

Multiple mating and offspring quality in *Lasius* ants

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Abstract Genetic diversity benefits for social insect colonies headed by polyandrous queens have received intense attention, whereas sexual selection remains little explored. Yet mates of the same queen may engage in sperm competition over the siring of offspring, and this could confer benefits on queens if the most successful sire in each colony (the majority sire) produces gynes (daughter queens) of higher quality. These benefits could be increased if high-quality sires make queens increase the percentage of eggs that they fertilize (unfertilized eggs develop into sons in social hymenopterans), or if daughters of better genetic quality are over-represented in the gyne versus worker class. Such effects would lead to female-biased sex ratios in colonies with high-quality majority gynes. I tested these ideas in field colonies of *Lasius niger* black garden ants, using body mass of gynes as a fitness trait as it is known to correlate with future fecundity. Also, I established the paternity of gynes through microsatellite DNA offspring analyses. Majority sires did not always produce heavier gynes in *L. niger*, but whenever they did do so colonies produced more females, numerically and in terms of the energetic investment in female versus male production. Better quality sires may be able to induce queens to fertilize more eggs or

so-called caste shunting may occur wherever the daughters of better males are preferentially shunted to into the gyne caste. My study supports that integrating sexual selection and social evolutionary studies may bring a deeper understanding of mating system evolution in social insects.

Keywords Genetic compatibility · Good genes · Polyandry · Sex ratio · Social insects

Introduction

The reasons why females mate with multiple males in many animals is a hotly debated issue in evolutionary biology (Boomsma and Ratnieks, 1996; Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000; Crozier and Fjerdingstad, 2001; Simmons, 2005). Having several mates costs females energy, increases the risk of predation, physical harm, exposure to deleterious seminal products, and induces immunity costs (Thornhill and Alcock, 1983; Chapman et al., 1995; Jennions and Petrie, 2000; Baer et al., 2006; den Boer et al., 2010). In social insects, such as ants, many bees and wasps, multiple mating by queens also reduces relatedness in colonies, which decreases the inclusive fitness for the helper daughters (workers) that rear the queen's brood (Bourke and Franks, 1995; Crozier and Pamilo, 1996). To compensate for these costs, most current theories for social insects suggest that an increased genetic diversity in colonies could bring benefits for queens (e.g., a reduced queen–worker conflict over colony sex ratios, better resistance to parasites and pathogens, or greater working efficiency, (Boomsma and Ratnieks, 1996; Schmid-Hempel, 1998; Crozier and Fjerdingstad, 2001)).

Clear support exists for several of these hypotheses in some social taxa; for example genetically diverse colonies

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have been found to perform better and resist parasites and pathogens better in bumble bees (Baer and Schmid-Hempel, 1999) and honeybees (Seeley and Tarpy, 2007; Mattila and Seeley, 2007). Similar results have been obtained for polyandrous *Acromyrmex* leafcutter ants (Hughes and Boomsma, 2004). That polyandry can bring colonies more diverse worker forces has been corroborated in several studies proving that workers from different patriline vary in size or shape (e.g., *Pogonomyrmex* harvester ants (Rheindt et al., 2003), leafcutter ants (Hughes et al., 2003), *Eciton* army ants (Jaffe et al., 2007)). Also, that multiple mating can bring colony sex ratios closer to the values that maximize queen fitness has found support in some wood ants (Sundström and Ratnieks, 1998), though this requires that colony sex ratios co-vary with the relatedness of workers to gynes versus males (Ratnieks and Boomsma, 1995). At the comparative level, finally, it has been found that species with multiple matri- or patriline per colony have more size and shape diverse workers (Fjerdingstad and Crozier, 2006; Hughes and Boomsma, 2008).

Nevertheless, tests for genetic diversity benefits of multiple mating by queens have often given variable or inconclusive results. For example, support for genetic survival benefits was not found in *Atta* fungus-growing ants (Fjerdingstad et al., 1998), polyandry did not boost the genetic diversity in colonies much in *Plagiolepis* (due to paternity skew) (Trontii et al., 2007), but a greater genetic diversity did increase colony survival and growth in *Pogonomyrmex* harvester ants (Cole and Wiernasz, 1999). Also, while queens of some wood ants gain sex ratio benefits from multiple mating (Sundström and Ratnieks, 1998), the closely related black garden ants, *L. niger* and other wood ants do not (Boomsma and Sundström, 1998; Fjerdingstad et al., 2002). These mixed findings may arise in part because the costs and benefits of polyandry are predicted to depend on the life history and ecological characteristics of species (Corley and Fjerdingstad, 2010), and such effects may help explain why some species evolve obligate polyandry and others do not.

It is, however, also likely that several selective factors affect a given species and that we have been overlooking some. Though it has still been little studied (exceptions e.g., Davidson, 1982; Abell et al., 1999; Baer and Boomsma, 2004; Fjerdingstad and Keller, 2004), a better comprehension of multiple mating in the social insects may be had by integrating sexual selection theory (Andersson, 1994; Eberhard, 1996; Arnqvist and Nilsson, 2000; Tregenza and Wedell, 2000; Simmons, 2005) with social evolution. Just because some aspects are absent in social insects (e.g., courtship feeding, nutritious spermatophores, territoriality (Boomsma and Ratnieks, 1996), queen mating strategies could still be shaped in part by selective pressures to obtain the genetically best or most compatible partner (Andersson, 1994; Jennions and Petrie, 2000; Simmons, 2005). Specifically,

if a queen is not capable of pre-copulatory evaluation of partner quality, she may benefit from having multiple partners compete inside her by way of their sperm, or cryptically select sperm more compatible with her own gametes (Eberhard, 1996; Keller, 2009).

It is in fact a common occurrence in social insects that one sire is more successful in fertilizing a queen's eggs than her other mates (Boomsma and Sundström, 1998; Boomsma and van der Have, 1998) and this could result from sperm competition (Baer and Boomsma, 2004; den Boer et al., 2010) or cryptic female choice/genetic compatibility effects. If so and if this is to favor the evolution of multiple mating by queens, we would expect that the male fertilizing the majority of the brood has offspring of greater quality (cf. Hosken et al., 2003; Keller, 2009). For male-haploid hymenopterans like ants this means that daughter queens (gynes) of majority sires should be of better quality, because sons carry no paternal genes and arise from unfertilized eggs (Bourke and Franks, 1995). Also, competition among sires for siring as many offspring as possible could lead to selection favouring seminal substances that make queens fertilize more of their eggs. Greater fertilization rates by queens would mean that more of their offspring would be female and could lead to a greater investment by colonies in females versus males. This would directly increase the reproductive success of males as males transmit genes only via daughters (Crozier and Pamilo, 1996). Such male manipulation may not be deleterious for queens, if population sex ratios are at the queens' Fisherian optimum of 1:1, as they are in some species (Fjerdingstad et al., 2002), so that producing sons or daughters gives the same genetic payoff for queens (haplodiploid sex ratio theory, Crozier and Pamilo, 1996). Increased female-bias may even benefit queen fitness if only high-quality or more compatible males are able to increase egg fertilization rates. Such benefits of producing more of the sex one can produce with a higher quality are an aspect of sex ratio theory that has so far been almost ignored by social insect researchers (but see Abell et al., 1999). Possible positive effects for queens might also result if the best sire's daughters end up over-represented in the gyne class (as opposed to the worker class) due to growing better or faster (Keller, 2009), again leading to female-biased sex ratios in colonies with better quality majority sires. Combined, if sperm competition among social insect males affect their siring success in a manner that bring queens benefits, we would expect that gynes fathered by majority sires are of higher quality especially whenever we find female-biased sex ratios in colonies.

To perform a first test of these ideas, I studied wild colonies of the black garden ant *L. niger*. Via pedigree analyses using microsatellite markers I assigned gynes to patriline in each colony, and then tested whether majority sires produced better gynes always or at least whenever colony sex ratios

were female-biased. I used the fresh body mass of sexually mature gynes before their mating flights as an estimator of gyne quality because this variable is known to co-vary positively with queen fecundity in many ants (e.g., Lachaud et al., 1999; DeHeer, 2002; in *L. niger*: Fjerdingstad and Keller, 2004) and with survival during colony founding in others (Wiernasz and Cole, 2004; see also Keller and Passera, 1989; Sundström, 1995). I first used an ANOVA to test whether majority gynes were always heavier than gynes sired by less successful fathers (minority sires), controlling for among-colony differences in body mass. Next, I performed correlation analyses to test whether heavier majority versus minority gynes did indeed characterize colonies producing mainly gynes, using both energetic and numerical sex ratios to estimate female production. I controlled my analyses for colony productivity, as it could potentially confound the expression of among-patriline differences in gyne body mass (Fjerdingstad, 2004, 2005).

Materials and methods

Sample collection, body mass estimation, and genetic paternity analyses

In July 1997 and 1998, *L. niger* gynes and males were collected in tent traps as they were leaving their natal colonies for mating-and-dispersal flights on the meadows of Lausanne University in Switzerland (Fjerdingstad et al., 2002). After killing by freezing at -25°C , I measured fresh body mass for approximately 20 gynes per colony (mean number of gynes \pm SD for 1997, 19.7 ± 0.7 for 23 colonies; for 1998, 19.5 ± 1.2 for 34 different colonies), using a Toledo-Mettler balance (accurate to the nearest μg). Measurements were highly repeatable ($r > 0.97$, $P < 0.0005$, $N = 39$).

Genetic analyses were then performed on DNA extracts of gyne heads, using two hypervariable microsatellite DNA markers L10-282 and L10-53 (as described by Fjerdingstad et al., 2002). The genotype data allowed me to establish the numbers of paternal males for each colony via pedigree analyses (Fjerdingstad et al., 1998, 2002), a procedure which is facilitated by the male-haploidy that characterizes ants. The great variability of the microsatellite markers meant that the risk of two fathers having identical genotypes was negligible (less than 0.1% (Fjerdingstad et al., 2002, 2003)). As reported by Fjerdingstad et al. (2002) multiple paternity was found in 59 and 62% of colonies in 1997 and 1998, respectively. For the purposes of the present study I focused on those colonies with multiple fathers where the gynes could be assigned unambiguously to patriline. I found 11 and 18 such colonies in 1997 and 1998, respectively. In more detail, nine colonies sampled in 1997 had two fathers,

Table 1 Paternity skew in *L. niger* colonies at Lausanne 1997, 1998

Year	Colony	$N_{\text{male } 1}$	$N_{\text{male } 2}$	$N_{\text{male } 3}$	$N_{\text{male } 4}$	B skew	$P_{B\text{skew}}$	
1997	17	17	3	0	0	0.22	0.003	
	19	18	1	0	0	0.37	0.0001	
	20	14	6	0	0	0.055	0.11	
	21	14	6	0	0	0.055	0.12	
	30	15	5	0	0	0.071	0.10	
	40	17	3	0	0	0.22	0.002	
	41	11	9	0	0	-0.02	0.83	
	43	13	7	0	0	0.02	0.26	
	44	11	7	1	1	0.14	0.002	
	47	15	5	0	0	0.1	0.04	
	101	11	8	1	0	-0.014	0.65	
	1998	A11	14	6	0	0	0.055	0.11
		A12	17	2	0	0	0.29	0.001
		A13	15	3	2	0	0.23	0.0004
A17		17	3	0	0	0.22	0.0025	
A24		14	5	1	0	0.19	0.0011	
A27		15	4	0	0	0.13	0.032	
A31		15	5	0	0	0.1	0.042	
A33		15	5	0	0	0.1	0.04	
A38		13	7	0	0	0.0083	0.36	
A42		12	4	0	0	0.094	0.07	
A44		11	9	0	0	-0.02	0.83	
A47		12	8	0	0	0.055	0.12	
A49		14	6	0	0	0.055	0.12	
A54		17	4	0	0	0.155	0.013	
A56	14	6	0	0	0.055	0.11		
A59	18	2	0	0	0.30	0.0004		
A76	13	7	0	0	0.02	0.26		
NT1	13	7	0	0	0.02	0.26		

Gynes were assigned to patriline through microsatellite DNA pedigree analyses (see Fjerdingstad et al., 2002) and paternity skew is measured by the B skew index (Nonacs, 2000), calculated using Nonacs (2003) skew calculator software. Significant P values are given in bold and those that remained significant after false discovery rate control (Verhoeven et al., 2005) are given in bold italics

one had three, and one colony had four fathers; in 1998, 16 colonies had two fathers while two colonies had been sired by three fathers (Table 1; see Fjerdingstad et al., 2002 for more details). I next tallied up which paternal male had sired how many of the gynes in each colony (Table 1).

Data analyses

Differences in siring success among the two fathers in each colony

To ensure that it was meaningful to assign sires to different paternity categories, I verified that the siring successes of

males mated to the same queen did differ significantly. I did this by estimating *B* skew values (Nonacs, 2000) for each colony and for the population using the software skew calculator (Nonacs, 2003). *B* skew is considered the state of the art skew estimator, and the associated *P* values (determined through simulations) take into account the likelihood of observing by chance the skew seen in the raw data, given the sample sizes (Nonacs, 2000). When interpreting results for all the colonies I applied false discovery rate control (Verhoeven et al., 2005) to control for multiple tests. In this skew analysis I included also gynes sired by any third and fourth paternal males (Table 1), almost all gynes were, however, sired by the most successful (majority) or the most common minority (minority male). For my further analyses ($N = 29$ colonies), I therefore used only gynes from the majority and minority patriline for each colony. This left me with 13.7 ± 2.6 (SD) and 5.3 ± 2.3 gynes per majority and minority sire, respectively, for each of 11 colonies in 1997, and 14.3 ± 2.0 majority gynes and 5.0 ± 1.9 minority gynes for each of 18 colonies in 1998 (Table 2).

Body mass of majority versus minority gynes

To test whether majority gynes were heavier than minority gynes after statistically removing among-colony variation in body mass, I did an analysis of variance (2-factor ANOVA) for each year (1997 and 1998) separately, using a trial version of the microsoft excel add-in Statistix1 1.8 2007 and ‘colony’ and ‘paternal type’ as factors. The factor ‘colony’ will adjust for differences in gyne mass due to environmental or maternal effects, and genetic variance components from maternal genes (Fjerdingstad, 2005). The interaction ‘paternal type \times colony’ tested for the presence of differences in gyne mass between patrilines regardless of their status as majority or minority sires. Different colonies were sampled in different years and therefore the 2 years had to be analyzed separately.

Differences in the body mass of majority versus minority gynes within colonies

First I calculated estimators of the difference in quality of majority versus minority gynes, the degree of siring success, and colony sex ratios. Differences in the body mass of majority versus minority gynes within colonies were expressed as relative values (%) after dividing the mean difference between these classes by the average mass of the minority gynes, i.e. as $(X_{\text{majority}} - X_{\text{minority}})/X_{\text{minority}}$ %. Siring success of the majority male in each colony was estimated as $n_{\text{majority}}/(n_{\text{majority}} + n_{\text{minority}})$, where n_{majority} is the number of gynes sired by the majority male in that colony and n_{minority} is the number of minority gynes.

Table 2 Numbers of majority and minority gynes measured in 1997 and 1998

Year	Colony	$N_{\text{majority male}}$	$N_{\text{minority male}}$
1997	17	17	3
	19	18	1
	20	14	6
	21	13	6
	30	14	5
	40	16	3
	41	11	9
	43	11	5
	44	11	7
	47	15	5
	101	11	8
1998	A11	14	6
	A12	18	2
	A13	15	3
	A17	17	3
	A24	13	5
	A27	15	5
	A31	15	5
	A33	15	5
	A38	13	7
	A42	12	4
	A44	11	9
	A47	14	6
	A49	14	6
	A54	17	3
	A56	14	6
	A59	18	2
	A76	13	7
NT1	13	7	

Thirdly, colony sex ratios were estimated by collecting all sexuals produced in tent traps during the entire (1997) or almost entire (1998) season (Fjerdingstad et al., 2002, 2003). For each colony, the numbers of gynes and males produced and their average body mass were measured (Fjerdingstad et al., 2002). On the basis of this I calculated the numerical sex ratio for each colony as $(N_f/(N_f + N_m))$. I also estimated the energetic investment sex ratio for each colony as $(N_f/(N_f + N_m(1/\text{cost ratio})))$, where the cost ratio takes into account the differences in gyne-male size and the higher metabolic rate of males (cost ratio = $(DW_f/DW_m)^{0.7}$, Boomsma and Isaaks, 1985; Boomsma, 1989). I based my estimations on the population mean dry mass of individual gynes and males (data from Fjerdingstad et al., 2002). The population-level investment sex ratio was not significantly different from 1:1 in my population of *L. niger* (Fjerdingstad et al., 2002) and therefore any colony-level investment larger than 0.50 is female-biased.

Lastly, the overall resource availability of a colony could affect the degree to which genetic differences in majority versus minority gyne quality differences are expressed. To overcome this, I statistically controlled all analyses for the total biomass of gynes and males produced by each colony, with correction for the greater metabolic rate of males (Boomsma and Isaaks, 1985; Boomsma, 1989). This was done by estimating colony productivity as $N_f + N_m$ (1/cost ratio)). Non-parametric tests were used when assumptions for parametric tests were not fulfilled, and one-tailed tests were used when expectations were unidirectional as was the case for correlations of majority versus minority gyne body mass with siring success and sex ratio.

Power analyses

The statistical power to detect genuine relationships between variables was determined for a range of effect sizes using *GPOWER* 3.1 (Faul et al., 2007, 2009) for analyses giving non-significant results.

Results

Paternity skew

Differences in the siring success of the mates of a queen as estimated through *B* skew values (Nonacs, 2000) were significant in tests for 44–45% of the colonies in both 1997 and 1998 (Table 1). Applying false discovery rate control (Verhoeven et al., 2005), 36% (1997) and 33% (1998) of the individual colony skew tests remained significant. Also, skew was highly significant overall in the population (colonies pooled across years, $B_{pop} = 0.11$, $P < 0.0001$; calculated using skew calculator, Nonacs, 2003). This means that it was meaningful to assign different paternity status to males varying in observed siring success. The skew tests were based on the offspring of all fathers per colony (Table 1), but nearly all gynes were sired by the most (majority male) or the second-most successful male (minority male). Therefore, only majority and minority gynes were used for the further analyses to ensure good sample sizes per patriline.

Body mass of majority versus minority gynes

Gynes sired by majority sires were not generally heavier than minority gynes in any year (1997: ‘paternal type’ $F_{1,194} = 0.72$, $P = 0.49$; 1998: ‘paternal type’ $F_{1,316} = 0.58$, $P = 0.45$). Only ‘colony’ (1997: $F_{10,194} = 19.0$, $P < 0.0005$; 1998: $F_{10,316} = 70.8$, $P < 0.0005$) and the interaction factors (‘paternal type by colony’) were significant (1997: $F_{10,194} = 3.6$, $P = 0.009$, 1998: $F_{10,316} = 1.9$,

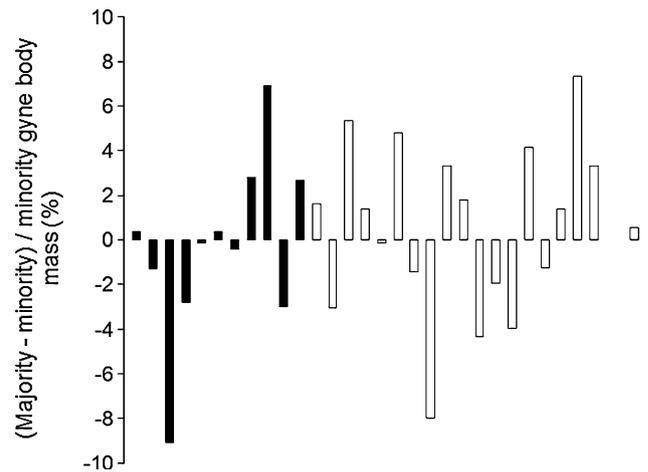


Fig. 1 Majority sires did not produce heavier gynes in *L. niger* ants. Instead majority gynes were heavier than minority gynes in some colonies and lighter in others, which held for both years (1997 black bars, 1998 white bars)

$P = 0.02$). The latter means that genetic differences between patrilines in gyne body mass exist (as established by Fjerdingstad, 2005), but they have varying directionality: in some colonies majority sires produce heavier daughters; in others minority males do (Fig. 1).

Differences in majority versus minority gyne body mass within colonies

Similar to the findings above (that majority sires do not always produce heavier gynes), I found that a greater siring success of the majority sire did not always mean that his daughters were heavier than those sired by the minority male (Fig. 2). This held also when controlling statistically for colony productivity (partial correlations 1997: $r = -0.47$, $df = 8$, $P = 0.09$; 1998: $r = 0.04$, $df = 15$, $P = 0.45$; P values combined following Fisher (1932): $\chi^2 = 3.78$, $df = 4$, $P = 0.44$).

Investment sex ratios were more female-biased in those colonies where majority sires did produce heavier gynes than did minority sires. This was shown by a positive correlation between sex ratios and the weight difference between majority and minority gynes within colonies (Fig. 3; test included all colonies). The patterns in each year were strikingly similar, and the relationship was significant in each year (Fig. 3). When controlling for colony productivity, the association remained significant for 1997 (partial correlation 1997: $r = 0.59$, $df = 8$, $P = 0.04$) and was marginally significant for 1998 (partial $r = 0.38$, $df = 15$, $P = 0.07$). Combining P values across years, the association between investment sex ratio and relative weight of majority gynes was significant in a one-tailed test ($\chi^2 = 9.1$, $df = 4$, $P = 0.03$). Also, the body mass advantage of

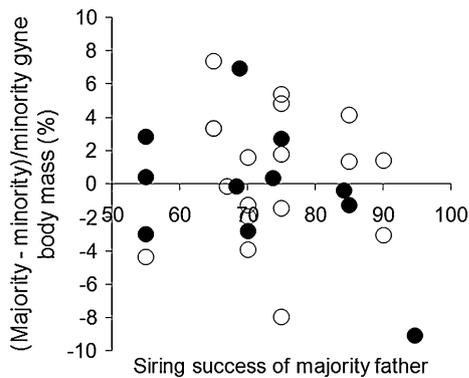


Fig. 2 Greater siring success of the majority sire did not invariably lead to his daughters being heavier than those sired by the minority male (Pearson correlations, *black circles* 1997: $r = -0.46$, $df = 9$, $P = 0.08$; *white circles* 1998: $r = 0.04$, $df = 16$, $P = 0.85$). This held when combining P values following Fisher (1932) ($\chi^2 = 3.9$, $df = 4$, $P = 0.42$)

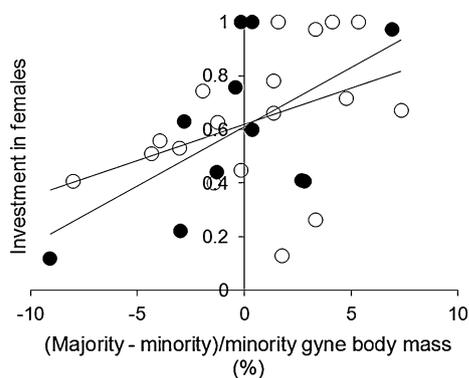


Fig. 3 Majority sires produced heavier gyne than did minority sires in those colonies that invested more in female production. This was shown by a positive correlation between sex ratio and the relative weight difference of majority and minority gyne within colonies, and this association was significant in each year (Pearson correlations, *black circles* 1997: $r = 0.57$, $df = 9$, $P = 0.04$; *white circles* 1998: $r = 0.41$, $df = 16$, $P = 0.045$) (combining P values across years: $\chi^2 = 10.1$, $df = 4$, $P = 0.04$, two-tailed test)

majority gyne was positively associated with numerical sex ratios in 1998 (Kendall's τ rank correlation, $\tau = 0.36$, $N = 18$, $P = 0.02$) but not in 1997 ($\tau = 0.27$, $N = 11$, $P = 0.12$), with the association being significant when combining tests across years ($\chi^2 = 9.3$, $df = 4$, $P = 0.025$, one-tailed test). The latter held when controlling for colony productivity (Kendall's partial τ not shown).

Power of test

For the 1997 ANOVA, I had a power larger than 87% to detect significance at $\alpha = 0.05$ in two-tailed tests for 'paternity type' effects explaining 15% or more (a medium effect following Cohen, 1988) of the variance in gyne body mass. For 1998 the corresponding power was 98%. For

'paternity type' effects explaining 10% or more of the variance in gyne body mass the powers were 71 and 90% for 1997 and 1998, respectively (estimations done using *GPOWER* 3.1, Faul et al., 2009). I assumed sample sizes of three majority and three minority gyne per colony for the power analyses, which is a very conservative way to deal with the unbalanced ANOVAs (the imbalance arising from paternity skew, see Table 1).

After pooling data across years for the correlation analyses ($N = 29$ colonies), my power was 92 and 74% to detect significances (at $\alpha = 0.05$) for correlations of $r = 0.50$ or 0.40, respectively, for a correlation of siring success and majority versus minority gyne body mass (using *GPOWER* 3.1, Faul et al., 2007) (assuming one-tailed tests).

Discussion

While some sires were more successful than others in *L. niger* ants (corroborating earlier findings by Boomsma and van der Have, 1998), majority sires did not invariably produce heavier gyne in the 2 years studied. Instead the heaviest gyne were produced by majority sires only in a subset of colonies and by minority sires in others. I therefore found no evidence that majority gyne are always of higher quality as regards body mass, which is one of the major fitness components affecting colony founding success in *L. niger* queens (Fjerdingstad and Keller, 2004). This lack of evidence for consistent majority-sire effects on gyne body mass cannot be attributed to a poor statistical power of my ANOVAs because I would have detected moderate (15%) true effects of paternity status on gyne weight with a high probability, especially in 1998 (power of 98%). Also for smaller effects (10%) I had a good power in particular for the 1998 dataset (90%). While my power of test was somewhat lower for the correlation analyses, the combined results suggest that an association of male siring success per se with gyne body mass in *L. niger* ants is absent or weak, although the data do not permit excluding very small effects.

My study therefore did not give significant support that sperm competition between mates of the same queen (or cryptic postcopulatory choice of sperm by queens, Eberhard, 1996) invariably bring benefits to polyandrous *L. niger* queens in the form of better daughters. These findings are stands in contrast with those for some other insects such as dungflies where superior sperm competitors do produce better offspring (faster at developing) (Hosken et al., 2003). The results for *L. niger* are, however, comparable to findings that attractive *D. simulans* fruit fly males, despite being better sires (Hosken et al., 2008), do not sire better daughters (Taylor et al., 2009; and their review). Equally, seedbeetle males producing good offspring have a lower

siring success than males with poor quality offspring (Bilde et al., 2009). Bilde et al. (2009) suggested that such may result from antagonistic co-evolution between males and females which could render multiple mating unfavorable for females. Mate order effects or differences in the numbers of sperm carried by the different mates of a queen may also affect which male achieves the greatest siring success. It is possible that environmentally induced differences (Fjerdingstad, 2005) in sperm numbers can explain why sires producing gynes of lower genetic quality (as regards body mass) sometimes experience a high siring success in *L. niger* ants.

In *L. niger*, however, males with a high siring success did produce better gynes in some colonies, making it possible that queens heading such colonies can profit by producing more high-quality gynes. Colonies where the heaviest gynes were produced by majority sires did in fact invest more in the production of female sexuals (Fig. 3) and these findings were consistent between years and held for both investment- and numerical sex ratios. Importantly, these results are not an artifact arising from an overestimation of the investment in gynes in colonies where the majority of the gynes are heavy. This is because I used population average values of gyne body mass to calculate gyne/male cost ratios which were used to estimate colony-specific investment in gynes (same procedure followed for colony productivity). Furthermore, the female-bias held when using numerical sex ratios. *L. niger* colonies therefore invest more resources in producing gynes versus males, whenever a majority sire's gynes were of higher genetic quality as regards body mass than daughters of the minority sire (among-patriline differences represent genetic variance (Fjerdingstad, 2005)).

In *L. niger* ants, population sex ratios are very close to the queen's optimum (at least in Sweden and Switzerland (Fjerdingstad et al., 2002)). This means that a queen in a given colony will profit equally from producing mainly females or males, and if she can produce particularly good gynes this should benefit her fitness (see Abell et al., 1999). As for workers, we would not expect them to be averse to producing more high-quality sister gynes because from the workers' point of view (even for those in multiple paternity colonies) population sex ratios in *L. niger* are too little female-biased. De facto, multiple-paternity *L. niger* colonies do not invest less in females than in males (Fjerdingstad et al., 2002), and this fits with population sex ratios being at the queen's optimum of 1:1 (Fjerdingstad et al., 2002, for theory see Ratnieks and Boomsma, 1995).

The association of colony sex ratio with the body mass of the majority sire's gynes could result from so-called 'caste shunting', where bigger larvae of more compatible sires are shunted preferentially into the gyne (as opposed to worker) developmental pathway (Keller, 2009). This, however, cannot work if *L. niger* sexuals (gynes and males) are reared

from a different batch of eggs (autumn) than the workers (spring), as appears likely (J.J. Boomsma, B. Seifert, pers. comm.). Stronger data are, however, needed on this issue. If 'caste shunting' occurs, the relative representation of sires in worker and gyne brood should differ. In *Formica* wood ants, however, no evidence exists that paternity representations differ between worker and gyne brood (Keller et al., 1997). An alternative mechanism, whereby a colony's relative investment in gynes can be increased whenever the majority male sires heavier gynes is through the culling of male brood (cf. Sundström et al., 1996). For this to work, workers must be able to assess the quality of the gyne brood, a capability which has yet to be demonstrated.

A more plausible hypothesis may be that *L. niger* queens in colonies with majority males siring heavy gynes display a higher fertilization rate during the season where sexual brood is produced. This would lead to female-biased sex ratios when a constant proportion of fertilized eggs become gynes, whereas unfertilized eggs invariably turn into haploid males (Crozier and Pamilo, 1996). Seminal substances produced by males of high genetic quality or more genetically compatible males may make queens fertilize more eggs and such effects would be in a male's fitness interest, as daughters are his only way of transmitting genes to future generations (Crozier and Pamilo, 1996). While this idea cannot be tested with my data, male accessory glands do produce substances acting on females in other insects (e.g. fruit flies Chapman et al., 1995; Bangham et al., 2002; see also Bilde et al., 2009; Green and Tregenza, 2009, bumblebees Baer and Schmid-Hempel, 2005) and may do so in ants (attine ants, Baer and Boomsma, 2004) where an intricate interplay of seminal and spermathecal substances affecting sperm has recently been reported (den Boer et al., 2010). Variation among *L. niger* colonies in primary (egg) sex ratios could be determined through chromosome staining tests (as in Aron et al., 1994).

Producing heavy gynes is likely to be particularly beneficial in social insects where the fate of young colonies depends strongly on queen quality (many ants with claustral colony founding by single queens; some wasps, Ross and Matthews, 1991; Wiernasz and Cole, 2004; Fjerdingstad and Keller, 2004, see also Keller and Passera, 1989). In fact, my findings for *L. niger* ants show similarities with the idea that multiple paternity colonies in yellowjacket wasps (*Vespa maculifrons*) invest more in female production because they can produce better daughter queens (Goodisman et al., 2007). In contrast, multiple mating cannot bring strong benefits in the form of better gynes in those social insects, e.g., several wood ants (Sundström, 1994; see also Meunier et al., 2008), where colonies exhibit split sex ratios such that multiple paternity colonies invest mainly in males (kin-selection theory, Crozier and Pamilo, 1996; Ratnieks and Boomsma, 1995).

Whether a scenario like the above (better sires causing female-biased colony sex ratios) brings benefit or not to polyandrous queens depends on whether such effects also occur in single mated queens. We cannot easily test this because a comparison of gyne body mass across single paternity colonies would confound the effects of environmental (V_E) and genetic variance (V_G) for body mass (Fjerdingstad, 2005). Unfortunately, ants are not easy to subject to controlled mating experiments or artificial insemination, nor can *L. niger* colonies be raised to produce sexuals in the laboratory. Although I cannot here decisively conclude that *L. niger* queens gain the benefit of better daughters from having multiple mates, my study strongly suggests that we should complement studies of eusocial evolution with sexual selection hypotheses to advance our understanding of the mating systems of social insects.

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