pharaonis* workers is generally very low (A.M. Schmidt, pers. obs.) and small groups are biologically more relevant study units because trophallaxis, grooming, and other characteristic social interactions can occur naturally (Hughes *et al*., 2002). The experimental groups consisted of 18 workers and 12 second to third instar larvae. The colonies were kept in boxes containing multiple tubes in which the ants keep their brood. We sampled from these nest tubes to obtain workers of intermediate age, which was feasible because younger workers take care of the brood and older workers are foragers (Berndt & Eichler, 1987).

Low genetic diversity groups were created from each of the original lineages U1, U3, and U5 (inbred lineages; Fig. 2). We further used these inbred lineages to create high inter-individual genetic diversity groups by mixing individuals from these three colonies (mixed groups; Fig. 2). As pharaoh ants are highly polygynous and generally display low levels of intraspecific aggression, one can normally combine worker ants from different colonies without inducing antagonistic interactions (Schmidt *et al.*, 2010). We used the high heterozygosity stock colony to create the high heterozygosity experimental groups (crossed groups, Fig. 2). Previous work (Schmidt *et al*., 2010) has shown that the three inbred laboratory lineages are genetically highly divergent ($F_{ST} = 0.601$; $P < 0.0001$) and have low levels of genetic diversity with an average allelic richness across four polymorphic microsatellite loci of only 2.1 ± 0.2 SD (based on standard samples of 30 workers). Our breeding and mixing design therefore meant that the allelic richness, as inferred from single colony data, became about twice as high, both in the mixed (4.3 ± 0.7) and crossed (4.1 ± 1.1) groups, relative to the original inbred lineages U1, U3 and U5. Furthermore, whereas intra-individual genetic diversity was similar in the U1, U3, U5, and mixed groups (average observed heterozygosity across loci, $H = 0.322 \pm 0.109$ SD), this had become almost twice as high (0.619 ± 0.051) in the crossed groups (estimation based on analysis in FSTAT 2.9.3.2; Goudet, 2002).

Each experimental group was made by carefully transferring six workers and four larvae at a time to 10-cm-diameter Petri plates (Fig. 2), resulting in a uniform procedure for setting up all groups of 18 workers and 12 larvae. Larvae were transferred first, because these exchange food with the workers and their presence may thus reduce the stress level of newly added workers (Berndt & Eichler, 1987). A total of 36 groups [18 treatment groups which were exposed to the fungal pathogen (see below) and 18 controls which were not exposed] were made for each

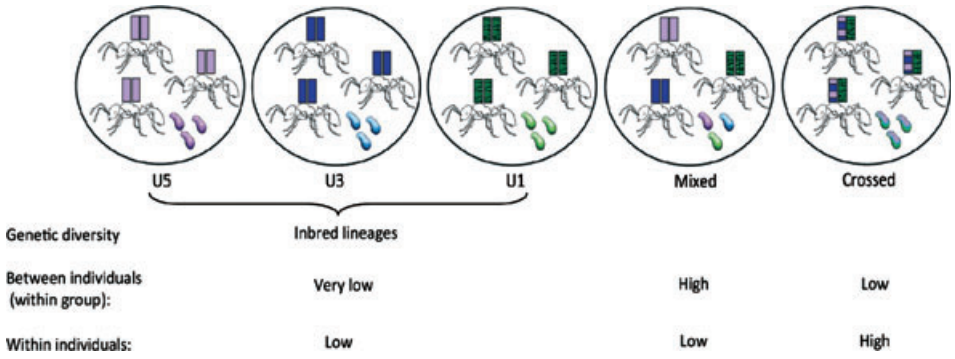

Fig. 2. Experimental setup and genetic diversity of the different treatment groups of *Monomorium pharaonis*. Three highly genetically differentiated, low-diversity and inbred source lineages (U5, U3, and U1; average allelic richness, $k' = 2.1 \pm 0.2$ SD; average heterozygosity, $H = 0.322 \pm 0.109$ SD, cf. Materials and methods) were used singly, mixed or crossed, thereby creating groups of different levels of intra- or inter-individual level of genetic diversity (mixed groups, $k' = 4.3 \pm 0.7$, $H = 0.322 \pm 0.109$; crossed groups, $k' = 4.1 \pm 1.1$, $H = 0.619 \pm 0.051$, cf. Materials and methods). Each worker in the figure represents six individuals and each larva in the figure represents four individuals. The bars of different colour represent alleles of different lineage origin as given in Fig. 1.

of the three low diversity colonies (U1, U3, and U5), as well as for each of the mixed and crossed group combinations.

Pathogen exposure

After transfer to Petri plates, the ants were given a water tube plugged with cotton wool as well as a small piece of paper to hide under. The treatment groups were exposed to conidiospores from the entomopathogenic fungus *Beauveria bassiana* which were added to the Petri plates on pieces of filter paper. The strain used was KVL 04-33, originally collected in Denmark from soil using *Tenebrio molitor* Linnaeus baits. We chose *B. bassiana* as the infecting agent because it is a generalist entomopathogen, has a worldwide distribution, is frequently used for biocontrol, and has very characteristic conidiospore balls, making infected individuals easily identifiable (Hajek & Leger, 1994; Schmid-Hempel, 1998). To the best of our knowledge no specific natural pathogens have previously been identified for pharaoh ants, but, as they are scavengers and eat dead insects, it is likely that they would naturally come across *B. bassiana* when foraging.

To simulate natural exposure to conidiospores, as through a contaminated food source, we placed the ant's normal food (dead, frozen mealworms, boiled egg yolk, boiled liver, and honey) on spore-soaked filter paper pieces. These were prepared by soaking 4-cm^{2}pieces in a high concentration spore suspension by dipping them directly into a water-filled agar plate on which the fungus had been cultured. The filter paper pieces were left to dry for *c*. 1 h at room temperature, and transferred to the Petri plates with the ants. To further increase exposure, a small portion of fungal hyphae and conidiospores (ca. 15 mm^3) was added on top of each piece of filter paper, thereby obtaining a dose of approximately 1.0×10^{11} ml^{-1}. All experimental groups had conidiospores added from the same two agar plates. Although this method of exposure may appear somewhat crude, we found it necessary to provide an environment with very high conidiospore concentrations, as pilot experiments showed that pharaoh ants are very resistant to smaller (size order 10^9 ml^{-1}), albeit still high doses. The control groups had filter paper pieces without conidiospores added. The germination rate of conidiospores was checked separately on an agar plate kept under similar conditions and found to be 100%.

The Petri plates were placed in fluon-coated trays, grouped according to treatment and type of genetic diversity and left undisturbed in 26°C climate cabinets for a week, after which the number of live and dead workers was counted. This timespan was chosen because it takes several days for the fungus to kill the ants. There were several worker escapes from the groups during the experiments, which meant that some groups lost some workers and others gained some. These exchanges only occurred between groups of the same type and treatment, and for the mixed groups no more than three ants escaped from or were introduced to a Petri plate, rendering any re-sorting according to group of origin highly unlikely. We therefore chose to include all experimental groups that still contained a total of 15–21 workers (including both dead and live individuals) by the end of the experiment to ensure similar sizes of experimental colonies in our analyses and minimal changes in group composition. Dead ants were inspected under a stereomicroscope, removed, and surface sterilised by dipping them in 70% alcohol, dH_2O, 10% NaClO and three subsequent changes of dH_2O. At this time, the number of surviving larvae was also counted. No dead larvae could be investigated further, as they are routinely cannibalised by the workers. During the experiment the humidity in the plates was not controlled externally, as the ants seek out relatively high levels of humidity and often nest close to or in the openings of water tubes anyway. This behaviour also provides an environment that facilitates germination of conidiospores. Dead ants were kept for six more days in high humidity boxes and were then inspected for fungal growth under a stereomicroscope. *Beauveria bassiana* was assessed as being the cause of death by the presence of hyphae and characteristic conidiospore balls on the cuticle.

Data analysis

A total of 138 experimental groups were analysed using the proportion of workers alive after 1 week to control for variation in worker number across the groups. The data were analysed in R version 2.10.1 (2009) using a Generalized Linear Model (GLM function) on the proportions of live workers and on the number of live brood, applying group and fungal treatment as fixed effects and using quasibinomial and poisson error structures for the proportion and count data, respectively. The effects of treatment, group type, and the interaction of these terms were further tested using analyses of deviance with F or χ^2 tests.

Results

At the time that dead ants were removed from the groups none of them had any fungal growth, but we found *B. bassiana* conidiospore balls on dead ants in 73% of the treatment groups when checked 6 days later, whereas none of the dead ants from the controls had any *B. bassiana* growth (Fisher's exact test, $P < 0.0001$). In the control groups, a total of 115 out of 1218 workers died whereas 174 out of 1115 workers died in the *Beauveria*-treated groups. Of the 174 ants that died in the fungus-treated groups, 99 had conidiospores (i.e. 57% in total, varying from 27 to 75% of the dead individuals in the different groups). The values are likely underestimates, because fungal growth may take longer to become visible, and sporulation is expected to increase over time (Sun *et al*., 2002). Overall, this confirms that the mortality differences found were largely caused by the infections with *Beauveria*.

All experimental colony types appeared to be adversely affected by the *B. bassiana* treatment and suffered increased worker mortality relative to controls (GLM on averages for the different group types; $F_{1,132} = 9.96$, $P < 0.002$; Fig. 3a). The groups differed in the proportion of workers surviving ($F_{4,133} = 10.23$, $P < 0.001$; Fig. 3a), and although there were significant effects of group type, there was no interaction between group type and treatment ($F_{4,128} = 1.36$, $P = 0.25$). Hence, the diversity treatment therefore did not have a detectable effect on group resistance to *Beauveria*. However, when using GLMs to compare the average survival of workers across group categories, the crossed groups had higher survival than the inbred U1 and mixed groups ($P < 0.02$), but not significantly higher than the inbred U3 and U5 groups. The mixed groups had significantly higher survival than the U1 groups, which had lower average survival than all other groups ($P < 0.001$, Fig. 3a). The inbred U5 groups had significantly higher survival than all other group types, except for the crossed groups for which the difference was not significant. There was no effect of fungal treatment on larval survival ($P = 0.188$, Fig. 3b), but a significant effect of group type ($P < 0.001$, Fig. 3b), with mixed groups having significantly fewer larvae surviving than all other groups ($P < 0.001$), and the crossed groups having significantly more larvae surviving than the other groups except U1 ($P = 0.008$). There was no significant interaction between group type and treatment ($P = 0.052$) for larval survival, although the U1 larvae did appear to be more adversely affected by the fungal treatment (Fig. 3b), an effect that could be direct or indirect via reduced care offered by infected workers.

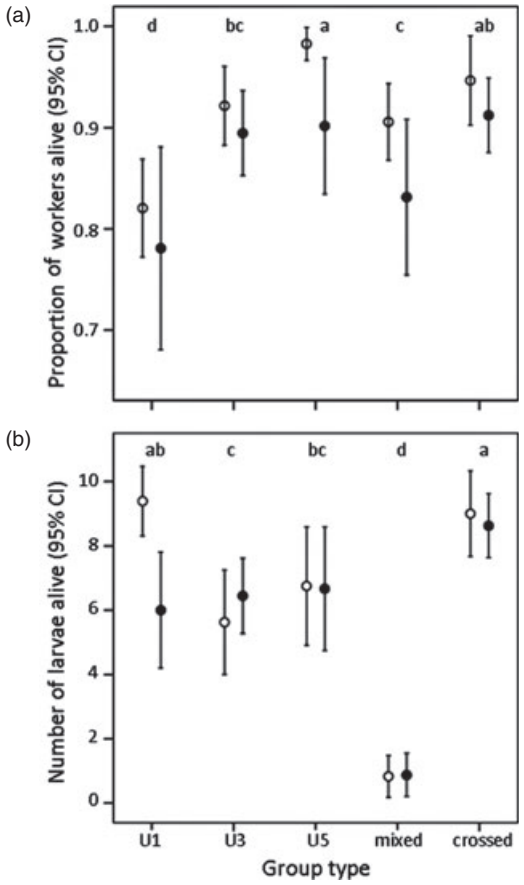

Fig. 3. Proportion of *Monomorium pharaonis* workers (a) and number of larvae (b) alive after 1 week. Open and filled symbols indicate controls and *Beauveria bassiana* treatments, respectively. The *B. bassiana*-treated groups had significantly lower worker ant survival. Number of replicates (without/with fungal treatment): U1, $n = 18/11$; U3, $n = 16/18$; U5, $n = 12/9$; mixed, $n = 12/17$; crossed, $n = 9/16$. Significant differences between control worker survival in the group types are indicated by different lower case letters. Means and 95% CIs are given in the figure.

No obvious pathogen avoidance behaviour was observed during the experiment. Although no systematic behavioural observations were made, it was striking that workers could be regularly observed walking over the filter paper infected by the very high doses of conidiospores that we used, rather than changing their course. In addition, 5–10% of the colonies nested under the spore-infected filter paper.

Discussion

We found a small, but significant, decrease in worker survival in all group types when exposed to *Beauveria bassiana*, but no consistent effect of fungal treatment on larval survival (Fig. 3). Furthermore, the decrease in worker survival was similar

across group types (i.e. there was no interaction between fungal treatment and group type), indicating that the degree of genetic variation within our study colonies did not affect disease resistance. We found considerable variation in control worker survival (Fig. 3a) across the different inbred control groups that were not exposed to the pathogen. For example, lineage U5 had considerably higher survival overall than lineage U1 (Fig. 3a). This suggests that the inbred lineages may vary in general survival rates. Whether this inter-lineage variation may also affect their abilities to resist a pathogen such as *B. bassiana* remained unclear, but it might be expected to be the case, as substantial inter-population or inter-colonial variation for disease resistance exists in other invertebrates such as *Daphnia* (Ebert *et al*., 1998; Altermatt & Ebert, 2008), *Drosophila* (Lazzaro *et al*., 2006) and *Bombus terrestris* (Shykoff & Schmid-Hempel, 1991).

Comparisons of control worker survival between the different inbred lineages, the mixed, and the crossed groups, showed that the crossed groups did significantly better than the U1 lineage and the mixed groups, although the U5 lineage appeared to have equivalently high levels of survival (Fig. 3a). This suggests that heterozygosity may provide survival benefits, as the mixed group did not appear to be doing better than could be expected from the relative colony contributions. However, we cannot exclude the possibility that survival in mixed groups was somewhat affected by subtle forms of within-group antagonism as a consequence of nestmate discrimination behaviour (d'Ettorre & Lenoir, 2010). Pharaoh ants are considered a unicolonial species (Wilson, 1971), and are therefore characterised by the formation of large colonies and at times low levels of aggression between distantly related colonies (Schmidt *et al*., 2010). It is thus conceivable that genetically different colonies may naturally merge, should they meet. Although we did not see any aggression while setting up the small experimental colonies, we did observe some transient aggression shortly after we combined very large numbers of individuals from the inbred lineages in other experiments (A.M. Schmidt and T.A. Linksvayer, pers. obs.). The observation that larvae in mixed groups were significantly less likely to survive (Fig. 3b) also indicates that there may have been some longer-term antagonism in these groups. This means that the observed worker and larval mortality in the mixed groups may be an overestimate relative to what it would be found in an established colony where within-colony discrimination would not be an issue. Cross-fostering pilot experiments have shown that workers readily adopt foreign larvae, but that their survival varies somewhat. However, this potential problem in the mixed groups does not diminish the finding that increased heterozygosity of individual ants resulted in survival rates as high as those of the inbred control colony with the highest survival.

Reber *et al.* (2008) showed that experimental mixing of workers from different monogynous (single queen) colonies of *Formica selysi* increased the mean resistance of group members when exposed to another pathogenic fungus *Metarhizium anisopilae*, but, interestingly, they found that survival in mixed control groups was reduced, consistent with our result. A possible way to exclude cryptic antagonistic interactions could be to rear worker pupae separately from their colonies to minimise the chance that they acquire colony-specific odours and subsequently display any discrimination behaviours (Vander Meer *et al*., 1998; Lenoir *et al*., 1999; Errard *et al*., 2006).

Another possible explanation for our finding of increased larval mortality in mixed groups irrespective of infection may be that mergers of different colonies result in some overall reduction in cohesion of social behaviour. Previous studies on other ant species suggested that successful colonies may have co-adapted behavioural complexes, with favourably-interacting worker, brood, and queen phenotypes (Foitzik *et al*., 2003; Linksvayer, 2007, 2008). It is therefore conceivable that adaptive social cohesion for which selection may have taken place in the different inbred lineages may not be optimally expressed when members of different inbred lineages are combined in new colonies. One could therefore speculate that interactions that enhance social immunity (Cremer *et al*., 2007) among colony members are somehow impaired in merged colonies, perhaps even to a point that natural fusion of genetically differentiated pharaoh ant colonies is selected against. However, a disruption of co-adapted gene complexes may be expected to affect the social cohesion of crossed lineages even more (Linksvayer, 2008), but here we observed a predominantly positive effect of crossing on worker and larval survival.

Whereas other studies have shown the occurrence of clear behavioural responses to pathogens, e.g. ants removing and covering up spores (Jaccoud *et al*., 1999) or termites actively avoiding spores or fungus-killed individuals (Kramm *et al*., 1982; Mburu *et al*., 2009), we did not observe any such responses. In fact, pharaoh ant workers often walked directly over or nested under the filter paper with high concentrations of conidiospores. If there are behavioural responses of pharaoh ants to *B. bassiana*, these must therefore be more subtle, post-exposure responses involving for example mutual grooming rather than avoidance. As much research has illustrated the importance of social context and behaviour in disease resistance (Rosengaus *et al*., 1998; Hughes *et al*., 2002; Traniello *et al*., 2002; Calleri *et al*., 2006, 2010; Yanagawa *et al*., 2008; Wilson-Rich *et al*., 2009; Evans & Spivak, 2010), investigating the behavioural interactions of ants in different types of groups such as the ones that we created would be an interesting way to obtain a better understanding of the underlying mechanisms of resistance.

The fungus-induced mortality was generally low compared to what might be expected from studies on other ant species and termites (Rosengaus *et al*., 1998; Hughes *et al*., 2002; Traniello *et al*., 2002; Hughes & Boomsma, 2004). In pilot experiments on pharaoh ants, using the same *Metarhizium* strains that previously were shown to be highly detrimental to leaf-cutting ants (e.g. Hughes *et al*., 2002), we found no fungus-induced mortality, which indicates that pharaoh ants are not very susceptible to these standard bio-control pathogens, independent of strain. Low mortality meant that detection of statistically significant differences in disease resistance was difficult in the current experiment. However, our results show that intercolony differences in general survival are significant (Fig. 3a) and that there can be costs to genetic diversity, as well as the potential benefits that are often discussed. Overall, our results therefore

question whether the genetic diversity for the disease resistance hypothesis, which has been a fruitful paradigm for social insects in general, does in fact apply in the pharaoh ant.

The present study shows that increasing the genetic variation in a group of ants does not always lead to increased survival, and that, if it does, it may matter whether genetic diversity is increased within or across nestmates. Apparently, groups with increased inter-individual diversity may not function optimally even in species where they might be expected to do so because workers can easily be transferred between nests, and the mixing of colonies may adversely affect the brood. This idea may have remained relatively unexplored, because measuring brood survival in mixed colonies relative to controls is difficult. As natural mixing of pharaoh ant colonies appears to be rare (A.M. Schmidt, unpubl. data), our results also indicate that gene flow between lineages may be much more restricted than the notion of unicoloniality may suggest, consistent with the high F_{ST} values previously found, irrespective of geographical distance (Schmidt *et al*., 2010). Pharaoh ants can thus be considered as rather extreme representatives of the polygyny syndrome in ants, which should reinforce scepticism towards ignoring the ways in which genetic diversity is created when we consider polyandry and polygyny as similar sources of colony-level genetic diversity (Keller & Reeve, 1994; Boomsma & Ratnieks, 1996; Schmid-Hempel & Crozier, 1999; Hughes *et al*., 2008c). Our results rather suggest that some form of enhanced colony-level genetic diversity, be this through increased intra- or inter-individual diversity, may not always be favourable for disease resistance. It is thus conceivable that an increased level of heterozygosity does not confer a long-term advantage to colonies and hybridization is selected against. Hybridization may simply not be advantageous in the case of pharaoh ants, and this may explain the low levels of diversity so far found in pharaoh ant colonies collected at numerous different localities, some of which where colony mergers would definitely be possible (Schmidt *et al*., 2010 and A.M. Schmidt, unpubl. data).

Finally, we should remain cautious that although advantages of disease or parasite resistance can be shown to apply in manipulation experiments, this does not necessarily mean that these advantages are substantial under field conditions. For example, the bumblebees of Baer and Schmid-Hempel (2003) have retained single mating of queens in spite of the documented colony-level costs in disease resistance, which may be because other benefits outweigh the costs of low genetic diversity. We infer that pharaoh ants have retained their inbred, highly structured populations because colony-level selection may have produced local adaptations and caused some colonies to have high levels of survival and resistance. Inbred colonies may thus not generally be particularly vulnerable if inbreeding also maintains other fitness advantages, such as adaptive brood tending behaviours, which may surpass the costs from vulnerability towards disease. Overall it therefore remains ambiguous whether the level of genetic diversity plays a crucial role in the general survival and disease resistance abilities of pharaoh ants, although it is clear that the genetic composition of their colonies does affect general survival.

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