

Daniel J. C. Kronauer · Stefanie M. Berghoff ·  
Scott Powell · A. Jay Denny ·  
Keith J. Edwards · Nigel R. Franks ·  
Jacobus J. Boomsma

## A reassessment of the mating system characteristics of the army ant *Eciton burchellii*

Received: 14 February 2006 / Accepted: 9 April 2006 / Published online: 5 May 2006  
© Springer-Verlag 2006

**Abstract** In a recent study, Denny et al. (2004a) showed that queens of the army ant, *Eciton burchellii*, mate with multiple males and presented estimates suggesting that they mate with more males than queens of any other ant species so far investigated. They also inferred that data were consistent with queens being inseminated repeatedly throughout their life, which would be exceptional among the social Hymenoptera and contradictory to predictions from kin selection theory. In the present study, we reanalyze these data using new software and supplement them with similar microsatellite data from other colonies of the same species. Mating frequencies in *E. burchellii* are indeed very high (mean observed and effective queen-mating frequencies of 12.9 each) but considerably lower than the previous estimates. We show that the number of patrilines represented in the first worker offspring of a young queen is lower than in older queens but suggest that this may be due to initial sperm clumping in the queen's sperm storage organ, rather than to repeated inseminations. Moreover, we found no evidence for repeated mating by genotyping sequential worker generations produced by a single old queen, showing that she did not obtain new inseminations despite ample opportunities for mating.

### Introduction

Queens of the army ant subfamilies Ecitoninae and Dorylinae have recently been shown to mate with many males (Denny et al. 2004a; Kronauer et al. 2004). This finding has advanced our understanding of the evolution of obligate multiple mating by females (polyandry) in eusocial Hymenoptera because this trait is only found in relatively few taxa (Strassmann 2001). However, these rapidly expanding insights into the occurrence of polyandry raise further questions about the quantitative details of army ant mating frequencies, necessitating standardization in analysis of genetic marker data to obtain precise estimates of mean mating frequencies, of confidence limits of these means, and of variation in paternity realized by males mating with the same queen. Only assessments of this accuracy will ultimately allow a deeper understanding of the selective forces that have shaped the mating systems of army ants.

The neotropical army ant, *Eciton burchellii*, was one of the first army ant species for which mating frequencies were estimated (Denny et al. 2004a). However, a later evaluation of the raw data prompted a reanalysis with more sophisticated statistical tools after correcting scoring errors, to see whether the reported very high estimate of effective mating frequency ( $m_e=22.6$ ; the number of equally contributing, unrelated fathers that would have produced the observed average relatedness among workers given as the harmonic mean of the values estimated following Nielsen et al. 2003) would be robust.

In the present study, we offer an analysis using the program MATESOFT (Moilanen et al. 2004), which was specifically designed to analyze complex data sets on multiple paternity. This allows an explicit quantitative comparison of queen mating frequencies of *E. burchellii* and other army ants, which were analyzed in the same way before (Kronauer et al. 2004, 2006). In addition, our study provides the first explicit genetic-marker test of the hypothesis that army ant queens mate repeatedly later in life (Raignier and van Boven 1955; Rettenmeyer 1963; Denny et al. 2004a).

---

D. J. C. Kronauer (✉) · J. J. Boomsma  
Institute of Biology, Department of Population Biology,  
University of Copenhagen,  
Universitetsparken 15,  
2100 Copenhagen, Denmark  
e-mail: djckronauer@bi.ku.dk  
Tel.: +45-353-21280  
Fax: +45-353-21250

S. M. Berghoff · S. Powell · A. J. Denny ·  
K. J. Edwards · N. R. Franks  
School of Biological Sciences, University of Bristol,  
Woodlands Road,  
Bristol, BS8 1UG, United Kingdom

S. Powell  
Universidade Federal de Uberlândia, Instituto de Biologia,  
Campus Umuarama Bloco 2D,  
38400-902 Uberlândia MG, Brasil

## Materials and methods

### Sample collection and genotyping

We reviewed the original data matrix and chromatogram files of Denny et al. (2004a); (Table 1) and scrutinized them according to the criteria of Kronauer et al. (2004). We greatly expanded this data set by analyzing similar samples of workers from 26 additional colonies that were sampled at four different sites in the Panama Canal area (9°10' N, 79°51' W) and were stored in 96% ethanol (Table 1). A total of 692 new workers were genotyped for the same eight microsatellite loci as in Denny et al. (2004a,b).

To specifically address the possibility of old queens mating repeatedly, we genotyped an additional 161 old and 72 young workers from colony E (Denny et al. 2004a) for four of these loci (Eb24, Eb14, Eb51, Eb42) and a subset of these for two additional loci (Eb10, Eb25). This colony had undergone a recent fission with the old queen being retained by one of the two daughter colonies. Males from foreign colonies (approximately 10–20 and identifiable because they had lost their wings) were observed in both the colony with the old queen (colony E) and the colony with the new queen (colony F) shortly after colony fission, and therefore, potentially had the opportunity to copulate with the respective queen. We thus genotyped a total of 201 old workers that had been produced by the mother queen before colony fission and before laying the reproductive brood (90 and 111 of which had accompanied the old and the young queen, respectively) and 92 young workers that the old queen had produced after colony fission and after the males had entered her colony during the subsequent nomadic phase. If any of these males had inseminated the old queen, we should be likely to detect new patrilineages among the young worker offspring. Likewise, the males that inseminated the queen during the two nomadic phases preceding colony fission should also be detected.

### Data analysis

The analysis of the data largely followed Kronauer et al. (2004). The data was partitioned into four different data sets (BCI, PLR, GIG, CHA), one for each sampled site (Table 1).

In a first step, we deduced the genotypes of the queen and her mates for all colonies with the computer program MATESOFT (Moilanen et al. 2004), after entering all known alleles at equal frequencies. The inferred queen genotypes were then duplicated and the inferred male genotypes “diploidized,” so that alternative father genotypes could be entered as heterozygotes. We then obtained an unbiased estimate of the real allele frequencies with the program FSTAT version 2.9.3.2 (Goudet 2002).

In a second step, we replaced the original uninformative background allele frequencies with the unbiased new estimates and ran the final analyses of mating frequency statistics in MATESOFT (the exception was the single

**Table 1** Observed ( $k_{\text{obs}}$ ) and effective ( $m_e$ ; Nielsen et al. 2003) mating frequency estimates for *Eciton burchellii* queens from the Panama Canal area

Site	Colony	Sample size	$k_{\text{obs}}$	$m_e$
BCI	A	20	13	17.4
BCI	B	20	10	10.6
BCI	C	20	13	17.4
BCI	D	20	14	23.9
BCI	E	287	17	12.4
BCI	F <sup>a</sup>	20	6	4.4
BCI	E59	24	11	10.7
BCI	E62	24	16	23.1
BCI	E68	24	9	9.6
BCI	E73	24	14	21.3
BCI	E74	24	11	14.3
BCI	E78	23	11	12.0
BCI	E79	23	11	12.1
BCI	E80	24	13	14.2
PLR	E08	40	20	28.1
PLR	E09	24	15	25.2
PLR	E10	24	12.1	13.2
PLR	E16	24	12	11.5
PLR	E17	24	14	19.2
PLR	E19A	16	9	10.1
PLR	E22	18	11	11.0
GIG	E27	30	15	14.5
GIG	E29	48	18	17.2
GIG	E38	24	13	16.3
GIG	E39	23	9	7.1
GIG	E40	24	13	12.6
GIG	E44	23	8	8.2
GIG	E45	24	15	23.1
GIG	E48	48	18.7	17.4
GIG	E50	24	11	12.4
GIG	E55	24	15	15.4
CHA	E18	24	9	4.5
Overall	31	816	12.9±0.5	12.9±1.1

Sites are Barro Colorado Island (BCI), Pipeline Road (PLR), Gigante (GIG) and Charco (CHA). Overall estimates are given as the arithmetic and harmonic mean for  $k_{\text{obs}}$  and  $m_e$ , respectively, with standard errors from jackknifing over colonies and do not include the young queen of colony F. For colonies A, B, C, D, E, and F, the revised estimates based on the data in Denny et al. (2004a) are given

<sup>a</sup>The genotypes of colony F could be explained by two nearly equally likely queens (*alt. p*=0.52 and 0.48, respectively). However, only the second alternative queen could have been a daughter of the queen of colony E and one of her recognized mates. The second alternative was, thus, assumed to be the correct queen genotype

colony sample from the CHA site, where we used uninformative allele frequencies throughout). When several alternative queen mothers could account for the offspring data, mating frequency estimates were averaged over alternative queens, weighting each estimate by the probability of the respective queen genotype determined in MATESOFT.

Estimates of average regression relatedness between individuals ( $r$ ) were calculated with the computer program RELATEDNESS version 5.0.8 (Goodnight and Queller 1998) using the algorithms of Queller and Goodnight (1989).

## Results

Eight worker genotypes each had to be removed from the newly sampled colonies E08 and E19A, because they were not compatible with a single queen having produced all offspring. The combined data set of 31 colonies of *E. burchellii* gave an average observed mating frequency of  $12.9 \pm 0.5$  (arithmetic mean  $\pm$  SE) and an effective mating frequency (Nielsen et al. 2003) of  $12.9 \pm 1.1$  (harmonic mean  $\pm$  SE).

The average effective mating frequency on Barro Colorado Island (BCI) ( $n=13$  colonies) was not significantly different from that on the three mainland sites ( $n=18$ ; two-sided  $t$  test;  $P=0.81$ ).

Both the observed and effective mating frequencies of the single young queen of colony F were lower than that of any other of the 31 queens (only the effective queen mating frequency of colony E18 was nearly as low) and were outside the 95% CI around the mean in both cases (reciprocals in case of  $m_e$ ). This phenomenon has already been recognized by Denny et al. (2004a).

Among the newly genotyped workers of colony E, four individuals could not be explained as offspring of the queen (they are most easily explained by 2-bp insertions in the maternal gamete at locus Eb14, in one case, and Eb24, in three cases) and were excluded from the data set. Two additional individuals (one of the old and one of the young workers) represented a unique patriline each. In each case, these would have been identical to an existing patriline if we assumed a 2-bp deletion at locus Eb14 and Eb51, respectively. Given the large number of samples from this colony and the apparently high mutation rate of some of the loci used, it seemed most appropriate to exclude these two individuals as well. After revising the data file from Denny et al. (2004a) and combining it with the additional worker genotypes, we detected a total of 17 patrilines. All of these were represented in the workers that had been produced by the queen before the colony fission ( $N=198$  workers), and no new patrilines were detected in the workers produced after the fission event ( $N=89$ ).

The young queen (F) belonged to a patriline of normal frequency in the mother colony (0.087 among all worker offspring genotyped), and the observed frequency of that patriline was slightly higher among workers that accompanied the old queen (0.102) than among those that accompanied the young queen (0.089). The average relatedness of workers to the new queen was not higher for workers that accompanied her after the fission ( $r=0.42$ ) than for those that stayed with the old queen ( $r=0.41$ ;  $P=0.45$ ; two-sided paired  $t$  test over all loci) or the average relatedness between workers in the colony ( $r=0.40$ ;  $P=0.65$ ; two-sided paired  $t$  test over all loci). There is,

thus, no indication of nepotistic discrimination between full sisters vs half sisters during colony fission.

## Discussion

Our data show that the earlier mating frequency estimates for *E. burchellii* (Denny et al. 2004a), were overestimations. Our present findings indicate that the mating frequency of *E. burchellii* queens is still one of the highest known for any eusocial hymenopteran species but similar to estimates for other army ants produced by the same methods (Kronauer et al. 2004, 2006).

While mating frequencies in honeybees can be considerably lower on islands where mating conditions may be less favorable (Neumann et al. 1999), we did not detect such an effect in the colonies from BCI. This means that queens on BCI are not limited by mating opportunities. Such a constraint could have been expected for small and potentially isolated populations (the *E. burchellii* population on BCI has been estimated to consist of approximately 50 colonies (Franks 1982)), if for example, flying males rarely or never disperse over the waters of the Panama Canal.

Worker nepotism towards full-sister queens during colony reproduction appears to be negligible in honeybees both at the stage when the leading queens are chosen (e.g., Chaline et al. 2005; Tarpy et al. 2004) and during swarming (Kryger and Moritz 1997), probably because nepotism is strongly selected against at the colony level. Absence of nepotism during colony fission is also in accord with our data on army ants.

The observation by Denny et al. (2004a) that the first sampled progeny of a newly inseminated queen (colony F) contains fewer patrilines than the average progeny of old queens was upheld. However, this finding might be explained by initial sperm clumping in the queen's sperm storage organ (spermatheca) and not by a lower mating frequency. This inference is supported by dissections of six newly mated callow *Eciton* queens by Whelden (1963), who found that spermatheca sperm was clumped into a number of discrete balls. On the other hand, he found a single homogeneous mass of sperm in most of the ca. 40 older queens that he dissected, or in a few cases, many small balls of sperm embedded in a homogeneous mass. This seems to confirm that sperm are initially transferred in packages and require time to mix. A similar effect is found in honeybees, where the contributions of different fathers to the offspring become less variable over time because sperm gradually become mixed in the spermatheca (Franck et al. 1999, 2002).

Whelden (1963) also found plugs in the posterior part of the spermathecal duct in three callow *Eciton* queens that had been recently inseminated, while such plugs were never found in old queens. These plugs are most likely mating plugs, which are also produced in other eusocial Hymenoptera (Boomsma et al. 2005) and provide further evidence that the old queens had not been recently inseminated. The function of these plugs is not known, but they obviously do not keep the queen from mating with

many males because the mating frequency is very high (Denny et al. 2004a; this study). They could, however, be analogues to mating plugs in honeybees, which also have a derived function (Woyciechowski et al. 1994).

While single foreign males can sometimes be found in colonies of *E. burchellii* (Rettenmeyer 1963; Schneirla 1971), the acceptance of many males may occur only in the period shortly after a fission event (based on the observation described from the colony studied here vs observations of many colonies that had not undergone fission recently by SMB, NRF, DJCK, and SP). This implies that old queens might have their best opportunity to mate again during a brief period after colony fission. Although tens of males had entered colony E, which contained the old queen after colony fission, we found no evidence that she was inseminated by any of these males. We admit that clumping of the hypothetical stored sperm from new matings might have reduced our detection power. However, our sample size of young workers is fairly large, sperm clumping is not complete as is evident from the ability of young queens to use sperm shortly after insemination, and sperm from recent copulations should be numerically overrepresented in the spermatheca because sperm from former mates has already been partially used up. It, therefore, seems unlikely that contributions from new males, if, in fact, present as sperm in the spermatheca, simply were not represented as new patriline in our sample of young worker offspring.

This leaves the question why dispersing males from other colonies are accepted into colonies that contain an old queen after fission, if she is not willing or allowed to mate? One explanation could be that it is the presence of brother males in both colonies for some time after the fission that suppresses aggressive behavior of workers also towards foreign males, so that these are accepted initially into both colonies, irrespective of whether the queen is receptive or not. What happens once the males get close to the resident queen is not known, but our results suggest that successful mating must be prevented by the workers close to the queen or the queen herself.

Although it has been suggested repeatedly that army ants may be exceptional among the eusocial Hymenoptera in that queens are inseminated repeatedly throughout their lives (Rettenmeyer 1963; Schneirla 1971; Raignier and van Boven 1955; Denny et al. 2004a), we conclude that there is no unequivocal evidence to support that this happens in the field. Serial mating of queens would be difficult to reconcile with kin-selection theory, which predicts that workers should prevent their mother from mating repeatedly to avoid dilution of their relatedness to her future reproductive offspring (Bourke and Franks 1995). We, therefore, propose that repeated queen mating hypotheses are formulated as alternatives that require explicit proof against the null hypothesis of serial mating being absent. Further experimental and dissection work will be needed to finally settle this issue in the army ants.

**Acknowledgements** This work was supported by grants from the Danish Research Training Council (DJCK), the Danish Natural Science Research Council, and the Danish National Research Foundation (JJB), the Leverhulme Trust (F/00 182/A1 to SMB, KJE and NRF), the Natural Environment Research Council (NER/S/A/2001/05997 to SP and NER/B/S/2002/00225 to AJD), and the Smithsonian Tropical Research Institute (SRA award to SMB and Predoctoral award to SP). We also thank the Smithsonian Tropical Research Institute for providing facilities and arranging for ANAM collection and export permits. All research reported in this paper complies with the present laws of Denmark and the UK.

## References

- Boomsma JJ, Baer B, Heinze J (2005) The evolution of male traits in social insects. *Annu Rev Entomol* 50:395–420
- Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, New Jersey
- Chaline N, Martin SJ, Ratnieks FLW (2005) Absence of nepotism toward imprisoned young queens during swarming in the honey bee. *Behav Ecol* 16:403–409
- Denny AJ, Franks NR, Powell S, Edwards KJ (2004a) Exceptionally high levels of multiple mating in an army ant. *Naturwissenschaften* 91:396–399
- Denny AJ, Franks NR, Edwards KJ (2004b) Eight highly polymorphic microsatellite markers for the army ant *Eciton burchellii*. *Mol Ecol Notes* 4:234–236
- Franck P, Coussy H, Le Conte Y, Solignac M, Garnery L, Cornuet JM (1999) Microsatellite analysis of sperm admixture in honeybee. *Insect Mol Biol* 8:419–421
- Franck P, Solignac M, Vautrin D, Cornuet JM, Koeniger G, Koeniger N (2002) Sperm competition and last-male precedence in the honeybee. *Anim Behav* 64:503–509
- Franks NR (1982) A new method for censusing animal populations: the number of *Eciton burchellii* army ant colonies on Barro Colorado Island, Panama. *Oecologia* 52:266–268
- Goodnight KF, Queller DC (1998) Relatedness 5.0.4. Goodnight Software, Houston. Available at <http://gsoft.smu.edu/GSoft.html>
- Goudet J (2002) FSTAT, a program to estimate and test gene diversities and fixation indices 2.9.3.2. Institute of Ecology, University of Lausanne, Lausanne. Available at <http://www.unil.ch/izea/software/fstat.html>
- Kronauer DJC, Schöning C, Pedersen JS, Boomsma JJ, Gadau J (2004) Extreme queen-mating frequency and colony fission in African army ants. *Mol Ecol* 13:2381–2388
- Kronauer DJC, Schöning C, Boomsma JJ (2006) Male parentage in army ants. *Mol Ecol* 15: 1147–1151
- Kryger P, Moritz RFA (1997) Lack of kin recognition in swarming honeybees (*Apis mellifera*). *Behav Ecol Sociobiol* 40:271–276
- Moilanen A, Sundström L, Pedersen JS (2004) MATESOFT: a program for deducing parental genotypes and estimating mating system statistics in haplodiploid species. *Mol Ecol Notes* 4:795–797
- Neumann P, Van Praagh J, Moritz RFA, Dustmann J (1999) Testing reliability of a potential island mating apiary using DNA microsatellites. *Apidologie* 30:257–276
- Nielsen R, Tarpay DR, Reeve HK (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Mol Ecol* 12:3157–3164
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43: 258–275
- Raignier A, van Boven JKA (1955) Etude taxonomique, biologique et biométrique des *Dorylus* du sous-genre *Anomma* (Hymenoptera Formicidae). *Ann Mus R Congo Belg* 2:1–359
- Rettenmeyer CW (1963) Behavioural studies of army ants. *Univ Kans Sci Bull* 44:281–465
- Schneirla TC (1971) Army ants: a study in social organization. Freeman, San Francisco

- Strassmann J (2001) The rarity of multiple mating by females in the social Hymenoptera. *Insectes Soc* 48:1–13
- Tarpy DR, Gilley DC, Seeley TD (2004) Levels of selection in a social insect: a review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement. *Behav Ecol Sociobiol* 55:513–523
- Whelden RM (1963) The anatomy of the adult queen and workers of the army ants *Eciton burchelli* Westwood and *Eciton hamatum* Fabricus. *J NY Entomol Soc* 71:158–178
- Woyciechowski M, Kabat L, Krol E (1994) The function of the mating sign in honey-bees, *Apis mellifera* L—new evidence. *Anim Behav* 47:733–735