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Inbreeding and disease resistance in a social insect: effects of heterozygosity on immunocompetence in the termite *Zootermopsis angusticollis*

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Recent research has shown that low genetic variation in individuals can increase susceptibility to infection and group living may exacerbate pathogen transmission. In the eusocial diploid termites, cycles of outbreeding and inbreeding characterizing basal species can reduce genetic variation within nestmates during the life of a colony, but the relationship of genetic heterogeneity to disease resistance is poorly understood. Here we show that, one generation of inbreeding differentially affects the survivorship of isolated and grouped termites (*Zootermopsis angusticollis)* depending on the nature of immune challenge and treatment. Inbred and outbred isolated and grouped termites inoculated with a bacterial pathogen, exposed to a low dose of fungal pathogen or challenged with an implanted nylon monofilament had similar levels of immune defence. However, inbred grouped termites exposed to a relatively high concentration of fungal conidia had significantly greater mortality than outbred grouped termites. Inbred termites also had significantly higher cuticular microbial loads, presumably due to less effective grooming by nestmates. Genetic analyses showed that inbreeding significantly reduced heterozygosity and allelic diversity. Decreased heterozygosity thus appeared to increase disease susceptibility by affecting social behaviour or some other group-level process influencing infection control rather than affecting individual immune physiology.

Keywords: isoptera; termite; life history; immunity; social behaviour

1. INTRODUCTION

Decreased heterozygosity can arise from life-history traits and impact disease resistance (Lively *et al.* 1990; Paterson *et al.* 1998; Lively *et al.* 2004; Pearman & Garner 2005). However, low heterozygosity does not compromise parasite and pathogen resistance in a consistent manner across taxa (Hanley *et al.* 1995; Wiehn *et al.* 2002; Giese & Hedrick 2003). In social insects, studies of the role of genetics in disease resistance must consider life-history traits that increase the relatedness of colony members and decrease heterozygosity (Shykoff & Schmid-Hempel 1991). Enhanced disease exposure and transmission rates thought to be associated with group living and high nestmate densities might further increase the risk of infection (Hamilton 1987; Rosengaus *et al.* 1998; Schmid-Hempel 1998). In monandrous ants, bees and wasps (order Hymenoptera), haplodiploid sex determination produces relatively high coefficients of relatedness that can increase the susceptibility of workers to infection (Sherman *et al.* 1988, 1998; Keller & Reeve 1994; Schmid-Hempel & Crozier 1999). Additionally, haploid males may be especially vulnerable to parasites due to their lower genetic variability at the individual level (Gerloff *et al.* 2003; O'Donnell & Beshers 2004; Vainio *et al.* 2004; Baer *et al.* 2005). Multiple mating can increase allelic diversity and polyandry in bees and ants has been demonstrated to improve resistance to infection (Baer & Schmid-Hempel 1999; Baer & Schmid-Hempel 2001; Tarpy 2003; Denny *et al.* 2004; Hughes & Boomsma 2004).

Termites (order Isoptera), in contrast, are diploid and typically monogamous and the life history of many basal species is characterized by alternating generations of outbreeding and inbreeding that can alter heterozygosity during a colony's life cycle (Thorne 1997; Husseneder *et al.* 1999; Thorne *et al.* 1999; Vargo 2003; DeHeer & Vargo 2004). Termite colonies generally are founded by dispersing winged primary reproductives followed by the development of secondary reproductives upon the death of the primaries (Abe & Higashi 2001). Secondary reproductives may be full siblings that mate with each other or a surviving parent (Thorne 1997), in either case contributing to colony growth through the production of inbred offspring. Cycles of outbreeding by primary reproductives and inbreeding by secondary reproductives are considered to be significant in termite sociobiology because of their consequences to nestmate relatedness

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(Hamilton 1978; Thorne 1997; Roisin 1999). These cycles could involve disease-related costs of decreased heterozygosity associated with inbreeding (Thorne & Traniello 2003) and a pathogen-related increase in mortality associated with outbreeding (Rosengaus & Traniello 1993; Calleri *et al.* 2005). Because the immune function of individuals and social interactions can both decrease the susceptibility of termite colonies to disease (Rosengaus *et al.* 1998, 1999*a*,*b*; Rosengaus & Traniello 2001; Traniello *et al.* 2002), we examined immunocompetence in the broad sense of Owens & Wilson (1999) and measured a diverse array of individual- and group-level phenomena that could be involved in infection control.

We experimentally evaluated whether heterozygosity in individual pseudergates ('false workers') and nymphs of the dampwood termite *Zootermopsis angusticollis* can influence immune function and how it might affect disease resistance through its interaction with sociality. We assessed immunocompetence by recording the survivorship of isolated and grouped termites challenged with fungi, bacteria and a non-pathogenic immune inducer (a nylon implant). Additionally, we estimated cuticular microbial loads of inbred and outbred offspring to index disease risk. We relate these measures of disease susceptibility to estimates of genetic variation in the inbred and outbred offspring whose disease resistance we examined. Our results suggest that inbreeding may not impact individual immunity, but may decrease the efficacy of group-level processes of disease resistance.

2. MATERIAL AND METHODS

(a) *Colony establishment*

Colonies of *Z. angusticollis* Hagen ($n=13$, *ca* 200–1000 individuals/colony) were collected from sites separated by approximately 100 m in Palo Alto Foothill Park, Palo Alto, California. Log nests were transferred to plastic tubs ($50\times30\times20$ cm) lined with moist paper towels, regularly sprayed with water, and maintained in the laboratory at 25 °C. Alates (winged reproductives), which we used to establish inbred and outbred colonies, emerged from most stock colonies within two months of collection.

To create inbred and outbred colonies, we paired female and male alates from the same stock colony (inbred, $n=22$ pairs) or different stock colonies (outbred, $n=23$ pairs). No stock colony was used more than three times as a source of alates. Because of the complexity of mating combinations (parent/offspring, sib/sib, multiple sibs) by primary and secondary reproductives, we used sibling primary reproductives in place of sibling secondary reproductives to produce inbred offspring because of the genetic equivalency of the two forms and ease of experimentation. Reproductives were haphazardly paired in Petri dish nests (60×15 mm) lined with moist filter paper containing *ca* 5.0 g of decayed wood and stacked inside covered plastic boxes ($30\times23\times10$ cm). The same source of wood was used as food for all colonies. Colonies were fed *ad libitum* and allowed to develop for approximately 3 years. To avoid compromising the health of colonies, we did not fully dissect nests to determine if primary or supplementary reproductives were present at the time that offspring were collected for genetic study. Nevertheless, microsatellite analysis of genetic variation showed that colonies established by sibling pairs were significantly different from colonies established with outbred primaries (see §3*a*). Twenty-four colonies, each containing *ca* 200–500 individuals ($n=11$ inbred colonies; $n=13$ outbred colonies), provided termites for study. Inbred and outbred offspring (pseudergates of instars IV–VII and nymphs) were haphazardly removed from their nests and temporarily housed by colony in covered plastic boxes ($15\times10\times6$ cm) containing moist paper towel and nest wood. Individuals from all colonies were used in assays of immunocompetence within 5 days of removal from their parent colony. Fungal, bacterial and nylon implant immune challenges for each colony occurred within this time period.

(b) *Microsatellite variation, relatedness and genetic diversity*

To estimate genetic variation and the degree of relatedness of inbred and outbred termites, individuals from each colony (17.3 ± 4.7, mean $\pm$s.d., $n=24$ colonies) were genotyped at five polymorphic microsatellite loci developed for *Z. angusticollis* according to the methods described in Dronnet *et al.* (2005). Four loci consisted of dimers and one consisted of a tetramer. The microsatellites were amplified, run on a LiCor automated sequencer and scored according to established methods (e.g., Vargo 2003). The number of alleles per locus ranged from three to six, with a mean ($\pm$s.d.) of 4.2 ± 1.3. The program FSTAT v. 2.9.3.2 (Goudet 2001) was used to determine the average number of alleles, observed heterozygosity and inbreeding coefficient (F_{IS}) for each group. Standard errors for F_{IS} were estimated by jackknifing over loci. The coefficient of relatedness among siblings was estimated with the program RELATEDNESS v. 5.0.8 (Queller & Goodnight 1989), and standard errors were estimated by jackknifing over colonies. We used a one-tailed *t*-test to determine if there were significant differences between inbred and outbred colonies.

(c) *Preparation of disease agents and experimental infections*

The entomopathogenic fungus *Metarhizium anisopliae* (original source: American Type Culture Collection, batch 93-09, media 325, ATCC no. 90448) was used to experimentally infect termites. This fungus naturally occurs with termites (Zoberi 1995) and has been cultured from *Z. angusticollis* cadavers found in freshly collected field colonies during a post-collection quarantine period (Calleri *et al.* 2005, 2006). A detailed description of the preparation of Tween 80 conidia suspensions is given in Rosengaus *et al.* (1998). In the present experiment, suspensions of 8.1×10^3 and 4.5×10^5 conidia ml^{-1}, which produce intermediate levels of mortality in isolated and grouped termites, respectively, were freshly prepared. The average germination rate ($\pm$s.d.) of conidia was $98.0\pm1.8\%$ ($n=30$ fields of vision).

Termites from the 11 inbred and 13 outbred colonies were individually exposed to a conidia-free 0.1% Tween 80 solution, an 8.1×10^3 (low dose) or 4.5×10^5 (high dose) conidia ml^{-1} suspension of *M. anisopliae* according to established protocol (Traniello *et al.* 2002; Calleri *et al.* 2005). Because we could not anticipate the number of individuals and their age distribution in each log nest we opened, sample sizes were lower for the high-dose conidia exposure experiments, which were carried out later in the study. Immediately after exposure, termites were either isolated (inbred control, $n=220$; low dose, $n=229$ individuals; high dose, $n=30$; outbred control, $n=260$ individuals, low dose, $n=270$ individuals, high dose, $n=30$ individuals) in a Petri dish (60×15 mm) lined with moist filter paper or

placed haphazardly in mixed-instar nestmate groups of 10 (inbred control replicates, $n=22$ groups; low dose, $n=22$ groups; high dose, $n=4$ groups; outbred control replicates, $n=25$ groups; low dose, $n=26$ groups; high dose, $n=4$ groups), in Petri dishes (110×15 mm) lined with moist filter paper. Termites were placed in isolation or in groups to determine the effect of genetic variation on physiological immunity and the efficacy of social mechanisms of disease resistance, respectively (Rosengaus *et al.* 1998; Traniello *et al.* 2002).

Pseudomonas aeruginosa (Strain P11-1) served as a model bacterial pathogen (Faulhaber & Karp 1992). Suspensions of 2.6×10^3, 3.5×10^4 and 3.7×10^6 were tested to determine their impact on termite survivorship. The suspension of 3.7×10^6 bacteria ml^{-1} was chosen for experimental inoculations because it was the only suspension to significantly impact mortality (see §3). To infect termites, individuals from the six inbred and seven outbred colonies were cold immobilized in 1.5 ml microcentrifuge tubes and placed with the ventral abdomen exposed on a piece of filter paper. While the termite was held with sterile featherweight forceps, the abdomen was swabbed with 75% ethanol and bacteria were introduced by dipping a sterile insect pin (size 00) in the suspension and piercing the intersegmental membrane between the fourth and fifth abdominal segments. Control termites were inoculated with Burns–Tracy solution in the same manner (Rosengaus *et al.* 1999*b*). Following inoculation with bacteria, termites were placed in isolation (inbred control, $n=50$; inoculated, $n=40$; outbred control $n=50$; inoculated, $n=40$) in Petri dishes (60×15 mm), lined with moist filter paper. Inoculated termites were also placed haphazardly in mixed-instar groups of 10 in Petri dishes (100×15 mm) lined with moist filter paper (inbred control replicates, $n=5$ groups; inoculated, $n=4$ groups; outbred control replicates, $n=5$ groups; inoculated, $n=4$ groups).

(d) *Survival analysis*

Following exposure to fungal conidia or inoculation with bacteria, termites were censused daily for 30 days. Because not all termites were infected on the same day, results were standardized by analysing survival data based on the time elapsed between pathogen exposure and each census. Survival parameters included the survival distribution (the time-course of survival), percent survivorship, and median survival time (LT_{50}). Survival distributions were compared and analysed with the Breslow Statistic (BS; Kaplan–Meier survival test; SPSS 1990). When multiple, pairwise statistical comparisons were made, the α-value of significance was adjusted accordingly (Rice 1989). Cox proportional regression analyses were also performed. This analysis generated the Wald Statistic (WS), which described the effect of the following variables on survival: colony of origin, instar (IV–VII or nymph), relatedness (inbred or outbred), treatment (isolated or grouped) and conidia dose (control, low or high). Two separate Cox proportional regression analyses were carried out because of the co-linearity of the variables colony of origin and relatedness. Colony of origin is known to influence survivorship (Rosengaus *et al.* 1998; Rosengaus & Traniello 2001). Here we are interested in the influence of inbreeding and outbreeding (relatedness) on survivorship rather than differences in survivorship within inbred or outbred colonies. We therefore attributed difference in the survivorship of inbred and outbred grouped termites to inbreeding.

During the census period, dead individuals were removed, surface sterilized and plated on potato dextrose agar (conidia-exposed termites) or tryptic soy agar (bacteria-inoculated termites) to confirm mortality was due to the intended infective agent (Rosengaus *et al.* 1998, 1999*b*). Confirmation rates for conidia-exposed termites ranged from 82 to 93% for isolated termites and 25–100% for grouped termites. Four percent of control termites confirmed positive for *M. anisopliae*, most likely as a result of natural infection. Confirmation rates for bacteria-inoculated termites ranged from 64 to 95% for isolated individuals and 40–87% for individuals in groups. One percent of control termites showed evidence of bacterial infection, although the causative agent(s) was not identified.

(e) *Induction of immunity with nylon monofilament implants*

Assessment of immune response to a non-pathogenic challenge was carried out by implanting *ca* 2 mm of nylon monofilament in the abdomen (Scientific Anglers, 3M, Tippet 2lb Test line, 0.1 mm diameter). Nylon can serve as an inert non-pathogenic challenge to cellular immunity (Siva-Jothy *et al.* 1998; Gerloff *et al.* 2003). Haemocytes attach to the nylon and initiate the prophenoloxidase cascade, which results in the deposition of melanin on the implant. Melanin deposition can then be quantified to estimate immune response (Konig & Schmid-Hempel 1995; Siva-Jothy *et al.* 1998). Termites from randomly selected parent colonies (inbred, $n=4$; outbred, $n=4$) were cold immobilized in separate 1.5 ml microcentrifuge tubes and placed with the ventral surface exposed on a dissecting microscope stage. The abdomen was swabbed with 75% ethanol. Nylon implants were sterilized with UV light (230 nm) for 90 s prior to implantation and forceps were sterilized with alcohol and flamed. Implants were inserted through the intersegmental membrane of the fourth and fifth segments. Termites were then placed individually in Petri dishes on moist filter paper for 4 days (inbred, $n=30$; outbred, $n=30$), after which surviving termites were cold immobilized and the implants removed and mounted in glycerol on microscope slides. Because melanin (the main product of encapsulation) autofluoresces at 488 nm, we quantified the degree of encapsulation of each implant using confocal fluorescence microscopy and imaging software (Image J, National Institutes of Health, Bethesda, MD, USA). Implants were optically sectioned lengthwise into 52, 5 μm slices for a total depth of 260 μm to ensure the entire implant was scanned. Under 200× magnification, we located the centre of each implant and scanned 500 μm lengthwise on either side to exclude implant ends that often had excess tissue or melanin deposition. A three-dimensional image was reconstructed from the 52 stacked images and the mean grey value, a measure of pixel density, was calculated for each implant (inbred, $n=25$; outbred, $n=26$). Pixel density served as a measure of the amount of melanin deposited on an implant and thus an estimate of the strength of the immune response. A *t*-test was used to compare the average degree of encapsulation of monofilaments implanted in inbred and outbred termites.

(f) *Estimation of cuticular microbial load*

Cuticular microbial loads were quantified from 10 termites/colony for inbred ($n=5$ colonies) and outbred ($n=5$ colonies) colonies according to protocols outlined in Cruse (1998)

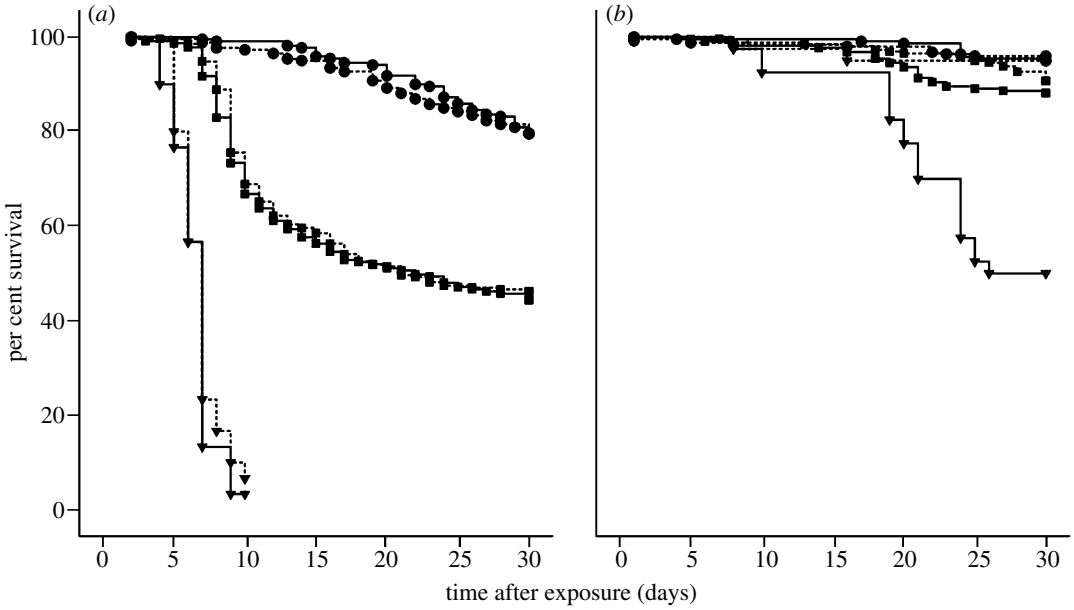

Figure 1. Survival distributions of (*a*) isolated and (*b*) grouped inbred (straight line) and outbred (dashed line) termites following exposure to conidia and in controls (control, filled circle; low dose, filled square; high dose, filled triangle).

Table 1. Survival parameters of conidia-exposed termites. (p denotes the significance of differences between survival distributions of inbred and outbred termites for each conidia dosage.) Different superscript letters following LT_{50} values denote significant differences between exposure treatments within inbred and outbred treatments (pairwise comparisons of survival distributions, Kaplan–Meier survival test). Isolated and grouped termites were analysed separately. The α-value of significance was adjusted ($p<0.016$) for multiple comparisons. Sample size for isolated termites is the number of individuals exposed. Sample size for grouped termites is the number of groups of 10.)

		isolated termites			grouped termites		
		control	low dose	high dose	control	low dose	high dose
inbred	LT_{50} (days, $\pm$s.d.)	$>30^{a}$	22 ± 4^{b}	7 ± 0^{c}	$>30^{a}$	$>30^{b}$	26 ± 0^{c}
	percent survival at 30 days	79.6	44.5	3.3	95.0	88.2	50.0
	n	220	229	30	22	22	4
outbred	LT_{50} (days$\pm$s.d.)	$>30^{a}$	21 ± 0^{b}	7 ± 0^{c}	$>30^{a}$	$>30^{a}$	$>30^{a}$
	percent survival at 30 days	79.6	46.3	6.7	96.0	90.8	95.0
	n	260	270	30	25	26	4
p		n.s.	n.s.	n.s.	n.s.	n.s.	$p<0.0001$

and Rosengaus *et al.* (2003). Haphazardly chosen colonies of *ca* 200–500 individuals were the source of termites used to estimate microbial loads. Estimates were taken within a day of removal from parent nests. To control for differences in termite size, each individual was weighed and its surface area (SA) estimated using Meeh's formula $SA=kW^{2/3}$, where W is mass (g) and k is a constant, which for termites is 12 (Sponsler & Appel 1990). Colony forming units (CFUs) cultured from cuticular washes were standardized for SA and compared using a Mann–Whitney U-test.

3. RESULTS

(a) *Genetic analysis*

Genetic data showed that offspring of sibling reproductives were significantly more homozygous than those of unrelated reproductives, as determined by the standard inbreeding coefficient, F_{IS} ($F_{IS}\pm$s.e.; inbred$=0.51\pm0.05$; outbred $=0.17\pm0.09$; $t_8=3.27$, $p<0.01$). Correspondingly, termites from inbred families had significantly fewer alleles per locus than those from outbred families (mean$\pm$s.d.; inbred$=1.68\pm0.38$; outbred$=1.93\pm0.26$; $t_{24}=1.40$; $p<0.025$) and had significantly lower heterozygosity than outbred families (mean $\pm$s.d.; inbred$=0.26\pm0.12$; outbred $=0.48\pm0.15$, respectively; $t_{24}=4.10$; $p<0.0005$). Finally, inbred termites were significantly more related to their siblings than outbred termites (mean $r\pm$s.e.; inbred$=0.74\pm0.05$; outbred$=0.63\pm0.04$; $t_{24}=1.82$; $p<0.05$). The results of the genetic analyses confirm that pairing nestmates to produce inbred colonies resulted in offspring of lower genetic diversity, increased homozygosity and elevated degrees of relatedness.

(b) *Survival of conidia-exposed inbred and outbred termites*

Survival distributions of isolated control and conidia-exposed inbred and outbred termites did not differ (BS$=$0.04, 0.4, 0.4, $p>0.05$ for control, low-dose and high-dose exposures, respectively, figure 1*a*, table 1). Additionally, differences in the survivorship of inbred and outbred grouped termites in the control and low-dose exposure treatment were not significant (BS$=$0.2, 1.1, $p>0.05$,

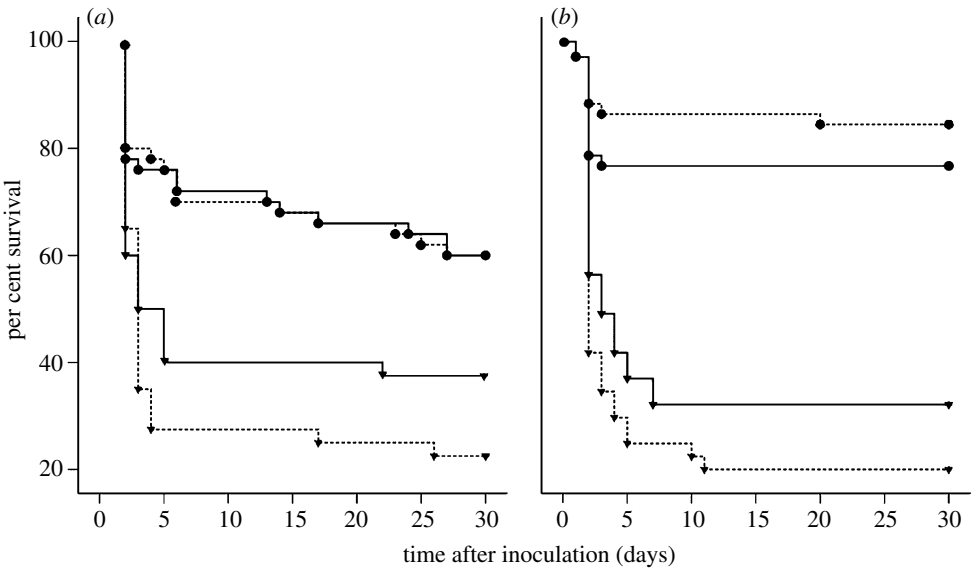

Figure 2. Survival distributions of (*a*) isolated and (*b*) grouped inbred (straight line) and outbred (dashed line) termites following inoculation with the bacterium *P. aeruginosa* (control, filled circle; inoculated, filled triangle).

figure 1*b*, table 1). However, at the higher conidia dosage, outbred grouped termites survived significantly longer than inbred grouped termites (BS = 17.9, $p < 0.0001$, figure 1*b*, table 1). Colony of origin, instar, treatment (isolated versus grouped) and conidia dosage were significant and independent predictors of mortality (WS = 95.2, 17.3, 336.1, 338.1, d.f. = 23, 4, 1, 2, $p < 0.002$, respectively). In a separate regression, instar, treatment and dosage were highly significant and independent predictors of mortality (WS = 25.9, 334.4, 369.9, d.f. = 4, 1, 2, $p < 0.001$, respectively). Relatedness was also a significant predictor of mortality (WS = 4.0, d.f. = 1, $p = 0.05$).

(c) *Survival of bacteria-infected inbred and outbred termites*

There were no significant differences between the survival distributions of isolated or grouped inbred and outbred termites inoculated with *P. aeruginosa* (isolated, BS = 0.0, and 0.9; grouped, BS = 1.1 and 2.1 for control and inoculated termites, respectively, $p > 0.05$, figure 2*a*,*b*). In a regression that included the variables colony of origin, instar, treatment (isolated versus grouped) and dosage (3.7×10^6 versus control), colony of origin and dosage were significant and independent predictors of mortality (WS = 24.0, 37.0, d.f. = 12, 1, $p = 0.02$, 0.001, respectively), whereas instar and treatment were not (WS = 5.4, 0.1, d.f. = 4, 1, $p = 0.25$, 0.71, respectively). In a separate regression, dosage was a significant and independent predictor of termite mortality (WS = 83.2, d.f. = 3, $p = 0.001$) but instar, treatment and relatedness were not (WS = 6.4, 0.1, 1.8, d.f. = 4, 1, 1, $p = 0.17$, 0.82, 0.18, respectively).

(d) *Encapsulation of nylon implants by inbred and outbred termites*

There were no significant differences in the degree of encapsulation of nylon monofilaments implanted in inbred and outbred termites (mean grey value ± s.d., inbred = 12001 ± 8393, outbred = 14947 ± 6959, d.f. = 49, $t = -1.3$, $p = 0.2$, *t*-test).

(e) *Cuticular microbial load estimates of inbred and outbred termites*

The cuticular microbial load (mean ± s.d.) of inbred termites (44 ± 53 CFU/mm^2, range, 0–266 CFU mm^{-2}) was on average approximately double that of outbred individuals (25 ± 43 CFU mm^{-2}; range, 0–244 CFU mm^{-2}). This difference was highly significant (d.f. = 1, $U = 8618.5$, $p < 0.001$, Mann–Whitney *U*-test).

4. DISCUSSION

The effect of reduced heterozygosity due to inbreeding on disease resistance in *Z. angusticollis* was related to the nature of the experimental infection and the solitary or social treatment of exposed termites. Inbred and outbred termites exposed to a low concentration of fungal conidia or inoculated with bacteria did not differ significantly in survivorship when maintained in isolation (figures 1*a*, 2*a*; table 1). This indicates that genetic variation did not affect the physiological immunity of individual termites. However, grouped outbred termites had greater survivorship than grouped inbred termites when exposed to a relatively high concentration of conidia (figure 1*a*,*b*; table 1). This pattern was not found for grouped termites challenged with bacteria (figure 2*a*,*b*). The reduction in heterozygosity associated with inbreeding therefore may have impacted the efficacy of social mechanisms of disease resistance such as allogrooming rather than physiological immunity at the individual level. Cuticular pathogen loads can be lowered and/or conidia inactivated during bouts of mutual grooming, which increase in frequency and are elevated in intensity after exposure to *M. anisopliae* (Rosengaus *et al.* 1998, 2000). A lower frequency of mutual grooming in inbred termites is suggested by their significantly higher cuticular microbial loads, although genetic variation could also affect cuticular hydrocarbon composition and thus impact colonization by microbes. Variation in survivorship in grouped (but not isolated) conidia-exposed termites support the finding of Rosengaus *et al.* (1998) and further demonstrates that disease susceptibility in *Z. angusticollis* can result from differences in social interactions rather than individual

immunity. The grouping of bacteria-inoculated termites had no impact on survival because this infection was internal and thus unlikely to be remedied by mutual grooming, although trophallaxis and other social interactions could enhance immune function (Traniello *et al*. 2002). We caution, however, that we can not rule out the possibility that an unidentified social effect contributed to the enhanced resistance of grouped termites.

Assessing innate (cellular) immunity with a nylon 'pseudoparasite' removed the confounding effects of a live pathogen and provided an estimate of the immune response (Konig & Schmid-Hempel 1995; Baer & Schmid-Hempel 2003; Gerloff *et al*. 2003). Our implant studies showed that cellular immunity was unaffected by the increased homozygosity that accompanied inbreeding in our experiments. There were no significant differences in the extent of encapsulation of nylon implants by inbred and outbred termites and isolated and grouped termites do not differ in their encapsulation ability (Traniello *et al*. 2002). This suggests that social processes did not affect this aspect of innate immunity.

Inbreeding and reduced heterozygosity are generally detrimental to fitness (Keller & Waller 2002) and can lower disease resistance (Cassinello *et al*. 2001; Acevedo-Whitehouse *et al*. 2003; Reid *et al*. 2003). In some clonal or hermaphroditic species, however, parasite resistance is not directly correlated with reduced heterozygosity, but varies with host genetic history (Hanley *et al*. 1995; Wiehn *et al*. 2002; Haag *et al*. 2003). The impact of inbreeding on disease resistance may also depend more on host/parasite genetics (Haag *et al*. 2003) or family (Wiehn *et al*. 2002) than the degree of inbreeding. Some models show that episodes of inbreeding for one or a few generations do not necessarily result in inbreeding depression and decreased immune function (Crow & Kimura 1970; Lande & Schemske 1985; Charlesworth & Charlesworth 1987). Our results indicate that limited amounts of inbreeding can occur in *Z. angusticollis* without detrimental physiological effects on immunity that can be associated with inbreeding depression. The influence of inbreeding, however, may be greater in other termites (DeHeer & Vargo 2006).

There are too few studies of the genetics of termite immunity to draw broad conclusions about the influence of allelic variation within individuals on disease resistance (Lamberty *et al*. 2001; Bulmer & Crozier 2004; Bulmer & Crozier 2006). It is nevertheless clear that heterozygosity within individuals can change through the colony life cycle. For example, *Z. nevadensis* alates appear to outbreed (Shellman-Reeve 2001) but secondary reproductives are common in *Zootermopsis* colonies that have lost one or both primaries (Castle 1934; Light & Illg 1945), probably reducing allelic variation in their offspring (Husseneder *et al*. 1999; Thorne *et al*. 1999; Vargo 2003; DeHeer & Vargo 2004). In addition, nesting ecology (Hamilton 1972), predation on alates (Lepage 1991; Matsuura & Nishida 2002) and disease risk (Rosengaus & Traniello 1993; Rosengaus *et al*. 2000; Calleri *et al*. 2005) may limit dispersal, which could also increase inbreeding (Roisin 1999). Cycles of inbreeding and outbreeding may have influenced the way in which termite colonies adapted to disease, outbreeding generating variation in disease resistance traits and inbreeding maintaining adapted disease-resistant genotypes selected for during colony growth (Thorne & Traniello 2003). Experimental studies have shown that outbred primary reproductives of *Z. angusticollis* incur higher mortality costs than inbred primary reproductives but colony growth is unaffected by inbreeding (Rosengaus & Traniello 1993; Calleri *et al*. 2005). Rosengaus *et al*. (1998) suggest an important role for sociality in the disease resistance repertoire of termites. Our results suggest that the inbreeding inherent in termite life history may have favoured the selection of individual physiological immunity able to cope with decreased heterozygosity, but that the infection-control benefits of grouping, such as allogrooming or other social effects, may be less resilient to decreased genetic variation.

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