

Polygyny and polyandry in small ant societies

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Abstract

Social insects, ants in particular, show considerable variation in queen number and mating frequency resulting in a wide range of social structures. The dynamics of reproductive conflicts in insect societies are directly connected to the colony kin structure, thus, the study of relatedness patterns is essential in order to understand the evolutionary resolution of these conflicts. We studied colony kin structure and mating frequencies in two closely related Neotropical ant species *Pachycondyla inversa* and *Pachycondyla villosa*. These represent interesting model systems because queens found new colonies cooperatively but, unlike many other ant species, they may still co-exist when the colony becomes mature (primary polygyny). By using five specific and highly variable microsatellite markers, we show that in both species queens usually mate with two or more males and that cofounding queens are always unrelated. Polygynous and polyandrous colonies are characterized by a high genetic diversity, with a mean relatedness coefficient among worker nestmates of 0.27 (± 0.03 SE) for *P. inversa* and 0.31 (± 0.05 SE) for *P. villosa*. However, relatedness among workers of the same matriline is high (0.60 \pm 0.03 in *P. inversa*, 0.62 \pm 0.08 in *P. villosa*) since males that mated with the same queen are on average closely related. Hence, we have found a new taxon in social Hymenoptera with high queen-mating frequencies and with intriguing mating and dispersal patterns of the sexuals.

Keywords: colony kin structure, mating frequencies, microsatellites, *Pachycondyla*, polyandry, primary polygyny

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Introduction

Insect societies are typically complex family groups with a significant degree of relatedness among individuals. It is unusual to find very low or even zero-relatedness, where group members are no more closely related to each other than pairs of individuals collected randomly from the population. This situation appears to be restricted to two instances: the first being the so-called unicolonial ants (e.g. Giraud *et al.* 2002), in which the borders between colonies have been lost and individuals can migrate freely between the nests in a population; and the second being founding associations, in which unrelated queens cooperate to start a new society. Such founding associations are temporary in most species and queen numbers are reduced to one after the emergence of the first workers, either through queen-

queen aggression or by the elimination or expulsion of some queens by the workers (reviewed by Bernasconi & Strassmann 1999).

The Neotropical ant species *Pachycondyla inversa* is one of the few species in which cofounding queens survive to colony maturity (Trunzer *et al.* 1998), and relatedness among nestmates may consequently be rather low. In the founding phase, queens associate and establish a social and reproductive dominance hierarchy by aggressive interactions, which leads to a division of labour: dominant queens stay in the nest and care for the brood, while subordinate queens forage for food (Kolmer & Heinze 2000). After workers have emerged, the frequency of aggression among queens decreases, but their original rank orders are still reflected in the maternity of workers (Kolmer *et al.* 2002). Reproduction appears to be more equally partitioned among nestmate queens in larger colonies (Heinze *et al.* 2001). Previous studies with multilocus DNA fingerprinting (banding patterns) and a single microsatellite locus suggested that queens of *P. inversa* are unrelated (Heinze *et al.* 2001). However, the resolution of these markers was not high enough

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to unambiguously decide whether queens are multiple or singly mated. Furthermore, no genetic data were available for the co-occurring sibling species *Pachycondyla villosa*, which shows a similar life history to *P. inversa* (D'Ettorre *et al.* 2005).

The study of the variation in queen-mating frequency in social Hymenoptera has received much attention in the last decades, because of its important effect on the relatedness among offspring and the genetic diversity of colonies (reviews by Bourke & Franks 1995; Boomsma & Ratnieks 1996; Crozier & Fjerdingstad 2001; Strassmann 2001; Fernández-Escudero *et al.* 2002; Baer & Boomsma 2004). However, the co-occurrence of polygyny and polyandry appears to be rare (cf. Pedersen & Boomsma 1999; Schmid-Hempel & Crozier 1999). Keller & Reeve (1994) suggested that, if there was a benefit from increased genetic variability among nestmates, either polygyny or polyandry would be sufficient to achieve such an increase.

We therefore investigated colony kin structure and mating frequencies of these two ant species. Using five microsatellite markers explicitly designed for *Pachycondyla* (Trindl *et al.* 2004), we show that nestmate queens in both species are unrelated and usually mate with more than one male.

Materials and methods

Ant collecting and housing

Colonies of *Pachycondyla inversa* and *Pachycondyla villosa* were collected from rotting cocoa pods and knotholes in an experimental cocoa plantation at Centro de Pesquisas do Cacau, near Itabuna, Bahia, Brazil. The area sampled was of about 300 m × 600 m. At the Regensburg laboratory, ants were housed under near-natural conditions (27 °C and 60% humidity, 12 L: 12 D photoperiod) in plastic boxes (20 cm × 10 cm × 6 cm) with a plaster floor. A chamber (2 cm × 3 cm × 1 cm) in the plaster, covered by a glass plate, served as nest. Colonies were fed diluted honey and pieces of cockroaches or crickets three times a week. The plaster was regularly wetted and a permanent water supply was provided near the feeding area.

Genetic analysis

In total, nine colonies of *P. inversa* and seven colonies of *P. villosa* were analysed using eggs, larvae, and legs of frozen queens and workers (Table 1, the two founding associations had no offspring). Genomic DNA was extracted from each queen and all the individuals present in the colony (some colonies were rather small and had few offspring, e.g. colony i5, see Table 1), using a modified cetyltrimethyl ammonium bromide (CTAB)-method (Sambrook & Russell 2001).

Nine of the *P. inversa* queens, four additional founding queens (x1, x2, x3, x4), two of the *P. villosa* queens and four

Table 1 Composition of the colonies used to study colony kin structure in *Pachycondyla inversa* and *Pachycondyla villosa*. The number of reproducing queens is based on matriline distributions detected by CERVUS 2.0 (Marshall *et al.* 1998) (see also Material and Methods)

<i>P. inversa</i> Colony label	No. of queens present	No. of reproducing queens	No. of offspring genotyped
i1	6	5 [3]	12 (5)
i2	3	2 [1]	21 (3)
i3	3	3 [2]	34 (10)
i4	3	3	13
i5	3	2	9
i6	3	—	f. a.
i7	2	2	21 (8)
i8	1	1 [1]	23* (2)
i9	1	1	22
<i>P. villosa</i> Colony label	No. of queens present	No. of reproducing queens	No. of offspring genotyped
v1	4	3 [2]	18 (7)
v2	2	2	21
v3	2	2	26
v4	2	2 [1]	45** (4)
v5	2	—	f. a.
v6	1	1	23
v7	1	1 [1]	17 (2)

The number of nonassignable offspring to any of the present queens is in parentheses; the minimum number of queens, which had mothered the nonassignable offspring as estimated from their genotype, is in square brackets. f. a., founding association (without offspring); * of which seven are virgin queens; ** of which 12 are virgin queens.

additional founding queens (z1, z2, z3, z4) were dissected under a stereomicroscope and their spermathecae was removed and used to extract and genotype sperm DNA, as described by Chapuisat (Chapuisat 1998). Dissected queens were also genotyped.

Five microsatellite loci (for *P. inversa* with 9–20 alleles per locus and expected heterozygosities between 0.61 and 0.93; for *P. villosa* with 3–18 alleles per locus and expected heterozygosities between 0.63 and 0.87) were amplified and analysed as described by Trindl *et al.* (2004).

Data analysis

Eggs and larvae with homozygous genotypes at all loci are presumably haploid males and were excluded from subsequent analysis (the probability at the colony level, based on allele frequencies, that a diploid individual was homozygote at all loci is 0.0000336 for *P. inversa* and 0.000468 for *P. villosa*).

Table 2 Relatedness among workers in *Pachycondyla inversa* and *Pachycondyla villosa* colonies (colony composition as in Table 1). Relatedness coefficients were assessed using the software RELATEDNESS 5.0.8 (Queller & Goodnight 1994); standard errors (SE) were obtained by jackknifing over loci

	Worker-worker relatedness \pm SE			
	Overall colonies	Monogynous colonies	Polygynous colonies	Within matrilines
<i>P. inversa</i>	0.357 \pm 0.023	0.628 \pm 0.022	0.273 \pm 0.031	0.608 \pm 0.033
<i>P. villosa</i>	0.428 \pm 0.057	0.657 \pm 0.066	0.310 \pm 0.049	0.623 \pm 0.082

F-statistics, allele frequencies and relatedness coefficients were calculated using the program RELATEDNESS 4.2.c and 5.0.8 (Queller & Goodnight 1994). Standard errors were obtained by jackknifing over loci. Relatedness between nestmate queens was calculated for the seven polygynous *P. inversa* colonies (i1–i7, Table 1) and the five polygynous *P. villosa* colonies (v1–v5, Table 1). Worker–workers relatedness was calculated both for polygynous and monogynous colonies, but not for founding associations, which had only few eggs (i6 and v5, Table 1). Matrilines were grouped using the software CERVUS 2.0 (Marshall *et al.* 1998) and double-checked by visual inspection of worker genotypes. These matriline distributions give an indication of the number of queens that are reproducing, compared to the number of queens present in the colony (Table 1). Moreover, for both species we calculated the effective mean number of queens per colony according to Seppä (1994), assuming the average relatedness of workers belonging to the same matriline, of nestmate queens, and of worker nestmates as estimated (cf. Table 2). The average relatedness of the mates of co-existing queens (r_{m1}) was estimated using the highest likelihood male genotypes as inferred by the software MATESOFT (Moilanen *et al.* 2004). We also estimated the average relatedness of mates of a single queen (r_{m2}), which is included in the calculation of the effective number of mates of a single queen (Seppä 1994; Ross 2001).

The possible differential contribution of nestmate queens to the offspring (skew) was calculated using the B-index (Nonacs 2000) in the program SKEW CALCULATOR 2003 (www.obee.ucla.edu/Faculty/Nonacs). This program also allows for an estimation of the probability level that the observed B values differ from zero (i.e. that reproduction is not randomly distributed between breeders).

Some colonies contained workers that could not be assigned to any of the present queens (Table 1) and were probably offspring of queens that died after the colony foundation (drifters or adopted workers are very unlikely because of the strong intercolonial aggression in these species). These workers were excluded from the analysis of relatedness within matrilines, but when possible we estimated the number of additional queens which would have had been in the colonies to explain their presence (cf. Table 1).

The observed mating frequencies of queens and the number of patrines were inferred from offspring genotypes using MATESOFT, which also calculates the nonidentification error f' . Queens that mothered only one or two offspring were not included in the MATESOFT mating frequency statistical analysis, because it would have been unlikely that all mates were reliably represented in such a small sample size.

Mating frequencies (total number of mates) determined from offspring genotypes can be underestimated through nonsampling error (when the number of offspring analysed is relatively small) and the nondetection error (by coincidence two males may have the same genotype at all loci) (Boomsma & Ratnieks 1996). Thus, we additionally calculated the effective paternities (m_e) following the method by Nielsen *et al.* (2003). The effective paternity is the number of equally contributing males giving the same diversity among the offspring as the observed mates, that is in the absence of reproductive skew. Queens, which mothered two or less offspring, had to be excluded from this analysis as well. The probability that two males shared the same genotype was very low: 0.000028 for *P. inversa* and 0.00094 for *P. villosa*, based on population allele frequency and at the individual level, calculated according to Boomsma & Ratnieks (1996).

Queen-mating frequencies were also estimated by sperm genotyping. These two methods are complementary: mother–offspring comparison takes the relative effective contribution of each male into account and sperm genotyping can avoid problems linked to possible temporal fluctuations in the contribution of mates and small offspring sample size (Gertsch & Fjerdingstad 1997; Chapuisat 1998). Alleles found in the sperm DNA were compared to the alleles of genotyped queens. When the sperm had exactly the same alleles as the queen at all loci, we assumed sperm DNA to be contaminated by queen DNA and excluded it from the analysis.

We attempted to test Keller & Reeve's (1994) prediction that queen-mating frequency should be negatively correlated with colony queen number. This is because increased genetic variation among offspring can be achieved by multiple mating in monogynous populations and by multiple maternity in polygynous populations. We looked at the relationship between the number of reproducing queens

per colony and the average number of matings per queen (see Discussion). We included those queens that were not present in the colonies but mothered the 'nonassignable offspring' (Table 1) and we used the observed number of mates as estimated by MATESOFT and by sperm typing when available.

Results

The population inbreeding coefficient F_{IT} was 0.004 ± 0.02 for *Pachycondyla inversa* and 0.033 ± 0.08 for *Pachycondyla villosa*, neither were significantly different from zero (t -tests $P > 0.05$), suggesting no substructuring of our study population.

Nestmate queens were found to be unrelated in both species. Queen–queen relatedness was on average -0.036 ± 0.02 in colonies of *P. inversa* (range -0.11 – 0.08) and 0.0241 ± 0.07 in *P. villosa* (range -0.14 – 0.28), neither were different from zero (t -test $P = 0.224$ and $P = 0.803$, respectively). Negative values presumably result from the large standard errors of single colony estimates.

Worker–worker relatedness was calculated at different levels: at the colony level (relatedness among nestmate workers) and at the matriline level (relatedness of sister workers within a matriline). Moreover, we considered polygynous and monogynous colonies separately. In *P. inversa*, the worker–worker relatedness at the colony level was lower in polygynous colonies (0.27 ± 0.03) than in monogynous colonies (0.63 ± 0.02). In *P. villosa* we found a similar pattern (0.31 ± 0.05 in polygynous colonies and 0.66 ± 0.07 in monogynous colonies). Results are summarized in Table 2, where estimates of relatedness among nestmate workers for all colonies (polygynous plus monogynous) are also given. At the matriline level, relatedness among sister workers was 0.61 ± 0.03 in *P. inversa* and 0.62 ± 0.08 in *P. villosa* (Table 2).

Even with the often limited number of workers available for the analysis, it appeared from queen and offspring genotypes that nearly all *P. inversa* queens (nonidentification error $f' < 0.000001$, observed proportion of double or multiple mated queens $D_{obs} = 1.00$, estimated proportion of double mated queens $D_{exp} = 1.00$) and 89% of *P. villosa* queens ($f' = 0.005$, $D_{obs} = 0.888$, $D_{exp} = 0.893$) were at least doubly mated. The average effective paternity (m_e), based on pedigree relatedness (Nielsen *et al.* 2003), is 2.38 ± 0.57 (harmonic mean \pm SE) for *P. inversa* and 1.67 ± 0.92 for *P. villosa* (see also Fig. 1). However, in both species, multiple males mated to the same queen were found to be on average closely related ($r_{m2} = 0.43 \pm 0.04$ for *P. inversa* and $r_{m2} = 0.42 \pm 0.08$ for *P. villosa*, both not significantly different from 0.5, the average relatedness expected for brothers; t -test $P = 0.474$ and $P = 0.462$, respectively). Hence, m_e based on regression relatedness and taking into account r_{m2} (Seppä 1994) is 1.98 for *P. inversa* and 1.78 for *P. villosa*.

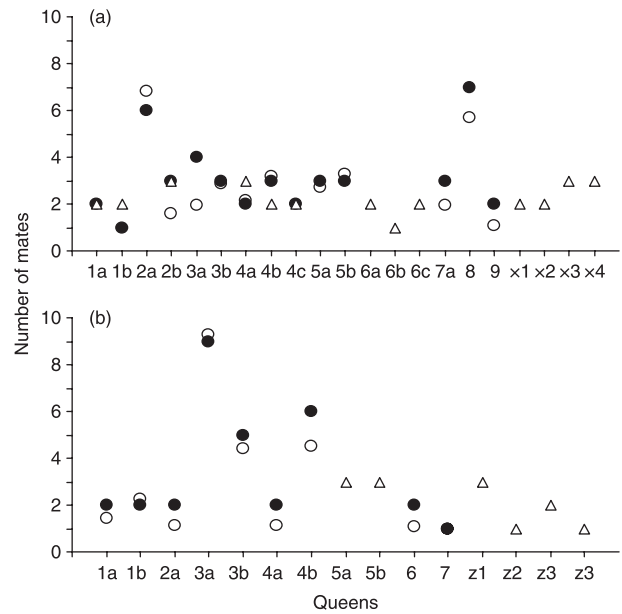


Fig. 1 Number of mates per queen in (a) *Pachycondyla inversa* and (b) *Pachycondyla villosa*. Observed paternity (filled circles) as estimated by the program MATESOFT (Moilanen *et al.* 2004) from the offspring genotype, when available. Effective paternity (open circles) was calculated according to Nielsen *et al.* (2003) only if the number of offspring was higher than two. Number of mates estimated by sperm typing of the seminal fluid stored in the queen's spermathecae (open triangles).

Males mated to different queens from the same colony were on average unrelated ($r_{m1} = 0.16 \pm 0.03$ for *P. inversa* and $r_{m1} = 0.17 \pm 0.08$ for *P. villosa*, t -test $P = 0.02$ and $P = 0.249$, respectively). Moreover, the queens and their mates were always unrelated (0.08 ± 0.03 for *P. inversa* and 0.03 ± 0.13 for *P. villosa*, neither was significantly different from zero, t -test $P = 0.270$ and $P = 0.410$, respectively), confirming the absence of inbreeding in these species.

Multiple mating is also evidenced by the analysis of sperm DNA from the queens' spermathecae, which in 12 of 13 *P. inversa* queens (92%) and in four of six *P. villosa* queens (67%) contained sperm from two or even three males (Fig. 1). The proportions of multiple-mated queens and the mating frequencies were lower than those inferred from offspring genotypes, but in the three *P. inversa* colonies (i1, i2 and i4), where both offspring and sperm could be analysed, estimates from the different methods were consistent.

From the analysis of worker genotypes, it appears that the number of queens present in a colony may be different from the number of reproducing queens (Table 1). The harmonic mean number of reproducing queens is 2.29 in *P. inversa* and 2.37 in *P. villosa*. Whereas the effective mean number of queens per colony is 1.90 for *P. inversa* and 1.57 for *P. villosa*. This indicates that there is a certain degree of skew in worker production. Using the B-index, we confirmed that queens may contribute unevenly to the offspring. In

three *P. inversa* colonies (i2, i3 and i7) the contribution of the queens to the worker offspring was significantly skewed ($B = 0.135, 0.108$ and 0.201 , respectively, all significantly different from random sharing of reproduction at $P < 0.05$). A similar pattern was found in *P. villosa* for three colonies (v1, v2 and v4, $B = 0.146, 0.385$ and 0.145 , respectively, all $P < 0.05$). Interestingly, in colony v4 we also found a significant skew in the production of virgin queens ($B = 0.458$, $P < 0.01$).

With the data available, we could not find any relationship between the number of reproducing queens per colony and the average number of matings per queen ($r_s = -0.055$ for *P. inversa* and $r_s = 0.229$ for *P. villosa*, both $P > 0.05$).

Discussion

The results of our study show that colonies in both *Pachycondyla inversa* and *Pachycondyla villosa* usually contain several unrelated queens, which mate multiply. We have therefore found a new taxon in social Hymenoptera with high queen-mating frequencies. Multiple mating is shown for the first time in the ant subfamily Ponerinae, where all other studies have as yet suggested single mating (e.g. Monnin & Peeters 1998; Kikuta & Tsuji 1999; Peeters *et al.* 2000; Doums *et al.* 2002; Viginier *et al.* 2004). Multiple mating has been documented in *Rhytidoponera* (Tay & Crozier 2001), but this genus has subsequently been shown not to be a member of the poneroid clade (Moreau *et al.* 2006).

Combining polygyny and polyandry should dramatically decrease the relatedness among nestmate workers, but this was not the case in our colonies for two reasons. First, we found that overall the number of reproducing queens was lower than the number of queens present in the colonies and that in some colonies there was a skew in the production of workers. Thus, polygyny appears to have a lower effect in decreasing relatedness among nestmate worker than in a situation where all queens reproduce and contribute equally to the offspring. Consequently, the mean relatedness coefficient among nestmate workers in polygynous and polyandrous colonies of *P. inversa* (0.25 ± 0.03 SE) and *P. villosa* (0.32 ± 0.05 SE) is in the range of that observed in several other social insects (cf. Hammond & Keller 2004; Fjerdingstad & Crozier 2006; Wenseleers & Ratnieks 2006), and not as low as that found in some queenless ants (Chapuisat & Crozier 2001). Second, multiple males mated to the same queen were typically closely related (brothers). This highly increases the relatedness among offspring of a multiple mated queen (relatedness within matriline: 0.61 ± 0.03 SE for *P. inversa* and 0.62 ± 0.08 SE for *P. villosa*).

We have documented a surprisingly high mating frequency of *Pachycondyla* queens both from mother-offspring analysis and sperm genotyping. This is in accordance with the results of a recent study showing worker policing by egg-

eating in *P. inversa* (D'Etterre *et al.* 2004). A mating frequency higher than two is one of the conditions for worker policing to occur (cf. Ratnieks 1988), since workers are expected to favour queen-produced males over worker-produced males, to which, under these conditions, they are on average less related (Foster & Ratnieks 2000). However, worker policing is also thought to occur in genetically more homogenous colonies probably to enhance colony efficiency (Ratnieks 1988; Hammond & Keller 2004).

High mating frequencies appear to be rare in social Hymenoptera and restricted mostly to a few taxa with large colony sizes, such as honey bees (Tarpy *et al.* 2004), leaf-cutting ants (e.g. Villesen *et al.* 2002), harvester ants (Cole & Wiernasz 1999; Volny & Gordon 2002; Gadau *et al.* 2003), and army ants (Kronauer *et al.* 2004, 2006), where it might provide the high genetic variation needed to cope with the high parasite and pathogen pressure on large colonies (Schmid-Hempel 1994; Schmid-Hempel & Crozier 1999; Tarpy 2003). *Pachycondyla inversa* and *P. villosa* are characterized by relatively small colony sizes (up to 200 workers), but they live in the humid, warm environment of rotting plant material, which is presumably full of parasites, pathogens and fungal spores.

Several other explanations for multiple mating have been proposed. For example, the sperm-limitation hypothesis — that multiple mating prevents queens from running out of sperm (Cole 1983; Fjerdingstad & Boomsma 2000) — does not appear to be applicable in *Pachycondyla* and several other polyandrous species with comparatively small colony sizes, such as *Nothomyrmecia macrops* (Sanetra & Crozier 2001) and *Cardiocondyla batesii* (Schrempf *et al.* 2004). Similarly, the convenience hypotheses — whereby females mate multiply because they are forced to do so by males (Alcock *et al.* 1978) — is unlikely as the large *P. inversa* and *P. villosa* queens can seemingly defend themselves against unwanted male mating attempts. Alternatively, as also suggested in *Myrmica* (Pedersen & Boomsma 1999) mating with more than one male might simply bear little costs for *Pachycondyla* queens, which are large, heavily sclerotized and have a potent sting, and seem to have a low predation risk during their nuptial flights.

High mating frequency in *Pachycondyla* can probably not be explained by a restricted dispersal of young queens, given that cofounding queens are unrelated and the F_{IT} values in both species suggest that queens do not mate close to the nest and relatedness data show that there is no inbreeding. The lack of detailed quantitative field studies on mating behaviour of these species does not allow us to unambiguously describe their reproductive strategy, but from our data and observations, we suggest the following scenario. *Pachycondyla* sexuals are produced year-round and asynchronously (P. D'Etterre, personal observation), so that any one time unmated young queens will be able to find males almost exclusively from one colony in a given

area. When a young queen would be ready to mate, she would leave the mother colony and disperse by flying. She would then land and release a sex pheromone to attract males (the 'female calling syndrome' is typical of many ponerine species, cf. Hölldobler & Bartz 1985). This pheromone would easily reach males in a nest close by, since *Pachycondyla* inhabits rather open nests such as rotten cocoa pods or holes in three branches and the density of nests can be high (about one nest per square meter, J. van Zweden & M. Fürst, personal observation). Thus, brother males would come out together and mate with the calling female. After mating, young queens start searching for a suitable nest site and associate with unrelated females to find a new colony (D'Ettorre *et al.* 2005). Significant genetic relatedness among males mated to the same queen has been documented in the ant *Myrmica sulcinodis* (Pedersen & Boomsma 1998), but in that species co-existing queens may be related and multiple males mate with different queens in the same polygynous colony.

It has been previously suggested that polygyny and polyandry rarely co-occur (Keller & Reeve 1994; Schmid-Hempel & Crozier 1999). However, queen number and queen mating frequency are positively associated in *Myrmica* ants (Pedersen & Boomsma 1999) and colonies of some leafcutter ants may be polyandrous and polygynous (Bekkevold *et al.* 1999). Pedersen & Boomsma (1999) suggested that restricted dispersal of young queens may promote, at the same time, both polygyny and polyandry and that moderate degrees of multiple mating may be a consequence of mating at low cost (mating occurring close to the nest) and mating in swarms with a highly male-biased sex ratio. We did not find any relationship between the number of reproducing queens per colony and the average number of matings per queen. Thus, the Keller & Reeve's (1994) prediction that queen-mating frequency should be negatively correlated with colony queen number is not supported by our data.

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References

- Alcock J, Barrows M, Gordh G (1978) The ecology and evolution of male reproductive behaviour in bees and wasps. *Journal of the Linnean Society*, **64**, 293–326.
- Baer B, Boomsma JJ (2004) Male reproduction investment and queen mating frequency in fungus growing ants. *Behavioral Ecology*, **15**, 426–432.
- Bekkevold D, Frydenberg J, Boomsma JJ (1999) Multiple mating and facultative polygyny in the panamanian leafcutter *Acromyrmex echinator*. *Behavioral Ecology and Sociobiology*, **46**, 103–109.
- Bernasconi G, Strassmann JE (1999) Cooperation among unrelated individuals: the ant foundress case. *Trends in Ecology & Evolution*, **14**, 477–482.
- Boomsma JJ, Ratnieks FLW (1996) Paternity in eusocial Hymenoptera. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **351**, 947–975.
- Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.
- Chapuisat M (1998) Mating frequency of ant queens with alternative dispersal strategies, as revealed by microsatellite analyses of sperm. *Molecular Ecology*, **7**, 1097–1105.
- Chapuisat M, Crozier R (2001) Low relatedness among cooperatively breeding workers of the greenhead ant *Rhytidoponera metallica*. *Journal of Evolutionary Biology*, **14**, 564–573.
- Cole BJ (1983) Multiple mating and the evolution of social behaviour in the Hymenoptera. *Behavioral Ecology and Sociobiology*, **12**, 191–201.
- Cole BJ, Wiernasz DC (1999) The selective advantage of low relatedness. *Science*, **285**, 891–893.
- Crozier RH, Fjerdingstad EJ (2001) Polyandry in social hymenoptera – disunity in diversity? *Annales Zoologici Fennici*, **38**, 267–285.
- D'Ettorre P, Heinze J, Ratnieks FLW (2004) Worker policing by egg eating in the ponerine ant *Pachycondyla inversa*. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **271**, 1427–1434.
- D'Ettorre P, Kellner K, Delabie JHC, Heinze J (2005) Number of queens in founding associations of the ponerine ant *Pachycondyla villosa*. *Insectes Sociaux*, **52**, 327–332.
- Doums C, Cabrera H, Peeters C (2002) Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventris*. *Molecular Ecology*, **11**, 2251–2264.
- Fernández-Escudero I, Pamilo P, Seppä P (2002) Biased sperm use by polyandrous queens of the ant *Proformica longiseta*. *Behavioral Ecology and Sociobiology*, **51**, 207–213.
- Fjerdingstad EJ, Boomsma JJ (2000) Queen-mating frequency and relatedness in young *Atta sexdens* colonies. *Insectes Sociaux*, **47**, 354–356.
- Fjerdingstad EJ, Crozier R (2006) The evolution of worker caste diversity in social insects. *The American Naturalist*, **167**, 390–400.
- Foster KR, Ratnieks FLW (2000) Facultative worker policing in a social wasp. *Nature*, **407**, 692–693.
- Gadau J, Strehl CP, Oettler J, Hölldobler B (2003) Determinants of intracolony relatedness in *Pogonomyrmex rugosus* (Hymenoptera; Formicidae): mating frequency and brood raids. *Molecular Ecology*, **12**, 1931–1938.
- Gertsch PJ, Fjerdingstad EJ (1997) Biased amplification and utility of spermatheca-PCR for mating frequency studies in Hymenoptera. *Hereditas*, **126**, 183–186.
- Giraud T, Pedersen J, Keller L (2002) Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences, USA*, **99**, 6075–6079.
- Hammond RL, Keller L (2004) Conflict over male parentage in social insects. *PLoS Biology*, **2**, E248.
- Heinze J, Trunzer B, Hölldobler B, Delabie JHC (2001) Reproductive skew and queen relatedness in an ant with primary polygyny. *Insectes Sociaux*, **48**, 149–153.

- Hölldobler B, Bartz SH (1985) Sociobiology of reproduction in ants. In: *Experimental Behavioral Ecology and Sociobiology* (eds Hölldobler B, Lindauer M), pp. 237–257. Sinauer & Associates, Sunderland, Massachusetts.
- Keller L, Reeve HK (1994) Genetic variability, queen number and polyandry in social Hymenoptera. *Evolution*, **48**, 694–704.
- Kikuta N, Tsuji K (1999) Queen and worker policing in the monogynous and monoandrous ant, *Diacamma spec.* *Behavioral Ecology and Sociobiology*, **46**, 180–189.
- Kolmer K, Heinze J (2000) Rank orders and division of labour among unrelated cofounding ant queens. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 1729–1734.
- Kolmer K, Hölldobler B, Heinze J (2002) Colony and population structure in *Pachycondyla cf. inversa*, a ponerine ant with primary polygyny. *Ethology, Ecology and Evolution*, **14**, 157–164.
- Kronauer DJC, Schöning C, Pedersen J, Boomsma JJ, Gadau J (2004) Extreme queen-mating frequency and colony fission in African army ants. *Molecular Ecology*, **13**, 2381–2388.
- Kronauer DJC, Berghoff SM, Powell S *et al.* (2006) A reassessment of the mating system characteristics of the army ant *Eciton burchellii*. *Naturwissenschaften*, **93**, 402–406.
- Marshall TC, Slate J, Kruuk L, Pemberton J (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.
- Moilanen A, Sundström L, Pedersen J (2004) MATESOFT: a program for deducing parental genotypes and estimating mating systems statistics in haplodiploid species. *Molecular Ecology Notes*, **4**, 795–797.
- Monnin T, Peeters C (1998) Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadricaps*. *Animal Behaviour*, **55**, 299–306.
- Moreau SC, Bell CD, Vila R, Archibald SB, Pierce NE (2006) Phylogeny of the ants: diversification in the age of angiosperms. *Science*, **312**, 101–104.
- Nielsen R, Tarpay DR, Reeve HK (2003) Estimating effective paternity number in social insects and the effective number of alleles in a population. *Molecular Ecology*, **12**, 3157–3164.
- Nonacs P (2000) Measuring and using skew in the study of social behavior and evolution. *American Naturalist*, **156**, 577–589.
- Pedersen J, Boomsma JJ (1998) Direct genetic evidence for local mate competition in ants. *Naturwissenschaften*, **85**, 593–595.
- Pedersen J, Boomsma JJ (1999) Positive association of queen number and queen-mating frequency in *Myrmica* ants: a challenge to the genetic-variability hypotheses. *Behavioral Ecology and Sociobiology*, **45**, 185–193.
- Peeters C, Liebig J, Hölldobler B (2000) Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. *Insectes Sociaux*, **47**, 325–332.
- Queller DC, Goodnight KF (1994) *RELATEDNESS 4.2 Goodnight Software*. Cold Spring Harbor Laboratory Press, Houston, Texas.
- Ratnieks FLW (1988) Reproduction harmony via mutual policing in eusocial hymenoptera. *American Naturalist*, **132**, 217–236.
- Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.
- Sambrook J, Russell D (2001) *Molecular Cloning*, 3rd edn. Cold Spring Harbor Laboratory Press, New York.
- Sanetra M, Crozier RH (2001) Polyandry and colony genetic structure in the primitive ant *Nothomyrmecia macrops*. *Journal of Evolutionary Biology*, **14**, 368–378.
- Schmid-Hempel P (1994) Infection and variability in social insects. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **346**, 313–321.
- Schmid-Hempel P, Crozier RH (1999) Polyandry versus polygyny versus parasites. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **354**, 507–515.
- Schrempf A, Reber C, Tinaut A, Heinze J (2004) Inbreeding and local mate competition in the ant *Cardiocondyla batesii*. *Behavioral Ecology and Sociobiology*, **57**, 502–510.
- Seppä P (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica lobicornis*: number, relatedness and longevity of reproducing individuals. *Journal of Evolutionary Biology*, **7**, 71–95.
- Strassmann JE (2001) The rarity of multiple mating by females in social hymenoptera. *Insectes Sociaux*, **48**, 1–13.
- Tarpay DR (2003) Genetic diversity within honey bee colonies prevents severe infections and promotes colony growth. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 99–103.
- Tarpay DR, Nielsen R, Nielsen DJ (2004) A scientific note on the revised estimates of effective paternity frequency in *Apis*. *Insectes Sociaux*, **51**, 203–204.
- Tay WT, Crozier RH (2001) Mating behaviour of *Rhytidoponera* sp. 12 ants inferred from microsatellite analysis. *Molecular Ecology*, **10**, 167–173.
- Trindl A, Heinze J, D'Ettoire P (2004) Isolation and characterization of five microsatellite loci in the ponerine ant *Pachycondyla inversa* (Hymenoptera, Formicidae). *Molecular Ecology Notes*, **4**, 583–586.
- Trunzer B, Heinze J, Hölldobler B (1998) Cooperative colony founding and experimental primary polygyny in the ponerine ant *Pachycondyla villosa*. *Insectes Sociaux*, **45**, 267–276.
- Viginier B, Peeters C, Brazier L, Doums C (2004) Very low genetic variability in the Indian queenless ant *Diacamma indicum*. *Molecular Ecology*, **13**, 2095–2100.
- Villesen P, Murakami T, Schultz TR, Boomsma JJ (2002) Identifying the transition between single and multiple mating of queens in fungus growing ants. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 1541–1548.
- Volny V, Gordon D (2002) Genetic basis for queen-worker dimorphism in social insects. *Proceedings of the National Academy of Sciences, USA*, **99**, 6108–6111.
- Wenseleers T, Ratnieks FLW (2006) Comparative analysis of worker reproduction and policing in eusocial Hymenoptera supports relatedness theory. *American Naturalist*, **168**, E163–E179.

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