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Fitness and the level of homozygosity in a social insect

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Abstract

To date very few studies have addressed the effects of inbreeding in social Hymenoptera, perhaps because the costs of inbreeding are generally considered marginal owing to male haploidy whereby recessive deleterious alleles are strongly exposed to selection in males. Here, we present one of the first studies on the effects of queen and worker homozygosity on colony performance. In a wild population of the ant *Formica exsecta,* the relative investment of single-queen colonies in sexual production decreased with increased worker homozygosity. This may either stem from increased homozygosity decreasing the likelihood of diploid brood to develop into queens or a lower efficiency of more homozygous workers at feeding larvae and thus a lower proportion of the female brood developing into queens. There was also a significant negative association between colony age and the level of queen but not worker homozygosity. This association may stem from inbreeding affecting queen lifespan and/or their fecundity, and thus colony survival. However, there was no association between queen homozygosity and colony size, suggesting that inbreeding affects colony survival as a result of inbred queens having a shorter lifespan rather than a lower fecundity. Finally, there was no significant association between either worker or queen homozygosity and the probability of successful colony founding, colony size and colony productivity, the three other traits studied. Overall, these results indicate that inbreeding depression may have important effects on colony fitness by affecting both the parental (queen) and offspring (worker) generations cohabiting within an ant colony.

Introduction

Understanding the fitness consequences of inbreeding in natural populations is crucial for analysing the effects of inbreeding depression on the evolution of a wide variety of traits such as sex-biased dispersal (Perrin & Mazalov, 2000), mate choice (Charlesworth & Charlesworth, 1987), sociality (Michod, 1993; Burland *et al.*, 2002) and population survival (Saccheri *et al.*, 1998). In a wide range of species, there is evidence that inbreeding reduces fitness (Keller & Waller, 2002) and, partly, recessive deleterious mutations seem to be the most important source of inbreeding depression (Charlesworth & Charlesworth, 1999).

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In social Hymenoptera, the negative effects of inbreeding stemming from the effects of partly recessive deleterious mutations is generally thought to be greatly reduced due to purging in males, which are haploid (reviewed in Werren, 1993). Importantly, in ants and other social Hymenoptera with a well-defined reproductive division of labour, there are many genes whose expression is caste specific (Graff *et al.*, 2007). Thus, genes, which are only expressed the in female castes (workers and queens), but not in males are less targeted by purifying selection. Such genes include, for example, important genes associated with queen fertility. Consistent with this view, inbreeding depression has been

found in one solitary Hymenoptera species (Henter, 2003), several other haplodiploid organisms (Perrot-Minnot *et al.*, 2004) and genes on the X chromosome of *Drosophila melanogaster* (Wilton & Sved, 1979).

An additional inbreeding load specific to Hymenoptera comes from the production of nonfertile diploid males (Ross *et al.*, 1993; Cook & Crozier, 1995). In many Hymenopterans, the gender of an individual is determined by a single sex-determining locus (Haig, 1998; Beye *et al.*, 2003; Evans *et al.*, 2004). Hemizygotes (i.e. individuals developing from unfertilized eggs) become males, whereas diploids become females if heterozygous at this locus and diploid males if homozygous (diploid males are viable but sterile in most species). Normally the sex-determining locus is highly polymorphic (Yokoyama & Nei, 1979; Ross *et al.*, 1993; Antolin *et al.*, 2003; Fujiwara *et al.*, 2004), but as a result of loss of variance at this locus, the production of sterile diploid males increases colony mortality during colony foundation in fire ants (Ross & Fletcher, 1986), reduces colony growth in bumble bees (Plowright & Pallett, 1979) and winter colony survival in honey bees (Tarpy & Page, 2002).

Apart from the effect of diploid males on colony performance, there is only one study in social Hymenoptera on how inbreeding affects colony performance. In *Bombus terrestris*, colonies with queens experimentally mated with a brother performed less well than outbred colonies (Gerloff & Schmid-Hempel, 2005). Although revealing that worker inbreeding decreases colony foundation success and colony size, the study did not investigate whether queen homozygosity also influenced queen fitness. In the present study, we examine the effects of both queen and worker homozygosity, as a surrogate of inbreeding, in a natural population of the ant *Formica exsecta* (Nylander 1846). Previous work on this and two other *Formica* species revealed that workers are on average more inbred than queens (Sundström, 1993; Sundström *et al.*, 2003; Hannonen *et al.*, 2004). In social Hymenoptera this is unexpected, because caste determination usually depends on developmental changes triggered by environmental factors, including the amount and quality of food provided to the brood (Wheeler, 1986) rather than genetic differences between the female brood developing into queens and workers (but see Cahan & Keller, 2003). Possible explanations for this difference in inbreeding between queens and workers in *F. exsecta* are that inbreeding may influence the likelihood that female larvae develop into queens vs. workers, or that inbred queens may be less proficient in colony foundation, leading to a lower inbreeding coefficient in queens than workers in established colonies (Sundström *et al.*, 2003).

The level of queen and worker homozygosity were estimated from genotype data at 10 microsatellite markers (Aparicio *et al.*, 2006). We tested for associations between the level of homozygosity and five fitness-related traits. Four of the traits (colony foundation success, the number of pupae produced, the relative investment in sexuals and the number of workers per colony) are expected to be influenced by queen fecundity (which may depend on queen homozygosity), and brood survival (which may depend on worker homozygosity). Colony foundation success is also expected to be affected by worker homozygosity as this correlates with diploid male production (Ross & Fletcher, 1986; Pamilo *et al.*, 1994). The fifth trait, colony life span, should be affected primarily by queen traits, which may depend on queen homozygosity.

The study was carried out on three islands in the Hanko archipelago in southern Finland, near Tvärminne Zoological Station. Based on our sample, over 96% of the colonies have a single queen. The number of colonies on each island was small (11, 30 and 62 on average across the study years), implying a small effective population size, as the number of nests largely corresponds to the number of breeding females. Accordingly, some inbreeding is expected even under random mating (Falconer & Mackay, 1996). The probability of mating among relatives is further increased by sex-ratio specialization (Sundström *et al.*, 1996) and restricted gene flow both among and within islands (Sundström *et al.*, 2003). The small effective population size and the spatial structuring of our study population suggest that homozygosity at a limited number of loci is likely to reflect, at least partly, genome wide homozygosity and therefore inbreeding (Slate *et al.*, 2004). Despite a long queen life span (on average 20 years, Pamilo, 1991), we were able to trace inbreeding depression over a large part of the colony life cycle because we have long-term data on colony survival, colony size, colony productivity and reproductive allocation (13 years for two islands, 7 years for the third island).

Material and methods

Samples

A total of 212 colonies were found and surveyed between 1993 and 2004. Every year, we searched for newly founded colonies and recorded which ones disappeared. After 2004, colony survival was monitored until 2007. The subpopulations on the islands Joskär and Kalvholmen were followed from 1993 to 2007 and the subpopulation on Furuskär from 2000 to 2007. From a random sample of 120 colonies among the 212 colonies found, we collected and genotyped 13.2 ± 0.4 (mean ± SE) adult workers at 10 microsatellite loci (see below). Of these, we excluded eight colonies because workers' genotypes suggested that they were headed by more than one queen and 10 colonies because too few workers amplified successfully. This left a total of 102 colonies (45 on the island of Joskär, 45 on Furuskär and 12 on Kalvholmen). From the 76 colonies that produced males, we also genotyped 5.2 ± 0.3 (mean ± SE) male pupae at all 10 loci.

Because it was not possible to collect queens from established colonies, we inferred their genotype from those of their worker and male offspring. Whenever we could collect male pupae from a colony, we directly inferred the queen genotype from her male offspring. For the 26 colonies that did not produce males and the 48 colonies where too few males amplified to reliably sample both maternal alleles at a given locus, the queen genotype at that locus or loci was inferred from her daughter workers. Queen genotypes were inferred following established procedures (Sundström *et al.*, 1996). The procedure is straightforward in colonies headed by a heterozygous queen that has mated once, as the genotype of the haploid father shows as linked alleles in the offspring. The number of workers genotyped allowed identification of the two maternal alleles of heterozygous queens with a high probability (the average probability for detecting all alleles at all loci of queens was 98.3%). The situation is more complex when the queen is homozygous at a target locus, or when she is mated with several males. When several queen genotypes were equally parsimonious, one genotype was selected randomly for the ambiguous locus. Although adding some noise, this procedure should not bias the results because the genotype was selected randomly. Moreover, the effect was relatively small as there were only few cases: one locus (of 10 loci) in 27 colonies, two loci in nine colonies and four loci in one colony. When the queen genotype was deduced from worker genotypes, it would theoretically be possible to overestimate the queen heterozygosity if a queen was wrongly assessed as being singly mated instead of doubly mated. However, the probability of failing to detect a mate was very low given that workers were genotyped at 10 highly polymorphic loci.

To estimate the effect of inbreeding on colony foundation success, we collected young queens immediately after mating flight. These 89 queens were found on two of the islands (2002: 33 queens on Joskär and seven on Furuskär; 2003: 13 queens on Joskär and 36 on Furuskär). All these queens were dissected and their spermatheca examined to assess mating status (73 of the 89 queens were mated). All these queens, as well as their spermathecal contents, were genotyped at the 10 microsatellite loci. Because queens collected after mating flight had not yet established a colony, the genotypes of their potential diploid/worker offspring were inferred from the queen's genotype and that of the sperm stored in their spermatheca. When the queen had mated with more than one male, we assumed that both males contributed equally to the brood, and worker homozygosity was estimated from the possible worker genotypes. Under natural conditions, it is likely that males do not contribute equally to worker production. However, assuming equal male contribution does not lead to biased worker heterozygosity, unless there would be a consistent difference in the relative male contribution and their genetic similarity to the queen (which our unpublished data do not suggest).

Microsatellite analyses

All the queens, their sperm and the workers were genotyped at 10 loci: Fe11, Fe13, Fe17, Fe37, Fe38, Fe42, Fe49 (Gyllenstrand *et al.*, 2002) and Fl21 (Chapuisat, 1996), P22 (Trontti *et al.*, 2003) and Fy3 (Hasegawa & Imai, 2004) (described in Table 1). Single PCR amplifications were carried out for Fe13, Fe17, Fl21, Fe37 and P22 in 10 μL of reaction solution of 10 mM Taq Buffer, 0.8 mM dNTPs, 0.3 μM of each primer, 0.25 units of DyNAzyme© (Finnzymes, Espoo, Finland) and 1 μL of template DNA extracted applying Chelex© (Biorad, Hercules, CA, USA) extraction protocol. A multiplex PCR amplification was carried out for Fe42 and Fe49 in 10 μL of reaction solution of 15 mM Finnzymes© Taq Buffer, 1 mM dNTPs, 0.15 μM of Fe42 primer, 0.45 μM of Fe49 primer, 0.25 unit of DyNAzyme© and 1 μL of template DNA extracted applying Chelex© extraction protocol. Fe11, Fy3 and Fe38 were amplified in 10 μL multiplex containing 15 mM Finnzymes© Taq Buffer, 1 mM dNTPs, 0.2 μM of each primer, 0.6 unit of DyNAzyme© and 1 μL of template DNA. PCR products were analysed using an automated sequencer ABI 377 for Fe13, Fe37, Fe42, Fe49 and Fl21, and Megabace 1000 (Amersham Biosciences, Uppsala, Sweden) sequencer for P22, Fe11, Fe17, Fe38 and Fy3.

We found evidence for null alleles at two loci: Fe49 and Fe17. Null alleles are obvious in a colony headed by a single heterozygous queen mated with a single male carrying a null allele. In such colonies, two worker genotypes are observed at similar frequency, each appearing homozygous for one of the queen's alleles at the locus in question. In all cases, the results from other loci confirmed that the colony was headed by a singly mated queen. Based on the frequency of colonies with a null allele and the number of colonies where such null allele could be detected, the estimated frequency of null

Table 1 Locus characteristics.

	Number of alleles	H_{exp}
Fe13	15	0.85
Fe37	9	0.74
Fe42	9	0.77
Fe49	17	0.83
Fl21	27	0.94
Fe11	2	0.36
Fe17	19	0.88
Fe38	43	0.94
Fy3	5	0.55
P22	11	0.82

H_{exp} is the expected heterozygosity under random mating.

allele was 2.8% for Fe 49 and 1.8% for Fe17. Excluding Fe49 and Fe17 from the analyses did not change the results, as expected, if null alleles are randomly distributed across individuals and colonies with respect to trait values. Hence, these loci were included in the analyses. No other locus showed evidence of null alleles based on the genotypes of nestmate workers.

Inbreeding level

Inbreeding was estimated as the individual average homozygosity at loci weighted by the allelic diversity of the locus, HL, following Aparicio *et al.* (2006). We chose this measure, because it has been shown to correlate better with average genome-wide inbreeding than other measures, particularly when there is variation in the polymorphism of the markers used (Aparicio *et al.*, 2006), as is the case with our microsatellite markers (Table 1). To determine whether HL reflects genome-wide homozygosity (inbreeding), we measured the level of inbreeding on sets of five loci, which we then correlated with the value for the remaining five loci (Balloux *et al.*, 2004). This procedure was repeated 10 000 times for both queens and workers, each time randomly selecting a set of five loci and the 95% CI was constructed based on the observed correlation coefficients, using $R = 2.6.1$ (freeware; availlable at www.r-project.org) script for resampling (J. Alho, pers. comm.). The homozygosity estimated with a randomly selected half of the loci was positively correlated with that estimated with the remaining half loci for both workers (Pearson correlation: $\rho = 0.17$, 95% CI: 0.09–0.24) and queens (Pearson correlation: $\rho = 0.08$, 95% CI: −0.01 to 0.15), the association being significant only for workers.

To compare our results with former studies, we estimated heterozygote deficit (F_{is}) separately for queens collected during mating flight, for their inferred workers, for queens heading established colonies and for workers of established colonies using the software FSTAT 2.9.3.2 (Goudet, 1995). A single worker was sampled randomly from each established colony or from the inferred worker offspring of queens collected after mating flight.

Fitness consequences of homozygosity

Colony foundation

To test whether queen homozygosity affects founding success, we compared the homozygosity of queens collected just after mating flight (i.e. before colony foundation) with that of queens heading established colonies (as inferred from their offspring). To test whether worker homozygosity affects the probability of colony establishment, we compared the homozygosity of the inferred diploid offspring produced by queens collected just after mating flight and the observed heterozygosity of workers in established colonies.

Colony survival

To assess whether queen and worker homozygosity affects colony survival, we tested for a negative correlation between homozygosity and colony 'age'. The 'age' of a colony was defined as the number of years it was found to be alive within the study period. Hence, for colonies still alive in 2007, colony 'age' was calculated as 2007 minus the year in which the colony was first discovered. For colonies that died during the study period, the colony 'age' was calculated as the year of death minus the year of first observation.

Colony size

To study the effect of queen and worker homozygosity on colony size after hibernation, we estimated in 1994, 1995, 1997, 1998, 2002, 2003 and 2004 the numbers of adult workers per colony by mark–recapture in late May and early June, as described by Sundström (1995). Over the whole study period, we marked 522 ± 21 (mean ± SE) workers per colony and recaptured 11 ± 0.4 marked and 63 ± 1.5 unmarked workers per colony per year. One or more colony size estimates were obtained for 95 of the 102 colonies. When more than one estimate was available, we used the average colony size in the analyses. To correct for an age effect on colony size, we included colony 'age' as a factor in the analyses.

Colony productivity

To study the impact of queen and worker homozygosity on colony productivity, we estimated by mark–recapture the total number of male, worker and queen pupae produced. The procedure was carried out in 1995, 1997, 1998, 2003 and 2004 when the main seasonal batch of pupae was produced. For each colony, we marked 340 ± 17 (mean ± SE) pupae and recaptured 12 ± 0.6 marked and 69 ± 2.3 unmarked pupae. We obtained at least one estimate for 69 of the 102 colonies. For colonies with multiple estimates, we used the average across years in the analyses. To take into account the strong correlation between colony size and colony productivity, colony size was included as a factor in the analyses.

Proportion of sexuals among pupae

To assess whether queen and worker homozygosity affects reproductive allocation, we compared the numerical proportion of sexual pupae (i.e. queens and males) among all pupae produced. We obtained estimates for 86 colonies in 1994, 1995, 1997, 1998, 2002, 2003 and 2004. The gender of a pupa was determined by morphological examination of 87.8 ± 5.7 (mean ± SE) pupae per colony (Liautard *et al.*, 2003). The proportion of sexuals was arcsine transformed for all statistical analyses. Colony 'age' was not included as a factor, because analyses showed that the proportion of sexuals was not significantly correlated with colony 'age' (see Results).

All statistical analyses were performed with the software S-PLUS 6.1 (TIBCO, Palo Alto, CA, USA). The assumptions of parametric tests were fulfilled. In each model, island (i.e. population) was included as a factor. We tested for the effects of queen and worker homozygosity in separate analyses to avoid problems that may arise depending on the order variables are entered in the analysis. We verified that the significant correlation between queen and worker inbreeding in the entire data set (see Results) did not confound our results based on established colonies only, by entering both variables in the same analysis. We did this analysis twice, once with queen homozygosity entered in the model before worker homozygosity, and second with the variables in reverse order. We also did not find any indication of collinearity between the measures of homozygosity (VIF = 1, and $t = 0.19$, $P = 0.84$ for the effects of one measure of homozygosity on the other).

Results

There was a significant heterozygote deficit among queens heading established colonies ($F_{is} = 0.04$, $n = 102$, 95% CI: 0.01–0.07), workers in established colonies ($F_{is} = 0.09$, $n = 102$, 95% CI: 0.01–0.19) and the inferred genotypes of workers of queens collected just after mating flight ($F_{is} = 0.10$, $n = 65$, 95% CI: 0.041–0.16), but not for queens collected after mating flight ($F_{is} = 0.05$, $n = 89$, 95% CI: −0.01–0.11). In established colonies, workers were significantly more homozygous than queens ($HL_{worker} = 0.24 \pm 0.01$, $HL_{queen} = 0.21 \pm 0.01$, t-test: $t_{202} = 2.1$, $P = 0.04$). By contrast, there was no significant difference between the queens collected after mating flight and their presumed workers ($HL_{worker} = 0.23 \pm 0.01$, $HL_{queen} = 0.24 \pm 0.02$, t-test: $t_{159} = 0.1$, $P = 0.91$).

There was no evidence that the level of queen or worker homozygosity affected the probability of successfully establishing a new colony. The homozygosity level of the queens collected just after mating flight ($HL_{queen} = 0.24$) was similar to that of queens in established colonies ($HL_{queen} = 0.21$, t-test: $t_{189} = 1.5$, $P = 0.13$). Similarly, the estimated heterozygosity of workers produced by queens collected just after mating flight ($HL_{worker} = 0.23$) was not significantly different from the value measured for workers collected in established colonies ($HL_{worker} = 0.24$, $t_{173} = 0.5$, $P = 0.64$.

Homozygosity did not differ among islands, neither for workers (one-way ANOVA: $F_2 = 0.22$, $P = 0.81$) nor for queens (one-way ANOVA: $F_2 = 0.25$, $P = 0.78$). Neither was the variation in average homozygosity across the study years significant (repeated measures ANOVA: workers – $F_{15,86} = 1.2$, $P = 0.26$; queens – $F_{15,86} = 1.4$, $P = 0.17$) (Fig. 1). However, queen homozygosity was significantly positively correlated with worker homozygosity (Pearson correlation: $r = 0.16$, d.f. = 173, $P = 0.031$).

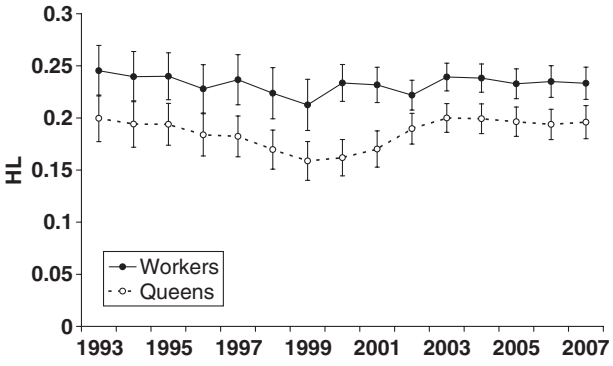

Fig. 1 Variation in the level of homozygosity among years.

The mean colony 'age' was 6.5 ± 0.4 years (mean ± SE). This value varied significantly among islands, with colonies on Furuskär being 'younger' (mean ± SE: 5.3 ± 0.4), because that island had been studied for fewer years than the others: Joskär (mean ± SE: 7.4 ± 0.7) and Kalvholmen (mean ± SE: 8.0 ± 1.4) (Table 2). Once the effect of island was factored out, worker homozygosity was not significantly correlated with colony 'age' (Table 2, Fig. 2), but queen homozygosity was significantly lower in older than in younger colonies (Table 2, Fig. 2). This suggests that queen homozygosity may reduce colony longevity with queen homozygosity explaining 6.8% of the variance in colony 'age'.

Colony size was not significantly associated with either queen or worker homozygosity, once the effects of colony 'age' and island had been factored out (Table 2, Fig. 2). Colonies contained on average 3495 ± 233 (mean ± SE) workers, with older colonies being significantly larger than younger ones (Table 2). The size of colonies varied significantly between islands (Kalvholmen: 4684 ± 652, mean ± SE; Furuskär: 3813 ± 450; Joskär: 2888 ± 222, Table 2). Similarly, once the effects of island and colony size were factored out, neither worker homozygosity, nor queen homozygosity, were significantly correlated with colony productivity (Table 2, Fig. 2). Colonies produced on average 1963 ± 144 (mean ± SE) pupae per year, and the production of pupae did not differ significantly among islands (Table 2), but increased significantly with the number of workers present in the colony (Table 2).

Among the brood produced in the spring, 66 ± 3% (mean ± SE) were sexuals. The proportion varied significantly among islands, with colonies on Furuskär investing more in sexuals (85 ± 4%, mean ± SE) than those on Joskär (55 ± 4%, mean ± SE) and Kalvholmen (51 ± 7%, mean ± SE) (Table 2). Once corrected for the effects of island ($F_{2,65} = 21.7$, $P < 0.001$), the proportion of sexual brood was not significantly correlated with colony productivity ($F_{1,65} = 1.5$, $P = 0.23$). Colony 'age' was not significantly correlated with investment in sexuals (Pearson correlation: $\rho < 0.01$, d.f. = 84,

Table 2 Summary table of all ANCOVA analyses.

	d.f.	SS	*F*	*P*(*F*)
Worker				
Dependent: colony age				
Island	2	128	4.2	0.02
HL_{worker}	1	26	1.7	0.19
Residuals	98	1496		
Dependent: colony size				
Island	2	37 089 688	4.0	0.02
Age	1	30 741 907	6.6	**0.01**
HL_{worker}	1	2 763 580	0.6	0.4
Residuals	90	416 223 150		
Dependent: colony productivity				
Island	2	5 250 162	2.5	0.09
Colony size	1	25 758 570	24.7	**< 0.001**
HL_{worker}	1	193 859	0.2	0.67
Residuals	64	66 805 258		
Dependent: proportion of sexuals				
Island	2	3.41	18.7	**< 0.001**
HL_{worker}	1	1.34	14.7	**< 0.001**
Residuals	82	7.49		
Queen				
Dependent: colony age				
Island	2	128	4.4	**0.01**
HL_{queen}	1	112	7.8	**0.006**
Residuals	98	1410		
Dependent: colony size				
Island	2	37 089 688	4.0	0.02
Age	1	30 741 907	6.7	**0.01**
HL_{queen}	1	5 584 234	1.2	0.27
Residuals	90	413 402 495		
Dependent: colony productivity				
Island	2	5 250 162	2.5	0.09
Colony size	1	25 758 570	24.7	**< 0.001**
HL_{queen}	1	312 303	0.3	0.59
Residuals	64	66 686 814		
Dependent: proportion of sexuals				
Island	2	3.41	16.4	**< 0.001**
HL_{queen}	1	0.31	3.0	0.09
Residuals	82	8.53		

Because four different traits were studied simultaneously, the significance level is 0.0125 based on Bonferroni correction. Significant results are shown in bold

$P = 0.92$). Once corrected for the effects of island (Table 2), there was, however, a significant association between worker homozygosity and colony investment in sexuals, with more homozygous colonies investing proportionally less in sexuals than less homozygous colonies (Table 2, Fig. 2). Worker homozygosity explained 11.0% of the variance observed in the proportion of sexual brood. In contrast, queen homozygosity was not significantly correlated with the proportion of sexual brood (Table 2, Fig. 2).

Discussion

Our results show that both the levels of queen and worker homozygosity can influence important components of the life history of the ant *F. exsecta*. In particular, worker homozygosity correlated with traits related to colony performance in terms of sexual investment, whereas queen homozygosity correlated with colony longevity. Queen and worker homozygosity appear not to play a major role in the success of colony establishment, as no significant difference in the level of homozygosity was found between queens collected just after mating flight and queens in established colonies (the same holds for the level of homozygosity of female offspring of these two types of queens).

There was a negative correlation between colony 'age' and the level of queen homozygosity. There are two possible explanations for this association. The first is that more inbred queens are less fecund, hence leading to smaller colony size and lower colony survival. However, this hypothesis seems unlikely, because queen homozygosity correlated with neither colony size nor productivity. The other possible explanation is that queen longevity is directly influenced by queen homozygosity. Such an effect is likely, in particular because deleterious genes affecting the lifespan of the long-lived queens are likely to be only under week purging selection in the very short-lived males. Indeed, males have an adult lifespan of only a few weeks, whereas queens can live and reproduce for over 20 years (Pamilo, 1991). Such a negative effect of homozygosity on queen lifespan could explain why queen homozygosity was lower than worker homozygosity in this as well as an earlier study in *F. exsecta* (Sundström *et al.*, 2003).

Worker homozygosity affected reproductive allocation, with higher homozygosity being associated with less resources being allocated to sexual brood and more to worker production. At least two possible hypotheses may account for this difference. The first is that higher homozygosity of female brood decreased the likelihood of development into queens. A key factor determining the developmental pathway of a female may be the level of juvenile hormone in growing larvae (Wheeler, 1986). It is conceivable that more homozygous larvae are less able to enter the queen developmental pathway, if a higher level of homozygosity curtails the production of juvenile hormone. This would also explain the observed difference in homozygosity between queens and workers. Alternatively, the effect may also be mediated by adult workers, as the process of caste determination also depends on the type and quantity of food provided to larvae. Thus, it is conceivable that worker homozygosity might decrease colony efficiency and consequently the ability of workers to raise sexual brood.

We also found a significant positive correlation between queen homozygosity and worker homozygosity. Interestingly, a similar correlation between parents and offspring inbreeding has been found in the song sparrow *Melospiza melodia* (Reid *et al.*, 2006). As pointed out by the authors, such a pattern can arise when immigrants mated elsewhere mix with a local inbred population. Alternatively,

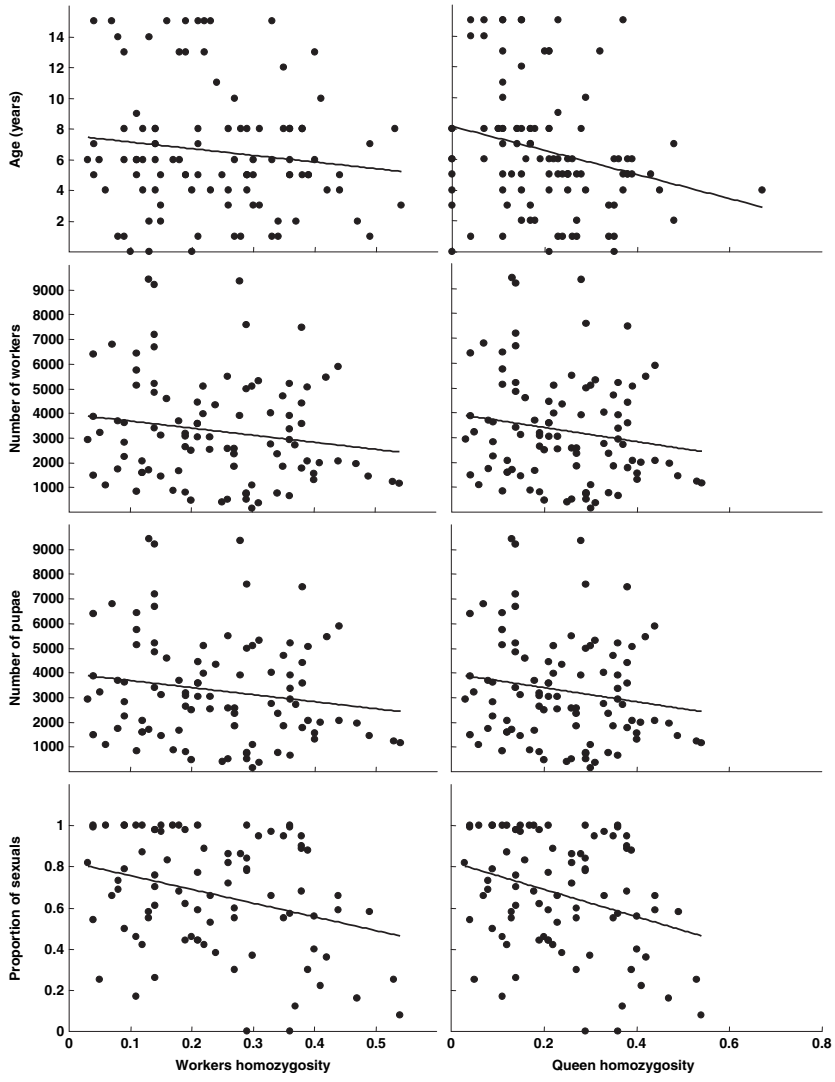

Fig. 2 Relationship between either worker or queen homozygosity and 'age' of colony (number of years), colony size (number of workers), colony productivity (number of pupae produced in the spring) and the proportion of sexual pupae among all pupae.

inbred females may have a propensity to mate with relatives, or both factors may come into play simultaneously, as seems to be the case with song sparrows (Reid *et al.*, 2006). At present we cannot discriminate between the two possibilities. *Formica exsecta* females tend to be philopatric and start new colonies near their natal colony (Sundström *et al.*, 2003), hence providing opportunities to mate with related males. A positive association between parental and offspring homozygosity would then be possible if more inbred queens would tend to disperse less and thus be more likely to mate with related males.

Neither queen homozygosity nor the homozygosity of their daughter workers differed between queens collected just before mating flight and queens in established colonies. This suggests that neither the genetic makeup of the queens nor that of their offspring impose strong selection during the colony founding stage through reduced queen fecundity or lower offspring viability. A major cost of inbreeding during colony founding is the production of diploid males, which frequently may lead to colony death (Ross & Fletcher, 1986). We found diploid males in two of the 102 colonies studied. Importantly, the cost incurred by the production of diploid males depends on how new colonies are initiated. Because *F. exsecta* queens temporarily parasitize established colonies of *Serviformica* species (Collingwood, 1979), the strength of selection resulting from diploid male production might be lower than in species where queens have to raise the first brood without the help of workers.

There are two possible explanations for the lack of a significant association between inbreeding and several of the traits measured. The first is that some traits are more influenced by inbreeding than others, as has indeed been demonstrated in several studies (e.g. Keller & Waller,

2002; Gerloff & Schmid-Hempel, 2005). Second, because the overall level of inbreeding was relatively low, the power of this study might not have been sufficient to detect a significant effect for all the traits measured.

Our results are also of interest regarding the standing problem of inferring correlations between inbreeding and fitness in the wild. There has been considerable controversy about whether the level of homozygosity at genetic markers truly reflects the level of true inbreeding (David, 1998; Balloux *et al*., 2004; Markert *et al*., 2004; Slate *et al*., 2004). There are several reasons why this is likely to be the case in our study. First, our study population is small and fragmented into local patches, leading to significant probability of sibmating (Sundström *et al*., 2003). Indeed, the overall inbreeding of queens and worker was significant. This, together with earlier findings on patterns of dispersal in the same population (Sundström *et al*., 2003), suggests that there is variation in the inbreeding level among individuals in different colonies. Second, the re-sampled homozygosity estimates obtained at two independent sets of five loci were positive correlated for workers (significantly so) and queens (not significantly so), suggesting that homozygosity indeed reflects true inbreeding (Balloux *et al*., 2004). There are two possible reasons why the correlation was significant for workers, but not queens. First, queen inbreeding (F_{is}) was lower than worker inbreeding necessarily reducing the effect size. Second, the test power is lower for the queen data as the variance in the estimate of homozygosity is higher for queens, because their estimate is based on a single individual per colony, whereas worker homozygosity was based on several individuals per colony.

Taken together, our study highlights the fact that inbreeding depression, at least as measured from the homozygosity at 10 highly variable loci, can affect fitness in social Hymenoptera. The effects may vary between species according to their social traits, as temporary social parasitism is likely to protect *F. exsecta* from effects of inbreeding that could be detrimental in other species. On the other hand, perennial ant colonies may show long-term effects that would not be seen in a species with annual colonies. In particular, it is notable that inbreeding depression can affect more than one generation at the same time in the same colony. Similar results have been obtained in oldfield mice (Margulis, 1998), highlighting the necessity of taking into account all the generations present in a social group when estimating the effects of inbreeding on lifetime fitness.

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