

Mating strategies of queens in *Lasius niger* ants—**is environment type important?**

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Abstract High relatedness among society members is believed important for the evolution of highly cooperative behaviours, yet queens of many social insects mate with multiple males which reduces nestmate relatedness and imposes also direct costs on queens. While theoretical models have suggested explanations for this puzzling queen behaviour, empirical studies fail to provide consistent answers especially for species with moderate levels of multiple mating. This may result from multiple mating only conferring benefits in some environments, as suggested by recent genetic variance theory and considerations on types of traits, direct costs and benefits. All concur in an expectation of higher levels of multiple mating in more complex or milder environments, and we perform a first, broad test of this idea by comparing mating strategies of queens in *Lasius niger* ants from northern (harsh, cold stressed) and southern populations (milder, greater bio-complexity). First, we collected new genetic data from Ireland and Southern France and then compared these to data on Swiss and Swedish populations. Queens from northern populations were near exclusively single mated and even at times inbred (in Ireland), whereas southern queens showed high levels of multiple mating, leading to more genetically diverse colonies in the south. Equally, paternity skew was greater in the north, as expected if northern queens only remate when their first mate transfers few sperm. Our findings are consistent with the idea that environment type may affect mating strategies in social insects and calls for an exploration of such effects.

Keywords Adaptation · Environment · Polyandry · Social insect · Sociogenetics

Social insects, such as ants, many bees and wasps, are characterized by cooperative foraging, nest defense and maintenance, and a division of labour between queens (which mate and lay eggs) and their workers (helper daughters that rear the brood). High relatedness in colonies is believed important for the evolution and maintenance of such a sophisticated sociality (Crozier and Pamilo 1996), yet in approximately 25% of all species queens have multiple mates (Page 1986; Keller and Reeve 1994; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996; Crozier and Fjerdingstad 2001; Kronauer et al. 2007; Hughes et al. 2008), which reduces relatedness in colonies and so the inclusive fitness of workers (Hamilton 1964). A recent comparative study found support that multiple mating may be able to evolve because workers are stuck in a helping role in some species, being unable to mate and store sperm (Hughes et al. 2008). This, however, overlooks the fact that a low fitness pay-off from helping may impose selection on female larvae to selfishly develop into gynes (young queens) rather than workers (imposing costs on the colony; cf. Wenseleers et al. 2003; see also Aron et al. 1999). Multiple mating can also be costly for queens, directly, via increased risk of disease or predation, increased energy expenditure, and is known to decrease immune responses (Baer et al. 2006). It is therefore puzzling that multiple mating is frequent and phylogenetically widespread (Hughes et al. 2008; see also Crozier and Page 1985; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Boomsma et al. 2009).

To understand the evolution of multiple mating by social insect queens considerable theoretical work has been done (reviews by Crozier and Page 1985; Boomsma and

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Ratnieks 1996; Crozier and Fjerdingstad 2001; see also Rüppest et al. 2008; Gove et al. 2009), but a long series of empirical studies has found varying levels of support for these models. For example, consistent support has not been found for the suggestion that queens mate multiply in order to attain sex ratios more favourable for them, in the context of worker–queen conflict (Ratnieks and Boomsma 1995; Sundström and Ratnieks 1998; Fjerdingstad et al. 2002) or for the sperm limitation hypothesis that multiple mating increases the lifetime fecundity of queens (Fjerdingstad et al. 1998; Fjerdingstad 2004; Kraus et al. 2004). Equally, variable support has been obtained for the most favoured set of hypotheses, those centered on potential benefits for colonies (and so queens) of having a genetically more diverse worker force. Worker caste diversity, colony growth and survival increases with genetic diversity from multiple mating in army ants and harvester ants, respectively (Jaffe et al. 2007; Cole and Wiernasz 1999, Wiernasz et al. 2008; see also Hughes et al. 2003; Jones et al. 2004; Fjerdingstad and Crozier 2006), and highly diverse colonies of leafcutter ants and honeybees (in some studies) show improved resistance to parasites (Tarpay 2003; Hughes and Boomsma 2004, 2005; Seeley and Tarpay 2007; Mattila and Seeley 2007) fitting theoretical expectations (Crozier and Page 1985; Sherman et al. 1988; Boomsma and Ratnieks 1996; Schmid-Hempel 1998; Crozier and Fjerdingstad 2001; see also Rüppest et al. 2008, Gove et al. 2009); however, studies on other social insects have failed to find support that increased genetic diversity due to multiple mating has clear benefits for colony and queen fitness (Fjerdingstad et al. 1998, 2003; Pedersen and Boomsma 1999; Fjerdingstad 2004, Trontii et al. 2007, Percy et al. 2009).

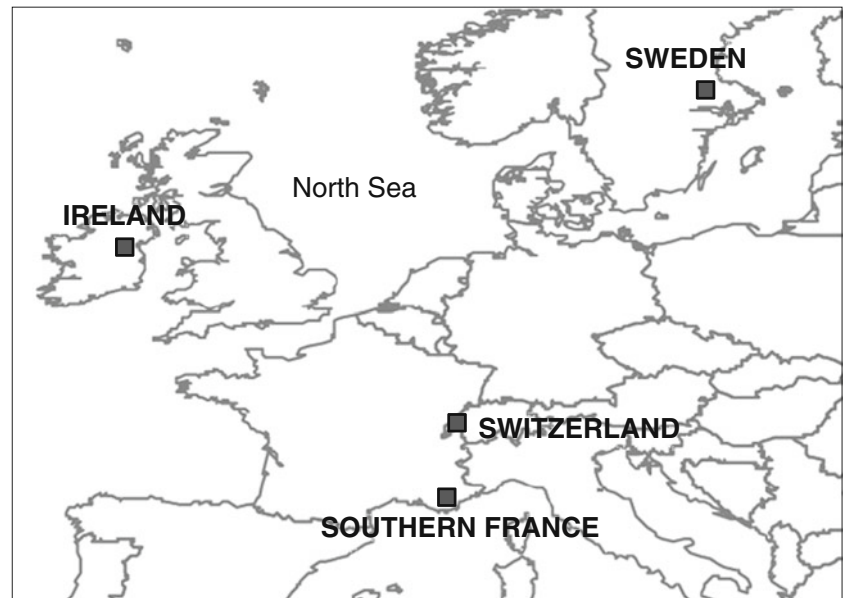
Advances in our understanding of queen mating strategies may be possible by considering the idea that selection on queen strategies could covary with the local environment. Indeed, a recent genetic variance model predicted that milder versus harsher environments would select differentially on queen strategies (Rüppest et al. 2008; see also Pamilo 1993). Rüppest et al.'s (2008) model assumes that in harsh environments only exceptionally good colonies survive, which imposes directional selection favouring single mating, whereas colonies close to average performance may do well enough to survive in milder habitats (Fig. 1 in Rüppest et al. 2008). This would confer an advantage on multiple-mated queens in milder habitats, as multiple paternity reduces variance in colony performance when genetic variation for household tasks exist (Rüppest et al. 2008). Another new simulation paper demonstrated that the number of tasks that a colony must perform affects the selective value of having multiple patriline in colonies (Gove et al. 2009). Notably, even moderate numbers of mates were found to increase colony fitness considerably under certain combinations of task numbers, environmental variability and behavioural

thresholds (Gove et al. 2009). Combined, these theoretical results suggest that multiple mating may bring queens and their colonies greater benefits in more benign *or* complex environments while single mating should be favoured in harsher *and* simpler environments.

One variable likely to greatly affect the harshness of the environment for social insects such as ants is summer temperature because it affects brood development and can dramatically delay colony growth (e.g. *Lasius niger* ants, Kipyatkov et al. 2004). It has indeed been shown, that the rate of brood development as a function of temperature shows latitudinal variation across Russia in a manner consistent with local adaptation to maximize usage of warm summer temperatures (Kipyatkov et al. 2004). Colder habitats in the north of Europe may also constitute comparatively simple environments for social insects. This is because Southern Europe generally enjoys a greater biodiversity as regards both flora and fauna than do northern regions (Gaston 2000), and colonies of social insects in southern environments may therefore need to deal with greater numbers of tasks (foraging on diverse resources, dealing with more competitor species, potentially more predator types and parasites, see e.g. Boomsma et al. 1982 on *Lasius* ants). This suggests the broad hypothesis that multiple mating may bring more benefits for queens and colonies of social insects in Southern European climates (milder and greater biotic diversity) than under northern skies (see also Rüppest et al. 2008). As a first step towards testing whether queen strategies covary with habitat characteristics, we can determine whether the number of mates per queen are greater in Southern Europe than in the north and if this leads to greater within-colony genetic diversity in the south. If support is found in such a broad test, specific factors responsible for among-habitat variation in queen mating behaviour can be pinpointed later via targeted analyses of specific environmental factors. Studies such as this have never been performed, in fact only one study ever tested for variation in the numbers of mates per queen among multiple populations, the one by Boomsma and van der Have (1998) on *L. niger* meadow ants in England, Holland and Denmark. Though a seminal study, the latter did not test whether observed differences in queen strategies covaried with habitat types.

We here use *L. niger* ants to test the hypothesis that queen mating strategies may covary with broad habitat type in social insects. These ants are well suited test organisms, not only because their mating strategies vary between regions (Boomsma and van der Have 1998), but because they occupy habitats varying greatly in the degree of climatic harshness, temperature and general biotic diversity (Gaston 2000) (from Southern Fennoscandia, Ireland to Northern Spain/Southern France and over to Eastern Russia; Seifert 1992). First, we collected new data on

Fig. 1 Locations of the Irish (Dublin) and Southern French (Cannes) populations sampled in summer 2007, along with the Swedish (Uppsala) and Swiss (Lausanne) populations studied earlier by Fjerdingstad et al. (2002)



queen mating strategies via microsatellite DNA analyses on worker offspring from two *L. niger* populations representing northern (Dublin, Ireland) and southern (Cannes, France) European habitats. Then we combined these with previous findings from another pair of north–south populations (Uppsala, Sweden and Lausanne, Switzerland, respectively; Fjerdingstad et al. 2002; 2003) to test whether southern populations as expected (Rüppell et al. 2008 and above) exhibit higher levels of multiple mating and if this translates into greater genetic diversity in colonies.

Materials and methods

Sample collection and genetic analyses

In summer 2007, one of us (E.J.F.) collected samples of workers from mature colonies of *L. niger* in populations in Dublin, Ireland (17 colonies) and Cannes, France (15 colonies) (Fig. 1). All samples were stored in RNAlater solution (Ambion Inc.) and shipped back to the US for storage at -25°C until genomic DNA was extracted with the DNeasy Blood and Tissue kit (Qiagen Inc.) and their insect protocol. Using four dinucleotide repeat microsatellite markers, L10-53, L10-282, L10-174 and L1-5 previously shown not to be linked (Fjerdingstad et al. 2002, 2003), we genotyped up to 20 workers per colony (Table 1). Primers were fluorescently labelled with 6-FAM, PET or VIC (Eurofins MWG Operon) or NED (Applied Biosystems Inc.). L10-53 and L10-282 (6-FAM and NED-labelled, respectively) were amplified together, as were L10-174 and L1-5 (PET and VIC, respectively). Reaction mixes for markers L10-282 and L10-53 consisted in 1x reaction buffer containing 1.5 mM MgCl_2 ,

300 μM dNTP, 0.4 μM and 0.8 μM primer, respectively, 0.04 U of Top Taq (Qiagen Inc.) per microlitre and double distilled water to a total reaction volume of 20 μl . Mixes for markers L10-174 and L1-5 were the same with 0.4 μM and 0.8 μM primer, respectively. PCR programmes comprised a 2-min initial denaturation at 94°C , followed by 30 cycles of 30 s of denaturation at 94°C , 40 s of annealing at 54°C and 30 s of elongation at 72°C , ending with a 20-min elongation at 72°C . The same programme was used for all loci.

After PCR, the two products per individual ant were mixed by transferring aliquots of 5 μl from each to a new tube/well and mixing with 45 μl of double distilled water. These diluted PCR products (four loci per ant) were then sent to the Nevada Genomics Center for fragment analysis on an ABI3730 DNA Analyzer, allowing separation and sizing of alleles. Based on resulting peak diagrams, alleles were designated using Genemarker 1.70 (SoftGenetics Corp.).

Pedigree analyses

All four microsatellite loci were highly polymorphic in both populations, with 14 to 19 alleles per locus and heterozygosities above 0.74 in all cases in the full data set. Using Microchecker 2.2.3 (van Osterhout et al. 2004) (on a dataset consisting in one worker per colony) we found no evidence that our loci had null alleles in either population (employing 95% confidence intervals for our tests), supporting that all genotypes were correctly readable. The high allelic diversity meant a very low probability of two unrelated males carrying the same alleles at all loci (patriline nondetection error; Pamilo 1993) in both populations (Ireland, 0.0009; Southern France, 0.001; based on allele frequencies obtained using Relatedness 4.2; Queller and Goodnight 1989). Our markers

Table 1 Variation in the number of mates and effective paternity between Irish and Southern French *L. niger* populations, estimated via offspring analysis using four microsatellite loci

Population	N_{colonies}	n_{workers} (mean \pm SD)	N mates/queen	Effective paternity		
				me_{ped}	$me_{\text{ped}, N}^a$	me_R^b
Ireland	17	17.9 \pm 3.2	1.18	1.08	1.08	1.00
Southern France	15	16.1 \pm 4.8	2.00	1.74	1.91	2.01
Sweden	52	10.0 \pm 3.0	1.11	1.09 ^c	1.11 ^c	1.00 ^c
Switzerland	61	19.4 \pm 2.1	1.73	1.40 ^c	1.44 ^c	1.63 ^c

For comparison, data are shown for populations in Sweden (Uppsala) and Switzerland (Lausanne) studied previously (Fjerdingstad et al. 2002)

^a Corrected using Nielsen et al.'s (2003) sample size unbiased method

^b me_R is 1 (and not lower) for Ireland (Dublin) despite regression relatedness of nestmate $r_R=0.77$ because effective paternity cannot be less than 1 (single mating) (Laidlaw and Page 1984)

^c Recalculated as arithmetic means from Fjerdingstad et al. (2002), $me_{\text{ped}, N}$ determined using Nielsen et al.'s (2003) sample size bias correction; note me_{ped} and $me_{\text{ped}, N}$ for Switzerland are based on only 59 colonies as patriline designation was not possible with certainty for two colonies (two different possible scenarios, but both giving the same number of fathers)

therefore allowed a powerful resolution of the sociogenetic structure of colonies.

From the genotypes of workers, we inferred parental genotypes for each colony and determined the numbers of mates per queen (as in Fjerdingstad et al. 1998; 2002). This was possible because males are haploid in ants (Crozier and Pamilo 1996) and so carry only one allele at each locus which is transmitted to all their offspring, leading to an across-locus association of paternal alleles. We assumed multiple paternity only when the data were not compatible with single paternity (like the 'narrow deduction option' in Matesoft, Moilanen et al. 2004). To support the correctness of our inferences, we also confirmed via χ^2 tests that worker genotypes were consistent with Mendelian segregation of alleles in heterozygous queens. Next, to assess the genetic effects of multiple mating which depend also on variation in siring success among mates of the same queen, we estimated effective paternity (me_{ped}) (Boomsma and Rattiéks 1996) based on the observed contribution of each father to the worker brood (Starr 1984). Given the paternity skews previously found for *L. niger* (Fjerdingstad et al. 2002), the average numbers of workers we analyzed per colony (Table 1) should ensure a strong power to detect all patrilines (Pedersen and Boomsma 1999). Nevertheless, sample sizes were limited for a few colonies and therefore we applied Nielsen et al.'s (2003) correction to the entire dataset to achieve sample size unbiased estimators of the effective numbers of fathers per colony. Finally, we calculated the expected nestmate relatedness of workers in each colony (R_{ped}) based on the effective paternity (me_{ped}).

Regression relatedness

To obtain independent estimates of the sociogenetic structure of colonies, we estimated nestmate relatedness

directly via the genetic similarity between members of the same colony as compared to the background population (Queller and Goodnight 1989). We used only the population in question as the background population due to the great geographical distance separating the Southern French and Irish populations (Fig. 1). Nestmate relatedness and effective paternity estimated via pedigree analyses and regression techniques were significantly positively correlated (results not shown) and gave the same qualitative results in all further tests. Therefore, we only present values and tests for pedigree effective paternity and regression relatedness in this paper.

Population genetics

As the degree to which multiple mating increases genetic diversity in colonies is reduced by inbreeding, we tested for evidence that such occurs. We based our tests on the first workers of each colony, first estimating allelic richness at each locus for both populations using FSTAT vs. 2.9.3.2 (Goudet 1995), then testing if genotype distributions fit those expected under random mating or showed a deficit of heterozygotes using GENEPOP 4.0 (Raymond and Rousset 1995). Finally, based on inferred parental genotypes, we tested directly whether queens were related with their mates, using Relatedness 4.2 (Queller and Goodnight 1989).

Comparisons of populations

We combined our data with those of two other *L. niger* populations from Uppsala in Sweden and Lausanne in Switzerland (Fjerdingstad et al. 2002, 2003) to determine whether the number of mates per queen and effective paternity were greater in southern populations (Rüppell et

al. 2008) and nestmate relatedness lower. Also, we tested whether multiple mating increased genetic diversity of colonies less in the north or in populations where this queen behaviour is rare, as we may expect if queens in such populations only remate when a mate transfers a subaverage sperm load (leading to high paternity skew; Boomsma and van der Have 1998). To test these hypotheses, we calculated for each population the mean B skew index (which represents the variance in siring success controlled for expected variance due to chance, Nonacs 2000), using the Skew Calculator 2003 (Nonacs 2003). For all tests, whenever our data did not meet the assumptions for parametric analyses, we employed non-parametric equivalents.

Results

Pedigree analyses

We found that a strategy of multiple mating dominated among queens in the population from Southern France, with ten out of 15 colonies (67%) having two or more mates per queen. By contrast, single mating prevailed among queens from the Irish population, with only two out of 17 (11.8%) colonies having multiple-mated queens (Fig. 2). These among-population differences in the prevalence of multiple mating in Ireland vs. Southern France were significant (Fig. 2; Mann–Whitney U test, $U=57.5$, $n_1=17$, $n_2=15$, $P=0.007$). Equally, the average number of fathers per colony was significantly higher in the Southern French than in the Irish population (Mann–Whitney $U=58.0$, $n_1=15$, $n_2=17$, $P=0.008$, Fig. 3). Thus, while our genetic analyses showed that all colonies contained only one queen, the number of mates per queen ranged from one to three in Ireland and from one to four in Southern France (Fig. 3). Effective paternity m_{ped} was equally significantly lower in Ireland (Table 1; range, 1–2.2) than in

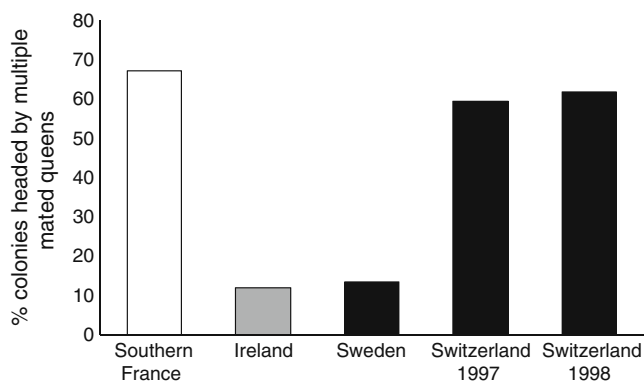


Fig. 2 Differences in the prevalence of multiple mating by *L. niger* queens in populations from Southern France, Ireland, Sweden and Switzerland (Swiss and Swedish data from Fjerdingstad et al. 2002)

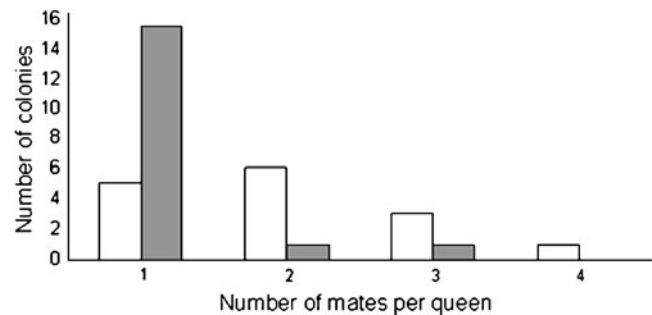


Fig. 3 Numbers of colonies headed by queens with one, two, three or four mates in Ireland (Dublin, dark bars) and Southern France (Cannes, light bars)

Southern France (range, 1–4.6) (Mann–Whitney $U=54.5$, $n_1=15$, $n_2=17$, $P=0.005$).

Regression relatedness

Average nestmate relatedness was much higher in the Irish population of *L. niger* ($r=0.77\pm 0.019$ SE by colony) than in the Southern French ($r=0.59\pm 0.046$ SE by colony) and this was significant (Mann–Whitney $U=55$, $n_1=15$, $n_2=17$, $P=0.005$). Additionally, in Southern France colonies headed by multiple-mated queens had a significantly lower nestmate relatedness ($r=0.54\pm 0.13$) than single paternity colonies ($r=0.77\pm 0.07$) (Mann–Whitney $U=3.0$, $n_1=5$, $n_2=10$, $P=0.007$, Fig. 4) (no test performed for Ireland as only two multiple paternity colonies were found, leading to a low statistical power). Thus, in the southern French population multiple mating on average reduced nestmate relatedness to 72% of the value for a colony of full siblings (0.75), which is 46% of the maximum possible reduction that multiple paternity can cause.

Population genetics

In the dataset of one worker per colony, allelic richness was great and heterozygosity very high for all loci and

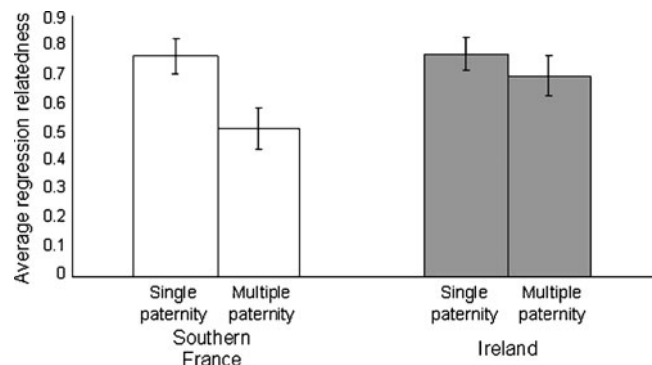


Fig. 4 Regression nestmate relatedness in colonies headed by single versus multiple-mated queens in Southern French (light bars) and Irish (dark bars) populations

populations (Table 2). Observed genotype distributions did not deviate significantly from Hardy–Weinberg expectations (Dublin, $\chi^2=13.3$, $df=8$, $P=0.10$; Cannes, $\chi^2=6.6$, $df=8$, $P=0.58$), and in direct tests for heterozygote deficit no evidence for inbreeding was found in Southern France ($F=0.006$, multilocus test, $P=0.45$; $P>0.16$ for all tests on individual loci). In Ireland, however, some inbreeding seemed to occur ($F=0.09$, $P=0.04$) as heterozygote deficit was significant for one locus (L10-174, $F=0.17$, $P=0.04$) and close to significance for another (L10-282, $F=0.10$, $P=0.08$), though not for the third and fourth (L1-5 and L10-53, $F=0.09$, $P=0.39$ and $F=-0.004$, $P=0.66$, respectively) (all tests using GENEPOP 4.0, Raymond and Rousset 1995). Pairwise relatedness analyses confirmed that queens and their mates were unrelated in Southern France (single-tailed t test for deviation from $r=0$) ($r=0.027$, $t_{14}=0.43$, $P=0.67$) but in Ireland a slight relatedness was found ($r=0.15$, $t_{16}=2.10$, $P=0.05$) (tests based on jackknifing over colonies), though this was not significant when using SE from jackknifing over loci ($t_3=1.65$, $P=0.20$). This slight relatedness between queens and their mates in Ireland is not likely to have led to underestimates of the numbers of mates in pedigree analyses because we had information from four independent loci.

Comparisons of populations

As predicted, the absolute number of mates per queen varied significantly between our four populations (Kruskal–Wallis test, $H=36.7$, $n_1=17$, $n_2=61$, $n_3=15$, $n_4=52$, $P<0.0001$) as did the frequency of multiple versus single mating (Kruskal–Wallis tests, $H=36.3$, $n_1=17$, $n_2=61$, $n_3=15$, $n_4=52$, $P<0.001$, Fig. 2). More specifically, queens in populations living at lower latitudes, Southern France and Switzerland, had significantly more mates than queens from Ireland (Mann–Whitney U test above and $U=277$, $n_1=61$, $n_2=17$, $P=0.001$, respectively) and Sweden ($U=168.5$, $n_1=15$, $n_2=52$, $P=0.00001$ and $U=816.5$, $n_1=61$, $n_2=52$, $P<0.00005$, respectively) (see also Fig. 2). By contrast, numbers of mates did not differ between the two northern (Ireland and Sweden; $U=438$, $n_1=17$, $n_2=52$, $P=0.92$) nor

between the two southern populations (Southern France and Switzerland, $U=385$, $n_1=15$, $n_2=61$, $P=0.30$). Equally, the effective number of mates was significantly lower in the north (Ireland and Sweden) than in Southern France (above and $U=173.5$, $n_1=52$, $n_2=15$, $P=0.00007$, respectively) or Switzerland ($U=261$, $n_1=17$, $n_2=59$, $P=0.001$ and $U=851$, $n_1=52$, $n_2=61$, $P<0.00005$, respectively), but did not differ between the two southern ($U=333$, $n_1=15$, $n_2=59$, $P=0.13$) or the two northern populations ($U=434.5$, $n_1=17$, $n_2=52$, $P=0.86$) (Note that two Swiss colonies were excluded here as two inferred pedigrees were possible for each, giving different effective paternities but the same numbers of fathers per colony). Conclusions held when applying false discovery rate control for multiple tests (Verhoeven et al. 2005, controlling for $n=6$ tests in each case).

Regression relatedness of nestmates also differed among the four populations (Kruskal–Wallis, $H=21.9.5$, $n_1=17$, $n_2=61$, $n_3=15$, $n_4=52$, $P<0.0001$), and this was due to differences among the Irish and Swiss (Mann–Whitney $U=221$, $n_1=17$, $n_2=61$, $P=0.0003$) and the Southern French and Swedish populations (Mann–Whitney $U=230$, $n_1=15$, $n_2=52$, $P=0.02$) as well as to the differences among Sweden and Switzerland ($U=987$, $n_1=52$, $n_2=61$, $P=0.001$) and the Irish and Southern French populations (above). By contrast, no significant differences in regression relatedness were found between Ireland and Sweden ($U=363$, $n_1=17$, $n_2=52$, $P=0.27$) or Southern France and Switzerland ($U=416$, $n_1=15$, $n_2=61$, $P=0.59$). Again, applying false discovery rate control (Verhoeven et al. 2005) did not change conclusions.

Paternity skew, which reduces the genetic impact of multiple mating, was significant in all populations (mean skew B [min; max]; Ireland, $B=0.21$ [0.12; 0.30], $P<0.001$; Sweden, $B=0.049$ [−0.05; 0.27], $P=0.0496$; Southern France, $B=0.049$ [−0.07; 0.29], $P=0.01$; Switzerland, $B=0.11$ [−0.08; 0.37], $P<0.001$; calculated using the Skew Calculator Nonacs 2003). Skew estimates for the different populations, however, appeared to be affected by the number of workers that had been genotyped for each multiple paternity colony. Thus, smaller sample sizes of workers were associated with lower skew estimates, and while this was not significant ($B=-0.13+0.14$, $t_2=1.7$, $P=0.23$) such effects are actually expected (Nonacs 2000). The residuals from this regression covaried significantly negative with mean numbers of mates per queen across our four populations (res $B=0.16-0.10$ * number of mates, $t_2=-4.1$, $P=0.026$, one-tailed test, Fig. 5).

Table 2 Allelic richness^a and observed heterozygosities (in brackets) at our four microsatellite DNA loci in the Southern French (Cannes) and Irish (Dublin) *L. niger* ant populations

	L10-53	L10-282	L10-174	L1-5
Ireland	10.4 (0.88)	12.4 (0.91)	12.4 (0.92)	10.3 (0.71)
Southern France	14 (0.94)	10 (0.90)	9 (0.85)	7 (0.74)

^a Estimated using FSTAT (Goudet 1995) assuming sample sizes of one worker for each of 15 colonies for each population

Discussion

Multiple mating by queens was far more frequent in southern populations of *L. niger* ants than in the north of Europe, and these differences in mating behaviour affected the levels of genetic diversity within colonies. Thus, colonies from Ireland

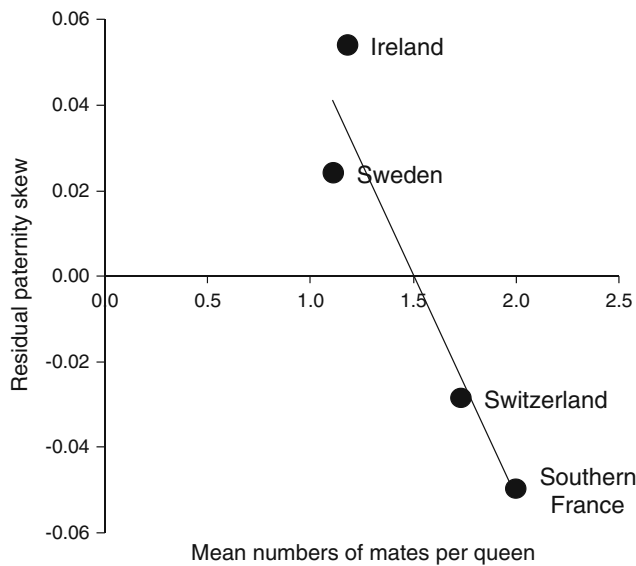


Fig. 5 Paternity skew, after controlling for variation in the number of workers genotyped per multiple paternity colony, was significantly greater in populations where queens had few mates (see text for statistics). Regression based on population averages for all multiple paternity colonies in each region, leading to a total of four population estimates of paternity skew

and Sweden showed greater average nestmate relatedness and a lower effective paternity than those in Southern France and Switzerland. This did not result from general differences among populations because the two southern populations did not differ between each other, nor did the northern pair. Consequently, genetic diversity was consistently greater in colonies from populations inhabiting Southern Europe, and this pattern was reinforced by the presence of a lower paternity skew in the south than at northern latitudes. The greater paternity skew in northern populations suggest that queens may only remate if their first mate transfers a very small sperm load while the low levels of inbreeding in Ireland supports that queens in the north behave in a manner reducing the levels of genetic diversity in colonies. Overall, our findings therefore support that *L. niger* ant queens living in regions that are climatically milder and on average biotically richer (the south, Gaston 2000) are more likely to have multiple mates, and, consequently, greater levels of genetic diversity in their colonies. These are the first findings, to our knowledge, consistent with the idea that mating strategies in social insects may be affected by the environments they inhabit.

Whether the pattern of mainly single mating in the north (Ireland, Sweden) and frequent multiple mating in the south (Southern France, Switzerland) results from several independent evolutionary events or from close coancestry of populations showing similar strategies, cannot be determined without population genetic analyses to establish the geographic and demographic history of *L. niger* populations. While we and

collaborators have started such a study (E. J. Fjerdingstad, S. Singh, M.K. Corley, J. Milander and G. Papacharalambous, unpublished data; using multiple microsatellite loci and mtDNA sequence data) which requires samples from a very wide area, some tentative conclusions can be made based on the geographic locations of our four populations. Ireland and Sweden, while both in the north of Europe, are separated by multiple water barriers (the Irish Sea, English Channel and North Sea/Baltic Sea) which makes it unlikely that the extreme similarities in queen mating strategies are due to ongoing direct gene flow. Data on queen strategies in *L. niger* populations occupying the landmasses between our four populations will of course be of great value and are under collection (E. J. Fjerdingstad, M.K. Corley, G. Jackman, T. Goodman, unpublished data).

Our four populations of *L. niger* ants are broadly consistent with recent models (Rüppell et al. 2008, Gove et al. 2009) that led us to predict that multiple mating by queens should be more frequent in milder or more complex habitats due to how habitat characteristics affect the fitness effects of genetic variance in worker performance. More multiple mating in the south is consistent with those regions constituting a milder habitat than northern ones as concerns a core aspect of ant colony success, brood development. Also, the generally greater levels of biotic diversity found at southernly latitudes across Europe as regards both flora and fauna make it plausible that *L. niger* colonies in Southern Europe occupy more complex environments (Gaston 2000). This is made more likely by the fact that *L. niger* ants are generalists who will make opportunistic use of a wide range of food resources, also showing a great ability to exploit a diversity of nesting sites (Seifert 1992; E.J.F. personal observation) and can be found both in near-monocultures as well as in plots heavily occupied by a diversity of other ants (myrmicines, formicines, personal observation E.J.F.; see also Boomsma et al. 1982). Studies quantifying habitat characteristics to pinpoint the specific environmental factors that vary across Europe and how these affect colonies in experimental studies would be of great interest and are feasible, though demanding.

Factors not related with genetic variance effects on worker forces may, however, also impose differential selection on queen mating strategies in ants and could lead to similar patterns of mating strategies across habitat types. For example, differences in climatic harshness may affect the direct costs of mating multiply, such as the energy and immune costs of sperm storage (Baer et al. 2006; Castella et al. 2009). In *Bombus terrestris* bumblebees, challenges to the immunity of individual workers induce fitness costs for the entire colony, and these costs are greater under harsher environmental conditions (Moret and Schmid-Hempel 2004). Harsher environments could likewise impose greater fitness costs when immunity is challenged in *L. niger*

founding queens. Equally, if harsher habitats (colder, wetter, with a shorter warm season, e.g. Ireland and Sweden) imply a greater risk of catastrophic colony extinction, life history theory (Stearns 1992) suggests that this may select for early sexual reproduction at small colony sizes, which would reduce the direct benefits of gaining many sperm when mating multiply (Cole 1983; Fjerdingstad et al. 1998; Fjerdingstad 2004; see also Boomsma and Ratnieks 1996, Kraus and Page 1998, Oldroyd and Fewell 2007). Finally, differences in colony density caused by habitat quality could affect the chance of queens obtaining multiple partners, but strong effects are less likely for *L. niger* because numerical sex ratios are heavily male biased (Fjerdingstad et al. 2002). The greater allelic diversity in Ireland than in Southern France for three out of four microsatellite loci (Table 2) also suggests that the former does not suffer from a dramatically smaller effective population size than the latter (further population genetics, E. J. Fjerdingstad, S. Singh, M.K. Corley, J. Milander, B. Meraj unpublished).

The association between environment and queen strategies we here report for *L. niger* ants supports that it is time to study the mating systems of social insects in the context of their environments. Diverse selective factors, acting at different life stages (single founding queens, colonies), may affect the evolution of insect queen mating strategies in habitats that vary in harshness, biotic richness and complexity. Exploring these theoretically (Rüppell et al. 2008; Gove et al. 2009) but also experimentally may considerably increase our understanding of the evolution of multiple mating by queens and the sociogenetic structure of colonies.

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Ethical standards The experiments described here complied with current laws of the European Union and the United States of America.

Conflict of interest None.

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