The relationship between multiple mating by queens, within-colony genetic variability and fitness in the ant *Lasius niger*

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Abstract

Multiple mating has been suggested to benefit social insect queens because high genetic variation within colonies might decrease the load imposed by sterile diploid males, enhance resistance to parasites and pathogens, and lead to a more effective division of labour and/or a wider range of tolerable environmental conditions. We tested these hypotheses in the ant *Lasius niger* with three population samples from Switzerland and Sweden. We found no diploid males in young or mature colonies suggesting a lack of diploid male load. Colonies with multiply-mated queens were not larger nor did they produce more sexuals than colonies with singly-mated queens. We did find a significantly lower frequency of multiple mating among newly mated queens than among the queens heading mature colonies in one population sample (Switzerland 1997). However, this result was not repeated in the other study population, or in the following year in the Swiss population.

Introduction

One of the more debated issues in evolutionary biology is why in many species females mate with multiple males. For females, the primary function of copulation is to fertilize their ova (Daly, 1978; Parker, 1979; Keller & Reeve, 1995; Jennions & Petrie, 2000; but see Arnquist & Nilsson, 2000). If one mating provides females with sufficient sperm, and the males provide little but gametes in mating, there are no obvious reasons why females should benefit from further matings. In social Hymenoptera (ants, bees and wasps), the number of matings per queen varies significantly within and between species (Boomsma & Ratnieks, 1996; Strassmann, 2001) and several hypotheses have been put forward to explain the adaptive significance of multiple mating (Crozier & Page, 1985; Keller & Reeve, 1994; Boomsma & Ratnieks, 1996; Schmid-Hempel, 1998; Crozier & Fjerdingstad, 2001). Four of these hypotheses propose that polyandry (mating with multiple males) is advantageous to queens because the resultant increase in genetic variation (GV) within colonies leads to an increased colony performance (Keller & Reeve, 1994).

The first of these four GV hypotheses holds that by increasing GV within colonies, polyandry allows a more complete expression of genetically based caste systems (Crozier & Page, 1985; Starr, 1985; Robinson & Page, 1989; Page & Robinson, 1991) which leads to a more efficient worker force (see also Bonabeau *et al.*, 1996). That workers from different matrilines and/or patrilines may specialize to some degree in specific tasks has been demonstrated in several bee, ant and wasp species (see Crozier & Fjerdingstad, 2001). However, it remains unclear whether higher genetic diversity translates into higher colony efficiency. The second hypothesis proposes that increased GV within colonies provides a means to expand the range of environmental conditions that a colony can tolerate (Crozier & Page, 1985). So far, no experiment has explicitly tested this hypothesis. The third hypothesis asserts that by increasing GV within colonies, polyandry reduces the risk that parasites or

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pathogens will diminish the worker force to the point of jeopardizing the colony's survival and reproduction (Hamilton, 1987; Sherman *et al.*, 1988, 1998; Schmid-Hempel, 1994, 1998). Conflicting results have been found for the effect of increased genetic diversity on the incidence of parasites and colony productivity with some studies showing a benefit of increased genetic diversity and others showing no such effect (Palmer & Oldroyd, 2000; Crozier & Fjerdingstad, 2001). Thus, it remains unclear whether increased genetic diversity does enhance parasite and pathogen resistance in wild colonies of social Hymenoptera (Kraus & Page, 1998; Sherman *et al.*, 1998).

The fourth hypothesis proposes that polyandry reduces the sex determination load. In Hymenoptera with complementary sex determination (Cook, 1993; Cook & Crozier, 1995), a young queen that mates with a male sharing one of her sex determination alleles (a 'matched' mating) will produce a diploid brood in which half of the offspring are diploid males instead of all being workers. Polyandry may be favoured when colony fitness is a decelerating function of the proportion of diploid males produced because multiple paternity reduces the variance in diploid male load (Page, 1980; Ratnieks, 1990; Pamilo *et al.*, 1994). Currently, there is virtually no information on whether colony fitness is a decelerating or accelerating function of the proportion of diploid males produced and whether diploid male production selects for polyandry (but see Crozier & Fjerdingstad, 2001).

Overall, the pace of theoretical studies on why females may benefit to mate multiply has by far exceeded that of empirical ones, making it difficult to evaluate whether the consequences of GV on colony performance is a selective factor affecting the mating behaviour of social insect queens. This is compounded by the fact that tests of the effect of GV have often provided inconsistent results (Crozier & Fjerdingstad, 2001).

In the present study we carry out a multifaceted study on the common European ant *Lasius niger* to test the predictions of all four GV hypotheses on colony performance. *Lasius niger* provides a good model system for such a study due to its life history. Mature colonies are large (up to over 50 000 workers, this study; Boomsma *et al.*, 1982) and long-lived (up to 29 years, H. Appel, in Kutter & Stumper, 1969), which should make them conspicuous to parasites and pathogens (Boomsma & Ratnieks, 1996). Also, workers exploit many and varied food sources (e.g. aphid secretions, small invertebrates, Collingwood, 1979; Seifert, 1996) and carry out a multitude of different tasks (e.g. aphid tending, nest construction, hunting, colony defense). Thus, all four GV factors may influence colony performance in this species. Previous studies also showed that colonies generally contain a single queen mated with one to four males (Van der Have *et al.*, 1988; Boomsma & Van der Have, 1998; Fjerdingstad *et al.*, 2002) hence providing a good system to test for an association between queen mating frequency and the survival, size and productivity of colonies.

To determine whether multiple mating increases colony survival, we compared the mating frequency of queens collected just after a mating flight and queens heading established colonies. Mating frequencies were determined by genetic analysis by microsatellite markers we specifically developed for this purpose. The relationship between queen mating frequency and colony size and productivity was tested by comparing worker number and the number of reproductive males and queens produced by mature field colonies headed by singly- and multiply-mated queens. Moreover, we screened these field colonies to determine the prevalence of adult diploid males and to examine the association between colony performance and diploid male load. Finally, we established incipient colonies in the laboratory from queens collected just after mating flights to investigate the production of diploid male brood to test whether the variance in colony size was lower in multiple-paternity colonies than in single-paternity colonies, as expected if there is a diploid male load (Crozier & Page, 1985; Pamilo *et al.*, 1994). The tests were replicated in two geographically separated populations, Lausanne (Switzerland) and Uppsala (Sweden) as well as in two separate years in Lausanne.

Material and methods

Field collections and laboratory rearings

Twenty-eight and 52 mature *L. niger* colonies were located on meadows in Lausanne (Biology Building, Lausanne University) and in Uppsala (at the Department of Genetics and the Biomedical Centre, Uppsala University), respectively, at the beginning of the reproductive season in 1997 (June–July in Lausanne, August–September in Uppsala). The total annual production of sexuals by these colonies was estimated through trapping (Lausanne) or excavation (Uppsala) as described in Fjerdingstad *et al.* (2002). Workers were also sampled from each colony. The sampling of sexuals and workers was repeated in Lausanne for another set of 34 colonies in 1998 as detailed in Fjerdingstad *et al.* (2002). The size of the worker force of mature colonies in Lausanne was estimated by mark-recapture studies on 21 colonies during July and August 1997. Nest mounds were disturbed thorougly to ensure unbiased sampling of all worker classes, ants were collected by a car vacuum cleaner, or shovel, from the disturbed mounds, placed in a bowl and marked with spray paint (Kiwi brands inc., Douglassville, PA, USA). The number of marked ants was counted and the ants were released onto the nest mound. Recaptures were made 2–3 days after marking and the unbiased colony size and standard deviation were estimated as in Boomsma (1982).

Young, newly mated queens were collected just after mating flights at four different sites near the University of Lausanne (Closelet, EPFL, Biology building, Renens) in July 1997, and at one site (Biology building) in July 1998, and on the campus of Uppsala Genetic Centre in August–September 1997. In both Uppsala and Lausanne the sites of collection were <2 km away from the site where mature colonies were collected. A subset of the queens collected in Uppsala was immediately frozen for later genetic analyses while the other queens (99 and 182 in Lausanne in 1997 and 1998, respectively, and 379 in Uppsala,) were placed individually in the laboratory in plastic nest boxes with water basins or in closed tubes at room temperature in darkness. Queens were supplied with water until the first workers eclosed after which colonies were fed honey water, pure water, and protein food (Keller *et al.*, 1989). Colonies were checked weekly until colony termination [i.e. four (Lausanne) or three (Uppsala) months after queen collection] to determine whether diploid males were produced or whether the incipient colonies produced only workers as they should if queens did not have a matched mating (Ross & Fletcher, 1986). At the end of the experiments we counted the number of workers per colony. In Lausanne the brood in each colony (workers, pupae, larvae, eggs) was also weighed.

Genetic analyses

Microsatellite markers were developed following the procedure of Ortius-Lehner *et al.* (2000). Of 5500 screened recombinant clones, 102 (1.85%) gave a positive hybridization signal and 11 of them were sequenced. In total 12 microsatellite sequences were identified. Primers were designed for four microsatellites. The remaining eight microsatellite loci were either too close to the cloning site or had a too short (<10) string of uninterrupted repeats.

The four loci had high levels of GV, the expected heterozygosity ranging from 0.89 to 0.91. Genepop exact tests (Raymond & Rousset, 1995) showed that all loci segregated independently, thus providing independent genetic information. No deviations from expected heterozygosity or Hardy–Weinberg proportions were detected. Finally, alleles of heterozygote queens showed Mendelian segregation among offspring in the colony samples. Primer sequences and details on the variation at the loci are given in Table 1. (See also Table 1 in Fjerdingstad *et al.*, 2002.)

Estimates of the genetic diversity for mature colonies were obtained through analysis of 20 (Lausanne) and 10 (Uppsala) female (workers or female sexuals) offspring per colony, using the microsatellite markers Ln10-53 and Ln10-282, as described in Fjerdingstad *et al.* (2002). The genetic data were used to reconstruct the mating frequency of colony queens which could be performed with high accuracy (Fjerdingstad *et al.*, 2002) because of the high variability of the markers. To test for the presence of diploid males in mature colonies we genotyped five males per colony in Lausanne and nine in Uppsala.

For the estimation of the mating frequency of newly mated queens, queen abdomens were dissected in a 0.9% NaCl-solution, a proteinase K-extraction buffer, or a phosphate-buffered saline/5% bovine serum albumin buffer (Reichard & Wheeler, 1995). Intact spermathecae were opened and the sperm balls were removed using insect pins and forceps. The sperm was then subjected to standard extraction using phenol–chloroform (Lausanne) or Chelex™ (Sigma-Aldrich, Stockholm, Sweden) (Uppsala) (Thorén *et al.*, 1995). DNA was also extracted from the flight muscles of 16 queens in Lausanne (1997) and all queens in Uppsala to verify that the spermathecal samples were not contaminated with maternal tissues. The sperm contents of spermathecae were amplified at two loci in Lausanne (Ln10-53, Ln10-282) and at four loci in Uppsala (Ln1-5, Ln10-53, Ln10-174, Ln10-282). The PCR conditions were as in Fjerdingstad *et al.* (2002). The loci were run on denaturing 6% polyacrylamide gels with running times 4000–5000 Vh for Ln10-53, 6000–8000 Vh for Ln10-282, 7000 Vh for Ln1-5 and 6000 Vh for Ln10-174.

The number of mates per young queen was estimated by the maximum number of allelic bands found in the

Table 1 Characteristics of the four microsatellite loci developed for *Lasius niger*.

Locus	Repeat motive	Primers (5′–3′)	Amplified fragment from the clone (bp)	T_a (°C)	*N* alleles	H_{exp}	H_{obs}
L1-5	$(CT)_4(TC)_{19}$	F: TTGTTATCAGAAAGGTATCCGC R: AAAGGCATAAACCAAAATTGC	272	51	15	0.89	0.82
L10-53	$(GT)_{27}$	F: ATTCTCCGCTGTTTGCCG R: CGGGCACAAAGAAAATTGG	138	55	26	0.91	0.94
L10-174	$(AC)_{24}(AT)_9$	F: CACGCTCGCGTTTACATAAC R: GAAAATCTTTGCCAATTCACG	234	53	20	0.91	0.91
L10-282	$(TC)_{15}TT(TC)_9$	F: TAAATCGGGAAAGTTCTCGC R: AAGAGAGTCCGTCAACACCG	265	53	22	0.89	0.85

T_a values are from OLIGO 4.1-program. Data on genetic variability are from 40 newly-mated queens collected in Uppsala in 1996.
The cloned sequences can be found in GenBank under accession numbers AF327042-AF327045.

spermathecal-PCR at any of the two (Lausanne) or four (Uppsala) loci (males are haploid in ants). The probability of detecting a double mating is equal to the probability that two males have different alleles at least at one marker locus, which equals one minus the multilocus homozygosity $1 - \Pi(1 - H_{exp})$, under the assumption of independent segregation of marker loci. The probability of detecting triple matings is lower because there is a higher probability that at least two males share an allele. The probability of not detecting one of the males is higher for studies based on spermatheca-PCR analyses than for offspring genotyping because in spermatheca-PCR, all males must have different alleles from each other at at least one locus to be detected. In contrast, when mate number is deduced from offspring genotypes it is possible to arrange the paternal alleles into haplotypes. Therefore, even if triple mating cannot be detected at any one locus, the multilocus genotypes may reveal three distinct types (e.g. A_A, A_B, B_B). Detection probabilities for triple mating were computed by DETECT, a program developed by P. Pamilo (unpublished) which computes from the allele frequencies in the marker loci the probabilities of sampling three distinct multilocus haplotypes (offspring analysis), or sampling at least at one locus three distinct single locus haplotypes (spermatheca analysis). The assumptions of the algorithm are random mating and random segregation of marker loci.

Detection probabilities for double mating in our material ranged from 0.99 to 0.9999, and for triple mating from 0.96 to 0.99. Thus, the variability of the marker loci was sufficient to get a good estimate of the number of mates per queen.

Analytical methods

Intracolonial regression relatedness was calculated by the program RELATEDNESS 5.05 (Queller & Goodnight, 1989; Goodnight, 1999) from genotypic data on workers and female brood. Statistical tests of fitness differences between singly- and multiply-mated queens were computed by software JMP3.1 (SAS Institute Inc., 1995) and SPSS 8.0 (SPSS Inc., 1997). We performed retrospective power analyses for nonsignificant tests using the program G*POWER (Erdfelder *et al.*, 1996) and then combined the separate estimates for each population sample into overall estimates for detecting among-paternity class differences in the fitness variables, productivity, brood mass, output of sexuals, and survival. For each fitness variable the overall power estimates were calculated as:

$$P_{\text{combined},i} = (1 - [(1 - P_{1,i}) \times (1 - P_{2,i}) \times (1 - P_{3,i})])$$

where $P_{1,i}$ represents the power of test for the first population sample with respect to an effect size of i% (of multiple-paternity colonies having a i% higher value for the fitness correlate or component considered), $P_{2,i}$ the power for the second population sample etc., and $(1 - P_{x,i})$ the probability of not detecting a difference of i% in population sample x. All the tests presented are two-tailed.

Results

Colony survival

The frequency of multiple mating in newly mated queens was 17% in Lausanne 1997 (17 of 100 queens), 65% in Lausanne 1998 (91 of 140), and 10.8% in Uppsala (four of 37) (Table 2). There were no significant differences in mating frequency between the four samples that constituted the Lausanne 1997 queens (Kruskal–Wallis test, Hadj = 1.933, d.f. = 3, $P > 0.40$), but the percentage of multiply-mated queens in Lausanne was significantly higher in 1998 than 1997 (Fisher's exact test $P < 0.0001$). Mating frequencies above two were detected only in Lausanne 1998 (three triple matings and one quadruple mating).

The fact that some queens mated with several males significantly affected colony genetic diversity (here quantified as $1 - r$ where '1' is the maximal possible relatedness, and r the observed relatedness between nestmate females). Colonies headed by multiply-mated queens had a 52, 100, and 113% higher genetic diversity than single-paternity colonies in Lausanne 1998, 1997, and Uppsala, respectively (values calculated from the data in Fjerdingstad *et al.* 2002).

All the colonies sampled in Lausanne ($n = 61$) produced sexuals. The percentage of multiple-paternity colonies was 60.7%, a value significantly higher than the percentage of multiply-mated queens collected after the mating flight in 1997 (17.0%) (Table 2) but not significantly different from the proportion of queens that were multiply mated in the sample collected after the mating flight in 1998 (65%; Table 2). In Uppsala only 33 of the 52 field colonies produced sexuals. Because these colonies were presumably larger and older than those not producing sexuals, we conducted two survival

Table 2 Frequency of multiple mating of newly-mated *Lasius niger* queens and queens heading established field colonies.

	Newly mated queens	Colony queens	*P*
Lausanne 1997	0.17 (100)	0.607 (61)*	<0.0001
Lausanne 1998	0.65 (140)		0.6327
Uppsala all	0.108 (37)	0.135 (52)	0.7570
Sexual producing colonies		0.182 (33)	0.499

*Represents the combined data for mature colonies collected in 1997 and 1998 in Lausanne Fjerdingstad *et al.* (2002), excluding colony C23 for which colony pedigree could not be resolved.
Given are the observed frequencies, and, in parentheses, the total sample sizes for Lausanne and Uppsala.
P values are from Fisher's exact test.

comparisons on the Uppsala data. First, we compared the mating frequency of queens collected just after the mating flight with that of queens heading colonies producing sexuals. Next, we did the same analysis but included all field colonies. Both analyses provided the same result, with no significant difference in the frequency of multiple mating between queens collected just after a mating flight and queens from established colonies (Table 2).

Colony size and productivity

On average 501 ± 235 (mean ± SD) workers were marked per colony, and 865 ± 566 were recaptured. We found an average of 20 ± 33 marked ants among the recaptured workers. Three colonies were excluded from further analysis because, in each, less than seven marked workers were recaptured, and thus unbiased colony size estimates were not possible (Boomsma, 1982). The average estimated colony size in the remaining 17 colonies was 24 247. There was considerable variation in colony size [range 3908–55 266, coefficient of variation (CV) 69%] but the average size of colonies with singly-mated queens (20 893) was not significantly different from that of colonies with multiply-mated queens (17 380; $t_{15} = -0.31$, $P = 0.75$). Similarly, there was no significant association between colony size and intracolonial relatedness among diploid brood, (workers or young female sexuals) (linear regression of log-tranformed values, $r = -0.18$, $t_{15} = -0.72$, $P = 0.48$). Variances in colony size did not differ significantly between paternity classes either (Levene's test, $F_{1,15} = 0.118$, $P = 0.74$).

There was great variation across colonies in the number of sexuals produced (CV = 89, 105, and 118% for Lausanne 1997, 1998, and Uppsala, respectively). The production of sexuals did not differ significantly between the two Lausanne samples (*t*-test on Box–Cox transformed values, $t_{60} = 1.3$, $P = 0.22$), nor between Uppsala and the pooled Lausanne data set (Wilcoxon test, $W = 1519$, $Z = -0.58$, $P = 0.56$; Table 3). There was also no difference in productivity between colonies with a singly- or multiply-mated queen, contrary to the prediction that increased genetic diversity translates into higher colony productivity (Table 3). Similarly, there was no significant relationship between productivity and intracolonial relatedness among females (Lausanne 1997: $\beta_{std} = 0.15$, $t_{27} = 0.78$, $P = 0.44$; Lausanne 1998: $\beta_{std} = 0.06$, $t_{32} = 0.33$, $P = 0.74$; Uppsala: $\beta_{std} = 0.11$, $t_{31} = 0.63$, $P = 0.53$). Variances of sexual production did not differ significantly between colonies headed by singly- and multiply-mated queens either (Table 3; Levene's test for homogeneity of variances: Lausanne 1997, $F_{25} = 0.10$, $P = 0.76$; Lausanne 1998, $F_{25} = 0.70$, $P = 0.68$; Uppsala, $F_{31} = 0.06$, $P = 0.82$).

Diploid male production

None of the 646 males collected from the 101 mature field colonies (59 in Lausanne and 42 colonies in Uppsala, on average five males per colony genotyped in Lausanne, nine in Uppsala) were heterozygous at any of the two loci. Because the expected heterozygosities of the microsatellite markers used were very high, the probability of diploid males remaining undetected was negligible ($P < 0.001$) indicating that mature diploid males are absent or very rare in *L. niger*. The complete lack of diploid males in mature colonies prevented us from testing for the impact of diploid male load on colony size and productivity.

Of the 660 newly-mated queens (99 in Lausanne 1997, 182 in Lausanne 1998, and 379 in Uppsala) that were placed in nest boxes, all except 10 Lausanne queens produced workers. The average number of workers ± SD recorded per colony was 22 ± 5 in Lausanne 1997, 24 ± 11 in Lausanne 1998, and 16 ± 6 in Uppsala. Not a single male was found in the brood of these 660 colonies. The fact that none of the 660 newly-mated queens produced diploid males suggests that none had a matched mating.

Brood production in colonies showed substantial variation (CV of worker number = 26% in 1997 and 42% in 1998, in Lausanne) but colonies headed by a singly-

Table 3 Mean ± SD sexual production of colonies with singly- and multiply-mated queens in Lausanne and Uppsala.

	Lausanne		
Colony class	1997	1998	Uppsala
All	7909 ± 7078 (28)*	5250 ± 5514 (34)	6121 ± 7220 (33)
Single paternity	10 884 ± 8421 (11)	5993 ± 6357 (13)	6601 ± 7687 (27)
Multiple paternity	5786 ± 5317 (16)	4791 ± 5034 (21)	3961 ± 4419 (6)
t	−1.9	−0.6	−0.8
P	0.07	0.43	0.55

*N_{All} for Lausanne 1997 includes the one colony (C23) for which pedigree was not resolved (Fjerdingstad *et al.*, 2002) but C23 is not included in paternity class comparisons.
Investment was estimated as total dry weight (mg) of sexuals sampled, with a correction for higher metabolic rates of males (Boomsma & Isaaks, 1985). Number of colonies analysed is given in parentheses.

Table 4 Brood mass and worker number in laboratory colonies with singly- and multiply-mated queens.

	Number of colonies singly-/multiply-mated	Mean singly/ multiply mated	t^*	P	Variances singly/ multiply mated	F_{Levene}†	P
Final brood mass (mg)							
Lausanne 1997	59/15	21.0/23.8	1.3	0.19	50.8/42.9	0.02	0.90
Lausanne 1998	44/84	21.8/22.4	0.39	0.70	68.9/100.2	0.005	0.95
Final worker number							
Lausanne 1997	82/17	21.8/23.3	0.97	0.33	37.2/18.2	0.52	0.47
Lausanne 1998	44/84	25.2/24.8	−0.23	0.82	96.5/119.5	0.003	0.95

*From linear regression analysis.
†Levene's test for homogeneity of error variances, based on medians.

Table 5 Overall power of our analyses to detect a significant advantage of multiple paternity colonies (maximal power is 100%).

	Power for different types of advantage of multiple paternity colonies (advantage in % : power in %)
Brood mass	20% : 98% 15% : 87% 10% : 69%
Worker number	20% : 99% 15% : 91% 10% : 67%
Sexual biomass	20% : 52% 15% : 41% 10% : 30%
Survival	20% : 59%
	Power for correlations (r) between nestmate relatedness and colony productivity (r : power in %)
Sexual biomass	−0.30 : 90% −0.25 : 80%

mated queen did not have a significantly lower productivity than did colonies with a multiply-mated queen (Table 4). Similarly the variance of productivity did not differ significantly between the two types of colonies (Table 4).

Power of the statistical tests

The large sample-sizes gave high overall power to detect a 20 or 15% difference in colony size (worker number of brood mass) between single- and multiple-paternity colonies (Table 5) (overall-power is based on combining the information from separate analyses on Lausanne 1997, 1998, and Uppsala data, see Materials and methods). Even a 10% difference would have been detected with a high probability (Table 5). The overall-power to detect a greater production of sexuals in multiple-paternity colonies was considerably lower because of the large variation across colonies in sexual productivity (Table 5). As for survival, a 20% survival advantage for multiple-paternity colonies would have been detected with medium power (Table 5). Power estimates for lower survival advantages were not possible because of the type of statistical test used (queens being not divisible). Finally, the power to detect correlations of $r = -0.30$ and -0.25 between intracolonial relatedness and sexual biomass was very high (Table 5).

Discussion

This study failed to demonstrate any beneficial effect of multiple mating on colony productivity in *L. niger*. Field colonies headed by multiply-mated queens were neither larger nor had a greater production of sexuals than colonies headed by singly-mated queens. Similarly, the experiments conducted in the laboratory showed no significant difference in productivity between incipient colonies headed by singly- and multiply-mated queens. Our failure to detect a greater productivity of colonies headed by multiply-mated queens is unlikely to merely result from the lack of power of the statistical analyses. Data were collected from a total of over 700 colonies and several of our tests were replicated, leading to a high power for most tests. Moreover, although the association was not significant, there was a tendency for colonies headed by singly-mated queens to have greater, not lower productivity.

The relationship between genetic diversity and colony productivity has been investigated in four other taxa of social insects. In the ant *Formica truncorum,* colony productivity was also found not to be significantly associated with the mating frequency of queens (Sundström & Ratnieks, 1998). By contrast, a positive correlation between genetic diversity and both growth rate and colony survival has been reported in the ant *Pogonomyrmex occidentalis* (Cole & Wiernasz, 1999; but see Cole & Wiernasz, 2000; Fjerdingstad & Keller, 2000). In this species the correlation between intracolonial relatedness and components of fitness was estimated to be high ($r = -0.50$, Cole & Wiernasz, 1999). There would have been a high probability to detect such a marked effect of genetic diversity on fitness in most of our analyses,

suggesting a genuine difference between *L. niger* and *P. occidentalis*.

The third species where the relationship between genetic diversity and colony productivity has been investigated is the honeybee *Apis mellifera*. In this species there have been inconsistent results with some studies showing a positive association between genetic diversity and colony fitness (Oldroyd *et al.*, 1992; Fuchs & Schade, 1994), whereas others found no significant association (Page *et al.*, 1995; Fuchs *et al.*, 1996; Kraus & Page, 1998; Neumann & Moritz, 2000). Interestingly, the studies that reported a significant effect of genetic diversity generally reported a strong association between GV and colony fitness (7.5–15 to 223% higher productivity of colonies headed by multiply mated queens, Fuchs & Schade, 1994). Again such a marked effect of genetic diversity on colony performance would have been unlikely to remain undetected with our sample size and the power of our analyses.

Finally, the fourth species where the effect of GV on colony fitness has been studied is the bumble-bee *Bombus terrestris* (Baer & Schmid-Hempel, 1999, 2001). In this species, colonies headed by queens artificially inseminated with the sperm of several males have higher productivity than colonies with singly-mated queens apparently because of lower parasite load in genetically heterogeneous colonies. As with other studies reporting a positive association between genetic diversity and colony performance, the association was strong (100% increase in colony productivity by colonies headed by multiply-mated queens, Baer & Schmid-Hempel, 1999) and within the range of effect that our study had high power to detect.

Altogether these data suggest genuine differences across species in the effect of genetic diversity on colony performance. In that respect it is interesting to note that several of the genera where significant effect of genetic diversity have been reported have peculiar features. Queens of *P. occidentalis* mate on average 6.8 times (Cole & Wiernasz, 2000), a value much higher than the average mating frequency of ants (Boomsma & Ratnieks, 1996; Crozier & Fjerdingstad, 2001; Strassmann, 2001). Importantly, some species of *Pogonomyrmex* have an unusual system of caste determination where queens mate with two types of males. Offspring fathered by queens and males of the same lineage develop into queens, whereas offspring fathered by males and queens from different lineages develop into workers (Helms Cahan *et al.*, 2002; Julian *et al.*, 2002; Volny & Gordon, 2002; Helms Cahan & Keller, in press). This peculiar system of caste determination selects for high mating frequency because only queens mated to both homo- and hetero-specific males succeed in initiating a new colony and producing female sexuals. It is unknown whether such a system may occur in *P. occidentalis* too. As for honey bees, the reported results are difficult to interpret because the experiments have been conducted on commercial strains whose amounts of GV may be far from natural levels. Finally, an interesting feature of *B. terrestris*, the third species where a positive effect of genetic diversity on colony performance has been reported, is that queens invariably mate once. Apparently, males deposit a mating plug after mating thus preventing females from re-mating (Duvoisin *et al.*, 1999). However, it is unclear why the mating plug is so efficient in this species given that they are usually only partly efficient in other species.

Another important finding of our study with regard to the potential benefits of multiple-mating on colony fitness is that the frequency of multiple mating by queens collected just after the mating flight in 1997 in Lausanne was significantly lower than among queens heading established colonies in the same population. This finding is consistent with a survival advantage for multiple-paternity colonies. However, this result should be interpreted with caution because it was not repeated in 1998 in Lausanne nor in the Uppsala population. Moreover, the finding of a strong between-year difference in mating frequency in Lausanne shows that the potential benefit of multiple mating on colony survival cannot simply be tested by comparing the frequency of polyandry in queens collected during a mating flight and in queens heading mature colonies. Such comparisons may lead to wrongly concluding that there is an effect of multiple mating on colony survival, when it may, in fact, only reflect temporal variation in the proportion of queens mating multiply during mating flights.

Factors influencing the proportion of queens that mate multiply during mating flights may include differences in operational sex ratio, with possibly higher mating frequency in mating swarms that are more male-biased. Alternatively, differences in temperature and weather conditions may influence the duration of the mating flight and the opportunity for multiple mating. The only other study which has so far compared mating frequency of queens between mating flights found that the mating frequency of *L. niger* queens from six populations was locally fairly constant (Boomsma & Van der Have, 1998).

Two previous studies have investigated colony survival as a function of intracolonial genetic heterogeneity. In *Atta colombica* there was no significant difference in queen mating frequency among incipient and mature colonies (Fjerdingstad *et al.*, 1998), whereas in *P. occidentalis* genetic diversity correlated positively with growth rate, which in turn correlated with survival (Cole & Wiernasz, 1999). However, the latter study did not demonstrate that increased genetic diversity resulted only from increased mating frequency of queens and not the presence of several queens in the same nest (Fjerdingstad & Keller, 2000, but see Cole & Wiernasz, 2000).

Another interesting finding of our study was that no diploid males were detected. Not a single of the 660 queens that successfully produced offspring in the laboratory in Sweden and Switzerland produced any

males. Moreover, all the males collected from established colonies had genotypes consistent with these males being haploid. This is in agreement with results of other studies where males of *L. niger* have been genotyped (Van der Have *et al*., 1988; J.J. Boomsma, pers. comm.). There are two general possible explanations for finding no diploid males. First, *L. niger* may have a sex determination mechanism that leads to no or very few diploid males. This may occur if there is a very large number of alleles at the sex locus, or if sex determination is based on multiple loci such that homozygosity of all of them is required for a diploid individual to develop into a male (Cook, 1993). Alternatively, *L. niger* may have a system of sex determination not based on sex alleles as probably is the case in some ants such as those which commonly inbreed (Buschinger, 1989; Keller & Passera, 1993).

The other possible explanation for the apparent lack of production of diploid males is that these males are not viable and/or are eliminated by workers or queens early in their development. Workers have been shown to destroy young diploid male larvae in *A. mellifera* (Woyke, 1963) and *A. cerana* (Woyke, 1980) but not in *Melipona* bees, (Camargo, 1982; Kerr, 1987) or in the ant *Solenopsis invicta* (Ross & Fletcher, 1986). In some *Formica* ants workers remove diploid males at least during periods where no sexuals are normally produced (Pamilo *et al*., 1994). Thus, the absence of diploid males in our colonies could be accounted for if these males are selectively eliminated from colonies during the egg or young larval stages at which time they cannot be visually distinguished from female brood by a human observer. This hypothesis, however, is inconsistent with our finding that colonies with singly-mated queens did not have a higher variance in productivity than did colonies headed by multiply-mated queens. A greater variance in productivity among colonies headed by singly-mated queens would be expected if male destruction did occur, because in such colonies, queens either produce 50% diploid males among their diploid offspring or no diploid males at all, whereas in colonies with a multiply-mated queen a high proportion of the colonies should produce some diploid males but these males would constitute a lower proportion of the diploid brood (0–25% in double-paternity colonies with one matched mating, depending on the relative sperm contribution of each male). Regardless of the ultimate reason for the lack of diploid males, the fact that they are not produced in detectable numbers suggests that a diploid male load is selectively unimportant or absent.

In conclusion, this study showed that the productivity of *L. niger* colonies is probably not influenced (or at best only weakly influenced) by the level of intracolonial genetic diversity. Clear positive effects of genetic diversity on colony productivity have now been reported in two species (*P. occidentalis* and *B. terrestris*), whereas no effect has been reported in two other species (*Formica truncorum* and *L. niger*). Both positive effects and lack of effects have been reported in *A. mellifera*, the fifth species where the association between genetic diversity on colony performance has been studied. Understanding whether genetic diversity affects colony performance is an important question and other studies like the one reported in this paper are warranted to determine why genetic diversity apparently affects colony performance differently across species. Reasons may include ecological differences between species (e.g. the types of parasites to which they are exposed and the variability of the environments that they have to deal with), affecting whether increased intracolonial genetic diversity provides advantages, has no effect, or even damages colony fitness (for instance decision-making may be more difficult in multiple-paternity colonies under some circumstances if patrilines have different behavioural thresholds, Page & Mitchell, 1998; and intracolonial diversity could make colonies more susceptible to diseases, the 'Bad apple' effect, Boomsma & Ratnieks, 1996). In any case, a better understanding of whether increased genetic diversity may provide benefits at the colony level can only be obtained if publication bias, with studies reporting a significant effect being more likely to be published than studies reporting no significant effect, is avoided.

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