Evolution, 58(6), 2004, pp. 1251–1260

GENETIC DIVERSITY AND DISEASE RESISTANCE IN LEAF-CUTTING ANT SOCIETIES

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Abstract.—Multiple mating by females (polyandry) remains hard to explain because, while it has substantial costs, clear benefits have remained elusive. The problem is acute in the social insects because polyandry is probably particularly costly for females and most material benefits of the behavior are unlikely to apply. It has been suggested that a fitness benefit may arise from the more genetically diverse worker force that a polyandrous queen will produce. One leading hypothesis is that the increased genetic diversity of workers will improve a colony's resistance to disease. We investigated this hypothesis using a polyandrous leaf-cutting ant and a virulent fungal parasite as our model system. At high doses of the parasite most patrilines within colonies were similarly susceptible, but a few showed greater resistance. At a low dose of the parasite there was more variation between patrilines in their resistance to the parasite. Such genetic variation is a key prerequisite for polyandry to result in increased disease resistance of colonies. The relatedness of two hosts did not appear to affect the transmission of the parasite between them, but this was most likely because the parasite tested was a virulent generalist that is adapted to transmit between distantly related hosts. The resistance to the parasite was compared between small groups of ants of either high or low genetic diversity. No difference was found at high doses of the parasite, but a significant improvement in resistance in high genetic diversity groups was found at a low dose of the parasite. That there is genetic variation for disease resistance means that there is the potential for polyandry to produce more disease-resistant colonies. That this genetic variation can improve the resistance of groups even under the limited conditions tested suggests that polyandry may indeed produce colonies with improved resistance to disease.

Key words.—*Acromyrmex*, herd immunity, *Metarhizium*, parasite, polyandry, social insect.

Received September 22, 2003. Accepted February 9, 2004.

The evolution of multiple mating by females (polyandry) represents a fundamental problem in behavioral ecology (Arnqvist and Nilson 2000; Jennions and Petrie 2000; Simmons 2001). Mating is generally assumed to be associated with significant costs to the female, including energy expended, exposure to predation, and the risk of being infected by sexually transmitted parasites (Daly 1978; Chapman et al. 1995; Crudgington and Siva-Jothy 2000). Polyandry is therefore a costly trait. Yet clear benefits to females of mating multiply have proved elusive. The problem is acute in the social insects, specifically those in the order Hymenoptera (ants, bees, and wasps; Crozier and Page 1985; Palmer and Oldroyd 2000; Crozier and Fjerdingstad 2001). Hymenopteran social insect queens typically mate only during brief nuptial flights. These represent the most vulnerable period of their lives as they are without their normal shield of protective workers, making polyandry relatively costly for these queens. In addition, encounters between males and females are extremely brief, generally lasting for little more than the duration of copulation. This makes a number of the material benefits that may explain polyandry in nonsocial animals, such as increased paternal care, almost certainly nonapplicable to social insects. Polyandry also reduces the high intracolonial relatedness that has been posited as a driving force in the evolution and maintenance of social behavior (Hamilton 1964). Yet polyandry is not uncommon in the social insects. It occurs in many taxa, and in five genera (honeybees [*Apis*], one of vespid wasps [*Vespula*] and three of ants [*Atta*, *Acromyrmex*, and *Pogonomyrmex*]) has been taken to extreme levels (Strassmann 2001). Honeybee queens, for example, can mate with over a hundred males (Wattanachaiyingcharoen et al. 2003). This, together with the probable costs, suggests that high levels of polyandry can have benefits to queen fitness.

Numerous benefits have been suggested to explain the evolution and maintenance of polyandry (Arnqvist and Nilson 2000; Jennions and Petrie 2000; Simmons 2001). These include a number that could potentially apply in some form in the social insects (Crozier and Page 1985; Palmer and Oldroyd 2000; Crozier and Fjerdingstad 2001). A key effect of polyandry is that it will result in a colony with a more genetically diverse worker population. Particular attention has become focused on the idea that this increase in worker genetic diversity may have some fitness benefit for the colony. Again, several mechanisms have been suggested by which this might occur. One that has gained strong support recently is the hypothesis that genetically diverse colonies may be more resistant to disease (Hamilton 1987; Sherman et al. 1988).

Disease is a substantial problem associated with living in groups (Schmid-Hempel 1998; Kraus and Ruxton 2002). The colony life of social insects makes them particularly vulnerable to the spread of diseases. In addition, the close relatedness between individuals may facilitate transmission and entail a high risk of the entire population being susceptible to a pathogen (Schmid-Hempel 1994, 1998). The impact of a parasite upon such a group may be reduced if a group is made up of multiple genotypes, and if these genotypes vary in their resistance to a parasite. Genetic variation for resistance to parasites has been found in many nonsocial organ-

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isms (Ebert et al. 1998; Carius et al. 2001; Little and Ebert 1999, 2000, 2001; Kover and Schaal 2002), and has recently been demonstrated in bumblebees and honeybees (Baer and Schmid-Hempel 2003; Palmer and Oldroyd 2003). Although the mechanism in these latter cases is not yet known, the cellular immune response in insects can vary between genotypes (Carlton et al. 1992; Cotter and Wilson 2002). This is thought to contribute to the well-established differences in immune response between bumblebee colonies (König and Schmid-Hempel 1995; Allander and Schmid-Hempel 2000; Mallon et al. 2003).

If groups consist of individuals that are genetically variable for resistance to parasites, then there are several ways by which this may benefit the group: (1) The transmission of a parasite between individuals may be hindered, because parasites adapted to infect one particular genotype may be less able to infect other genotypes. In a genetically diverse population, this can result in a "herd immunity" effect, where the proportion of susceptible individuals is lower than the threshold needed for the disease to maintain itself in the population through transmission (Anderson and May 1985). (2) Quite apart from the effect on transmission, the probability of at least some members of the group being resistant to, and thus surviving, a parasitic infection will be higher. Consequently the variation between colonies in the proportions of individuals surviving will be reduced. This will make being genetically diverse beneficial to colonies, providing that the mean proportion of individuals surviving is above the threshold needed for the colony to survive. (3) Susceptible individuals may benefit from the defense mechanisms of resistant individuals, for example through allogrooming or the allotransfer of antimicrobial defenses. (4) The adaptation of parasites to the host colony over successive generations will be hindered if the colony consists of multiple genotypes.

However, the evidence for the genetic diversity/disease resistance hypothesis, as with most of the other posited benefits of polyandry, is conflicting. In an important study in which queens of the bumblebee, *Bombus terrestris*, were artificially inseminated with sperm from multiple males, it was found that genetically diverse colonies were less afflicted by disease than low diversity colonies and that this was then associated with improved fitness (Baer and Schmid-Hempel 1999, 2001). This appears to be at least partly due to the key parasites being less able to transmit between different genotypes of bees (Shykoff and Schmid-Hempel 1991). It has also been shown that there is less intercolony variation in disease prevalence between genetically diverse colonies of the honeybee, *Apis mellifera*, than between low diversity colonies (Tarpy 2003). On the other hand, two studies on honeybees failed to find any effect of genetic diversity on resistance to disease (Page et al. 1995; Neumann and Moritz 2000). In addition, a number of studies, while not directly investigating the relationship between genetic diversity and disease, have investigated whether it improves colony fitness. Although some have found colony fitness benefits to result from polyandry (Oldroyd et al. 1992; Fuchs and Schade 1994; Page et al. 1995; Cole and Wiernaz 1999), others have not (Fuchs et al. 1996; Sundström and Ratnieks 1998; Neumann and Moritz 2000; Costa and Ross 2003; Fjerdingstad et al. 2003). The evidence for the genetic diversity/disease resistance hypothesis is thus currently limited to just two species (*Apis mellifera* and *Bombus terrestris*), of which one (*Bombus terrestris*) is obligately monoandrous (Schmid-Hempel and Schmid-Hempel 2000; Baer et al. 2001).

One group that has never been investigated, but that has considerable potential as a model system, is the leaf-cutting ants (Hymenoptera: Formicidae: Attini: *Atta* and *Acromyrmex*). Leaf-cutting ants have large, long-lived colonies, in which they culture a mutualistic fungus on harvested leaf material as an important food source (Weber 1972; Hölldobler and Wilson 1990). The queens are highly and obligately polyandrous. *Atta* queens have been estimated to mate with approximately three males on average (Fjerdingstad et al. 1998; Fjerdingstad and Boomsma 2000), whereas queens of *Acromyrmex octospinosus* and *Acromyrmex echinatior* typically mate with nine or 10 males (Ortius-Lechner et al. 2003; Sumner et al. 2004a).

We used laboratory colonies of *Acromyrmex echinatior* to examine the genetic diversity for disease resistance hypothesis for polyandry. *Acromyrmex* ants are not known to be infected by any specialized parasites, and so we used the generalist fungal entomopathogen *Metarhizium anisopliae* var. *anisopliae* as the model parasite. *Metarhizium* is a virulent generalist parasite of many insect species, including both *Atta* and *Acromyrmex* leaf-cutting ants (Alves and Sosa Gómez 1983; Humber 1992; Jaccoud et al. 1999; Hughes et al. 2002; Poulsen et al. 2002a; Hughes et al. 2004). It is semelparous and produces transmission stages (spores) only after host death. We first determined whether the key assumption for the hypothesis was met, that is, whether there was genetic variation for resistance. We then examined how relatedness affected disease transmission, and whether group genetic diversity influenced disease resistance.

Materials and Methods

General Methodology

Monogynous colonies of *Acromyrmex echinatior* were collected from Gamboa, Panama, in 1996 and 2000 and subsequently maintained in the laboratory on a diet of bramble leaves and rice at 25°C and 70% relative humidity. For experimental replicates, adult, small worker ants (head width: 1.0–1.4 mm) of similar cuticular coloration were removed from the fungus gardens of colonies to reduce variation in age between ants. The sampled ants were initially placed individually into plastic pots (diameter: 2.5 cm, height: 4 cm) with an ad libitum supply of water and 10% sucrose water. To allow assessment of an individual's genotype, a single middle leg was carefully removed from each ant. Preliminary experiments had confirmed that this procedure did not effect the survival of ants within the time spans of the experiments, nor their susceptibility to the experimental parasite. DNA was extracted from the ant legs using Chelex (Bio-Rad, Herlev, Denmark) beads and amplified at four microsatellite loci polymorphic for this species: Ech1390, Ech3385, Ech4126, and Ech4225 (Ortius-Lechner et al. 2000). The reactions were carried out in 20-μl volumes of 1 μl DNA, 1 × reaction buffer, 0.2mM dNTPs, 0.5U of Taq polymerase and 0.25, 0.35, 0.35, and 2.0 μM of the Ech1390, Ech3385, Ech4126, and Ech4225 primers respectively. The DNA was amplified

by multiplexing all four primers in Hybaid PCR Express Thermal Cyclers (AH Diagnostics, Aarhus, Denmark) using a touchdown temperature program. This had an initial denaturing step of 94°C for 4 min followed by two touchdown sequences of six cycles each (first sequence: 92°C for 30 sec, 65.0–64.0°C decreasing at 0.2°C per cycle, and 72°C for 30 sec; second sequence: 92°C for 45 sec, 55.0–52.5°C decreasing at 0.5°C per cycle, and 72°C for 45 sec). These were then followed by a sequence of 20 cycles in which the denaturing temperature was 92°C for 45 sec, 52°C for 30 sec, and 72°C for 45 sec. A final elongation step of 72°C for 60 min completed the amplification process. Polymerase chain reaction products were run on 5% polyacrylamide gels with an ABI (Applied Biosystems, Foster City, CA) 377 automatic sequencer. Allele sizes were scored by comparison with internal size markers and the genotypes of the colony queens and their multiple mates were inferred from the multilocus offspring genotypes.

The strain of *Metarhizium anisopliae* var. *anisopliae* used as the experimental parasite was KVL 02–73, which had been isolated from a soil sample collected at the same site in Gamboa, Panama, as that from which the ant colonies had been collected (Hughes et al. 2004). The strain is highly pathogenic to *A. echinatior* (Hughes et al. 2002; Hughes and Boomsma 2004). The monospore isolate of this strain (KVL 02-73) was used in the first and third experiments, whereas the original multispore isolate of this strain (KVL 01-106) was used in the second experiment (see below). For the experiments, spore (conidia) suspensions were made up from recently sporulating culture plates in a 0.05% solution of Triton-X surfactant. The suspensions were quantified using a hemocytometer and diluted to the required concentrations. The viability of spores was checked by measuring the germination of spores on media plates (Lacey and Brooks 1997) and was >95% in all cases. Ants were held in forceps and quantities of 0.5 μl were applied to their thoraxes using a micropipette (Hughes et al. 2002; Hughes and Boomsma 2004). Control ants had 0.5 μl of a 0.05% Triton-X solution applied in the same way. After application, ant mortality was assessed daily and any dead individuals were removed. As control and treated ants were treated identically, with the exception of the presence of *Metarhizium* spores, any additional mortality amongst treated ants can be considered to be a result of the presence of the parasite. Additionally, the cadavers were surface sterilized (Lacey and Brooks 1997), placed in petri dishes with wet cotton wool and monitored for the appearance of external features (conidiophores and conidia) diagnostic of a *Metarhizium* infection. Control ants never exhibited any symptoms of *Metarhizium* infection.

Genetic Variation for Resistance

To establish whether patrilines within colonies varied in their resistance to the parasite, workers were genotyped from each of three colonies (Ae33, 48, and 132). They were maintained in isolation in individual pots and treated either with a control solution of 0.05% Triton-X or a dose of 1.0×10^6 *Metarhizium* spores/ml. The survival of the ants was monitored for 14 days following application, and 20 days after the end of this experimental period cadavers were assessed for the presence of *Metarhizium* spores. The dose and time periods were chosen based on previous work. Following the results of this experiment, it was repeated using a lower dose of *Metarhizium* (5.0×10^4 spores/ml). The results for each experiment (high dose; low dose), and for each of the three experimental colonies, were analyzed separately. The survival of ants in the different patrilines was analyzed using Cox proportional-hazards regression models. The proportions of ant cadavers sporulating with *Metarhizium* were compared using *G* tests for heterogeneity, or Fisher's exact tests when the frequencies were low. Analyses were carried out using SPSS 11.0.

Relatedness and Transmission

The transmission of a parasite might be expected to occur more readily between more genetically similar hosts. To test this, *A. echinatior* workers from the three experimental colonies used above were infected with the multispore isolate of the *Metarhizium* strain, because this will have had greater genetic variability within it than the monospore isolate. Spore suspensions were then created from a number of the sporulating cadavers of ants of known genotype. Each cadaver was placed into an individual vial with 1 ml of 0.05% Triton-X solution and vortexed for 1 min to remove the *Metarhizium* spores into suspension. The cadaver was then removed and the concentration of spores in the suspension quantified with a hemocytometer. The suspensions were centrifuged for 5 min to concentrate the spores and then the supernatant was removed or added to as required to achieve final concentrations of 1.0×10^5 spores/ml. The result was suspensions of *Metarhizium* spores that had previously been cycled a single time through ants of known genotype. These suspensions were applied to ants of known genotype to give four levels of genetic similarity between the source cadaver of the spores and the experimental ant: full-sibling ($n = 67$), half-sibling ($n = 53$), nonsibling conspecific ($n = 60$), and congeneric species ($n = 60$). A similar number of ants ($n = 76$) were treated with a control solution of 0.05% Triton-X. Ants for the first three treatments and the controls were obtained from the three experimental colonies of *Acromyrmex echinatior* that were used in the first experiment. The individuals used came from the two most abundant patrilines in each colony, with the representation of these patrilines being matched for treatment. Panamanian *Acromyrmex octospinosus* and *A. echinatior* are closely related species (Sumner et al. 2004b), and workers of *A. octospinosus* were used for the congeneric treatment. Ants were taken from three laboratory colonies (AoGP, AoAH, and Ao35) that had been collected at the same site in Gamboa, Panama, as that from which the *A. echinatior* colonies and the *M. anisopliae* var. *anisopliae* strain had been collected. Following application, ant survival was recorded for 14 days, and 20 days after this period the cadavers were examined for the occurrence of *Metarhizium* sporulation. Ant survival was analyzed with the Cox proportional-hazards regression model as before. Pairwise differences between treatments were resolved using Kaplan-Meier survival analysis and the Breslow statistic, with the significance level being adjusted with the Bonferroni procedure to correct for multiple

comparisons. The sporulation rates were examined with a G test for heterogeneity as before.

Effect of Group Genetic Diversity on Resistance

Finally, we examined the impact of group genetic diversity on resistance to disease. Ants from all three experimental colonies of *Acromyrmex echinatior* were genotyped and sorted into groups of three ants of either high or low genetic diversity. Ants from any particular patriline were assigned equally to each level of diversity. Low diversity groups contained three ants from the same patriline, whereas high diversity groups contained ants from three different patrilines. Eighty low and 80 high diversity groups were created in this way and all three of the ants in each group were then treated with a 0.5-μl dose of a *Metarhizium* suspension containing 1.0×10^6 spores/ml. In addition, a further 12 low and 12 high diversity groups were created and treated with a control solution of 0.05% Triton-X. The survival of the ants was monitored for a 14-day period, and 20 days after this, ant cadavers were scored for the presence of sporulating *Metarhizium*. The experiment was later repeated with a lower dose of 5.0×10^4 spores/ml using 54 replicate groups of each diversity level, with an additional 12 groups of each diversity level again acting as controls. Each experiment (high dose; low dose) was analyzed separately using repeated-measures analyses of variance to compare the number of ants surviving per group over the course of the experiment. Data was log $(x + 1)$ transformed prior to analysis, and, as the assumption of a circular variance-covariance matrix structure was not met, the Huynh-Feldt adjustment to the degrees of freedom was used (SPSS 2001). In the low dose experiment, the occurrence of allogrooming behavior was also recorded immediately before application and at 5, 15, 30, 45, and 60 min afterwards. At each time point the number of replicate pots in which at least one ant was engaged in allogrooming was recorded, and the numbers of pots with or without allogrooming at the five time points after application were compared using a G test for heterogeneity.

Results

Genetic Variation for Resistance

Patrilines did not vary in survival when exposed to the control treatment, with greater than 90% surviving at the end of the experimental period in all cases (Ae33: Wald = 4.21, df = 6, $P = 0.649$, $N = 89$; Ae48: Wald = 4.82, df = 6, $P = 0.568$, $N = 97$; Ae132: Wald = 3.20, df = 6, $P = 0.525$, $N = 80$). However, there was significant variation between patrilines in their survival when exposed to the parasite, with the nature of this variation differing between the two doses tested (Fig. 1). At the high dose of the parasite most patrilines were similarly susceptible, and in colony Ae33 there was no significant difference in survival between the patrilines (Wald = 2.59, df = 6, $P = 0.858$, $N = 199$; Fig. 1A). In both colonies Ae48 and Ae132, though, there was at least one patriline that was more resistant than the others (Ae48: Wald = 13.6, df = 6, $P = 0.035$, $N = 203$; Ae132: Wald = 10.8, df = 5, $P = 0.029$, $N = 195$). At the low dose of the parasite, greater variation between patrilines was expressed (Fig. 1B). In both Ae33 (Wald = 13.07, df = 6, $P = 0.042$, $N = 87$) and Ae48 (Wald = 16.4, df = 6, $P = 0.012$, $N = 84$) this variation was significant, whereas in Ae132 it was not (Wald = 4.40, df = 5, $P = 0.354$, $N = 107$). There was a similar difference between doses in the proportion of ant cadavers sporulating, with there being greater variation between patrilines within colonies in the proportion sporulating at the low dose than at the high dose where most cadavers sporulated (Fig. 2). At neither of the doses and in none of the colonies was the variation between patrilines significant, although at the low dose the small sample sizes means that the tests have limited power (Fisher's exact values for low dose: Ae33 = 5.19, $P = 0.578$; Ae48 = 9.58, $P = 0.126$; Ae132 = 5.38, $P = 0.2348$; G_{Het} for high dose: Ae33 = 8.58, $P = 0.284$; Ae48 = 2.57, $P = 0.922$, Ae132 = 8.54, $P = 0.129$).

Relatedness and Transmission

The survival of ants differed significantly between the treatments (Wald = 25.6, df = 4, $P < 0.001$; Fig. 3A), and also between the experimental colonies (Wald = 6.97, df = 2, $P = 0.031$). Ants exposed to *Metarhizium* suffered significantly greater mortality than those exposed to the control solution. *Acromyrmex octospinosus* ants survived *Metarhizium* passaged through an *A. echinatior* worker significantly better than *A. echinatior* ants survived *Metarhizium* passaged through a more or less genetically similar ant of the same species. However, this may simply have been due to *A. octospinosus* being more resistant to *Metarhizium* than *A. echinatior*. There were no differences in survival between the conspecific, half-sibling, and full-sibling treatments (Fig. 3A). The proportion of ant cadavers sporulating increased with the genetic similarity of the treated ants to the source ants (Fig. 3B), although the differences were marginally nonsignificant at the 5% level ($G_{Het} = 7.67$, df = 3, $P = 0.053$).

Effect of Group Genetic Diversity on Resistance

The survival of ants was significantly lower in groups of ants treated with *Metarhizium* than in those treated with the control solution in both the high dose ($F_{14,2408} = 31.4$, $P < 0.001$) and low dose ($F_{14,1680} = 15.9$, $P < 0.001$) experiments (Fig. 4). Survival did not change significantly over time in the control groups in either experiment (high dose: $F_{14,252} = 2.21$, $P = 0.096$; low dose: $F_{14,252} = 1.73$, $P = 0.171$), nor was it affected by the diversity of the groups (high dose: $F_{14,252} = 0.359$, $P = 0.984$; low dose: $F_{14,252} = 0.622$, $P = 0.603$). In both the high ($F_{14,2156} = 159.0$; $P < 0.001$) and low ($F_{14,1428} = 82.9$; $P < 0.001$) dose experiments, the survival of ants treated with *Metarhizium* decreased over time. In the high dose experiment this decrease over time did not differ between the group diversities ($F_{14,2156} = 0.339$; $P = 0.779$), nor was there any overall difference in survival ($F_{1,154} = 0.644$; $P = 0.424$). However, in the low dose experiment, the overall survival of ants in high diversity groups was significantly greater than of those in low diversity groups ($F_{1,102} = 6.64$; $P = 0.011$) and the change in survival over time also differed greatly, although not quite significantly, between the two levels of diversity ($F_{14,1428} = 2.82$; $P = 0.054$).

There was no difference between the levels of diversity in

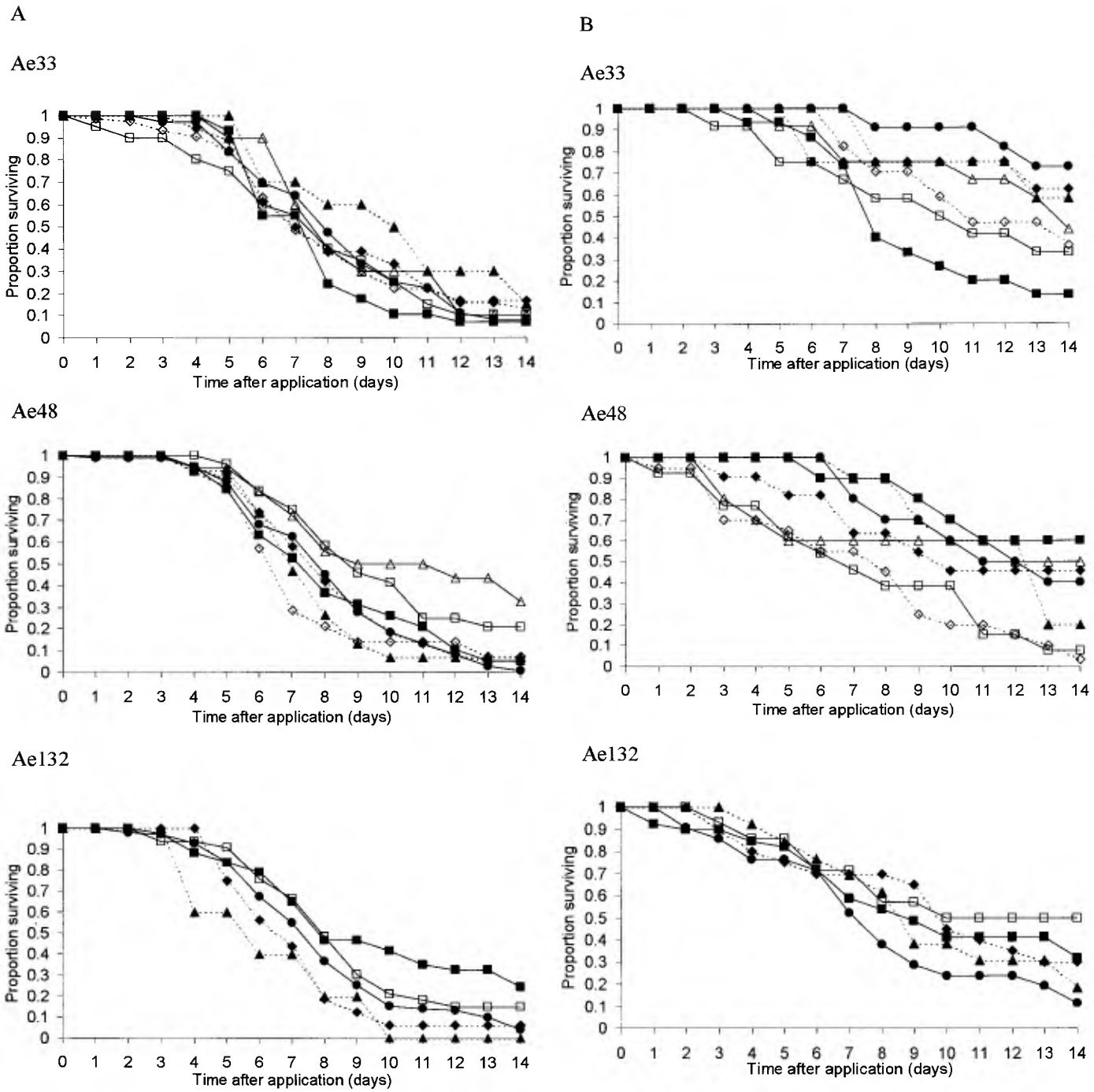

FIG. 1. Survival of ants from different patrilines within three colonies that were exposed to either a high (Fig. 1A) or low (Fig. 1B) dose of the *Metarhizium* parasite and maintained in isolation. Different symbols represent different patrilines within colonies (patriline 1: closed square; patriline 2: closed diamond; patriline 3: closed circle; patriline 4: closed triangle; patriline 5: open square; patriline 6: open diamond; patriline 7: open triangle).

the variation in survival (Levene's test for homogeneity of variances; high dose: $F_{1,158} = 0.141$, $P = 0.708$; low dose: $F_{1,106} = 2.28$, $P = 0.134$). There was also no effect of genetic diversity on the probability of an ant cadaver sporulating, with approximately 62% sporulating in all cases (low dose: $F_{1,106} = 0.017$, $P = 0.897$; high dose: $F_{1,158} = 0.042$, $P = 0.838$). In the low dose experiment, the frequency of allogrooming was observed to increase rapidly from a very low level prior to application to around 25% of pots having at least one ant allogrooming by 15 min after application of *Metarhizium* (Fig. 5). However, there was no difference in the frequency of allogrooming after exposure to *Metarhizium* between the different diversity groups ($G_{Het} = 3.58$, df = 4, $P = 0.47$).

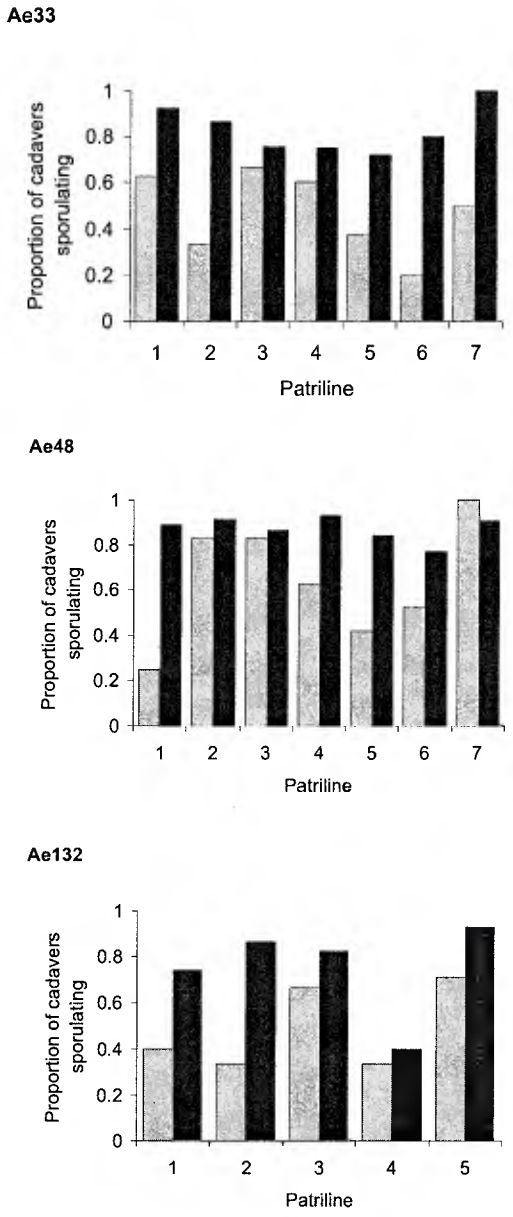

FIG. 2. Sporulation of the *Metarhizium* parasite from cadavers of *Acromyrmex* ants from different patrilines within three colonies that were exposed to either a high (dark shading) or low (light shading) dose of *Metarhizium* and maintained in isolation.

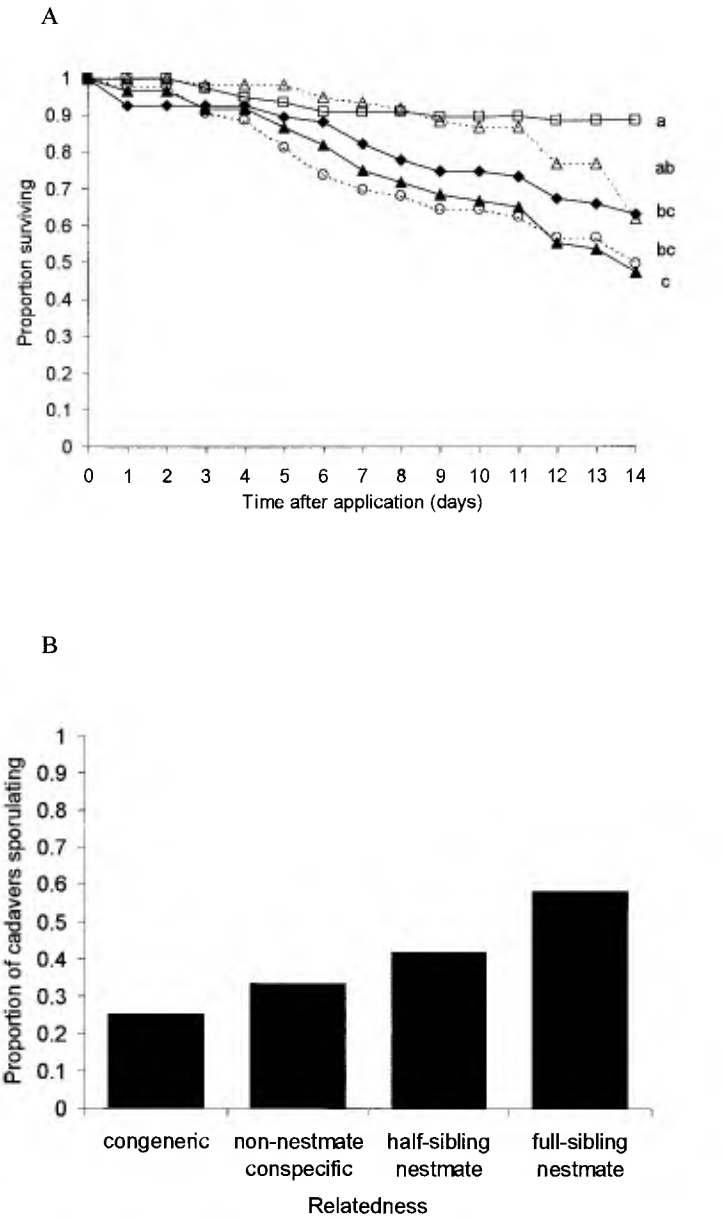

FIG. 3. (A) Survival of ants treated with *Metarhizium* isolates that had previously been cycled through *Acromyrmex* ants that were either full-siblings (closed diamond), half-siblings (open circle), non-nestmate conspecifics (closed triangle) or congenerics (open triangles) of the treated ants, or with a control solution (open squares). Different letters indicate treatments whose survival distributions differed significantly after correction with the Bonferroni procedure. (B) Sporulation of the *Metarhizium* parasite from the cadavers of treated ants 20 days after the end of the experimental period.

DISCUSSION

The results demonstrate that there is genetic variation in *Acromyrmex echinatior* for resistance to the parasite *Metarhizium anisopliae*. Patrilines within colonies varied in their resistance and, as individuals within these patrilines shared the same maternal genes and environmental cues, this can only have been caused by differences in their paternal genotype. Similar variation in resistance to disease has been found in the honeybee *Apis mellifera* (Palmer and Oldroyd 2003) and the bumblebee *Bombus terrestris* (Baer and Schmid-Hempel 2003). The key assumption for the genetic diversity/disease resistance hypothesis is therefore met in two polyandrous and one monoandrous social insect species. Given that genetic variation for disease resistance is also common among other taxa (Ebert et al. 1998; Little and Ebert 1999, 2000, 2001; Carius et al. 2001; Kover and Schaal 2002), it seems likely that it may be the case for many other social insects as well.

Interestingly, the nature of the variation in resistance depended on the dose of parasite to which individuals were exposed. For example, certain patrilines appeared unusually

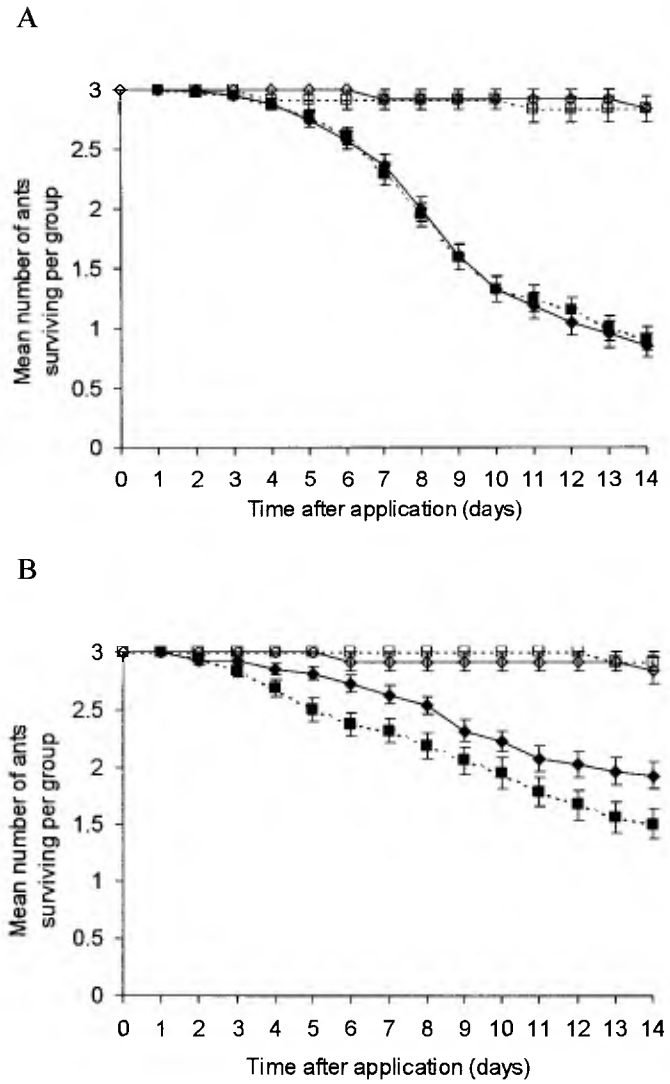

FIG. 4. Mean (±SE) survival at end of experimental period of ants exposed to either a high (Fig. 4A) or low (Fig. 4B) dose of the *Metarhizium* parasite (filled symbols), or a control solution (open symbols) and maintained in groups of three individuals of either high (diamonds) or low (squares) genetic diversity.

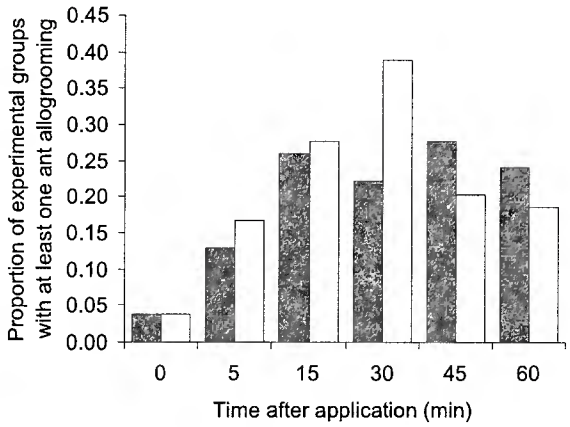

FIG. 5. Occurrence of allogrooming in groups of three ants of either high (shaded columns) or low (open columns) genetic diversity that had all been exposed to a low dose of the *Metarhizium* parasite.

resistant at the high dose but not at the low one. The differences need to be treated with caution because of the limited sample sizes in the low dose treatment, and because the two doses were tested in separate experiments. However, some differences between the doses are to be expected. Parasite virulence is dependent upon the density of parasites infecting a host in *Metarhizium* (Milner and Prior 1994; Vestergaard et al. 1995; Hughes et al. 2002), as well as in many other parasites (Anderson and May 1982; Ebert et al. 2000). The outcome of the parasite virulence versus host resistance interaction will therefore depend upon the density of the parasite to which the host is exposed. The manner in which parasite density acts is likely to itself vary between different resistance mechanisms. In leaf-cutting ants, resistance mechanisms include self-grooming, the production of antibiotic secretion, and both humoral and cellular components of the immune system (Kermarrec et al. 1986; Gillespie et al. 1999; Hughes et al. 2002; Poulsen et al. 2002a; Sumner et al. 2003). It is quite likely that these may differ in their effectiveness against high and low doses of parasite. For example, the effectiveness of antibiotic secretions are unlikely to be influenced by parasite dose, whereas high within-host densities of parasites may saturate the immune response or negate it with the large quantity of toxins that some parasites, such as *Metarhizium*, produce (Boucias and Pendland 1998).

If genotypes do differ in their resistance to a particular parasite, then it would be expected that a parasite that is adapted to exploit one genotype of host might be less successful at exploiting other host genotypes, with their different resistance attributes. This is a fundamental feature of most models of host-parasite dynamics and has been demonstrated in many empirical studies (Kaltz and Shykoff 1998; Lively and Dybdahl 2000; Schmid-Hempel and Ebert 2003). In bumblebees it has been found that transmission of the parasite *Crithidia bombi* is greater between related host individuals than unrelated ones (Shykoff and Schmid-Hempel 1991). However, we did not find this effect in the *Acromyrmex-Metarhizium* system (Fig. 3), in spite of the described host genetic variability for resistance. Almost certainly this was because the parasite used was a generalist. Although it did contain some genetic variability and had been passaged once through particular host genotypes prior to the experiment, it would appear that this was not sufficient for parasite genotypes to become differentially adapted to these host genotypes.

When ants were placed in groups of high or low genetic diversity and challenged with the parasite there was an effect of group diversity on ant survival. As with the genetic variation for resistance found in the first experiment, this effect depended on the dose of parasite. At the high dose there was no difference between the levels of diversity, but at the low dose, ant survival was significantly greater in the high diversity groups. Given that relatively little variation in resistance between patrilines was expressed at the high parasite dose in the first experiment, the lack of an effect of group diversity at the high dose is unsurprising. Greater variation in resistance is expressed at the low dose, and it is here that

group genetic diversity had an effect. High genetic diversity groups therefore appear to be better able to survive low doses of the *Metarhizium* parasite.

It is worthwhile to consider which of the hypothesized benefits outlined earlier may be involved in this enhancement of disease resistance. Because all of the ants in the groups were challenged with the parasite, this effect does not result from differences in transmission. There was no difference in the variation in ant survival between the two levels of diversity, which is surprising given the results of the first experiment. The resistance to disease of social insects, including leaf-cutting ants, can be improved by the presence of nestmates as a result of allogrooming (Rosengaus et al. 1998; Hughes et al. 2002) and the enhancement of immunocompetence (Traniello et al. 2002). *Acromyrmex* ants can actively apply the antibiotic secretions from their metapleural glands to their fungus garden (Fernández-Marin et al. 2003), and it seems likely that these secretions are also transferred between nestmates by contact and grooming, as occurs with cuticular hydrocarbons (Soroker et al. 1995; Lenoir et al. 2001). The occurrence of a resistant genotype may therefore also benefit susceptible genotypes in the group, which could result in the greater survival of individuals in high diversity groups that was seen. Although the groups were not found to differ in the frequency of grooming, certain genotypes may still be more effective at the behavior than others.

Another leading hypothesis for polyandry in social insects is that it may improve the division of labor within the colony (Crozier and Page 1985; Palmer and Oldroyd 2000; Crozier and Fjerdingstad 2001). Genetic variation for caste determination is well known in honeybees (Robinson 1992) and is suggested in several ant species (Stuart and Page 1991; Snyder 1992; Carlin et al. 1993; Fraser et al. 2000). It has recently been demonstrated in *A. echinatior* that individuals of different patrilines have different propensities to develop into either small or large workers (Hughes et al. 2003). It has also been reported that the patriline of an *Acromyrmex* worker can influence its probability of engaging in particular tasks (Julian and Cahan 1999). Both behavior and caste are likely to influence resistance to disease (Keller 1995). The small worker caste of *Acromyrmex* has been shown to be both more (Hughes et al. 2002) and less (Kermarrec et al. 1990) resistant to disease, with the difference appearing to depend upon the specific host-parasite dynamics. The antibiotic-producing metapleural glands of the small worker caste of leaf-cutting ants are disproportionately large, and these ants are also more effective at grooming than are large workers (Wilson 1980; Kermarrec et al. 1986; Bot and Boomsma 1996; Hughes et al. 2002). Several studies have suggested that it is the small worker caste that plays the main role in defending the colony against disease (Kermarrec et al. 1986; Jaccoud et al. 1999; Hughes et al. 2002; Poulsen et al. 2002b). Any genetic variation for caste determination and task performance is therefore also likely to impact a colony's resistance to parasites. These two hypotheses for polyandry in social insects (genetic diversity for division of labor and genetic diversity for disease resistance) may consequently be interlinked.

It is worth emphasizing two limitations of this study that may mean that greater effects of group diversity could occur under natural conditions. Critically, the ant groups consisted of only three individuals. Natural *Acromyrmex* colonies can contain many thousands of ants (Weber 1972; Wetterer 1999), and even slight increases in group size beyond three individuals can result in the improved survival of ants exposed to *Metarhizium* (Hughes et al. 2002). The limited group size also meant that the high diversity groups were restricted to only containing individuals from three different patrilines. As *Acromyrmex* queens typically mate with about nine or 10 males (Ortius-Lechner et al. 2003; Sumner et al. 2004a), the genetic diversity of these high diversity groups was far lower than would be the case in natural colonies. Therefore, there is likely to be a greater effect of group genetic diversity on resistance in natural colonies exposed to a multitude of more and less virulent parasites. Future work examining more natural conditions or other, possibly less virulent, parasites is likely to prove highly interesting.

Nevertheless, the results suggest that *Acromyrmex* queens may benefit from polyandry because it allows them to produce more genetically diverse colonies that are more resistant to disease. Although the precise mechanisms may vary, this means there is now data supporting the genetic diversity/disease resistance hypothesis in three species: *Bombus terrestris* (Shykoff and Schmid-Hempel 1991; Baer and Schmid-Hempel 1999, 2001, 2003), *Apis mellifera* (Palmer and Oldroyd 2003; Tarpy 2003), and *Acromyrmex echinatior* (this study). Any effect will depend upon the specific host-parasite interaction. However, it does seem that the genetic diversity/disease resistance hypothesis may be a possible explanation for the evolution of polyandry in at least some social insects.

Acknowledgments

We would like to thank L. Thomsen, R. Eliasen, and J. Eilenberg for their help in the collection and culturing of the *Metarhizium* isolates, and S. Mathiesen, K. A. Palmer, and D. Pedersen for their assistance with the ant cultures and experiments. We are also grateful to E. A. Herre and the Smithsonian Tropical Research Institute for providing facilities in Gamboa and to the Instituto Nacional de Recursos Naturales Renovables for permission to export them from Panama to Denmark. This research was supported by a Marie Curie Fellowship awarded to WOHH under the European Community program Human Potential (HPMF-CT-2000-00543), by a Carlsberg Foundation grant to the same author, and by several grants from the Danish Natural Science Research Council to JJB.

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Corresponding Editor: L. Keller