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Population genetic structure, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa*

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In social Hymenoptera, within-colony relatedness is usually high due to the haplodiploid sex-determining system. However, factors such as the presence of multiple reproductive queens (polygyny), multiple queen matings (polyandry) or worker reproduction result in decreased relatedness among workers and the brood they rear, and consequently dilute their inclusive fitness benefits from helping. Here, we investigated population genetic structure, mating system, worker reproduction and parthenogenesis in the desert ant *Cataglyphis sabulosa.* Analysis of worker genotypes showed that colonies are headed by a single queen, mated with 1–5 males. The inbreeding coefficient within colonies and the levels of relatedness between the queens and their mates were positive, indicating that mating occurs between related individuals. Moreover, the mates of a queen are on average related and contribute equally to worker production. Our analyses also indicate that colonies are genetically differentiated and form a population exhibiting no isolation-by-distance pattern, consistent with the independent foundation of new colonies (that is, without the help of workers). Finally, both ovarian dissections and genetic data on the parentage of males show that workers do not reproduce in queenright colonies; however, they lay both haploid (arrhenotokous males) and diploid (thelytokous females) eggs in queenless colonies. In contrast to the congeneric species *C. cursor*, where new queens are produced by thelytokous parthenogenesis, female sexuals of *C. sabulosa* result from classical sexual reproduction.
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Introduction

In social insects, workers give up most or all of their own reproduction to help relatives in their colonies to reproduce. Hamilton (1964) kin selection theory provided the evolutionary background for this altruistic behaviour. Relatedness between colony members is pivotal in kin selection, because it directly influences the benefits from indirect fitness. In social Hymenoptera, within-colony relatedness is usually high. This is largely a consequence of the haplodiploid sex-determining system, whereby males typically result from unfertilized, haploid eggs and females from fertilized, diploid eggs (Cook and Crozier, 1995). In colonies headed by a single, once-mated queen, workers are thereby more closely related to their sisters ($r = 0.75$) than to their own male and female offspring ($r = 0.5$).

However, two primary factors are known to alter the colony kin structure and, hence, within-colony relatedness: the number and relatedness of reproducing queens in a nest, and the number and relative contribution of male mates of the queen to colony offspring (Crozier and Pamilo, 1996). Multiple queen colonies (polygyny) have been reported in many ant species. Under polygyny, within-colony relatedness is directly affected by the relatedness among queens and it may approach zero when queens are unrelated. Moreover, colony kin structure may vary over time due to the replacement of old queen(s) by newly inseminated ones. Multiple mating by queens (polyandry) also lowers relatedness, but not as dramatically as polygyny, because the offspring workers still remain half sisters and their relatedness never drops below 0.25.

In ants, both polygyny and polyandry have been shown to influence many aspects of social behaviour and reproduction (Bourke and Franks, 1995; Crozier and Pamilo, 1996). For instance, polygyny is commonly associated with reduced mating flights, readoption of newly mated queens into their natal nest and colony fission, whereby queens found new colonies dependently, that is, with the help of a worker force (Hölldobler and Wilson, 1990). By contrast, under monogyny, reproduction usually relies on large mating flights, and newly mated queens found their colonies independently, without the assistance of workers. Variations in mating and dispersal strategies have important consequences on the genetic structure, at both the colony and population levels (Ross, 2001).

Queen-mating frequency also directly influences workers' reproductive interest concerning, for example,

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male parentage (Ratnieks, 1988). In many ant species, workers are not completely sterile and can lay unfertilized (haploid) eggs that will develop into males. In colonies headed by a single, once-mated queen, workers may promote their fitness by rearing sons ($r = 0.5$) and nephews (other workers' sons, $r = 0.375$) rather than queen's sons ($r = 0.25$). However, when workers are the offspring of a queen that is effectively mated to more than two males, they are still more related to their own sons, but on average less related to other workers' sons ($r < 0.25$), than to the queen's sons. On relatedness grounds alone, workers should prevent each other from reproducing to favour the production of queen's sons (Ratnieks, 1988). Reduction in the reproductive success of other workers may result from two 'policing' mechanisms (Ratnieks *et al*., 2006): selective oophagy of worker-laid eggs and/or aggression towards individual workers with activated ovaries.

The ant genus *Cataglyphis* comprises about a hundred species distributed in the Old World, in arid habitats including deserts and saline and dry plains (Agosti, 1990). Although the genus has been the focus of many behavioural studies (for example, Wehner and Menzel, 1969; Collett *et al*., 1998), the population structure and reproductive strategies have remained poorly studied. Recent genetic analyses in the monogynous species *C. cursor*, however, have revealed a unique blend of peculiar biological traits among ants, including (1) colony reproduction by budding (Lenoir *et al*., 1988), which is typical of the polygynous syndrome, (2) a level of polyandry higher than that usually reported in ants (Pearcy *et al*., 2004a) and (3) the conditional use of sexual and asexual reproduction for the production of non-reproductive and reproductive offspring, respectively, with workers resulting from fertilized eggs and new queens being produced asexually by thelytokous parthenogenesis (Pearcy *et al*., 2004a). Thus, by using alternative modes of reproduction, queens of the ant *C. cursor* increase the transmission rate of their genes to their reproductive female offspring while maintaining genetic diversity in the worker force. Whether such life-history traits are species specific or also occur in other species belonging to the genus *Cataglyphis* remains unknown.

We investigated population genetic structure, mating system, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa* (Kugler, 1981). This species belongs to the group *bombycinus*, which is phylogenetically distant from the group *cursor* (Agosti, 1990). Using polymorphic DNA microsatellites, we first estimated within-colony genetic relatedness and inbreeding and investigated the dispersal strategy by testing for a possible correlation between geographical and genetic distances among colonies (that is, isolation by distance). We also determined the frequency of colonies with single and multiple matrilines on the basis of genetic data. Second, we determined queen-mating frequency from field collections and from laboratory mother-offspring genetic combinations. Third, we investigated the possibility of worker reproduction by combining genetic and physiological (ovarian development) data. More particularly, we determined male parentage to assess whether workers are reproductively active in the presence of a functional queen. In addition, we explored the possibility of arrhenotokous and thelytokous parthenogenesis by sampling the number of male and female larvae produced by orphaned workers. Finally, we tested whether queens of *C. sabulosa* use sex for the production of workers and parthenogenesis for the production of new queens, as previously described in the congeneric species *C. cursor*.

Materials and methods

Field collection and sampling

Cataglyphis sabulosa is found in sand dunes in the southern Coastal Plain of Israel, Northern Sinai and the Arava Valley (Shalmon, 1981). Nests are usually established close to vegetation spots and extend up to 1.5-metres deep in the sand. Nuptial flights occur in the mornings at the end of April or early May (Shalmon, 1981). Males fly from their natal nest and land at the entrance to a foreign colony. Copulation occurs close to nest entrance. New colonies are founded independently; freshly mated queens fly away, land, shed their wings and start digging a new nest.

Twenty-nine colonies of *C. sabulosa* were sampled at Nizzanim Park (Ashdod, Israel), in early May 2005 and 2006, at the time of the nuptial flights. Males and females were seen flying around in both years. The study area was approximately 100×80 metres. Colonies whose entrances were too close together were not excavated to prevent mixing of individuals from different nests. Care was taken not to miss any room or gallery that might contain individuals. Adult ants (queens, sexuals and workers) as well as brood at various stages (eggs, larvae and both worker and sexual pupae) were collected. A sample of workers and all adult sexuals from each colony were immediately stored in 98% ethanol for subsequent genetic analyses. The remaining part of each nest was brought to the laboratory; the number of workers, queens and sexual offspring per colony was counted. Colonies were maintained under laboratory conditions ($31 \pm 2\,^{\circ}C$ and natural photoperiod 12:12 h light:dark) and fed on maggots, sugar water and grapes. They were surveyed twice a week, and all adult sexuals emerging from the pupae were collected and deep-frozen.

Sample extraction and microsatellite analysis

We used five microsatellite loci previously characterized for the species *C. cursor* (cc54, cc60, cc63a, cc96 and cc100; Pearcy *et al*., 2004b). Individual ant DNA was extracted by homogenization in a digestive solution (100 mM NaCl, 50 mM Tris, 1 mM EDTA, 0.5% SDS and 200 $\mu g\,ml^{-1}$ proteinase K (Biogene, Kimbolton, UK) and incubated for 12 h at 55 °C. Genomic DNA was purified by phenol/chloroform and precipitated with ethanol following standard protocols, and then suspended in 100 µl of distilled water. Amplifications were carried out in a 10 µl volume containing 2 µl of DNA extract, 0.19 mM of each dNTP, 0.20 µM of each primer, 1 × Taq buffer (containing 15 mM $MgCl_2$) and 0.03 U Taq DNA Polymerase (Qiagen, Venlo, The Netherlands). Amplifications were performed in an MJ Research PTC-200 thermal cycler with the following parameters: after an initial denaturing step of 3 min at 94 °C, the PCR consisted of 15 cycles of 25 s at 94 °C, 40 s at 65 °C, with a decrease of 0.5 °C per cycle and 40 s at 72 °C, followed by 20 cycles of 25 s at 94 °C, 40 s at 56 °C and 40 s at 72 °C, with 10 more minutes at 72 °C for the final extension. Amplified

fluorescent fragments were visualized using the automated Applied Biosystems Prism 3100 Sequencer (Applied Biosystems, Foster City, CA, USA). The size of the different alleles was determined using the Rox 350HD internal size standard and the GENESCAN version 3.2.1 analysis software (Applied Biosystems).

Cross-species amplification of microsatellite markers may result in null alleles due to mutations at primer sites, and allele drop-outs are frequent with microsatellites. This may induce incorrect assignment of genotypes and greatly bias population genetic analyses. To control for such genotyping errors, we used the software Micro-Checker (Van Oosterhout *et al*., 2004). Basic statistics were tested with GENEPOP ON THE WEB.

Genetic relatedness and social structure

A total of 8–25 workers ($x \pm$ s.d. $= 19.6 \pm 3.6$; $n = 570$) and the queen, when found during collection ($n = 20$), were typed from each colony ($N = 29$). Allele frequencies and *F*-statistics were estimated using *FSTAT* (Goudet, 2001). Individuals within colonies are related and so do not represent independent samples. Therefore, we estimated the inbreeding coefficient F (the correlation between pairs of genes within individuals) through a two-level (individual and colony) hierarchical *F*-analysis of variance using GDA 1.1 (Lewis and Zaykin, 2001). Confidence intervals were obtained by bootstrapping over loci 1000 times. The frequency of sib-mating α was estimated from $F = \alpha/(4-3\alpha)$ (Pamilo, 1985). Isolation by distance was investigated by plotting $(F_{st}/(1-F_{st}))$ coefficients between pairs of colonies against the ln of geographical distance. Significance of correlation coefficient between genetic differentiation and geographical distance was assessed with Mantel test. Relatedness coefficients r were estimated using the algorithm of Queller and Goodnight (1989) implemented in the program RELATEDNESS (version 5.0.8). Colonies were weighted equally and standard errors were obtained by jackknifing over colonies. The inbreeding corrected coefficient of relatedness r^* was estimated according to the equation of Pamilo (1985),

$$r^* = (r - 2F/(1+F))/(1 - 2F/(1+F))$$

Number of matrilines per colony

The minimum number of queens in each colony was determined from field observations and by comparing the pedigree of the queen and the workers at all loci. If the queens had not been sampled ($n = 9$), the genotype of the presumed single queen was inferred from those of workers. Individuals were assigned as belonging to different matrilines if they did not share an allele with the queen for at least at one locus. Assignment of individuals to matrilines was confirmed with the maximum-likelihood methods implemented in the program COLONY 1.2 (Wang, 2004).

Mating frequency

The absolute number of mating per queen (M_p) (that is, minimum number of males inferred from worker genotypes) was estimated from field collections, on the basis of worker genotypes of a single matriline ($x \pm$ s.d. $= 19.6 \pm 3.7$, $N = 29$ colonies; Table 1). This was easily achieved, as all colonies sampled were monogynous (see results). In addition, queen-mating frequency was assessed from pedigree analyses from mother–offspring combinations under laboratory conditions. One year after collection, a sample of 41 callow workers ($x \pm$ s.d. $= 10.3 \pm 4.0$; Table 1) and the mother queen from each of four laboratory-reared colonies were genotyped. As development from the egg to the adult stage lasts up to 2 months (personal observation), all callows have definitely originated from eggs laid by the colony queen in the laboratory.

The effective number of mating per queen ($M_{e,p}$) was estimated following Boomsma and Ratnieks (1996),

$$M_{e,p} = 1/\sum p_i^2$$

where p_i is the proportional contribution to the brood of the ith mate. This value was corrected for non-sampling error due to limited sample size, according to Pamilo (1993),

$$\pi = ((N\sum p_i^2) - 1)/(N-1)$$

where N is the number of offspring typed. This estimated pedigree-effective mate number was also corrected for inbreeding, according to Pedersen (personal communication),

$$M_{e,p,i} = \left(\pi\left(1 - \prod_{k=1}^{l}(F + (1-F)(1-H_{\exp,k}))\right)\right)^{-1}$$

where π is $M_{e,p}$ corrected for sampling error, F is the inbreeding coefficient and $H_{\exp,k}$ is the expected heterozygosity at the kth locus in the absence of inbreeding.

In addition, because two males may bear the same alleles at all loci studied, we estimated this non-detection error for each colony, according to Boomsma and Ratnieks (1996),

$$P_{\text{non-detect}} = \prod_j \sum_i f_{ij}^2$$

where f_{ij} is the frequency at the population level of the allele carried by the jth male at the ith locus.

Reproductive skew (that is, the unequal contribution of each father in worker production) of a given colony was quantified with the B index (Nonacs, 2000),

$$B = \sum_{i=1}^{N}\left(p_i - \frac{n_i}{N_t}\right)^2 - \left(1 - \frac{1}{\bar{N}}\right)\Big/k$$

where p_i is the proportion of offspring sired by the ith male and K is the total number of offspring. n_i is the time male i spends in a colony, N_t is the sum of the n_i for the colony and $\bar{N} = N_t/n_{\max}$ is a weighted mean group size, where $n_{\max}$ is the maximum time any male could be present; in our study, n_i is equal for all males as all males were assumed to be present the same length of time. The B index is equal to zero for randomly distributed reproduction, positive when skew is higher than random and negative when more evenly distributed than random. The B index combines observed variance with the expected binomial variance and adjusts for group size and productivity. Reproductive skews and the associated probability level for each group and across

Table 1 Queen-mating frequency and paternity skew

Colony	*Size*	*nw*	*nm*	*nf*	M_p	$M_{e,p}$	$M_{e,p,i}$	*Paternity skew*
IS01	80	22	0	0	4	2.92	3.56	0.059
IS03	221	23	17	11	4	2.80	3.39	0.075
IS04	212	19 (8)	11	1	3 (2)	2.09	2.47	0.111
IS05	82	16	0	0	1	1.00	1.11	—
IS06	175	21	0	0	4	3.71	4.76	−0.016
IS07	238	25	0	14	4	2.96	3.58	0.058
IS08	315	15 (15)	0	9	2 (2)	1.92	2.29	−0.013
IS09	128	20	0	0	2	1.60	1.84	0.100
IS11	264	8	30	0	3	1.68	2.07	0.177
IS12	303	19	1	0	2	1.99	2.35	−0.025
IS13	290	15	29	4	3	2.53	3.15	0.018
IS14	143	22	1	0	3	2.18	2.57	0.095
IS15	138	22	0	0	5	4.03	5.24	0.012
IS16	369	19 (12)	0	1	2 (2)	1.76	2.04	0.042
IS17	358	17 (6)	0	0	4 (2)	3.11	3.98	0.028
IS19	144	21	0	6	4	3.53	4.49	−0.002
IS20	106	24	0	11	2	2.00	2.32	−0.021
IS21	296	23	5	1	3	2.53	3.02	0.033
IS22	282	22	5	2	3	2.78	3.38	−0.004
IS23	193	20	0	0	5	3.23	4.06	0.070
IS24	177	20	0	1	5	4.65	6.40	−0.025
IS25	47	17	0	0	5	3.18	4.08	0.068
IS26	193	22	0	0	4	3.61	4.58	−0.007
IS27	59	14	2	0	2	1.85	2.20	0.005
IS28	171	22	0	6	3	2.55	3.06	0.029
IS29	48	18	0	0	2	1.67	1.93	0.071
IS30	192	21	0	0	2	1.96	2.29	−0.014
IS31	66	21	0	0	3	2.55	3.07	0.027
IS32	80	22	0	0	2	1.94	2.25	−0.006
Mean (1)		19.65 10.3						
± s.e.		0.66 0.74						
Mean (2)	185.72	21.07 ± 4.78			2.54 ± 1.16	2.30	2.75	0.034
± s.e.	17.71	0.84			0.21	0.20	0.22	0.009

The size of colonies (total number of workers), the number of workers (nw), males (nm) and sexual females (nf) typed, the absolute number (M_P) and effective number ($M_{e,p}$) of matings and paternity skew are given for each field colony. Data obtained from mother–offspring combination genetic analyses are given in parentheses. Paternity skew values take Bonferroni correction for multiple comparisons into account. Mean (1) is calculated from field samples or from laboratory samples. Mean (2) is calculated from the combination of both field and laboratory samples. Harmonic means are given for M_P and $M_{e,p}$.

all groups were calculated using the program SKEW CALCULATOR 2003 developed by P. Nonacs.

Worker reproduction

Male parentage: We investigated the possibility of worker reproduction by directly comparing the genotype of the males produced with the queen's genotype. Ninety-seven males were typed from six colonies ($x \pm$ s.d. $= 16.2 \pm 11.2$). Sons of queens must carry a queen-derived allele at all loci and, as a group, they should not display more than two alleles at a single locus. Sons of workers may carry with equal probability an allele derived from either the mother or the father of the worker. Any male that carries a non-queen allele is a worker's son. However, because sons of workers may carry queen alleles at all loci by chance, this probability of non-detection was estimated following Foster *et al*. (2001),

$$P_{\text{non-detect}} = \sum_{i}^{n} p_i(0.5^{li})$$

where n is the number of patrilines in the nest, p_i is the proportional contribution of the ith father to the brood and l_i is the number of informative loci analysed at the ith patriline. An informative locus is one where the queen and her mate have different alleles so that the workers carry an allele that the queen does not.

Ovary activation: We also examined worker reproductive activity by means of ovarian dissection. A sample of 152 adult workers ($x \pm$ s.d. $= 19 \pm 18.6$) from eight queenright colonies producing males and/or females were dissected in ethanol and their level of ovarian activity were described according to the following scale of ovarian development: stage 1, reduced ovarioles; stage 2, slightly expanded ovarioles, one with a small yolky oocyte; stage 3, expanded ovarioles, one with a fully developed oocyte and the others with smaller yolky oocytes; stage 4, all ovarioles have one fully developed oocyte and smaller yolky oocytes. This scale was determined on the basis of preliminary studies of ovarian activation of workers from queenless colonies.

Parthenogenesis: We explored the possibility of worker reproduction by thelytokous parthenogenesis by determining the sex of a sample of 21 larvae originating from worker reproduction in three colonies orphaned from at least 10 months ($x \pm$ s.d. $= 7.0 \pm 5.3$). In *C. sabulosa*,

the eggs complete development and reach the adult stage within 5 weeks under laboratory conditions (personal observation), so that all the eggs sampled were undoubtedly worker-produced. In ants, the sex of larvae cannot be determined on the basis of external morphological characters. We therefore used flow cytometry, a method that allows us to distinguish between haploid males and diploid females on the basis of their nuclear DNA content (Aron *et al*., 2003). Flow cytometry analyses were performed on a Partec PA (Germany) flow cytometer after treatment of larvae with a diamidino-4′,6-phénylindol-2 dichlorhydrate staining solution.

Thelytokous parthenogenesis by queens

To examine whether queens of *C. sabulosa* use asexual reproduction (that is, thelytokous parthenogenesis) for the production of new queens, as previously described in the species *C. cursor* (Pearcy *et al*., 2004a), we compared the pedigree of the queen with that of her sexual daughters. A sample of 61 sexual females ($x \pm$ s.d. $= 8.7 \pm 3.5$) from seven colonies was analysed. New queens harbouring only alleles identical to those of their mother may be fathered by a male with no diagnostic allele (no allele distinct from those of the queen) or may be parthenogenetically produced. To estimate the probability that a queen had mated with a male with no diagnostic allele, we determined for each queen and each locus the probability that a male would share one of the queen's alleles, following Pearcy *et al*. (2004a), $P_{\text{non-detection}} = (f_{1i} + f_{2i})$ when the queen is heterozygous at the ith locus, where f_{1i} and f_{2i} are, respectively, the frequency of the first and the second allele at the ith locus in the studied population, and $P_{\text{non-detection}} = (f_{1i})$ when the queen is homozygous, that is, to the population frequency of the allele (f_{1i}).

To obtain the overall probability of absence of any diagnostic allele, the values obtained for each of the loci were multiplied for each queen. Using this method, the probability of queens mating with a male harbouring no diagnostic allele at any of the loci was low, ranging from 0.002 to 0.036 across colonies.

Results

Genetic relatedness and social structure

Of the five microsatellite markers studied, one showed null alleles (cc54) and was excluded from the analysis. The other four markers showed no linkage disequilibrium; they ranged from 3 to 11 alleles, with a mean observed heterozygosity $H_o = 0.60$ (range: 0.44–0.74) and a mean expected heterozygosity $H_e = 0.63$ (range: 0.47–0.77). A significant departure from Hardy–Weinberg equilibrium was found in the studied population. The fixation index F was significantly different from 0 (mean $\pm$ s.e. $= 0.06 \pm 0.02$; 95% CI: 0.04–0.09). Inbreeding was also supported by the positive, though non-significant, relatedness between the queens and their pedigree-estimated mates $r_{q-m} = 0.16$ (two-tailed t-tests, $N = 29$, $t = 1.778$, $P = 0.086$). The frequency of sib-mating reached a value of $\alpha = 0.20$. F_{st} estimate (0.27 ± 0.03; 95% CI: 0.21–0.30) indicated a high genetic divergence between nests. There was no significant correlation between genetic differentiation between pairs of colonies and the geographical distance (Mantel test: matrices correlation $r = -0.02$; $P = 0.314$), indicating no isolation-by-distance pattern in the study population.

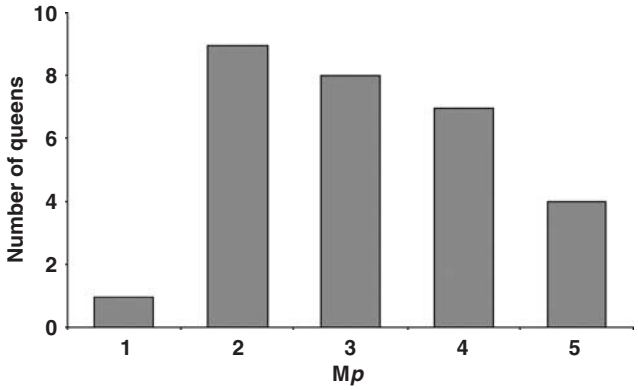

Figure 1 Distribution of the absolute number of mating (M_p) per queen ($n = 29$) in *C. sabulosa*.

The mean within-colony genetic relatedness r_{w-w} among nestmate workers was 0.49 ($SE_{jackknife} = 0.05$) and the inbreeding corrected relatedness estimate r_* was 0.44. These values were significantly lower than the 0.75 expected under monogyny and monandry (two-tailed t-tests, $N = 29$, both $P < 0.001$).

Number of matrilines per colony: A single queen was found in 20 colonies out of the 29 excavated; no queen was collected in the remaining nine colonies. All workers sampled were unambiguously assigned to the queen present in each nest. Similarly, in colonies where queens were not collected, worker genotypes were compatible with single maternity. In line with these results, the average colony relatedness between the workers and the queen r_{q-w} equals 0.53 (± 0.05; 95% CI: 0.43–0.63), not significantly different from 0.5 expected under strict monogyny (two-tailed t-test, $t = 0.26$, $N = 29$, $P = 0.801$).

Mating frequency: Pedigree analyses from both workers and queens collected in the field and from mother–offspring combinations under laboratory conditions were consistent with multiple-mated queens (Table 1). Across the 29 colonies sampled on the field, we found that queens had mated with at least 1–5 males (Figure 1). Similar estimates of queen-mating frequency were obtained from mother–offspring genetic combinations from laboratory-reared colonies. When both field and laboratory results were pooled, the absolute number of patrilines per colony was on average $M_p = 2.54 \pm 0.21$, the effective number of patrilines was $M_{e,p} = 2.30 \pm 0.20$ and the effective number of patrilines corrected for inbreeding and sampling error was $M_{e,p,i} = 2.75 \pm 0.22$. The average probabilities of non-detection due to two males bearing the same alleles at all loci ($P_{\text{non-detect}} = 0.01 \pm 0.01$) were reasonably low. Mating frequency was not correlated with colony size (Pearson's correlation: $r_P = -0.003$, $N = 29$, $P = 0.986$). The estimated relatedness among the male mates of a single queen was on average $r_{m-m} = 0.11$ ($SE_{jackknife} = 0.03$; 95% CI: 0.05–0.17), a value significantly different from 0 (two-tailed t-test: $t = 3.43$, $N = 28$,

$P = 0.002$). No significant paternity skew was observed in the studied population (Table 1).

Across the 29 colonies sampled, there was a positive and significant association between colony size and total sexual productivity (Spearman rank correlation, $r_s = 0.47$, $P = 0.01$; Table 1).

Worker reproduction

Male parentage was investigated in the six colonies that had produced at least four adult males (IS03, IS04, IS11, IS13, IS21, IS22; Table 1). All males typed showed a single allele at each locus, indicating that they were haploid. None of the 97 males examined carried non-queen alleles, suggesting that the workers had not produced them. The average probability of non-detection error, due to worker-derived males carrying queen alleles at all loci, over all colonies, was 0.35. Therefore, sons present in the sample should be detected with a probability of about 65%. Whether workers produce females by thelytokous parthenogenesis cannot be determined by genetic analyses, because worker-produced females are expected to bear the same genotype as queen-offspring (workers and gynes). However, none of the 152 workers dissected had expanded ovaries or developing eggs; their ovarioles were reduced and empty. These results are consistent with the complete absence of worker reproduction in queenright colonies of *C. sabulosa.*

Flow cytometric analyses of the 21 larvae developing from worker-laid eggs in the three orphaned colonies showed that nine larvae were haploid (males; 2, 4 and 3 larvae in IS03, IS11 and IS28, respectively) and 12 were diploid (females; 3 and 9 larvae in IS03 and IS11, respectively). Thus, workers of *C. sabulosa* can produce both males by arrhenotokous parthenogenesis and females by thelytokous parthenogenesis. No adults emerged from these worker-produced diploid larvae, so we were unable to determine whether they would have developed into workers or queens.

Thelytokous parthenogenesis by queens

In the seven colonies producing at least four sexual females (IS03, IS07, IS08, IS13, IS19, IS20, IS28; Table 1), the genotype of all such females differed from that of the queen for at least one locus, indicating that new queens are not produced by thelytokous parthenogenesis. Consistent with this result, the relatedness between the queens and their sexual daughters $r_{q-f} = 0.41$ ($SE_{jackknife} = 0.09$; 95% CI: 0.22–0.60) did not differ from that between the queens and the workers $r_{q-w} = 0.53$ ($SE_{jackknife} = 0.05$; 95% CI: 0.43–0.63) (paired *t*-test: $t = 0.03$, $N = 7$, $P = 0.975$).

Discussion

Our genetic data show that *C. sabulosa* colonies are the product of a single, multiple-mated queen. We found no evidence of polygyny in our populations; all workers could be assigned to the single resident queen. Most queens (97%) mate more than once and there are no significant differences in the apportionment of offspring among patrilines in most colonies. Therefore, genetic diversity beyond that typical of simple Hamiltonian families in colonies of *C. sabulosa* is solely because of polyandry. Queens mate with up to five different males, with an average effective mating frequency of $M_{e,p} = 2.30$. This estimate is comparable, although lower, with that previously reported in the species *C. cursor*, where queens mate with 2–8 males, and the effective mating frequency reaches 3.79 (Pearcy *et al*., in preparation). Multiple mating is relatively uncommon in ants where effective queen-mating frequency is usually lower than 2 (Boomsma and Ratnieks, 1996; Crozier and Fjerdingstad, 2001). Although queens from a large number of species can mate twice (Boomsma and Ratnieks, 1996), higher levels of polyandry are restricted to only a few genera, including *Atta* and *Acromyrmex* leafcutter ants, *Pogonomyrmex* harvester ants, and *Neivamyrmex*, *Eciton*, *Dorylus*, *Aenictus* army ants (see Kronauer *et al*., 2007 and references therein). Whether multiple mating in *Cataglyphis* is restricted to a few species or is a feature of the genus awaits further studies.

Multiple mating reduces relatedness among colony members compared with monandry and, hence, lessens the benefits from indirect fitness associated with reproductive altruism. Several hypotheses have been proposed to account for selection of polyandry in social insects (reviewed by Crozier and Fjerdingstad, 2001). A first set of hypotheses suggests that increased genetic diversity could enhance colony fitness through improving colony-level resistance to pathogens, by facilitating genetic polyethism, by decreasing the genetic load due to production of sterile diploid males or by reducing within-colony relatedness asymmetries and, hence, the costs associated with conflicts between queen(s) and workers over male production or the sex ratio of reproductive offspring. The second set of hypotheses suggests that multiple mating has been selected to increase the lifetime supply of sperm, to increase sperm competition or because it could be more costly to resist male sexual attempts than to mate. At least two of these suggestions seem unlikely for *C. sabulosa*. (i) In Hymenoptera, with complementary sex determination, individuals that are homozygous at the sex determination locus (or loci) develop into diploid males rather than females (Cook and Crozier, 1995). Production of diploid males imposes a cost on colony fitness because rearing efforts are wasted on individuals that do not work, and as diploid males are usually sterile, they also do not reproduce (Ratnieks, 1990). Not a single adult diploid male was found in our sample of 97 males, suggesting that they are infrequent or even absent. On the other hand, the lack of adult diploid males does not exclude the possibility that they are produced but removed by workers before maturation, as has been documented in the honeybee (Woyke, 1963; Ratnieks, 1990). To the best of our knowledge, there is no direct empirical evidence to date of selective removal of diploid male brood in ants (but see Kronauer *et al*., 2007 for a possible test). Thus, at this point, it seems unlikely that polyandry in *C. sabulosa* serves to reduce the genetic load associated with production of diploid males. (ii) The sperm limitation hypothesis predicts that queens should mate with multiple males to acquire more sperm (Cole, 1983). The amount of sperm stored has been considered as a potentially limiting factor primarily in species with very large and long-lasting colonies. Colonies of *C. sabulosa* are small and probably rarely exceed 300 individuals (Table 1). Moreover, this

hypothesis suggests a positive association between colony-size and queen-mating frequency ($M_{e,p,i}$), which was not found in our study population (Spearman rank correlation, $r_s = 0.07$, $P = 0.72$). Altogether, these data make the sperm limitation hypothesis improbable for the species.

One interesting finding of this study is that the mates of a single queen were related to each other. Such a relatedness pattern was reported in other ant species (for example, *Plagiolepis pygmaea*; Trontti *et al*., 2007). Moreover, a significant proportion of males (20%) copulated with their sisters. This is consistent with the positive relatedness between the queens and their pedigree-estimated mates, which results in a significant level of inbreeding in the population. As the males are capable of flying and do swarm, one cannot exclude the possibility that some of the males had already mated in their natal nest before dispersing, in search of other females with which to mate. Our genetic analyses indicate no significant isolation by distance in the population studied, which is also in agreement with field observations that queen *C. sabulosa* fly away after copulation and exhibit a solitary mode of colony foundation (Shalmon, 1981). Overall, the mating and dispersal strategies of *C. sabulosa* greatly differ from that reported in *C. cursor*, where (i) populations are outbred, females mate close to the nest entrance and with foreign males only, and (ii) mated queens do not fly, but (iii) they found colonies dependently in the close vicinity of their natal nest, which results in a strong local population genetic structure (Pearcy *et al*., 2004a).

Our data also show that workers of *C. sabulosa* can lay eggs that produce males by arrhenotokous parthenogenesis and females by thelytokous parthenogenesis. However, we found no evidence of worker reproduction in queenright colonies. None of the 152 workers dissected from male and/or female sexual producing colonies had activated ovaries, and there was no genetic evidence that any male was derived from a worker-laid egg. Conversely, workers do reproduce and raise their brood in queenless colonies. This indicates that worker reproduction, if any, is rare in the presence of the queen in *C. sabulosa*, in common with *C. cursor* (Cagniant, 1980). With an estimated nestmate worker genetic relatedness of 0.49, workers are on average equally related to queen- and worker-derived males. On the basis of relatedness values only, there should be no selection for worker policing to evolve and workers could reproduce. The same argument holds true for thelytokous worker reproduction. This is because workers' sisters (queen offspring) and nieces (daughters of other workers) are on average equally related to any focal worker, regardless of the degree of polyandry (Greeff, 1996). Thus, there should be no selection on workers to prefer rearing one above the other. Moreover, unlike arrhenotokous worker reproduction, thelytokous worker reproduction does not inflict a cost upon the colony queen due to differences in relatedness, because the latter is equally related to her daughters and granddaughters. Therefore, on relatedness grounds, one should not expect queens to resist thelytokous worker reproduction (Greeff, 1996). The absence of workers with activated ovaries suggests that the lack of worker reproduction is not merely a consequence of extant worker- or queen-policing, but rather that worker reproduction is not profitable, either on an individual or on a colony-level basis. Factors other than relatedness effectively act as a brake on worker reproduction. Queens sometimes monopolize male production either by forcibly preventing workers from reproducing or by chemical control (Hölldobler and Wilson, 1990). This explanation seems unlikely in *C. sabulosa*. We never observed directed aggression of queens towards workers. On the other hand, chemical manipulation of workers by the queens should not be evolutionarily stable, as workers should be selected to ignore such signals (Keller and Nonacs, 1993). Another, more convincing, explanation is that workers refrain from reproducing because of costs to the colony (Ratnieks, 1988; Hartmann *et al*., 2003; Hammond and Keller, 2004; Ratnieks *et al*., 2006). For example, reproductive workers spend time in hierarchical interactions and egg laying, at the expense of foraging or brood rearing. Workers may also be unable to distinguish between worker-laid and queen-laid eggs, in which case they may mistakenly replace queen-laid diploid eggs with their own males, hence reducing production of the worker force. Worker reproduction may also translate into the production of excess offspring. The extent of worker reproduction should therefore depend largely on its effects on colony efficiency, which is expected to vary across species (Hammond and Keller, 2004).

The ability of workers to reproduce by both modes of parthenogenesis has been reported in several other ant species: *Cerapachys biroi* (Tsuji and Yamauchi, 1995), *Platythyrea punctata* (Heinze and Hölldobler, 1995), *Messor capitatus* (Grasso *et al*., 2000) and *Cataglyphis cursor* (Cagniant, 1980). In these species, worker reproduction reportedly occurs in queenless colonies only. In *C. cursor*, the production of unfertilized diploid eggs has been shown to result from automictic parthenogenesis, with the central fusion of polar nuclei obtained at the end of the gametogenesis (Pearcy *et al*., 2006). Whether the same mechanism underlies the parthenogenetic production of females by workers of *C. sabulosa* remains to be verified. It has been suggested that thelytokous parthenogenesis in *C. cursor* was selected to counter high queen mortality and to allow workers to replace the queen when she dies (Lenoir *et al*., 1988). Recent genetic analyses support the assumption that queen replacement is indeed a common phenomenon in this species (Pearcy *et al*., 2006). Given the level of heterozygosity of the markers and the positive inbreeding coefficient in our study population, these data do not allow us to completely exclude this possibility in *C. sabulosa*. However, in the 29 colonies sampled, all genotyped workers and sexuals were compatible with being offspring of the resident queen, suggesting that queen replacement is uncommon in this species. It is also unclear whether diploid eggs laid by workers can develop into female sexuals or whether they give rise to workers only. These aspects certainly merit further studies.

Unlike queens of *C. cursor*, which produce new queens by thelytokous parthenogenesis and workers by normal sexual reproduction (Pearcy *et al*., 2004a), *C. sabulosa* queens do not use such alternative modes of reproduction for the production of reproductive and non-reproductive offspring. New queens and workers are both produced by sexual reproduction. It has been

suggested that the conditional use of parthenogenesis for new queen production would primarily occur in dependent-founding species, because the presence of workers compensates for the negative effects of increased inbreeding on founding queens due to thelytoky (Pearcy *et al*., 2004a). In line with this prediction, the production of female sexuals by queens through thelytokous parthenogenesis has been reported in three ant species with a dependent mode of colony foundation: the monogynous ant *C. cursor*, and two polygynous species, *Wasmannia auropunctata* (Fournier *et al*., 2005) and *Vollenhovia emeryi* (Ohkawara *et al*., 2006). As mentioned earlier, our genetic analyses confirm that colony reproduction in *C. sabulosa* proceeds through flight dispersal and independent colony founding. Further studies on population genetics and mating strategies in other *Cataglyphis* species may allow us to determine whether multiple-mating and thelytokous parthenogenesis originated relatively recently in the evolution of the genus, or whether they are ancestral characters.

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References

Agosti D (1990). Review and reclassification of *Cataglyphis* (Hymenoptera Formicidae). *J Nat Hist* **24**: 1457–1505.

Aron S, De Menten L, Van Bockstaele D (2003). Brood sex ratio determination by flow cytometryin ants. *Mol Ecol Notes* **3**: 471–475.

Boomsma JJ, Ratnieks FLW (1996). Paternity in eusocial Hymenoptera. *Philos Trans R Soc London Ser B* **351**: 947–975.

Bourke AFG, Franks NR (1995). *Social Evolution in Ants*. Princeton University Press: Princeton, NJ.

Cagniant H (1980). La parthénogenèse thélytoque des ouvrières de la fourmi *Cataglyphis cursor*: étude en élevage de la productivité de sociétés avec reine et de sociétés sans reine. *Insect Soc* **27**: 157–174.

Cole BJ (1983). Multiple mating and the evolution of social behavior in the Hymenoptera. *Behav Ecol and Sociobiol* **12**: 191–201.

Collett M, Collett TS, Bisch S, Wehner R (1998). Local and global vectors in desert ant navigation. *Nature* **394**: 269–272.

Cook JM, Crozier RH (1995). Sex determination and population biology in the hymenoptera. *Trends Ecol Evol* **10**: 280–286.

Crozier RH, Fjerdingstad E (2001). Polyandry in social Hymenoptera—disunity in diversity? *Annales Zoologici Fennici* **38**: 267–285.

Crozier RH, Pamilo P (1996). *Evolution of Social Insect Colonies*. Oxford University Press: Oxford.

Foster KR, Ratnieks FLW, Gyllenstrand N, Thorén PA (2001). Colony kin structure and male production in *Dolichovespula* wasps. *Mol Ecol* **10**: 1003–1010.

Fournier D, Estoup A, Orivel J, Foucaud J, Jourdan H, Le Breton J *et al*. (2005). Clonal reproduction by males and females in the little fire ant. *Nature* **435**: 1230–1234.

Goudet J (2001). *FSTAT, a Program to Estimate and Test Gene Diversities and Fixation Indices (version 2.9.3)*. University de Lausanne: Switzerland.

Grasso DA, Wenseleers T, Mori A, Le Moli F, Billen J (2000). Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. *Ethol Ecol Evol* **12**: 309–314.

Greeff JM (1996). Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Phil Trans R Soc Lond B* **351**: 617–625.

Hamilton WD (1964). The genetical evolution of social behavior I&II. *J Theor Biol* **7**: 1–52.

Hammond RL, Keller L (2004). Conflict over male parentage in social insects. *PLoS Biology* **2**: e248.

Hartmann A, Wantia J, Torres JA, Heinze J (2003). Worker policing without genetic conflicts in a clonal ant. *Proc Natl Acad Sci USA* **100**: 12836–12840.

Heinze J, Hölldobler B (1995). Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant *Plathytyrea punctata*. *Naturwissenschaften* **82**: 40–41.

Hölldobler B, Wilson EO (1990). *The Ants*. Springer: Berlin.

Keller L, Nonacs P (1993). The role of queen pheromones in social insects: queen control or queen signal? *Anim Behav* **45**: 787–794.

Kronauer DJC, Johnson RA, Boomsma JJ (2007). The evolution of multiple mating in army ants. *Evolution* **61**: 413–422.

Kugler J (1981). A new species of *Cataglyphis* (Hymenoptera; Formicidae) from Israel and Sinai. *Israel J Entomol* **15**: 83–88.

Lenoir A, Quérard L, Pondicq N, Berton F (1988). Reproduction and dispersal of the ant *Cataglyphis cursor* (Hymenoptera Formicidae). *Psyche* **95**: 21–44.

Lewis P, Zaykin D (2001). Genetic data analysis (GDA): computer program for the analysis of allelic data. Version 1.1.

Nonacs P (2000). Measuring and using skew in the Study of Social Behavior and Evolution. *Am Nat* **156**: 577–589.

Ohkawara K, Nakayama M, Satoh A, Trindl A, Heinze J (2006). Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. *Biol Lett* **2**: 359–363.

Pamilo P (1985). Effect of inbreeding on genetic relatedness. *Hereditas* **103**: 195–200.

Pamilo P (1993). Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity* **70**: 472–480.

Pearcy M, Aron S, Doums C, Keller L (2004a). Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* **306**: 1780–1783.

Pearcy M, Clemencet J, Chameron S, Aron S, Doums C (2004b). Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. *Mol Ecol Notes* **4**: 642.

Pearcy M, Hardy O, Aron S (2006). Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity* **96**: 377–382.

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 (1990). The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. *Behav Ecol Sociobiol* **26**: 343–348.

Ratnieks FLW, Foster KR, Wenseleers T (2006). Conflict resolution in insect societies. *Annu Rev Entomol* **51**: 581–608.

Ross KG (2001). Molecular ecology of social behaviour, analyses of breeding systems and genetic structure. *Mol Ecol* **10**: 265–284.

Shalmon B (1981). A preliminary note on the biology of *Cataglyphis sabulosa* Kugler (Hymenoptera: Formicidae) in the Southern Arava Valley, Israël. *Isr J Entomol* **15**: 103.

Trontti K, Thurin N, Sundstrom L, Aron S (2007). Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea*. *Behav Ecol* **18**: 298–303.

Tsuji K, Yamauchi K (1995). Production of females by parthenogenesis in the ant *Cerapachys biroi*. *Insect Soc* **42**: 333–336.
Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004). Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**: 535–538.
Wang J (2004). Sibship reconstruction from genetic data with typing errors. *Genetics* **166**: 1963–1979.
Wehner R, Menzel R (1969). Homing in the ant *Cataglyphis bicolor*. *Science* **164**: 192–194.
Woyke J (1963). What happens to diploid drone larvae in a honeybee colony. *J Apic Res* **2**: 73–75.