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Multiple mating increases the sperm stores of *Atta colombica* leafcutter ant queens

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Abstract Multiple mating is likely to be costly for ant queens and yet it is common. Whether multiple mating brings benefits to queens that outweigh the costs has, therefore, received considerable theoretical attention. Empirical tests of hypotheses have been scarce and no clear evidence has been reported. We tested the "multiple-mating-for-more-sperm" hypothesis on individual young queens in a natural population of the leafcutter ant *Atta colombica*, a monogynous ant characterised by very large colonies and high colony longevity. We found that the number of sperm stored by queens was positively correlated with the number of mates per queen estimated through mother-offspring analysis with microsatellite DNA markers. Queen sperm stores increased on average by 30 million sperm for each additional mate. Life-history information for *Atta* indicate that the number of stored sperm observed is likely to constrain the reproductive lifespan of queens in nature. Multiple mating, despite costs, may therefore enhance the fitness of *Atta* queens because it enables them to store more sperm.

Key words Fecundity · Fitness · Microsatellites · Multiple mating · Social insects

Introduction

In many animals females mate with several males (Thornhill and Alcock 1983; Smith 1984; Keller and Reeve 1995) although they often appear to gain no advantage by doing so. Mating multiply may require increased energy expenditure by females and may expose them to increased risk of disease and predation. This is especially likely for the many ants in which queens mate on physically demanding nuptial flights under a heavy mortality pressure (Bourke and Franks 1995, pp. 368–369; in *Atta cephalotes c.* 52% of swarming queens fall prey to birds, Dix and Dix, unpublished work, cited in Fowler et al. 1986). In such species queens may have to carry out several flights from the ground to the nuptial swarm in order to secure additional matings (Reichardt and Wheeler 1996). Also, the copulations themselves may take substantial time (between 1 and 13 min per mating; Woyciechowski 1990; Fortelius 1994; Reichardt and Wheeler 1996) during which queens are exposed to predators. Even so, queens of some ant species are known to commonly mate with two or more males (Reichardt and Wheeler 1996; Fjerdingstad et al. 1998; review in Boomsma and Ratnieks 1996).

Two sets of hypotheses have been put forward to explain multiple mating in ants. The first is based on the fact that multiple mating often leads to multiple paternity of the queen's offspring and thus to increased genetic diversity within her colony. Such increased genetic diversity within colonies has been hypothesised to enhance colony fitness, for instance by increasing disease resistance or worker task efficiency or by lowering the load of diploid, sterile males (which result when a paternal male shares a sex locus allele with the queen) (Page 1980; Crozier and Page 1985; Sherman et al. 1988; Pamilo et al. 1994; Schmid-Hempel 1994; Page et al. 1995). In addition, multiple paternity makes the genetic value of male and female reproductives more equal for the workers (as it is for the queen). This reduces worker-queen fitness conflict over the sex ratio (Moritz 1985), and may enhance the fitness of the queen (Ratnieks and Boomsma 1995; Sundström and Ratnieks, in press). So far very few empirical tests of these hypotheses on multiple mating have been carried out (only Pamilo et al. 1994 and Fjerdingstad et al. 1997) and no support for them has been reported.

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The second hypothesis is connected to the fact that queens only mate before the start of reproduction (Bourke and Franks 1995), usually on a single nuptial flight. The number of sperm stored during the mating flight thus places an upper limit on a queen's maximum lifetime output of fertilised eggs (e.g. Tschinkel 1987), and may constrain the size of her worker force and, eventually, the number of reproductive offspring produced. It was therefore hypothesised that ant queens mate with several males in order to obtain larger sperm stores (Hamilton 1964; Cole 1983). The hypothesis was criticised by Crozier and Page (1985) who argued that males should be selected to have enough sperm to satisfy the sperm requirements of a queen. However, this criticism cannot *a priori* invalidate the "mating-for-sperm" hypothesis, since male sperm content might be constrained by selection on other components of male fitness (Fjerdingstad and Boomsma 1997), and since males may parcel out their sperm between different queens (Boomsma 1996; Reichardt and Wheeler 1996; Boomsma and Sundström 1998).

Across ant species with single-queen colonies, the number of mates per queen is positively correlated with the species-specific size of mature colonies (number of workers) (Cole 1983; Boomsma and Ratnieks 1996), as would be expected under the "mating-for-sperm" hypothesis. However, no test has been made of whether individual ant queens actually gain more sperm by mating multiply or whether queens are likely to live long enough to make use of any such increased sperm stores. In this paper we present the first direct test of the "multiple-mating-for-more-sperm" hypothesis to consider these aspects.

As test species we used the Panamanian leafcutter ant *Atta colombica*. Our earlier work has shown that queens of this species mate with a high and variable number of males (average 3, range 1–5) (Fjerdingstad et al. 1998) and store very large numbers of sperm (previously reported range: 57–255 million) (Fjerdingstad and Boomsma 1997). The variation among queens in the numbers of sperm stored is substantial (coefficient of variation, CV = 0.42) (Fjerdingstad and Boomsma 1997) and much larger than in an obligatorily single mating ant species, *Solenopsis invicta*: (CV = 0.08) (Glancey and Lofgren, 1985). Whereas mortality of young *A. colombica* colonies is very high, successfully founded colonies grow to contain millions of workers (queen offspring), are longlived (10–16 years) and may have a reproductive output of tens of thousands of new queens (Weber 1972; Fowler et al. 1986; normally ant males arise from unfertilised eggs and thus require no expenditure of sperm by the queen, review in Cook 1993). Each colony contains only a single mother queen and all daughter queens disperse to mate and found colonies independently (Weber 1972; Fjerdingstad et al. 1997). Therefore, in *Atta colombica*, a queen's colony and all its resources will be lost if she runs out of sperm.

Methods

We examined 22 *A. colombica* queens and samples of their worker offspring (mean 13, range 8–18) from 22 young, established colonies in a natural population near Gamboa in Panama. The number of sperm stored by individual queens was assessed by dissecting out the spermathecae (sperm storage organs) and applying cell count chamber techniques (Fjerdingstad and Boomsma 1997). Queens and samples of their offspring were genotyped at two hypervariable dinucleotide microsatellite DNA loci for which markers had previously been developed (Etta5-6TF, Etta7-8TF; Fjerdingstad et al. 1998). Sperm and genotype data on 5 of the 22 colonies were obtained specifically for this study. The data for the other 17 colonies were gathered in another context in two previous separate studies (Fjerdingstad and Boomsma 1997; Fjerdingstad et al. 1998), but are analysed together for the first time here.

The number of mates per queen was estimated by reconstructing paternal genotypes on the basis of queen and worker offspring genotypes (Fjerdingstad et al. 1998). This is straightforward due to the haploidy of ant males since, for each locus, a male gives the same allele to all of his offspring. We also assessed the effective number of mates per queen. This parameter takes into account not only the absolute number of mates per queen but also any variation (paternity skew) in the proportional representation of the mates among the queen's offspring (Starr 1984). We applied the probabilistic correction of Pamilo (1993) to adjust the effective mate numbers for the effect of limited and varying sample size.

We tested distributions of variables for deviations from normality using Shapiro-Wilk tests (Shapiro and Wilk 1965), and examined data for heteroscedasticity through residual plots. The sample size corrected effective mate numbers were logarithmically transformed to attain normality. No other data distributions deviated from normality. All data were homoscedastic. We therefore used parametric tests (Pearson product moment correlation and linear regression). One-tailed tests were used for examining the prediction of a positive relationship between the number of sperm stored and the number of mates per queen. Although our estimates of sperm store size and queen mating frequency both had error components, we applied a model I regression analysis. This could be done because the aim was to *predict* the number of sperm stored based on the number of mates per queen (Sokal and Rohlf 1995, p. 543).

Results

The dissections and sperm counts showed that, among the 22 young *A. colombica* queens examined here, the average queen had stored 100×10^6 sperm (range: 7–234 million, SD = 56×10^6). Twelve different alleles at Etta5-6TF were found in queens, 14 in queens' mates. Eleven alleles at Etta7-8TF were found in queens and 9 in queens' mates. The observed heterozygosity in the sample of 22 queens was 0.82 for Etta5-6TF and 0.95 for Etta7-8TF. Due to this hypervariability of our genetic markers, the probability of being able to genotypically distinguish (detect) different paternal males was very high: 0.98 (Pamilo 1993; Fjerdingstad et al. 1998; see the latter for tests on the study population of the assumptions behind this error estimation method). This means that our estimate of the number of mates per queen was scarcely affected by genetical non-detection error (Pamilo 1993; Boomsma and Ratnieks 1996).

Our genetic paternity data on queens' offspring showed that the average queen had mated with 2.8 males

(SD = 0.85; range 1–5, mode 3). The relationship between the number of sperm stored per queen and the number of mates per queen was positive but not significant ($r = 0.35$, $P = 0.06$) (Fig. 1). A small part of the variation in the detected number of mates per queen ($r^2 = 0.16$, $P = 0.03$) (Fig. 2) was due to variation in the number of offspring analysed for each queen i.e. due to error arising from finite and varying sample size (non-sampling error; Pamilo 1993; Boomsma and Ratnieks 1996). For the range of sample sizes used here (8–18) this error could be adjusted for by using the residual variation in number of mates (Fig. 2) instead of the detected number of mates.

Using these residuals, we found that the number of sperm stored by individual queens was significantly positively correlated with the number of mates per queen (Fig. 3). *Atta colombica* queens which had mated with more than one male stored more sperm, and gained *c*. 29 million sperm per average additional mate. The number of sperm stored was also positively correlated with the logarithm of the effective number of mates (figure 4).

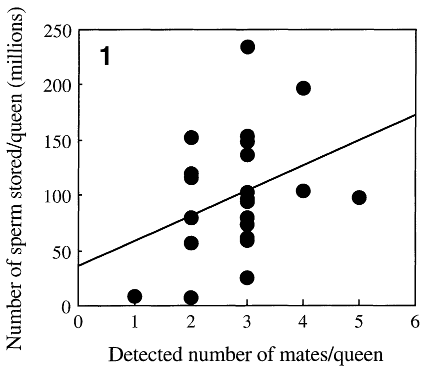

Fig. 1 Number of sperm stored per *Atta colombica* queen in relation to the number of mates detected per queen: $r = 0.35$, $P = 0.06$, NS. The least squares regression is $y = 36 \times 10^6 + 23 \times 10^6\ x$, where y = number of sperm stored and x = the number of mates detected

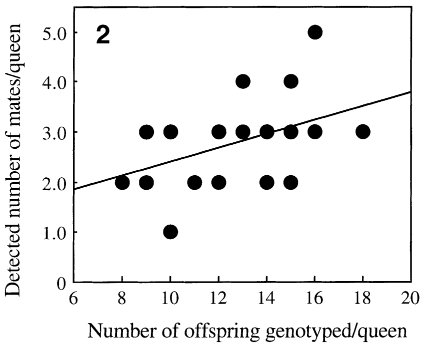

Fig. 2 Number of mates detected per queen as a function of the number of worker offspring genotyped per queen; $r = 0.41$, $P = 0.03$. The least squares regression is $y = 0.98 + 0.14\ x$, where y = number of mates, and x = number of offspring genotyped

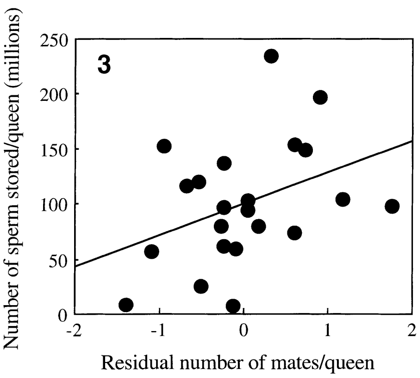

Fig. 3 Number of sperm stored in relation to the residual number of mates per queen (Fig. 2): $r = 0.39$, $P = 0.04$, one-tailed t-test. The least squares regression is: $y = 100 \times 10^6 + 29 \times 10^6\ x$, where y = number of sperm and x = residual number of mates per queen

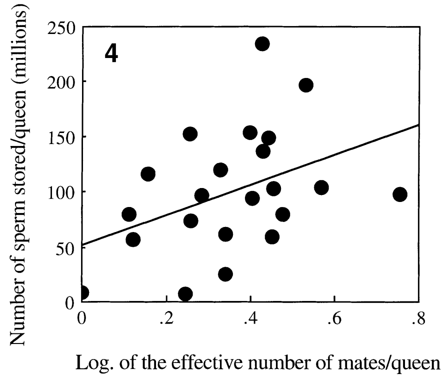

Fig. 4 Number of sperm stored in relation to the logarithm of the effective number of mates per queen: $r = 0.41$, $P = 0.03$. The least squares regression is: $y = 52 + 135 \log x$, where y = number of sperm and x = sample size adjusted effective number of mates per queen

Discussion

Our study showed that *A. colombica* queens obtain larger sperm stores when they mate with more males (Fig. 3, 4). This finding is based on analysis of mate number estimates adjusted for bias due to unequal sample offspring sizes. However even without correcting for limited and varying sample sizes, a positive linear relationship between the number of sperm and the number of mates was found and was close to being significant (Fig. 1).

The average sperm gain from the first mating of *Atta colombica* queens was c. 60 million (Fig. 3), whereas the gain from additional matings was *c*. 30 million. As the sperm complement of an average *A. colombica* male in the Gamboa population was 100×10^6 (Fjerdingstad and Boomsma 1997), this corresponds to, respectively, 60% and 30% of the sperm that an average unmated male has. Assuming that *A. colombica* males mate only once, this would imply a sperm storage efficiency similar to the one found for the obligatorily single mating ant *Solenopsis invicta* (65%) (Glancey and Lofgren 1985). The decline in sperm storage efficiency of *A. colombica* queens with additional matings may be due to limitations in the capacity of the spermatheca, but polynomial regression analysis (result not shown here) found no significant curvilinear component (Fig. 3). Alternatively males may be mating multiply as well and parcelling out their sperm between several queens as was found for another leafcutter ant by Reichardt and Wheeler (1996).

Skew in the representation of the different mates (paternity skew) of a multiply mated queen has been found in a considerable number of ant species (review in Boomsma and Ratnieks 1996). Such skew may result if the different mates of a queen are not equally succesful in transferring sperm to the queen or might arise later due to intra-spermathecal events such as differential sperm death. The number of sperm stored by a queen should then be more closely related to the effective number of mates than to the absolute number of mates. Genetic paternity skew is, however, rather low in *Atta colombica* (Fjerdingstad et al. 1998) and the effective number of mates per queen explained hardly more of the variance in the number of sperm, than did the absolute number of mates (cf. Figs. 3 and 4).

A considerable amount (83–85%) of the variance in the number of sperm stored per queen could not be explained by the linear relationship with either the effective or the absolute number of mates (Fig. 4 and 3, respectively). Part of the scatter in Figs. 3 and 4 may, however, be artifactual. Estimating the number of sperm stored by queens involves some error since sperm may clump (but see Fjerdingstad and Boomsma 1997, for statistical testing of the reliability of sperm counts). Furthermore, the queens examined in this study did not all come from the same age cohort. Variation in the number of sperm stored by queens of different cohorts has been found in *A. texana* (Moser 1967) and might be due to stochastic year-to-year variation in the sperm content of males or in the length of nuptial flights. Our earlier work (Fjerdingstad and Boomsma 1997), moreover, has shown that the number of sperm produced and carried by individual males varies tenfold in the Gamboa population of *A. colombica*. Therefore the sperm gains per mate may vary considerably, especially if some males also mate multiply.

To assess whether obtaining larger sperm stores through multiple mating is likely to allow *A. colombica* queens a longer reproductive lifespan in nature, we have approximated the sperm expenditure of an *A. colombica* queen on the basis of life history data on this and other *Atta* species. In natural populations, *Atta* colonies that survive the founding stage may live for 10–16 years and may contain 2.5–5 million workers when mature (Weber 1972; Fowler et al. 1986). For a conservative approximation of the sperm requirements of an average queen we assume: (a) a queen lifespan of 10 years; (b) that 3–10 sperm are needed to fertilise the number of eggs required to produce one viable, adult worker offspring (estimated from data on the fire ant *Solenopsis invicta* and the honeybee *Apis mellifera*; Tschinkel and Porter 1988); (c) that the average life span of a worker in the wild is 6 months (average *A. sexdens* worker longevity has been estimated as 4 months in the protected laboratory environment, but *A. colombica* forager worker longevity has been estimated to be as short as 2–3 weeks; review in Fowler et al. 1986); (d) that colony growth is logistic (Fowler et al. 1986) with an average colony size of 5000 workers in the 1st year, 150,000 in the 2nd year, 500,000 in the 3rd year, 1 million in the 4th year, and 2 million during the next 6 years; (e) that a colony matures at an age of 5 years; and (f) that such a mature colony will produce 500–5,000 new queens per year (Fowler et al. 1986), requiring the same number of sperm per egg as do workers (male ants arise from unfertilised eggs, review in Cook 1993; and so do not require the expenditure of sperm by their mother). The resulting conservative estimate is that a queen which lives and reproduces for 10 years would need at least 80–270 million sperm.

In our population, queens would on average have to mate with at least two males to avoid having too few sperm for such a lifespan of 10 years (a residual of −0.7 in Fig. 3 is approximately equivalent to two mates and would ensure a sperm supply of *c.* 80 million). The available life history data therefore indicate that, in natural populations, the number of sperm stored by an *A. colombica* queen is likely to constrain her reproductive lifespan and hence output. This implies that multiple mating may be an adaptive strategy for *A. colombica* queens in spite of mortality costs.

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