Multiple paternity in the leafcutter ant *Atta colombica* — a microsatellite DNA study

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An evolutionary understanding of the causes, correlates and consequences of multiple paternity in eusocial Hymenoptera (all ants, some bees and some wasps) relies on accurate estimation of this parameter at the species, population and colony level. We developed dinucleotide microsatellite DNA markers in order to study in detail the degree of multiple paternity in a population of the monogynous Panamanian leafcutter ant *Atta colombica*. These microsatellite markers were highly polymorphic such that nondetection caused by finite allelic diversity was very low (0.016). Hence, accurate information on the patterns of multiple paternity could be obtained. Genetic analysis revealed that in 33 out of 36 colonies two or more males had contributed genetically to the offspring. The mean number of fathers per colony was 2.6 and the mean effective paternity was 2.31. These are the highest values of effective paternity yet reported for any ant species. We examined the patterns of paternity frequency in young and old colonies to test if within-colony genetic diversity is related to colony survival, but found no support for any relationship. Our results confirm previous nongenetic studies showing high levels of multiple mating by queens of higher leafcutter ants. We discuss our findings in relation to known patterns of polyandry and paternity in other eusocial Hymenoptera.

Keywords: genetic diversity, multiple mating, polyandry, relatedness, social insects, worker policing.

Introduction

Multiple paternity, caused by multiple mating of queens, is one of the major factors affecting within-colony relatedness in the eusocial Hymenoptera (ants, some bees and some wasps). Multiple paternity lowers the relatedness of workers to the new queens (gynes) and thus decreases the inclusive fitness payoff for workers that help to rear gynes (Hamilton, 1964). Therefore it affects the optimal sex allocation by workers (Boomsma & Grafen, 1991; Pamilo, 1991a,b). It lowers the relatedness of workers to each other and thus influences whether worker production of males will be selectively favoured or not (Ratnieks, 1988). Multiple mating is likely to be costly for young queens in terms of time, energetic expenditure and increased predation risk (on *Atta* see Fowler *et al*., 1986). The fitness of colonies with multiple fathers may, however, be higher if increased genetic diversity brings benefits to compensate for these costs (review in Boomsma & Ratnieks, 1996). Alternatively, multiple mating may be selected for because it enables queens to store more sperm and obtain a higher lifetime reproductive success (Cole, 1983).

High degrees of multiple paternity are rather rare in eusocial Hymenoptera (review in Boomsma & Ratnieks, 1996). Thus far, high levels of effective multiple paternity have been genetically documented in *Apis* honeybees (Estoup *et al*., 1994; Moritz *et al*., 1995; Oldroyd *et al*., 1995) and in two *Vespula* yellowjacket wasps (Ross, 1986). Nongenetic studies, however, have shown multiple mating by queens in some species of the neotropical *Atta* and *Acromyrmex* leafcutter ants (Kerr, 1961; Corso & Serzedello, 1981; Reichardt & Wheeler, 1996).

An understanding of which selective factors may favour multiple mating and multiple paternity in some social insect species but not in others, requires detailed studies of the inter- and intraspecific

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patterns of paternity. This necessitates accurate genetic markers. Allozyme variation is low in most eusocial Hymenoptera (Graur, 1985), but microsatellites (Tautz, 1989) usually show high levels of variation (Evans, 1993; Hughes & Queller, 1993; Estoup *et al.*, 1994) making these excellent tools for paternity studies (Estoup *et al.*, 1994).

We developed microsatellite primers for two dinucleotide repeat loci for the monogynous (single queen per nest) Panamanian leafcutter ant *Atta colombica* and genotyped workers and queens from young and mature colonies in a natural population. Our genetic markers enabled a high resolution study of multiple paternity at the level of colony, age class and population. We show that *A. colombica* is characterized by substantially higher levels of multiple paternity than yet genetically documented in any ant. We discuss our findings in relation to the patterns of paternity known for other eusocial Hymenoptera.

Materials and methods

Development of microsatellite markers

The primers were developed as in Thorén *et al.* (1995) with minor modifications. Two thousand recombinant clones were screened for dinucleotide repeat microsatellites; 49 positives were found. Ant DNA inserts in two of these 49 clones were sequenced and the presence of microsatellites was confirmed. Primers were designed to be complementary to the flanking regions of the two loci and purchased from Operon Technologies. The two loci were named *Etta5–6TF* and *Etta7–8TF* (Table 1).

Genotyping procedure

DNA extractions, PCR and gel electrophoretic separation were according to Thorén *et al.* (1995) with the following modifications: (i) the incubation stage at 55°C during DNA extractions was prolonged to 2.5 h; (ii) supernatants were not transferred to new tubes. PCR mixes were as in Thorén *et al.* (1995) except that for *Etta7–8TF* the $MgCl_2$ concentration was increased to 1.2 mM. The PCR amplification programme for *Etta5–6TF* was 3 min at 94°C, 25–30 cycles of 30 s at 94°C, 30 s at $T_m = 60°C$ and 30–40 s at 72°C, and a final step of 10 min at 72°C. The *Etta7–8TF* locus was amplified in a touchdown PCR (Don *et al.*, 1991) with 3 min at 94°C, 24 cycles of 30 s at 94°C, 30 s at $T_m = 59°C - 0.5°C/cycle$, 30–40 s at 72°C, then 15 cycles at $T_m = 47°C$, and a final 10 min at 72°C. PCR products were subjected to polyacrylamide gel (6 per cent) electrophoretic separation for 3000 or 5000 volt hours. Dried gels were incubated with X-ray films for 2 to 4 days. All genotypes were read two or three times by rescoring films. One allele was chosen to be labelled 100, the allele with one more repeat was called 101, the allele with one repeat less was called 99, etc. After the first few gels, one or two known genotypes were always run as standards for every 10–20 samples. Approximately 20 per cent of the samples amplified only weakly or not at all and were rePCR'd once or several times. Some could not be brought to amplify. Analysis of the presumed sibling and maternal genotypes of these samples ascertained that amplification failures could not be caused by homozygosity for null alleles (cf. Pemberton *et al.*, 1995).

Samples analysed

Samples of *A. colombica* were collected by nest excavations near Gamboa in the canal zone of Panama in 1992–94. Samples of nestmate workers were obtained from 24 young colonies (3 months to 2 years old); from 21 of these 24 colonies we obtained the mother queen as well. Nestmate worker samples were collected also from 12 mature colonies, i.e. from colonies which were either producing sexuals (new queens and males) or were of a similar size to those that did, most likely at least 5 years old. The queen could only be found in one of these 12 colonies (mature nests are very large).

Table 1 Details of the two microsatellite loci used for the genetic analysis of *Atta colombica*, the primers designed to amplify these loci, and the allelic diversity of the loci

Locus	Cloned core sequence	Sequence of PCR primers	Size of PCR product of cloned allele (bp)	No. of alleles	Observed heterozygosity
Etta5–6TF	$(AG)_{24}$	5′-CAGCTCTCGTAGAAGAGTAGCATG-3′ 5′-CTGAACTTCGCCCAGCG-3′	153	15	0.89
Etta7–8TF	$(AC)_{27}$	5′-GGGAGATATTAGATGAATATATCC-3′ 5′-TATTCGCAAGTGTTGTATTATG-3′	155	13	0.92

Polygyny (multiple queens per colony) has been reported for one *Atta* species, *A. texana* (Moser, 1967; Mintzer, 1990), but was never found in excavations of more than 80 incipient to two-year-old colonies of *A. colombica* (Fjerdingstad, unpubl. data). A small number of male offspring were analysed in three of the mature colonies: three, three and seven male genotypes for *Etta5–6TF*, and one, three, and eight male genotypes for *Etta7–8TF*. We found that male alleles were always present in nestmate workers' genotypes and in the inferred (see below) or observed queen genotype. This is consistent with monogyny because Hymenopteran males come from unfertilized eggs and so carry only maternal alleles. Also, it was never necessary to assume more than a single queen genotype to explain the offspring genotypes.

The *Etta5–6TF* genotypes of five workers from one young colony were incompatible with those of the presumed siblings and the queen. The entire colony was excluded from the paternity analysis. In two young colonies we found one worker incompatible at both loci (queen genotyped in one colony and not in the other). These two individuals were excluded from the analysis. Brood raiding or queen supersedure may have caused these allelic inconsistencies. Mutation appears unlikely to have been the cause because it seems improbable that (a) several mutations should occur during gametogenesis in individual queens, and (b) that some of these gametes should be mutants at more than one locus.

Data analysis

An average of 13.4 (range: 8–19, mode: 14) worker offspring were analysed per colony for 36 colonies. Where queens were not available ($n = 14$), their genotypes were inferred from the offspring under the assumption of monogyny. The number and two-locus genotypes of fathers were inferred from the two-locus genotypes of queens and worker offspring. By chance, paternal males may have identical two-locus genotypes and thus be indistinguishable in such a genetic analysis (nondetection error, cf. Boomsma & Ratnieks, 1996). Following Pamilo (1993), we approximated the average nondetection error P_{ID} by summing, over all possible male two-locus types, the expected probability of sampling two identical males by chance. That is we estimated P_{ID} as:

$$P_{ID} = \sum_{j=1}^{z} \sum_{h=1}^{w} (p_j q_h)^2, \tag{1}$$

where p_j and q_h indicate the frequency of allele j in the first locus, *Etta5–6TF*, and allele h in the second locus, *Etta7–8TF*, and where $j = 1, 2, 3, \ldots, z$ and $h = 1, 2, 3, \ldots, w$ indicate the different alleles. This method assumes that there is random mating in the population, that there is no linkage disequilibrium among the alleles of the marker loci, and that paternal males are not related. We tested these assumptions by examining the allele and genotype frequency distributions of queens and males with the GENEPOP 2 program (Raymond & Rousset, 1995a,b). This program provides unbiased estimates of the *P*-values for exact tests using a Markov chain method. The sample size for all tests was 36 for queens and 95 for queen mates. One thousand dememorization steps were carried out, and 50–450 batches of 1000 iterative estimates were made for each *P*-value (Raymond & Rousset, 1995b).

Estimation of effective paternity and nestmate worker relatedness

The effective paternity frequency takes into account both the number of fathers and the proportional representation (paternity skew) of these fathers among the offspring (Starr, 1984) and is the parameter of importance for within-colony genetic diversity and nestmate relatedness. We estimated effective paternity m_e and nestmate relatedness on the basis of two different methods: the two-locus analysis used for estimating the absolute number of fathers (see above) and the identity-by-descent method (IBD) (Queller & Goodnight, 1989).

The two-locus method

Using the two-locus genotype information to divide a queen's worker offspring into patriline (common father) subgroups, we directly obtain an estimate of the effective paternity (Starr, 1984) of that colony:

$$m_{e,y} = \frac{1}{\Sigma y_l^2}. \tag{2}$$

The summation is over the squared proportional contributions (y) of all patrilines (l). This observed effective paternity is, however, a biased underestimate which only approaches the correct value for large sample sizes (Morton *et al.*, 1971; Nei & Roychoudhury, 1974; Pamilo, 1993). This is because both the observed skew among the representation of different fathers and the number of fathers detected is constrained by the number offspring examined (review in Boomsma & Ratnieks, 1996). We proba-

bilistically corrected the observed $m_{e,y}$ to the values they should reach if an infinite number of offspring was examined. This was carried out according to Pamilo (1993):

$$m_{e,p} = \frac{1}{\Sigma p_l^2} = \frac{1}{(N\Sigma y_l^2 - 1)/(N-1)}, \qquad (3)$$

where N is the number of offspring analysed for the colony.

On the basis of the two-locus effective paternity estimates, keeping paternal haploidy in mind and assuming monogyny, we estimated the relatedness among nestmate workers (cf. Page, 1986) as:

$$R = 0.25 + (1/2m_e). \qquad (4)$$

The identity-by-descent method (IBD)

This method is applied directly to the obtained worker offspring genotypes, and estimates the relatedness among nestmate workers by assessing the allelic similarity of an individual's nestmates with the background population (other colonies) relative to the similarity of the individual itself to the background population (Queller & Goodnight, 1989). The colony-specific IBD-relatedness estimate is then:

$$R_r = \frac{\Sigma\Sigma\Sigma(P_y - P^*)}{\Sigma\Sigma\Sigma(P_x - P^*)}, \qquad (5)$$

where P_x is the frequency of an allele at a specific allelic position and locus in an individual, P_y is the frequency of the allele in the nestmates of this individual, and P^* is the frequency of the allele in the background population, i.e. in the other colonies. The expression sums over allelic positions, loci and individuals.

From the IBD-relatedness estimates and assuming monogyny we estimated the IBD-based effective paternity $m_{e,r}$ by using eqn (4).

Results

Both microsatellite loci were highly polymorphic. Fifteen alleles were found for *Etta5–6TF* and 13 for *Etta7–8TF* (Fig. 1). The detected heterozygosity in queens (genotyped and inferred) was about 0.9 for both loci (Table 1). No deviation from Hardy–Weinberg proportions was found for queen genotypes (Hardy–Weinberg probability test, $P \pm SE = 0.50 \pm 0.01$ for *Etta5–6TF*; and $P \pm SE = 0.3 \pm 0.01$ for *Etta7–8TF*; overall: $P = 0.48$; GENEPOP 2, Raymond & Rousset, 1995b). Allele frequencies in queens

were not significantly different from those of inferred fathers ($P \pm SE = 0.1 \pm 0.007$ for *Etta5–6TF*, and $P \pm SE = 0.69 \pm 0.01$ for *Etta7–8TF*; GENEPOP 2, Raymond & Rousset, 1995b). There was no evidence that the two microsatellite loci were in
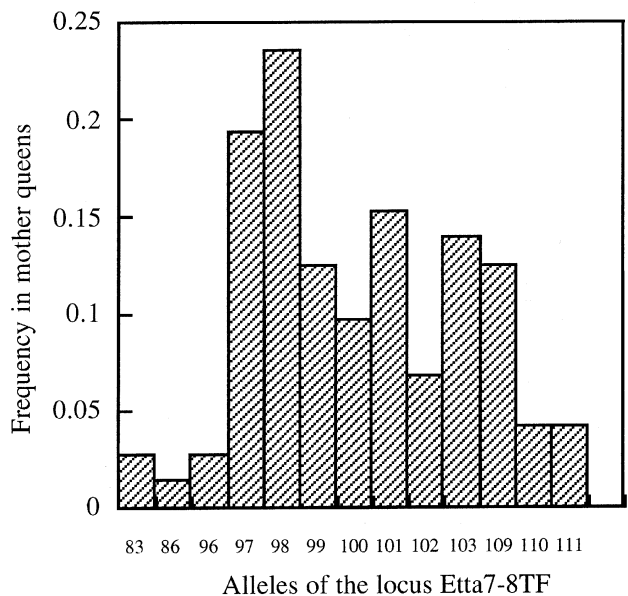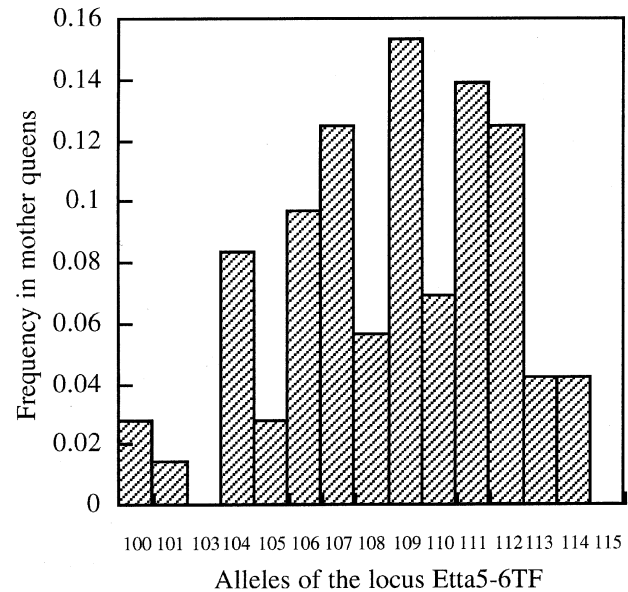

Fig. 1 Frequency in *Atta colombica* queens of alleles at the (AG) repeat locus *Etta5–6TF* and the (AC) repeat locus *Etta7–8TF*. Alleles are ordered by increasing length (for nomenclature see Materials and methods). Alleles 103 and 115 at *Etta5–6TF* did not occur in queens, but were found in paternal males at very low frequency.

linkage disequilibrium in males (Fisher's exact test, $P \pm SE = 0.83 \pm 0.01$; GENEPOP 2, Raymond & Rousset, 1995b). The free segregation of alleles into offspring of doubly heterozygous queens could not be tested as the number of offspring sampled per queen was too low to allow for a statistical test.

Results such as the above should be interpreted with some caution: in six colonies it was *parsimoniously* assumed that queens were single- or at most double-mated and heterozygous, although they could have been homozygous and multiple-mated. The IBD-relatedness estimates for these colonies, however, fit quite well with the relatedness values calculated on the basis of the parsimonious paternity estimation (Spearman rank correlation: $r_s = 0.74$, $n = 6$, $P = 0.07$, one-tailed test) and much less well with the least parsimonious estimate ($r_s = 0.3$, $P > 0.25$, NS, one-tailed test). We tested the general reliability of our parsimonious paternity procedure by reconstructing the number and genotypes of fathers for colonies where queens had also been genotyped, but without making use of this queen genotype information. We obtained the exact correct inferences of queen genotypes and paternal genotypes and numbers in twenty out of 22 times (91 per cent). In two cases the number of fathers was underestimated.

No evidence was found for relatedness among paternal males. The average IBD relatedness ($\pm SD$) among mates of the same queen was 0.03 ± 0.22 (SD based on jackknifing over colonies, Goodnight, 1992; $t = 0.60$, $P > 0.50$, NS, two-tailed t-test). The available evidence thus suggests that there is random mating in our *A. colombica* population, that paternal males are unrelated and that the two marker loci are not in linkage disequilibrium. We could therefore calculate according to Pamilo (1993) the expected population-level nondetection error resulting from chance identity of paternal alleles. On the basis of the allele frequencies in queens and males we estimated this error to be only 0.016 (eqn 1). The expected frequency of the most common two-locus male genotype was only 0.04. Therefore, nondetection error will have affected (lowered) our paternity frequency estimates very slightly if at all.

The mean number of fathers ($\pm SD$) detected per colony was 2.6 ± 0.90, and the largest number was five (Table 2, Fig. 2a). Out of 36 queens, 33 (92 per cent) had mated with two or more males, showing that multiple paternity is typical of *A. colombica*. Males appeared to share paternity unequally in several cases but in general the observed skew was not strong (Table 2). Because of unequal and limited

sample sizes in the different colonies of a given paternity frequency class, it was not possible to test if this skew was statistically significant. There was a slight, but nonsignificant, tendency for the detected number of fathers to be positively associated with the number of offspring analysed per colony (Spearman rank correlation analysis: $r_s = 0.11$, $n = 36$, $P > 0.25$, NS, one-tailed test). Similarly, effective paternity $m_{e,y}$ showed a nonsignificant tendency for a positive association with the number of offspring sampled (Spearman rank correlation analysis: $r_s = 0.18$, $n = 36$, $P > 0.10$, NS, one-tailed test). Applying Pamilo's (1993) probabilistic sample size correction slightly increased the effective paternity estimates: from $m_{e,y} = 2.06$ to $m_{e,p} = 2.31$ (Table 3, Fig. 2b). Harmonic means of the two estimates were lower ($m_{e,y} = 1.83$ and $m_{e,P} = 1.97$) as expected when effective paternity varies between colonies (Table 3). Effective paternity frequency $m_{e,r}$ as estimated through identity-by-descent analysis (IBD) gave an effective paternity value of 1.87. This latter value should be compared with the harmonic mean of $m_{e,y}$ (Table 3); because both these estimates are not corrected for sample size; the two were indeed very similar. The range of IBD-based effective paternity values was much larger (Table 3) than the range based on the direct two-locus analysis. Colony-specific IBD-relatedness estimates are very sensitive to the exact allelic composition of colony members and are only accurate if data from many loci are included (cf. Queller & Goodnight, 1989). For example, homozygosity of the queen will increase the IBD-relatedness estimate for workers of her colony, whereas heterozygosity of the queen will decrease it.

The average nestmate worker relatedness for the 36 colonies (Table 3) based on the estimates of harmonic mean effective paternity and on the IBD analysis all gave values close to 0.50 (Table 3). Colony-specific nestmate relatednesses, however, varied from 0.34–0.75 (two-locus-based relatedness values), and from 0.29–0.83 (IBD values) (Table 3). The colony-specific worker relatedness values of our *A. colombica* population thus covered a large part of the total possible range (0.25–0.75) for diploid offspring in monogynous colonies.

We compared the number of fathers of young and mature colonies (Fig. 2). The arithmetic mean number of fathers ($\pm SD$) in young colonies ($n = 24$) was 2.8 ± 0.9, and in mature colonies ($n = 12$) it was 2.4 ± 1.0, but the paternity frequency distributions did not differ significantly between age classes (two-tailed Mann–Whitney U-test, Zar (1984, pp. 138–143); $U' = 173$, $P > 0.20$, NS). The harmonic

mean effective paternity $m_{e,p}$ of young colonies was 2.1; that of mature colonies was 1.8. Also here, the distributions did not differ significantly among age classes (two-tailed Mann–Whitney U-test, $U' = 167$, $P > 0.20$, NS).

Discussion

Patterns of paternity in A. colombica *and other eusocial Hymenoptera*

The microsatellite markers we developed for *A. colombica* proved to be very polymorphic. The nondetection error was less than 2 per cent. Even elaborate allozyme studies such as the one by Pamilo (1993) on the wood ant *Formica aquilonia* (six loci were used) have suffered from considerably larger nondetection errors (Pamilo, 1993; 15–17 per cent). Such error is not easily corrected for in systems with high levels of multiple paternity (Pamilo, 1993), because one cannot determine whether an apparent paternity skew is genuine or caused by the cumulative effect of two genetically identical paternal males. Only sample size constrained our paternity estimations, but the effect was probably small. Pamilo's (1993) probabilistic correction also allowed us to adjust our effective paternity estimates for sample size error. Our markers therefore allowed a very accurate description of the colony-level patterns of paternity in the studied population of *A. colombica*.

Our study shows that multiple paternity is very frequent in *A. colombica* (Fig. 2). Although the average number of fathers per colony was almost three, variation in paternity shares among fathers (Table 2) meant that the effective paternity frequency was only two (harmonic mean). The previously known range of average effective paternity frequencies of ants was 1–1.5 (review of 19 species in Boomsma & Ratnieks, 1996.) Therefore, our results for *A. colombica* are the highest values so far genetically documented for any ant species.

Only 8 per cent of the *A. colombica* colonies showed single paternity, whereas single mating was found in 21–75 per cent of queens in the next most polyandrous ant species known to date: *Lasius niger* (van der Have *et al.*, 1988; Boomsma & Ratnieks, 1996), *Formica truncorum* (Sundström, 1993) and *F.*

Table 2 The sums of squared paternity shares ($\Sigma y_{j,h}^2$) in colonies of *Atta colombica* with different numbers of fathers (2, 3, 4 or 5)

	Number of fathers per colony							
	2		3		4		5	
	$\Sigma y_{j,h}^2$	N	$\Sigma y_{j,h}^2$	N	$\Sigma y_{j,h}^2$	N	$\Sigma y_{j,h}^2$	N
	0.51	19	0.56	14	0.34	15	0.23	16
	0.87	14	0.41	16	0.34	15		
	0.59	14	0.40	14	0.32	12		
	0.52	11	0.39	15	0.33	13		
	0.51	15	0.60	10				
	0.53	19	0.55	16				
	0.67	14	0.40	15				
	0.52	10	0.51	10				
	0.76	14	0.69	12				
	0.51	14	0.35	9				
	0.72	12	0.46	10				
	0.63	8	0.43	9				
	0.77	15	0.40	14				
			0.49	18				
			0.49	16				
Obs. mean	0.63	13.7	0.48	13.1	0.33	13.8	0.23	16
Exp. mean	0.50		0.33		0.25		0.20	

N, the number of worker offspring analysed.
Given also are the observed arithmetic means per colony type, and the expected Sums of squared paternity shares if there is no bias among the detected paternal males.

aquilonia (Pamilo, 1993). Only for one other ant (*F. aquilonia*, Pamilo, 1993) has it been found that some queens use sperm from as many as five males. Although exceptionally high for ants, the levels of multiple paternity in *A. colombica* are much lower than those found in honeybees, where the mean
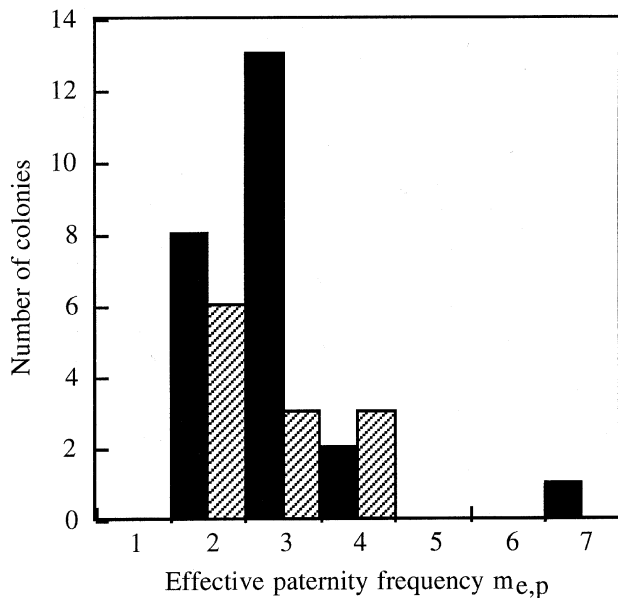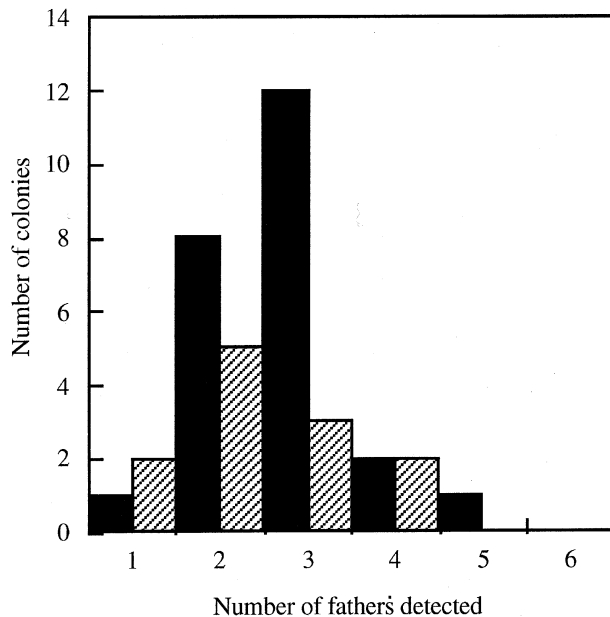

Fig. 2 Number of fathers and effective paternity $m_{e,p}$ in young (black bars) and mature (hatched bars) *Atta colombica* colonies as detected by two-locus genetic analysis.

number of fathers ranges from 10 to 30 (Estoup *et al.*, 1994; Moritz *et al.*, 1995; Oldroyd *et al.*, 1995), and where five is the lowest number of fathers reported for any honeybee colony with a naturally inseminated queen (Oldroyd *et al.*, 1995). Also, paternity frequencies in yellowjacket wasps are higher, ranging from 5.5 to 9.5 (Ross, 1986; Ross & Matthews, 1991), although single-paternity colonies may occur (Ross, 1986).

The levels of polyandry detected in *A. colombica* may nevertheless be very costly for queens. Mortality for honeybee queens on nuptial flights is only a few percent (Ratnieks, 1990), but has been estimated to be as high as 52 per cent for swarming *Atta* queens (Dix & Dix, unpublished, on *A. cephalotes*, cited in Fowler *et al.*, 1986; reviews in Weber, 1972; Fowler *et al.*, 1986). We may therefore predict that we will never find such extreme levels of polyandry in *Atta* as in honeybees.

Degree of multiple paternity and colony age in *A. colombica*

If high within-colony genetic diversity confers a fitness advantage, for instance in relation to disease resistance or worker task efficiency (Hamilton, 1987; Sherman *et al.*, 1988; Crozier & Page, 1985; and, e.g., Oldroyd *et al.*, 1992), then less diverse colonies would be expected to have higher mortality rates. Thus, the genetic diversity of mature (older) colonies which have survived years of selection should be higher than that of young colonies. We found no evidence of this: neither the number of fathers nor the effective paternity differed significantly between young and mature colonies (Fig. 2). Detecting paternity frequency variation among age classes belonging to different cohorts may, however, be difficult. Queen mating frequency may vary over years because of chance variation in the operational sex ratio of mating swarms or in the length of flights. For example, in *Lasius niger* bad weather is known to shorten nuptial flights (Boomsma & Leusink, 1981).

Our present data are thus probably too limited to reject the hypothesis that high genetic diversity, as brought about by multiple paternity, may have colony survival benefits for *A. colombica*. All higher leafcutter ants have extremely populous colonies (Fowler *et al.*, 1986) and live in an obligatory symbiosis with a clonal fungus that is quite susceptible to microorganisms and is chemically defended by the ants (Weber, 1972; Knapp *et al.*, 1994). Therefore, both the polyandry-for-sperm hypothesis (Cole, 1983) and the genetic-diversity–disease-resist-

Table 3 The number of fathers per colony ($\pm$SD), and different estimates of effective paternity frequency (m_e) and nestmate worker relatedness ($R\pm$SD) in *Atta colombica*

	Detected no. of fathers	Effective paternity frequency			Nestmate worker relatedness		
		$m_{e,y}$	$m_{e,p}$	$m_{e,r}$	R_y	R_p	R_r
Arithmetic mean	**2.64 + 0.90**	**2.06**	**2.31**	3.12	0.52 + 0.10	0.51 + 0.11	**0.52 + 0.14**
Harmonic mean	**2.29**	**1.83**	**1.97**	1.87			
Range	**1–5**	**1–4.4**	**1–5.71**	0.86–13.86	0.36–0.75	0.34–0.75	**0.29–0.83**

The subscripts *y*, *p* and *r* indicate, respectively, that the estimate has not been corrected for sample size, has been corrected for limited sample size (Pamilo, 1993), or is based on IBD relatedness estimates. Bold type indicates values estimated directly from offspring (and queen) genotypic or allelic data. Normal type represents values estimated indirectly through eqn (4).

ance hypotheses (Hamilton, 1987; Sherman *et al.*, 1988) may have power to explain why higher leafcutter ants show such high levels of polyandry (Kerr, 1961; Corso & Serzedello, 1981; Reichardt & Wheeler, 1996; this study).

Effective paternity and worker reproduction

Workers of many Hymenoptera can produce sons from unfertilized eggs (see, e.g., Bourke, 1988). However, when the effective paternity frequency is larger than two, workers are more closely related to brothers ($R = 0.25$) than to nephews ($R < 0.25$), and are therefore expected to prevent each other from producing males in queenright colonies ('worker policing', Ratnieks, 1988). Workers of at least one *Atta* species, *A. cephalotes*, can produce eggs, although it remains uncertain whether such eggs can produce viable offspring (review in Weber, 1972). Effective paternity in *A. colombica* is close to two on average, with colony-specific values scattered on either side (see Fig. 2b). Thus *A. colombica* would seem a good choice for a study on the possible existence of facultative worker policing.

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