

ORIGINAL ARTICLE

Dorte Bekkevoold · Jane Frydenberg
Jacobus J. Boomsma

Multiple mating and facultative polygyny in the Panamanian leafcutter ant *Acromyrmex echinator*

Received: 2 September 1998 / Received in revised form: 3 February 1999 / Accepted: 7 February 1999

Abstract Queen mating frequency of the facultatively polygynous ant *Acromyrmex echinator* was investigated by analysing genetic variation at an (AG)_n repeat microsatellite locus in workers and sexuals of 20 colonies from a single Panamanian population. Thirteen colonies were found to be monogynous, 5 colonies contained multiple queens, whereas the queen number of 2 colonies remained unresolved. Microsatellite genotypes indicated that 12 out of 13 queens were inseminated by multiple males (polyandry). The mean queen mating frequency was 2.53 and the mean genetically effective paternity frequency was 2.23. These values range among the highest found in ants, and the results are in keeping with the high mating frequencies reported for other species of leafcutter ants. Consistent skew in the proportional representation of different patriline within colonies was found, and this remained constant in two consecutive samples of offspring. Dissections showed that all examined queens from multiple-queen colonies were mated egg-layers. The mean relatedness value among nestmate workers in polygynous colonies was lower than that for monogynous colonies. No diploid males were detected in a sample of 70 genotyped males. Worker production of males was detected in one queenless colony. We discuss our findings in relation to known patterns of multiple maternity and paternity in other eusocial Hymenoptera.

Key words Relatedness · Leafcutter ants · Multiple mating · Facultative polygyny

Introduction

Relatedness between colony members is a fundamental factor when studying the evolution and maintenance of social behaviour in Hymenoptera in the light of kin selection theory (Hamilton 1964). A tight kin structure with highly related female nestmates has been hypothesised to at least partly explain the origin and differentiation of worker behaviour in eusocial Hymenoptera. Colony-level variation in relatedness is also expected to influence decisions made by workers regarding sex ratio allocation (Boomsma and Grafen 1990) and the maternity of male offspring (queen vs worker produced) raised (Ratnieks 1988). The two primary factors affecting nestmate relatedness in the eusocial Hymenoptera are (1) the number and relatedness of queens sharing reproduction in the colony and (2) the number and relative contributions of haploid males to colony offspring (i.e. the genetically effective number of males with which each queen mates prior to colony foundation).

Multiple-queen (polygynous) colonies are found in many species of ants, and have been shown to exert significant alterations in colony kin structure (Ross and Fletcher 1985; Herbers 1993), although cases of functional monogyny in multiple-queen colonies have also been reported (e.g. Heinze 1993). The hypotheses for the evolution of stable polygynous colony structures are numerous, and have predominantly been based on kin selection modified by specific ecological constraints (reviews in e.g. Herbers 1993; Bourke and Heinze 1994). Stable polygyny normally involves the adoption of related newly inseminated queens into existing colonies (Rissing and Pollock 1988). However, exceptions to this general pattern may occur in some leafcutter ants, in which associations of unrelated foundress queens may remain intact when the colony matures (*Atta texana*: Mintzer and Vinson 1985; *Acromyrmex versicolor*: Rissing et al. 1989).

Like polygyny, multiple mating by queens (polyandry) lowers average nestmate relatedness and is generally

D. Bekkevoold (✉) · J. Frydenberg · J.J. Boomsma
Department of Ecology and Genetics
Institute of Biological Sciences, Ny Munkegade
Building 540, University of Aarhus
DK-8000 Aarhus C, Denmark
e-mail: dorte.bekkevoold@biology.aau.dk
Tel.: +45-89423347; Fax: +45-86127191

considered costly to queens in terms of energy expenditure and predation risk during mating (Bourke and Franks 1995). Multiple mating by queens may be an adaptive trait if singly mated queens risk eventual sperm depletion (Cole 1983; Fjerdingstad and Boomsma 1998), or if colonies with genetically more diverse offspring have a selective advantage (recently reviewed by Boomsma and Ratnieks 1996). Hypotheses of the latter type fall into four general subcategories, where fitness advantages to multiply mated queens accrue by (1) a genetically more diverse worker force with a more efficient division of labour (Crozier and Page 1985; Page and Robinson 1991), (2) a genetically more diverse worker force that is more resistant to infectious diseases (Hamilton 1987; Sherman et al. 1988; Shykoff and Schmid-Hempel 1991; Schmid-Hempel 1994), (3) reduced colony-level genetic load due to production of diploid (sterile) males (Crozier and Page 1985; Pamilo et al. 1994), and (4) reduced worker-queen conflict over sex allocation ratio and male parentage (Starr 1984; Moritz 1985; Queller 1993; Moritz et al. 1995; Ratnieks and Boomsma 1995). A considerable number of ant species have been reported to mate multiply, but most of these reports have been observations of multiple copulations, whereas documented cases of high effective queen mating frequencies in ants are rare (see Boomsma and Ratnieks 1996 for a review). Ant species or populations where both polyandry and polygyny significantly reduce relatedness among nestmate workers have so far only been reported in *Formica* (Pamilo 1993; Sundström 1993).

This study describes the results of an investigation of parentage in both field- and laboratory-raised workers of the Panamanian leafcutter ant *Acromyrmex echinator*, originally described as a form of *Acromyrmex octospinosus* (Wheeler 1937) but recently elevated to species status (Schultz et al. 1998). The examined population of *A. echinator* contained both monogynous and polygynous colonies, and we show that polygyny is likely to reduce relatedness among nestmate workers. Our study further demonstrates that queens mate multiply, resulting in effective paternity frequencies substantially higher than those reported for most other multiply mated species of ants. We conclude that this combination of traits makes *A. echinator* highly suitable for future detailed tests of hypotheses for the evolution and maintenance of multiple queen mating in ants.

Methods

Field collections and sampling

Twenty colonies of *A. echinator* were dug up in a remnant of secondary-growth forest in Gamboa, Panama in April–May 1993 and 1994, and January–February 1996. In 7 of these colonies, all queens, alates (winged females and males) and samples of workers were collected, frozen and later stored at -70°C . In 13 other colonies, all queens were collected alive along with a number of workers and fragments of their symbiotic fungus. Additional

samples of workers from these colonies were collected and frozen immediately. The live colonies were transported back to Aarhus and maintained in the laboratory at a constant temperature of 25°C and a relative humidity of 60–70% in the rearing room (humidity in the fungus garden close to 100%) so that new broods of workers and alates could be collected over the following years. Seven of the 20 colonies were affected by the presence of a newly discovered social parasite, *Acromyrmex insinuator* (Schultz et al. 1998). In these colonies, most or all sexual offspring collected were the progeny of one or several parasite queens, while most of the workers (including all large workers analysed in this study) were daughters of the host queen. A detailed account of the impact of this social parasite will be reported elsewhere.

The queens from two polygynous (two queens per colony) laboratory colonies were dissected to elucidate their reproductive status. All four queens had enlarged spermathecae and considerable ovarian development with dark yellow bodies (corpora lutea), showing that all were egg-layers. At the time of dissection, the colonies of these queens had been kept at mature size in the laboratory for 4 and 5 years, respectively. No other queens from polygynous colonies were available for dissection for this experiment, but based on the observations from these two colonies it was assumed that polygyny in multiple-queen colonies of *A. echinator* is functional.

Molecular methods

Molecular data were obtained from field-collected workers from each of the 20 colonies. In addition, samples of workers from 11 of the 13 colonies brought into the laboratory were analysed 6–36 months (mean \pm SD = 24 ± 15 months) after transfer. From each of the colonies sampled and frozen in the field, the genotypes of 15 workers, the queen(s), 5 males, and 5 females (when available) were analysed. From each colony kept in the laboratory, 25–30 workers were collected for analysis, along with 15 workers collected in the field and 5 alates of each sex (when available).

DNA extractions from individual ants were carried out according to the CTAB procedure following Doyle and Doyle (1987). Genetic DNA polymorphism was investigated with the microsatellite primer pair Etta5–6TF designed by Fjerdingstad et al. (1998). The primers were originally designed to amplify microsatellite DNA from the leafcutter ant *Atta colombica*, but proved to amplify polymorphic DNA fragments in *A. echinator* as well. The primers were designed to match sequences flanking a core sequence of (AG)_n repeats, and amplified DNA products of 100–200 bp in length.

For each individual ant, PCR was carried out by setting up 6- μl reactions with 2 pmol of each primer (one primer being end-labelled with γ -³³P-dATP), 0.2 mM dTTP, 0.2 mM dGTP, 0.2 mM dCTP, 0.2 mM dATP, 0.24 units DynAZyme II DNA polymerase and 1 μl of template DNA solution (~ 3 ng DNA/reaction). The DNA was amplified in a Peltier Thermal Cycler, programmed to denature at 94°C for 3 min followed by 40 cycles of 40 s at 94°C , 40 s at 58°C , 40 s at 72°C . The last elongation step was lengthened to 10 min. The amplification products were diluted with 2.5 μl formamide loading dye, denatured by heating to 95°C and thereafter separated electrophoretically on 6% denaturing polyacrylamide gels (0.2 mm) run at 58 W for 2 h. Radiograph films were exposed to the dried gels, developed and the genotype of each individual scored. Radiographs of the electrophoresis gels showed amplification products of four different sizes (183, 187, 189 and 191 bp), signifying that the locus Etta5–6TF had four segregating alleles in this sample of *A. echinator*.

Analysis of mating frequency

Five of the 20 colonies had coexisting *A. echinator* queens (4 colonies with two, and 1 colony with four queens). In 2 additional colonies, the exact queen number remained unresolved due to inadequate sampling. These 7 colonies were excluded from the analysis of queen mating frequency, since the genetic resolution was

insufficient to distinguish colony offspring produced by one multiply mated queen from those produced by two or more singly mated queens.

The mating frequency of queens (expressed as the number of putative patriline lines observed within a colony) and the parental genotypes were inferred from the observed genotypes of queens and their offspring (Pamilo 1993). When queen genotypes were not available (due to non-destructive sampling), these could be reconstructed, either from worker genotypes ($n = 4$) or on the basis of colony male offspring ($n = 5$) (assuming that workers do not normally lay viable eggs themselves; see Results for a justification of this assumption).

Once the queen genotype was identified, the number of paternal genotypes necessary to explain all observed offspring genotypes could be established. The only shortcomings of this procedure were that multiple mating could not be detected if mates had identical genotypes (Pamilo 1982; Boomsma and Ratnieks 1996), and that some paternal genotypes remained uncertain when a heterozygous queen had diploid offspring with an identical heterozygous genotype (Pamilo 1982). In cases like this, mating frequencies of queens were estimated parsimoniously, so that when there was uncertainty about whether a colony had a heterozygous queen that had mated once, or a homozygous queen mated twice, the former was always assumed. By following this procedure, mating frequency may thus have been underestimated in some cases.

Estimates of effective queen mating frequency (m_e) address the relative contribution of each father to colony offspring. If the queen's mates do not contribute equally to her offspring (paternity skew), the effective mating frequency is lower than the absolute number of males and average relatedness between colony offspring is higher (Starr 1979, 1984; Boomsma and Ratnieks 1996). Effective queen mating frequency was estimated for each colony following Pamilo (1993), firstly as the harmonic-mean mating frequency ($m_{e,p} = 1/\sum p_i^2$, where p_i is the proportional contribution of male i) and secondly corrected for binomial sampling error ($m_{e,y} = 1/[(N\sum p_i^2 - 1)/(N - 1)]$, where N is the number of offspring analysed for each individual colony}. Assuming random mating, and following the notation of Pamilo (1993), the relatedness (g) among nestmates then follows from the relationship $g = 0.25 + 1/2m_e$. This equation gives colony-level relatedness estimates based on pedigree information, inferred via parent-offspring analysis.

An alternative method for analysing queen mating frequency is by estimating the regression relatedness values among female nestmates (b_{ff}) via the identity-by-descent method (Queller and Goodnight 1989). This method was applied directly to the observed worker genotypes via the computer program Relatedness 4.2c (Goodnight and Queller 1995). Independent estimates of the genetically effective queen mating frequency could thus be produced via the relationship $b_{ff} = 0.25 + 1/2m_e$.

Results

Even with the relatively limited information from a single marker locus with four alleles it was possible to detect multiple mating in 12 of the 13 queens from monogynous colonies. The observed number of patriline lines detected per colony varied between 1 and 4 (Fig. 1) with an arithmetic mean mating frequency of 2.53. Estimates of effective queen mating frequency ($m_{e,p}$) gave an average of 2.09. When corrected for sampling error ($m_{e,y}$), this average came out at 2.23 (Table 1).

The observed worker genotypes from field and laboratory samples of the same colony were very similar, indicating that no or very few queens had been missed during field excavations. In all colonies but one (where a

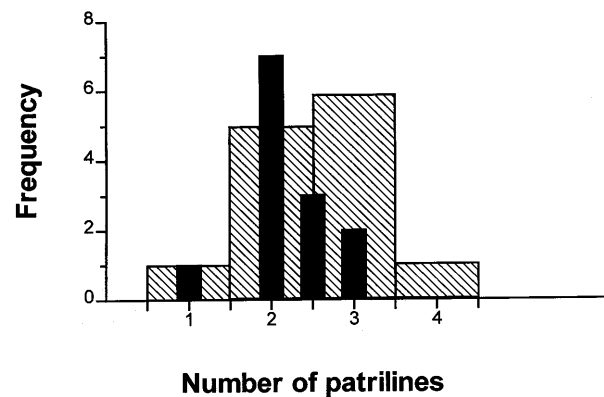


Fig. 1 Observed number of patriline lines (m , hatched bars) and effective number of patriline lines ($m_{e,y}$, black bars) in 13 monogynous colonies of *Acromyrmex echinator*

rare patriline was detected in the field, but not in laboratory-produced offspring), all patriline lines found in the field were also found after transfer to the laboratory. Furthermore, the effective number of patriline lines ($m_{e,p}$, monogynous colonies only) did not differ significantly between field- and laboratory-reared offspring (two-tailed Wilcoxon's signed rank test: $n = 7$, $P = 0.297$). Regression relatedness among nestmates (b_{ff} , all laboratory colonies irrespective of queen number) was also similar for field- and laboratory-collected individuals (two-tailed Wilcoxon's signed rank test: $n = 11$, $P = 0.123$; Fig. 2), so that the genetic data for field- and laboratory-collected offspring were pooled in the subsequent analyses. The average regression relatedness (\pm SE) among nestmate workers over all 20 colonies (in total 571 worker offspring) was 0.347 ± 0.052 . Relatedness estimates per colony on the basis of 9–43 genotyped individuals per colony (mean \pm SD = 28.5 ± 12) varied between 0.108 and 0.718. In spite of the large standard errors associated with these colony-level estimates, the regression relatedness estimates (b_{ff}) for monogynous colonies corresponded relatively well with pedigree relatedness estimates (g) obtained from the estimated effective mating frequencies (one-tailed $r = 0.581$, $P < 0.018$), as did the mean values taken across colonies (Table 1).

Table 1 The mean number of detected patriline lines per colony (m), the harmonic mean effective paternity ($m_{e,p}$) and the sample-size-corrected effective paternity ($m_{e,y}$) based on 13 monogynous colonies of *Acromyrmex echinator*. Also given are the average pedigree relatednesses (g) corresponding to the three estimates of queen mating frequency, and the mean regression relatedness of female offspring (b_{ff}) calculated for the 13 monogynous, and 5 polygynous colonies. Values are the mean \pm SE

	m	$m_{e,p}$	$m_{e,y}$
Mean mating frequency	2.53 ± 0.21	2.09 ± 0.13	2.23 ± 0.14
Range	1–4	1–2.92	1–3.08
Mean colony g	0.45	0.49	0.47
Mean b_{ff} (monogyny)	0.40 ± 0.04		
Mean b_{ff} (polygyny)	0.26 ± 0.05		

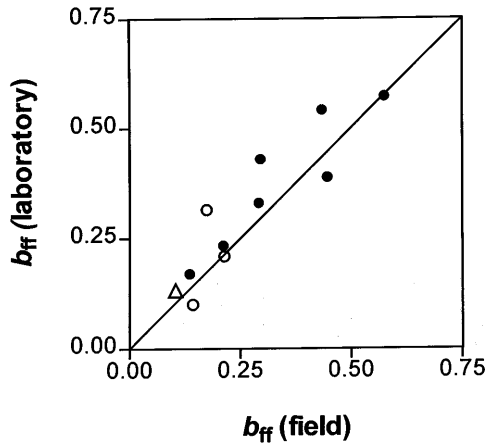


Fig. 2 Relationship between the regression relatedness values (b_{ff}) estimated from worker samples collected in the field and in the laboratory. Monogynous colonies are indicated by closed circles, polygynous colonies by open circles, and one colony with unknown queen number by a triangle

Worker genotype frequencies did not indicate any inbreeding ($F_{IS} = -0.020$, jack-knife SE = 0.053). Compared to the worker allele frequencies, the estimated allele frequencies in putative fathers were overall more uniform (relative frequencies for 571 workers from 20 colonies: 0.072, 0.335, 0.409 and 0.184; and in 34 putative fathers from 13 monogynous colonies: 0.176, 0.265, 0.265 and 0.294), probably indicating that patriline with common genotypes were missed more frequently than those with the rare genotypes. The discrepancy in allele frequencies between putative fathers and offspring could also result from the parsimonious method used for estimating queen mating frequency when the queen genotype was unknown ($n = 4$). The non-detection error of patrilines due to limited variation at the marker locus can be quantified precisely only for populations with single and double queen mating (Pamilo 1982; Pedersen and Boomsma, in press). However, as a crude approximation, one may use the sum of the squared population-wide allele frequencies among males as an average probability for each observed patriline to have been two patrilines carrying an identical father allele (Pamilo 1993; Boomsma and Ratnieks 1996). This average non-detection error was between 26 and 41%, depending on whether the observed allele frequencies among putative fathers or queens were used, respectively, implying that, on average, roughly one queen mate with a significant share in worker paternity was missed in each colony. The distribution of patrilines and the effective paternity in Fig. 1 are therefore both underestimates.

As expected (Pamilo 1982, 1993; Boomsma and Ratnieks 1996), there was a positive association between the number of offspring analysed per colony and the detected number of patrilines (one-tailed $r = 0.527$, $n = 13$, $P < 0.032$), whereas the colony-specific

regression relatedness estimates were independent of sample size (one-tailed $r = -0.190$, $n = 13$, NS). This suggests that, even though the exact mating frequencies of queens were underestimated when sample sizes were small, most of the missing mating types were uncommon and did not greatly affect the estimates of relatedness among nestmates. This is further illustrated by the fact that there appeared to be a consistently skewed distribution of patriline frequencies (Fig. 3), although the sample sizes were too small to test the significance of this skew statistically. The relative frequencies of individual patrilines remained constant between samples of field- and laboratory-reared workers (homogeneity tests of the representation of individual patrilines performed separately for all colonies, $P > 0.1$ for all tests). Extrapolating the skew pattern of Fig. 3 to include the one extra queen mate that was probably missed in each colony, an overall Σp_i^2 estimate of 0.34 was obtained, giving an estimate of $m_{e,y}$ of 3.14 and an estimate for g of 0.41, which was close to the estimate of $b_{ff} = 0.40$ found in monogynous colonies. Note that this inference does not exclude the possibility that more fathers with small contributions to the worker offspring were missed.

Based on the female and male offspring genotyped in this study (7 monogynous and 3 polygynous colonies produced males) none of the 20 colonies could be shown to be effectively polygynous. Still, estimates of nestmate relatedness generated by the identity-by-descent method did show a marginally significant lower average relatedness for colonies with more than one *A. echinator* queen (mean $b_{ff, polygyny} \pm SE = 0.26 \pm 0.05$) than for colonies with a single queen (mean $b_{ff, monogyny} \pm SE = 0.40 \pm 0.04$; one-tailed $t = 2.058$, $df = 16$, $P = 0.056$).

In one queenless laboratory colony, males were produced that differed in genotype from that of the deceased, genotyped queen. The genotype of these males was compatible with one of the worker patrilines, indicating that the males were worker produced. None of the 70 genotyped males was diploid.

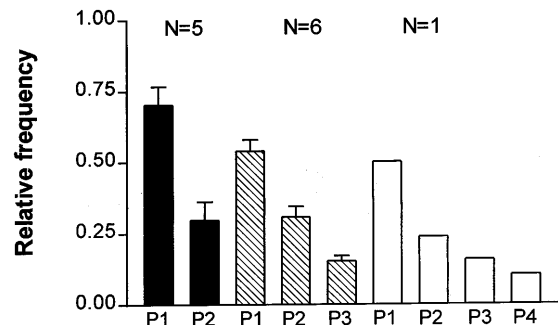


Fig. 3 Mean (+SE) relative frequencies of the most to the least common patrilines ($P1 > P2 > P3 > P4$), in colonies with two observed patrilines (black bars, $n = 5$), three observed patrilines (hatched bars, $n = 6$), and four observed patrilines (open bars, $n = 1$)

Discussion

Multiple queen mating and paternity in ants

The effective queen mating frequencies (m_e) reported in this study are minimum estimates, as a maximum of only four queen mates could be detected with the available molecular tools. The estimate suffers from a non-detection error, resulting in an expectation of detecting approximately two out of three queen mates. The regression relatedness estimates (b_{R}) give some idea of overall colony relatedness patterns within the population of *A. echinator*, but this approach will also only give an accurate estimate of m_e with a much higher number of polymorphic marker loci (Queller and Goodnight 1989). None the less, the correction based on an overall non-detection error of approximately 1/3 produced an estimate of pedigree relatedness (g) similar to the independently estimated average regression relatedness (b_{R}). Even with the relatively large standard errors associated with single-locus estimates of regression relatedness, it could still be shown that within-colony relatedness remained constant over time (Fig. 2).

The m_e values reported here range among the highest reported for any eusocial hymenopteran. Leafcutter ants are generally considered to have high mating frequencies, although earlier estimates were mostly based on observations of copulation or sperm counts (e.g. Moser 1967; Weber 1972). Recently, however, Reichardt and Wheeler (1996) showed that multiple mating is common in the desert leafcutter ant *A. versicolor* by combining sperm counts and genetic paternity analysis of offspring from two colonies. Another study using genetic markers and sperm storage analysis (Fjerdingstad and Boomsma 1997, 1998; Fjerdingstad et al. 1998) showed that m_e is close to 2 in a population of the leafcutter ant *A. colombica* living in the same area as our *A. echinator* population. In another sympatric and exclusively monogynous Panamanian population of *A. octospinosus*, the queen mating frequency was found to be 6.1, with nestmates having an average regression relatedness (\pm SE) of 0.326 ± 0.044 (Boomsma et al. 1999), a value similar to the average relatedness level reported here for *A. echinator* ($t = 0.3101$, $df = 40$, NS).

Comparable genetic marker studies of eusocial bees and wasps have revealed high levels of multiple paternity in several *Apis* honey bees (> 10 mates per queen; Estoup et al. 1994; Moritz et al. 1995; Oldroyd et al. 1997), and in two species of vespine wasps (*Vespa maculifrons*: $m_e = 7.1$; Ross 1986; *V. squamosa*: $m_e = 3.3$; Ross and Carpenter 1991). However, the levels of multiple paternity found in ants have so far been considerably lower. Pamilo (1993) detected up to six fathers per brood in colonies of the highly polygynous *Formica aquilonia*, but the overall variance between patriline contributions within and among colonies was so high that the population-wide m_e did not exceed 1.5. Other genetic mother-offspring comparisons in ants have so far also produced

estimates of m_e below 1.5 (see Boomsma and Ratnieks 1996 for a review).

Genetic diversity and microparasites

According to the genetic diversity hypothesis of Keller and Reeve (1994), queen mating frequency is predicted to correlate negatively with colony queen number across ant species. The hypothesis assumes that high genetic variability has a selective advantage per se, and that polygyny and polyandry can be different evolutionary strategies for maintaining high within-colony genetic diversity. Queens in multiple-queen societies are therefore predicted to avoid costly multiple matings, since the within-colony genetic variation is already high owing to polygyny. The results from our study do not seem to support this hypothesis in general, as effective mating frequencies above two would probably not be expected to occur when polygyny is as common (28%) as in our *A. echinator* population. Nevertheless, it remains possible that future studies will show that queen mating frequency is lower in polygynous than in monogynous colonies of *A. echinator*, or that the across-species differences in (facultative) polygyny between *A. echinator* and *A. octospinosus* are correlated with differences in queen mating frequency.

Ants have unique metapleural glands capable of producing substances with antibiotic activity (e.g. Veal et al. 1992). If microbial pathogens are a major cause of colony mortality, a genetically more diverse worker force may be able to produce a wider spectrum of antimicrobial substances. This effect could be especially significant in leafcutter ants, since these not only need protection from entomopathogens, but also must protect the clonal symbiotic fungus on whose survival they are entirely dependent (Boomsma et al. 1999). Leafcutter ants furthermore display distinct defensive behaviour when inoculated with entomopathogenic fungi (Diehl-Fleig and de Paula Lucchese 1991). In this context it is interesting to note that metapleural glands of individual workers of *A. echinator* and *A. octospinosus* are disproportionately large and variable in size (Bot and Boomsma 1997).

Facultative polygyny and social parasitism

Twenty-eight percent of the colonies in our sample were polygynous. This, along with high degrees of multiple mating, gives a uniquely high variation in colony kin structure not known to exist in single populations of other species of ants, bees or wasps (see Fig. 2). The genetic data presented here could not reveal information about the relatedness among, and the reproductive contributions of queens in polygynous colonies. However, the marginally significant lower nestmate relatedness found in polygynous colonies may imply that at least some coexisting queens are unrelated. In the laboratory, newly mated *A. echinator* queens willingly

initiate colonies in association with other young queens (A.N.M. Bot, personal communication). *Acromyrmex* queens forage for leaves prior to the emergence of the first brood of workers (Weber 1972; Rissing et al. 1989; Diehl-Fleig and de Araújo 1996), and colony initiation success seems to rely heavily on the efficiency with which a queen tends and nourishes her fungus garden (Diehl-Fleig and de Paula Lucchese 1992). Associations of co-founding queens therefore greatly benefit from enhanced provisioning rates during colony foundation, irrespective of relatedness (Rissing et al. 1989). Polygyny by foundress association is common in ants, but in most cases the association becomes unstable as the colony matures (reviewed by Rissing and Pollock 1988). Only one species, the desert leafcutter ant *A. versicolor* (Rissing et al. 1989), has been reported to have a pleometrotic colony structure that is not reduced to monogyny after the emergence of the first workers. Whether the facultatively polygynous colony structure observed in *A. echinator* is the result of such foundress associations or of secondary adoption of daughter queens therefore remains an interesting question for future investigation.

Several authors have described polygyny as a weak case of intraspecific parasitism (Elmes 1973; Buschinger 1990; Bourke and Franks 1991). Furthermore, Hölldobler and Wilson (1990) have pointed out that polygyny may necessitate a relaxation of kin discriminatory abilities, leading to a weaker aggressive response to non-relatives. Polygyny based on colonies normally accepting relatives as extra queens could thus lead to the sympatric evolution of 'parasite' queens that gain entrance to existing colonies irrespective of relatedness (Elmes 1973). Colony residents are expected to evolve counter-discrimination against such parasitic behaviour, but such responses are unlikely to be completely effective. In this context, it is interesting to note that a social parasite, *A. insinator*, closely related to *A. echinator*, can reproduce successfully in colonies of *A. echinator* but not in colonies of the exclusively monogynous and apparently less related congener *A. octospinosus* living in the same locality (Schultz et al. 1998).

Acknowledgements We thank Kirsten Pedersen and Camilla Håkansson for assistance in the laboratory, the Smithsonian Tropical Research Institute for providing working facilities in Panama, and Panama's Instituto Nacional de Recursos Naturales Renovables for permission to sample and export ants. Jes S. Pedersen and Chris Starr gave constructive comments on the manuscript. D.B. was supported by a travel grant from The Obel Foundation, and J.J.B. by several grants from the Carlsberg Foundation and the Danish Natural Science Research Council.

References

- Boomsma JJ, Fjerdingstad, EJ, Frydenberg J (1999) Multiple paternity, relatedness and genetic diversity in *Acromyrmex* leafcutter ants. *Proc R Soc Lond B* 266:249–254
- Boomsma JJ, Grafen A (1990) Intraspecific variation in ant sex ratios and the Trivers-Hare hypothesis. *Evolution* 44:1026–1034
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Proc R Soc Lond B* 351:947–975
- Bot ANM, Boomsma JJ (1997) Variable metapleural gland size-allometries in *Acromyrmex* leafcutter ants (Hymenoptera: Formicidae). *J Kans Entomol Soc* 69[Suppl] 375–385
- Bourke AFG, Franks NR (1991) Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. *Biol J Linn Soc* 43:157–178
- Bourke AFG, Franks NR (1995) *Social evolution in ants*. Princeton University Press, Princeton, NJ
- Bourke AFG, Heinze J (1994) The ecology of communal breeding: the case of multiple-queen leptothoracine ants. *Phil Trans R Soc Lond B* 345:359–372
- Buschinger A (1990) Sympatric speciation and radiative evolution of socially parasitic ants: heretic hypotheses and their factual background. *Z Zool Syst Evol Forsch* 28:241–260
- Cole BJ (1983) Multiple mating and the evolution of social behavior in the Hymenoptera. *Behav Ecol Sociobiol* 12:191–201
- Crozier RH, Page RE (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. *Behav Ecol Sociobiol* 18:105–115
- Diehl-Fleig E, Araújo AM de (1996) Haplometrosis and pleometrosis in the ants *Acromyrmex striatus* (Hymenoptera: Formicidae). *Insectes Soc* 43:47–51
- Diehl-Fleig E, Paula Lucchese ME de (1991) Reações comportamentais de operárias de *Acromyrmex striatus* (Hymenoptera: Formicidae) na presença de fungos entomopatogênicos. *Rev Bras Entomol* 35:101–107
- Diehl-Fleig E, Paula Lucchese ME de (1992) Nest foundation by *Acromyrmex striatus* (Hymenoptera, Formicidae). In: Billen J (ed) *Biology and evolution of social insects*. Leuven University Press, Leuven, pp 51–54
- Doyle JJ, Doyle J (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19: 11–15
- Elmes GW (1973) Observations on the density of queens in natural colonies of *Myrmica rubra* L. (Hymenoptera: Formicidae). *J Anim Ecol* 42:761–771
- Estoup A, Solignac M, Cornuet J-M (1994) Precise assessment of the number of patrines and of genetic relatedness in honeybee colonies. *Proc R Soc Lond B* 258:1–7
- Fjerdingstad EJ, Boomsma JJ (1997) Variation in size and sperm content of sexuals in the leafcutter ant *Atta colombica*. *Insectes Soc* 44:209–218
- Fjerdingstad EJ, Boomsma JJ (1998) Multiple mating increases the sperm stores of *Atta colombica* leafcutter ant queens. *Behav Ecol Sociobiol* 42:257–261
- Fjerdingstad EJ, Boomsma JJ, Thorén P (1998) Multiple paternity in the leafcutter ant *Atta colombica* – a microsatellite DNA study. *Hereditas* 80:118–126
- Goodnight KF, Queller DC (1995) *Relatedness 4.2c*. Goodnight Software, Houston
- Hamilton WD (1964) The genetical evolution of social behaviour. I and II. *J Theor Biol* 7:1–52
- Hamilton WD (1987) Kinship, recognition, disease, and intelligence: constraints of social evolution. In: Itô Y, Brown JL, Kikkawa J (eds) *Animal societies: theories and facts*. Japan Scientific Societies Press, Tokyo, pp 81–102
- Heinze J (1993) Queen-queen interactions in polygynous ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 335–361
- Herbers JM (1993) Ecological determinants of queen number in ants. In: Keller L (ed) *Queen number and sociality in insects*. Oxford University Press, Oxford, pp 236–293
- Hölldobler B, Wilson EO (1990) *The ants*. Springer, Berlin Heidelberg New York
- Keller L, Reeve HK (1994) Genetic variability, queen number, and polyandry in social Hymenoptera. *Evolution* 48:694–704
- Mintzer AC, Vinson SB (1985) Cooperative colony foundation by females of the leafcutting ant *Atta texana* in the laboratory. *J NY Entomol Soc* 93:1047–1051

- Moritz RFA (1985) The effect of multiple mating on the worker-queen conflict in *Apis mellifera*. *Behav Ecol Sociobiol* 16:375–377
- Moritz RFA, Kryger P, Koeniger G, Koeniger N, Estoup A, Tinkeg S (1995) High degree of polyandry in *Apis dorsata* queens detected by DNA microsatellite variability. *Behav Ecol Sociobiol* 37:357–363
- Moser JC (1967) Mating activities of *Atta texana* (Hymenoptera, Formicidae). *Insectes Soc* 3:295–312
- Oldroyd BP, Clifton MJ, Wongsiri S, Rinderer TE, Sylvester HA, Crozier RH (1997) Polyandry in the genus *Apis*, particularly *Apis andreiiformis*. *Behav Ecol Sociobiol* 40:17–26
- Page RE, Robinson GE (1991) The genetics of division of labor in the honey bee. *Behav Ecol Sociobiol* 9:45–50
- Pamilo P (1982) Multiple mating in *Formica* ants. *Hereditas* 97:37–45
- Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity* 70:472–480
- Pamilo P, Sundström L, Fortelius W, Rosengren R (1994) Diploid males and colony-level selection in *Formica* ants. *Ethol Ecol Evol* 6:221–235
- Pedersen JS, Boomsma JJ (in press) Multiple paternity in social Hymenoptera: estimating the effective mate number in single-double mating populations. *Mol Ecol*
- Queller DC (1993) Worker control of sex ratios and selection for extreme multiple mating by queens. *Am Nat* 142:346–351
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43:258–275
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* 132:217–236
- Ratnieks FLW, Boomsma JJ (1995) Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. *Am Nat* 145:969–993
- Reichardt AK, Wheeler DE (1996) Multiple mating in the ant *Acromyrmex versicolor*: a case of female control. *Behav Ecol Sociobiol* 38:219–225
- Rissing SW, Pollock GB (1988) Pleometrosis and polygyny in ants. In: Jeanne RL (ed) *Interindividual behavioral variability in social insects*. Westview, Boulder, Colo, pp 179–221
- Rissing SW, Pollock GB, Higgins MR, Hagen RH, Smith DR (1989) Foraging specialization without relatedness or dominance among co-founding ant queens. *Nature* 338:420–422
- Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. *Nature* 323:798–800
- Ross KG, Carpenter JM (1991) Phylogenetic analysis and the evolution of queen number in eusocial Hymenoptera. *J Evol Ecol* 4:117–130
- Ross KG, Fletcher DJC (1985) Comparative study of genetical and social structure in two forms of the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* 17:349–356
- Schmid-Hempel P (1994) Infection and colony variability in social insects. *Phil Trans R Soc Lond B* 346:313–321
- Schultz TR, Bekkevoeld D, Boomsma JJ (1998) *Acromyrmex insimulador* new species: an incipient social parasite of fungus-growing ants. *Insectes Soc* 45:457–471
- Sherman PW, Seeley TD, Hudson KR (1988) Parasites, pathogens, and polyandry in social hymenoptera. *Am Nat* 131:602–610
- Shykoff JA, Schmid-Hempel P (1991) Parasites and the advantage of genetic variability within social insect colonies. *Proc R Soc Lond B* 243:55–58
- Starr CK (1979) Origin and evolution of insect sociality: a review of modern theory. In: Hermann HR (ed) *Social insects*, vol I. Academic Press, New York, pp 427–464
- Starr CK (1984) Sperm competition, kinship and sociality in the aculeate Hymenoptera. In: Smith RL (ed) *Sperm competition and the evolution of animal mating systems*. Academic Press, Orlando, Fla, pp 428–464
- Sundström L (1993) Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae). *Behav Ecol Sociobiol* 33:345–354
- Veal D, Trimble JE, Beattie AJ (1992) Antimicrobial properties of secretions from the metapleural glands of *Myrmecia gulosa* (the Australian bull ant). *J Appl Bacteriol* 72:188–194
- Weber NA (1972) Gardening ants: the attines. *Mem Am Phil Soc* 92:1–146
- Wheeler WM (1937) *Mosaics and other anomalies among ants*. Harvard University Press, Cambridge, Mass

Communicated by P. Pamilo