

Mating system and population structure in the desert ant *Cataglyphis livida*

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Abstract We investigated population genetic structure, mating system, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis livida*. Pedigree analyses at polymorphic microsatellite loci show that colonies are headed by a single queen, and that queens are mated with two to eight males. No inbreeding was found in the population sampled. Colonies are genetically differentiated and exhibit no isolation-by-distance pattern, consistent with independent foundation of new colonies. Workers do reproduce and lay haploid (arrhenotokous) eggs in queenless colonies; conversely, we found no evidence of worker reproduction in queenright nests. In contrast with *C. cursor*, where new queens are produced by thelytokous parthenogenesis, female sexuals and workers of *C. livida* arise from classical sexual reproduction. We discuss the parallels and contrasts between the mating system and population structure in *C. livida* and the other *Cataglyphis* species studied so far.

Keywords Ants · Thelytoky · Polyandry · *Cataglyphis* · Social structure

Introduction

In social Hymenoptera, within-colony relatedness is usually high due to the haplodiploid sex-determining system,

whereby males arise from haploid eggs and females from diploid ones. However, factors such as the presence of multiple reproductive queens (polygyny), multiple queen mating (polyandry) or worker reproduction induce a reduction in the relatedness among workers and the brood they rear and, hence, dilute the inclusive fitness benefits from helping. The study of mating systems and social structure allows determining how these factors affect relatedness values and the sociogenetic organisation of colonies.

The ant genus *Cataglyphis* comprises a hundred species distributed in the Old World, from Mauritania to the Gobi Desert. Most species develop in opened habitats, including deserts and dry salted plains (Agosti, 1990). The genus has been the focus of several studies, mostly concerning adaptation and resistance to highly demanding biotopes. For example, mechanisms of thermoregulation and orientation in very hot, desertic areas have drawn a lot of attention (thermoregulation: Wehner et al., 1992; Gehring and Wehner, 1995; Cerda and Retana, 2000; navigation: Wehner and Menzel, 1969; Ziegler and Wehner 1997; Collett et al., 1998; Cerda, 2001; Wohlgemuth et al., 2001; Wolf and Wehner, 2005; Knaden and Wehner, 2005; Wehner et al., 2006; Sommer et al., 2008; Steck et al., 2009).

In contrast, mating system and colony social organisation of desertic ants remain largely unstudied. To date, detailed genetic analyses of the reproductive strategy and population structure have been documented for a few species only (*Cataglyphis cursor*, Percy et al., 2004a; Clémencet et al., 2005; *C. mauritanica* and *C. bicolor*, Knaden and Wehner, 2006; *C. sabulosa*, Timmermans et al., 2008). These studies revealed three remarkable features. First, species greatly differ in their dispersal strategies. In ants, social structure is usually associated

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with the mode of dispersion (Bourke and Franks, 1995; Crozier and Pamilo, 1996; Ross, 2001). Single-queen (monogynous) colonies produce new queens that usually disperse from their natal nest during mating flights and initiate new colonies independently (i.e., with no worker assistance). In contrast, new queens from multiple-queen (polygynous) colonies tend to mate in or close to their natal nest; then, young mated queens leave the natal nest to found a new colony dependently (i.e., with the help of a worker force), what ensures sustained colony growth and survival. Dispersion by colony budding limits female dispersal, what usually results in a pattern of isolation-by-distance. In line with these syndromes, in the polygynous *C. mauritanica* colonies reproduce by budding and populations are genetically structure (Knaden and Wehner, 2006), while in the monogynous *C. sabulosa* and *C. bicolor* colonies are founded independently and no isolation-by-distance pattern occurs (Knaden and Wehner, 2006; Timmermans et al., 2008). However, although colonies of *C. cursor* are strictly monogynous, young queens mate close to their natal nest and colony reproduction proceeds by budding (Lenoir et al., 1988; Clémencet et al., 2005; Hardy et al., 2008). Second, queens of *C. sabulosa* and *C. cursor* are polyandrous with effective mating frequencies of 2.30 and 3.79, respectively (Timmermans et al., 2008; Pearcy et al., 2009). No data are available for the other species of *Cataglyphis* studied so far. Multiple mating by queens appears to be uncommon in ants; queen mating frequency is usually lower than 2 (Boomsma and Ratnieks, 1996; Strassmann, 2001; Crozier and Fjerdingstad, 2001; Hugues et al. 2008). Higher levels of polyandry seem 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), as well as *Cardiocondyla* (Lenoir et al., 2007) and *Plagiolepis* (Trontti et al., 2007). Third, the most remarkable feature of *C. cursor* is that queens use alternate modes of reproduction for the production of the worker and queen castes (Pearcy et al., 2004a). While workers are produced by normal sexual reproduction from fertilised eggs, new queens are almost exclusively produced by thelytokous parthenogenesis. This allows queens to increase the rate of transmission of their genes to their sexual female offspring, while maintaining a genetic differentiation in the worker caste. Such a conditional use of sexual and asexual reproduction was not detected in the other *Cataglyphis* species studied so far.

Another characteristic of the reproductive biology of *Cataglyphis* ants is the ability of workers to reproduce by both arrhenotokous and thelytokous parthenogenesis (Cagniant, 1980; Pearcy and Aron, 2006; Timmermans et al., 2008). Worker reproduction was reported in

queenless colonies only. It has been suggested that thelytokous parthenogenesis could be selected for to counter queen mortality and to allow workers to replace the queen when she dies (Lenoir et al., 1988; Pearcy et al., 2006).

To determine whether such life-history traits also occur in other species belonging to the genus *Cataglyphis*, we performed a detailed analysis of the mating system and population genetic structure of the desert ant *Cataglyphis livida*. This species belongs to the group *albicans*, which is phylogenetically distant both from the group *cursor* (*C. cursor*) and from the *bombycinus* (*C. sabulosa*) (Agosti, 1990). Using polymorphic DNA microsatellites, we investigated the population structure, the number of queens present in each colony, and queen mating frequency. We also tested whether queens of *C. livida* take advantage of both sexual and asexual reproduction by selectively using sexual reproduction for production of workers and parthenogenesis for production of new queens, as occurs in the species *C. cursor*. Finally, we examined worker reproduction in two ways: first, we determined male parentage in order to assess whether workers are reproductively active in the presence of a queen; second, we tested the possibility of arrhenotokous and thelytokous parthenogenesis by sampling male (haploid) and female (diploid) larvae produced by orphaned workers.

Materials and methods

Collections and sampling

Cataglyphis livida was sampled from a large population (150 × 200 m) in sand dunes in Tel Aviv (Israel), in early May 2005 and 2006 at the time of nuptial flight. Seventeen colonies were completely excavated and all the workers, brood, sexuals and queens were collected. In some colonies, the majority of male and female adult sexuals had already left. The mean number of workers per colony ± SD was 147 ± 98 ($N = 17$). A sample of workers and all adult sexuals from each colony were immediately stored in 98% ethanol for subsequent genetic analyses. All colonies were then transferred to the laboratory; they were maintained under standard conditions (28 ± 2°C and natural photoperiod 12 h:12 h light:dark) and fed on maggots and sugar water.

Genetic analyses

Five microsatellite loci (cc11, cc26, cc65, cc80 and cc99; Pearcy et al., 2004b) were used to determine the genotypes of pupae and adults. Individual ant DNA was extracted by the same protocol as previously reported for other *Cataglyphis* species (Timmermans et al., 2008). Polymerase

chain reaction (PCR) were performed in three different mix (cc26 + cc65, cc11 + cc80 and cc99), each in 10.0 μ l volumes containing 1.0 μ l of extract of DNA; 1.0 μ l of Buffer 10 \times ; 0.2 μ l of dNTP mix 10 mM; 0.2 μ l of each primer (10 μ M); 0.05 μ l of Taq DNA polymerase (5 U/ μ l) (Qiagen, Venlo, The Netherlands); and 2.0 μ l Q solution only for mix 1 and 2; 0.75 μ l and 0.38 μ l of MgCl₂ 25 mM only for mix 2 and 3, respectively. Loci were amplified using the following PCR program: an initial denaturation step of 3 min at 94°C; 40 cycles of 25 s at 94°C, 40 s at 53°C, 40 s at 72°C and a final elongation step of 10 min at 72°C. PCR products were genotyped using an automated Applied Biosystems Prism 3100 Sequencer (Applied Biosystems, Foster City, CA, USA). The size of the different alleles was determined using the GENESCAN version 3.2.1 analysis software (Applied Biosystems). Control for genotyping errors due to null alleles and allele dropouts was performed with Micro-checker (Van Oosterhout et al. 2004). Linkage disequilibrium and basic statistics were tested with GENEPOP ON THE WEB.

Population structure

A total of 472 workers (adults and pupae) were genotyped at the 5 microsatellites loci ($X \pm SD = 27.8 \pm 12.9$; $N = 17$). Allele frequencies and F statistics were estimated using F_{STAT} (Goudet, 2001). Individuals within colonies do not represent independent samples because they are related to each other. Therefore, we estimated the inbreeding coefficient F 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 1,000 times. Life-for-life relatedness values (r) were calculated with the program RELATEDNESS 5.0.8 (Queller and Goodnight, 1989). Colonies were weighted equally and standard errors were obtained by jackknifing over colonies. Isolation-by-distance was investigated by plotting $[F_{st}/(1 - F_{st})]$ coefficients between pairs of colonies against the \ln of the geographical distance (Rousset, 1997). Significance of correlation coefficient between genetic differentiation and geographical distance was assessed with Mantel test using the algorithm SPAGeDi (Hardy and Vekemans, 2002).

Number of matriline per colony

The minimum number of queens in each colony was determined from field observations and by comparing the pedigree of the queen (when collected) and the workers at all loci. When queens were not sampled ($N = 4$), we inferred the pedigree of the supposed single queen from the genotype of the workers. Individuals were assigned as belonging to a different matriline if they did not share an

allele with the queen at least at one locus. Assignment of individuals to matriline was confirmed with the maximum-likelihood method implemented in the program COLONY 1.2 (Wang, 2004).

Queen mating frequency

The number of mating by queens was estimated from mother-offspring combinations under laboratory conditions and field collection. Six out of the 13 queens collected laid eggs in the laboratory. They were used for pedigree analyses from mother-offspring combinations; a sample of 64 worker pupae were collected ($X \pm SD = 10.7 \pm 8.2$, $N = 6$) and the mother queen from each colony was genotyped. For the six queens sampled, estimates of queen mating frequency from laboratory rearing and from field collection (134 worker pupae, $X \pm SD = 22.4 \pm 9.0$, $N = 6$) were not different (see “Results”), and they were pooled for data analyses. For the remaining 11 colonies, queen-mating frequency was estimated from field collection only. Overall, our estimate of queen-mating frequency was based on the genotype of 472 workers (adults and pupae from both laboratory rearing and field collection) from a single matriline ($X \pm SD = 27.8 \pm 12.9$, $N = 17$; Table 1).

We calculated the absolute number of mating per queen (M_p) from pedigree analyses. We also estimated the effective number of mating per queen ($M_{e,p}$) following Starr (1984),

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

where p_i is the proportional contribution to the brood of the i th mate.

We used the unbiased estimate of $\sum p_i^2$ corrected for non-sampling error after Pamilo (1993),

$$\sum p_i^2 = \left(\left(N \sum y_i^2 \right) - 1 \right) / (N - 1)$$

where N is the number of workers genotyped in the colony and y_i is the observed male contributions of the i th male that mated with the queen.

Because two males may bear the same alleles at the five loci studied, we estimated the non-detection error for each colony by calculating the probability that two mates bear the same alleles using Boomsma and Ratnieks (1996),

$$P_{\text{non-detect of queen's mates}} = \sum_j \prod_i f_{ij}$$

where f_{ij} is the frequency at the population level of the allele carried by the j th male at the i th locus.

Reproductive paternity skew for each colony was calculated with Nonacs' (2000) B index, as implemented in SKEW CALCULATOR 2003.

Table 1 Queen mating frequency and paternity skew

Colony #	Size	n_w	n_m	n_f	M_p	$M_{e,p}$	Skew
IL01	138	33	15	4	5	4.64	0.014
IL02	59	15	17	3	3	2.65	0.036
IL05	230	18	10		4	4.40	-0.026
IL08	291	59			8	6.99	0.017
IL10	263	45			5	4.31	0.031 [∇]
IL11	333	24			4	3.46	0.035
IL12	174	47			6	3.95	0.084 [∇]
IL14 ^a	131	25	15	15	6	6.02	-0.003
IL16	80	29			6	5.65	-0.009
IL17	44	30		17	6	6.06	-0.003
IL19	37	35		16	3	2.21	0.115 [∇]
IL20 ^a	140	13	19		4	3.04	0.065
IL21	47	8	6	3	2	2.07	-0.031
IL27 ^a	50	32	1	2	5	4.44	0.023
IL28 ^a	81	25	3	8	4	3.72	0.016
IL35	117	9	4	11	2	1.95	0.000
IL37	281	25			2	2.05	-0.013
Total	–	472	90	79	–	–	–
Mean	146.8	27.8	10	9	3.73	3.39	0.021
SD	98.2	12.9	7	6	–	–	–
SE	–	–	–	–	0.41	0.38	0.009

The size of colonies (total number of workers), the number of workers (N_w), males (N_m) and sexual females (N_f) typed, the absolute number of mating (M_p), the effective number of mating ($M_{e,p}$) and estimation of Nonacs' B index paternity skew are given for each field colony. Harmonic means are given for M_p and $M_{e,p}$.

^a Colonies for which no queen was collected. The observed reproductive skew values were tested for significant differences from zero. Paternity skew values take Bonferroni correction for multiple comparisons into account; noted values (∇) indicate a significant skew. Colonies IL08, IL10 and IL11 were used for analyses of worker reproduction in queenless conditions, 1 year after collection

Worker reproduction

First, we investigated the possibility of worker reproduction in queenright colonies by comparing the genotype of the males produced with that of the queen. Ninety adult males were found and genotyped ($X \pm SD = 10.0 \pm 6.7$, $N = 9$). 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 either from the mother or from the father of the worker. Therefore, any male that carry 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 according to Foster et al. (2001),

$$P_{\text{non-detect of worker's sons}} = \sum_i^n p_i (1 - 0.5^{l_i})$$

where n is the number of patriline in the nest, p_i the proportional contribution of the i th father to the brood and l_i is the number of informative loci analysed at the i th patriline.

Second, we tested whether workers can reproduce through thelytokous parthenogenesis, by determining the sex of worker-produced larvae in queenless colonies. A sample of 19 larvae from 3 colonies orphaned from about 1 year were genotyped ($X \pm SD = 6.3 \pm 4.5$, $N = 3$). Male larvae were defined as those appearing homozygous at all five loci and female larvae as those with at least one heterozygous locus.

Thelytokous parthenogenesis by queens

To examine whether queens of *C. livida* use asexual reproduction (i.e., thelytokous parthenogenesis) for the production of new queens, we compared the pedigree of the queens with that of her sexual daughters. We genotyped 79 sexual females from 9 colonies ($X \pm SD = 8.8 \pm 6.1$). To estimate the probability that a queen had mated with a male who has for each locus an allele identical to queen's allele, we determined for each queen and each locus the probability that a male would share one of the queen's alleles by following Percy et al. (2004a):

1. $P_{\text{non-detection}} = \frac{1}{2}(f_{1i} + f_{2i})$ when the queen is heterozygous at the i th locus, and f_{1i} and f_{2i} being, respectively, the frequency of the first and the second allele at the i th locus in the studied population, and
2. $P_{\text{non-detection}} = (f_{1i})$ 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.

Results

Population structure and within-colony relatedness

All the five microsatellite markers showed no evidence of null alleles. In the study population, these loci have between five and seven alleles and expected heterozygosity ranges from 0.58 to 0.75 (mean \pm SE 0.68 ± 0.03). There was no evidence for linkage disequilibrium (P values for each locus pair ranged from 0.06 to 0.98), indicating independence of the marker loci. No significant deviation from Hardy–Weinberg expectations was found. The fixation index F (mean \pm SE) was 0.01 ± 0.04 and did not differ significantly from zero (two-tailed t test, $t = 0.30$, $N = 17$, $P = 0.77$). The absence of inbreeding was also

supported by relatedness between the queens and their pedigree-estimated mates, $r_{m-q} = -0.05$ ($SE_{\text{jackknife}} = 0.10$) not different from 0 (two-tailed t test, $t = 0.50$, $N = 17$, $P = 0.62$). F_{st} estimates averaged 0.21 ± 0.02 , indicative of a genetic divergence between nests. There was no significant correlation between genetic differentiation between pairs of colonies and the geographical distance (Mantel test: matrices correlation $r = 0.07$; $P = 0.43$); thus, no isolation-by-distance pattern occurred in the study population.

The mean within-colony genetic relatedness among nestmate workers was $r_{w-w} = 0.42$ ($SE_{\text{jackknife}} = 0.05$). This value was significantly lower than the relatedness of 0.75 expected under monogyny and monandry (two-tailed t test, $t = 6.60$, $N = 17$, $P < 0.001$).

Number of matriline per colony

From the 17 colonies excavated, 13 contained a single queen, whereas in 4, we could not find the queen. Among the queenright colonies, all workers sampled could be assigned to the queen present in each colony. Similarly, in colonies where queens were not collected, worker genotypes were compatible with a single matriline. The average relatedness between the workers and the queen in each colony r_{q-w} was 0.46 ($SE_{\text{jackknife}} = 0.14$, $N = 17$), not significantly different from 0.50 expected under strict monogyny (two-tailed t test, $t = 1.00$, $P = 0.33$).

Queen mating frequency

The distribution of genotypes in parent-offspring combinations was consistent with queens being multiply mated (Fig. 1). Queens were found to be mated with up to eight different males. For the 6 queens reproducing under laboratory conditions (see “Materials and methods”), the mean number of fathers ($\pm SE$) detected per colony was $M_p = 3.83 \pm 0.70$ and the effective number of mating per queen reached a value of $M_{e,p} = 2.12 \pm 0.57$ ($N = 64$ worker pupae, $N = 6$ colonies). Estimation of queen mating frequency from field samples for the same six colonies (worker pupae and adults, $N = 134$, $N = 6$) gave similar results; queens were mated with two to six males, with $M_p = 3.83 \pm 0.70$ and $M_{e,p} = 2.83 \pm 0.46$. Combining the data from both laboratory and field collection, we obtain values of $M_p = 3.73 \pm 0.41$ and $M_{e,p} = 3.39 \pm 0.38$ (Table 1). Error due to two males bearing the same alleles at all loci was negligible ($P_{\text{non-detect of queen's mates}} = 0.013 \pm 0.002$); it is therefore unlikely that our data were affected by this potential source of error. The estimated relatedness among the mates of a single queen was on average $r_{m-m} = 0.17 (\pm 0.03)$ and was significantly different from 0 (two-tailed t test: $t = 5.67$, $N = 17$, $P < 0.001$).

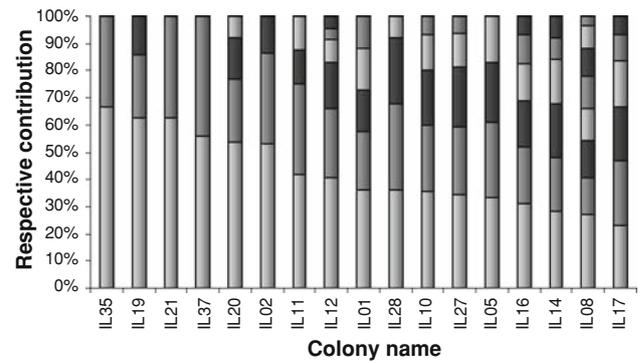


Fig. 1 The frequency distributions of patriline (offspring sired by different males). Patriline are shown by alternate shading patterns. Colonies were ordered by decreasing proportion of the largest patriline

Only in 3 colonies of the 17 colonies investigated, there was a significant deviation from equal father contribution ($P = 0.021$, $P < 0.001$ and $P < 0.001$) (Table 1). No association occurred between the number of mates per queen and colony size ($r = 0.17$; $P = 0.51$).

Worker reproduction

None of the 90 males ($N = 9$ colonies) examined carried non-queen alleles, supporting that worker reproduction is absent or rare in queenright colonies. However, the average probability of non-detection error due to worker-derived males carrying queen alleles at all loci, over all colonies, remains high and equal to 0.71. In short, any worker-produced male present in the sample should be detected with a probability of about 29%. No diploid male was observed in our sample.

We found no evidence of reproduction by thelytokous parthenogenesis by workers. All 19 worker-produced larvae in queenless colonies showed a single allele at each of the 5 loci typed, indicating that they were haploid and would have developed into males. The probability of misclassifying female larvae as male (i.e., the probability of a female larva to be homozygous at all loci) was low and equal to 0.003.

Thelytokous parthenogenesis by queens

Comparison of the pedigree between young sexual females and their mother ($N = 79$ females, $N = 9$ colonies) indicated that sexual daughters were not produced by thelytokous parthenogenesis in *C. livida*. All females sampled showed a different allele from those of the genotype of their mother at least at one locus, consistent with classical sexual reproduction. In line with this result, The average relatedness between the queens and their sexual daughters r_{g-q} was 0.38 ± 0.04 , a value not different

from the relatedness between the queens and their worker daughters ($r_{w-q} = 0.46 \pm 0.14$) (two-tailed t test, $t = 0.282$, $N = 9$, $P = 0.78$). Thus, queens of *C. livida* do not conditionally use sexual and asexual reproduction for the production of workers and sexual females, respectively. The probability of queens mating with a male harbouring no diagnostic allele at any of the loci was very low, ranging from 0.0001 to 0.0045 across colonies.

Discussion

Our data show that colonies of *C. livida* are strictly monogynous, and that queens mate with two to eight different males. Like most monogynous ant species, where colony reproduction is associated with nuptial flights and independent colony founding (Ross, 2001), population structure in *C. livida* shows a lack of inbreeding and no isolation by distance. Our genetic analyses also suggest that workers do not reproduce in queenright colonies. It should be noted, however, that the probability of detection of worker-produced male was low (29%), so that one may not completely exclude the possibility that workers father a proportion of the males. Conversely, workers do clearly reproduce and raise their brood in queenless nests. In this situation, the brood laid by the workers revealed to be haploid males; we did not find any worker-produced female. This indicates that workers are unable to reproduce by thelytokous parthenogenesis in this species. Likewise, none of the 79 female sexuals reared were produced by thelytokous parthenogenesis but by classical sexual reproduction, showing that queens of *C. livida* do not—or cannot—take advantage of both sexual and asexual reproduction for production of workers and new queens, respectively.

There are many parallels and some contrasts between the mating system and population structure in *C. livida* and the other *Cataglyphis* species studied so far.

First, monogyny and polyandry is also the rule in *C. cursor* and *C. sabulosa*, where queens mate with four to eight males and one to five males, respectively. Consequently, relatedness values within-colonies is similar in the three species studied (*C. cursor* $r_{w-w} = 0.42$, *C. sabulosa* $r_{w-w} = 0.49$, *C. livida* $r_{w-w} = 0.42$) (Pearcy et al., 2004a; Timmermans et al., 2008). However, while *C. bicolor*, *C. livida* and *C. sabulosa* show dispersal patterns typical of monogynous species (i.e., nuptial flights and no isolation-by-distance; Knaden and Wehner, 2006; Timmermans et al., 2008), females of *C. cursor* mate close to the nest entrance and found colonies with the help of a worker force in the close vicinity of their natal colony, which results in a strong local population genetic structure (Pearcy et al., 2004a; Clémencet et al., 2005; Pearcy and Aron, 2006). An

interesting finding of this study is that a proportion of the male-mates of a queen are related to each other in *C. livida* ($r_{m-m} = 0.17$). Two explanations may account for this result. Queens could have mates in their natal nest with their brothers, before departure for their nuptial flights. Partial intranidal mating occurs in *C. sabulosa*, where about 20% of the males copulate with their sisters before dispersing in search of other females (Timmermans et al., 2008). This mating strategy causes a significant level of inbreeding in the population. Such a scenario seems unlikely in *C. livida*, because queens are not related to their male mates ($r_{m-q} = -0.05 \pm 0.10$) and no inbreeding was detected. Alternatively, nuptial flights could occur in several successive swarms each drawn from few colonies, thereby increasing the probability of related males copulating with the same female. A temporal separation of adult males and females from the same colony would prevent kin-mating and favours outbreeding, as reported in *Acromyrmex heyeri* (Diehl-Fleig, 1993). Successive swarms involving alates from few colonies occur in other ant species, like *Messor aciculatus* (Hasegawa and Yamaguchi, 1994), where it promotes local mate competition and female-biased population sex ratio. Sex ratio data are not available for *C. livida*. However, in agreement with the second explanation, field observations show that nuptial flights proceed by successive pulses of small swarms over several days, rather than by a single, mass mating flight in this species (unpublished data).

Second, like other species of *Cataglyphis*, workers of *C. livida* have retained their reproductive potential, yet our genetic analyses suggest that they do not reproduce in the presence of the queen. Notwithstanding, we may not completely exclude the possibility that workers do lay eggs, but that these eggs are killed later by other workers. When queens disappear, in contrast, workers rapidly start to lay eggs that will develop into males by arrhenotokous parthenogenesis. Kin selection predicts that workers should forgo direct reproduction if it is balanced by fitness benefits from raising related offspring and maintaining an efficient and productive colony (Hammond and Keller, 2004). On relatedness grounds, worker reproduction in monogynous colonies should be favoured when the queen is singly or doubly mated, as workers are on average more related to their sons ($r = 0.5$) and nephews (males from sister workers $r = 0.375$, from half-sister-workers $r = 0.25$) than to their brothers (males produced by their mother, $r = 0.25$) (Ratnieks, 1988). When the queen mates with more than two males, the mean relatedness between workers and the male offspring of other workers declines, causing workers to favour queen-produced males over worker-produced males. Therefore, workers may inhibit the reproductive efforts of other workers (through worker- or egg-policing) or they may exhibit reproductive

self-restraint (Ratnieks, 1988; Wenseleers et al., 2004). In *C. livida*, the estimated nestmate worker genetic relatedness reaches 0.42, so that workers are on average slightly more related to queen than worker-derived males. Hence, there should be selection for worker-policing and/or reproductive self-restraint behaviour. Other factors such as maximising colony productivity could also favour reproductively restrained workers (Cole, 1986; Ratnieks, 1988; Hartmann et al., 2003). For example, reproduction of workers may be costly in terms of colony productivity, because of the time and energy devoted to egg laying rather than brood rearing or foraging. This may be particularly detrimental in species with small colony-sizes, as is the case for *C. livida*. The lack of worker-produced adult males in *C. livida* is therefore consistent with the predictions of kin selection theory both on genetic and ergonomic grounds.

Third, in contrast with *C. cursor* and *C. sabulosa*, workers of *C. livida* seem unable to reproduce by thelytokous parthenogenesis. All worker-produced brood in queenless nests was haploid. This result requires confirmation, as our sample size was relatively small ($N = 19$ larvae analysed). To date, worker reproduction by thelytokous parthenogenesis has been shown in a few ant species only, including *Pristomyrmex pungens* (Itow et al. 1984), *Cerapachys biroi* (Tsuji and Yamauchi, 1995; Ravary and Jaisson, 2004), *Platythyrea punctata* (Heinze and Hölldobler, 1995), *Messor capitatus* (Grasso et al., 2000), and both *C. cursor* (Cagniant, 1980) and *C. sabulosa* (Timmermans et al., 2008). In the three-first species (*P. pungens*, *C. biroi* and *P. punctata*), queens are rare or even absent, and worker reproduction by thelytoky is obligatory. In queenright ants (*C. cursor*, *C. sabulosa* and *M. capitatus*), it has been argued that thelytokous parthenogenesis was selected for to allow workers to replace the queen when she dies (Lenoir et al., 1988; Grasso et al., 2000; Percy et al., 2006). This interpretation should be considered with caution. Queen replacement is indeed a common phenomenon in *C. cursor*, in which queen longevity seems reduced (Percy et al., 2006). In contrast, in *C. sabulosa* queen replacement seems rare (Timmermans et al., 2008). To our knowledge, no data on queen life expectancy is available for *M. capitatus*. Alternative explanations such as phylogenetic constraints on worker reproduction certainly merit further studies.

Fourth, among the *Cataglyphis* species studied to date, the ability of queens to conditionally use sexual and asexual reproduction for the production of workers and new queens seems restricted to *C. cursor*. Such an aptitude does occur neither in *C. sabulosa*, nor in *C. livida*. To date, queen reproduction by thelytokous parthenogenesis has been documented in three polygynous ants, *Wasmannia auropunctata* (Fournier et al., 2005) *Vollenhovia emeryi* (Ohkawara et al., 2006) and *Mycocetopus smithii* (Himler

et al., 2009), and in the monogynous species *C. cursor* (Percy et al., 2004a). The reason for such a difference in reproductive strategies among species remains unknown. Future molecular studies could reveal other cases of asexual queen reproduction in ants, and should help deciphering both its proximate and ultimate causes.

In short, in the three *Cataglyphis* species studied so far, colonies are headed by a single, multiply mated queen and workers do reproduce in queenless nests. However, they can differ in the mode of colony foundation, the conditional use of sexual and asexual reproduction by queens, and the ability of workers to reproduce by thelytokous parthenogenesis. How reproductive system and sociogenetic organisation evolved in the genus awaits further studies in a phylogenetic context.

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References

- Agosti D. 1990. Review and reclassification of *Cataglyphis* (Hymenoptera, Formicidae). *J. Nat. Hist.* **24**: 1457-1505
- Boomsma J.J. and Ratnieks F.L.W. 1996. Paternity in eusocial Hymenoptera. *Phil. Trans. R. Soc. Lond. B* **351**: 947-975
- Bourke A.F.G. and Franks N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey. 529 pp
- 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
- Cerda X. and Retana J. 2000. Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits. *Oikos* **89**: 155-163
- Cerda X. 2001. Behavioural and physiological traits to thermal stress tolerance in two Spanish desert ants. *Etologia* **9**: 15-27
- Clémencet J., Viginier B. and Doums C. 2005. Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. *Mol. Ecol.* **14**: 3735-3744
- Cole B.J. 1986. The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behav. Ecol. Sociobiol.* **18**: 165-173
- Collett M., Collett T.S., Bisch S. and Wehner R. 1998. Local and global vectors in desert ant navigation. *Nature* **394**: 269-272
- Crozier R.H. and Pamilo P. 1996. *Evolution of Social Insect Colonies*. Oxford University Press, Oxford. 306 pp
- Crozier R.H. and Fjerdingstad E. 2001. Polyandry in social Hymenoptera—disunity in diversity? *Ann. Zool. Fenn.* **38**: 267-285
- Diehl-Fleig E. 1993. Sex ratio and nuptial flight pattern of the leaf-cutting ants *Acromyrmex heyeri* and *A. striatus* (Hymenoptera, Formicidae). *Insect. Soc.* **40**: 111-113
- Foster K.R., Ratnieks F.L.W., Gyllenstrand N. and Thorén P.A. 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. and Keller L. 2005. Clonal reproduction by males and females in the little fire ant. *Nature* **435**: 1230-1234

- Gehring W.J. and Wehner R. 1995. Heat-shock protein-synthesis and thermotolerance in *Cataglyphis*, an ant from the Sahara Desert. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 2994-2998
- Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices. University de Lausanne, Switzerland. <http://www2.unil.ch/popgen/softwares/fstat.htm>
- Grasso D.A., Wenseleers T., Mori A., Le Moli F. and Billen J. 2000. Thelytokous worker reproduction and lack of *Wolbachia* infection in the harvesting ant *Messor capitatus*. *Ethol. Ecol. Evol.* **12**: 309-314
- Hammond R.L. and Keller L. 2004. Conflict over male parentage in social insects. *PLoS Biol.* **2**: e248
- Hardy O.J. and Vekemans X. 2002. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes* **2**: 618-620
- Hardy O.J., Percy M. and Aron S. 2008. Small-scale spatial genetic structure in a ant species with sex-biased dispersal. *Biol. J. Linn. Soc.* **93**: 465-473
- Hartmann A., Wantia J., Torres J.A. and Heinze J. 2003. Worker policing without genetic conflicts in a clonal ant. *Proc. Natl. Acad. Sci. U.S.A.* **100**: 12346-12840
- Hasegawa E. and Yamaguchi T. 1994. Population structure, local mate competition, and sex-allocation pattern in the ant *Messor aciculatus*. *Evolution* **49**: 260-265
- Heinze J. and Hölldobler B. 1995. Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant *Plathytyrea punctata*. *Naturwissenschaften* **82**: 40-41
- Himler A.G., Caldera E.J., Baer B.C., Fernandez-Marin H. and Mueller U.G. 2009. No sex in fungus-farming ants or their crops. *Proc. R. Soc. B* **276**: 2611-2616
- Hugues W.O.H., Ratnieks F.L.W. and Oldroyd B.P. 2008. Multiple paternity of multiple queens; two routes to greater intracolony genetic diversity in the eusocial Hymenoptera. *J. Evol. Biol.* **21**: 1090-1095
- Itow T., Kobayashi K., Kubota M., Ogata K., Imai H.T. and Crozier R.H. 1984. The reproductive cycle of the queenless ant *Pristomyrmex pungens*. *Insect. Soc.* **31**: 87-102
- Knaden M. and Wehner R. 2005. Nest mark orientation in desert ants *Cataglyphis*: what does it do to the path integrator? *Anim. Behav.* **70**: 1349-1354
- Knaden M. and Wehner R. 2006. Fundamental difference in life history traits of two species of *Cataglyphis* ants. *Front. Zool.* **3**: 21
- Kronauer D.J.C., Johnson R.A. and Boomsma J.J. 2007. The evolution of multiple mating in army ants. *Evolution* **61**: 413-422
- Lenoir A., Quérard L., Pondicq N. and Berton F. 1988. Reproduction and dispersal of the ant *Cataglyphis cursor* (Hymenoptera Formicidae). *Psyche* **95**: 21-44
- Lenoir J.-C., Schrempf A., Lenoir A., Heinze J. and Mercier J.-L. 2007. Genetic structure and reproductive strategy of the ant *Cardiocondyla elegans*; strictly monogynous nests invaded by unrelated sexuals. *Mol. Ecol.* **16**: 345-354
- Lewis P.O. and Zaykin D. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0
- 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. and Heinze J. 2006. Clonal reproduction and genetic caste differences in a queen-polymorphic ant, *Vollenhovia emeryi*. *Biol. Lett.* **2**: 359-363
- Pamilo P. 1993. Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity* **70**: 472-480
- Percy M., Aron S., Doums C. and Keller L. 2004a. Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* **306**: 1780-1783
- Percy M., Clemencet J., Chameron S., Aron S. and Doums C. 2004b. Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. *Mol. Ecol. Notes* **4**: 642
- Percy M. and Aron S. 2006. Local resource competition and sex-ratio in the ant *Cataglyphis cursor*. *Behav. Ecol.* **17**: 569-574
- Percy M., Hardy O. and Aron S. 2006. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity* **96**: 377-382
- Percy M., Timmermans I., Allard D. and Aron S. 2009. Multiple mating in the ant *Cataglyphis cursor*: testing the sperm limitation and the diploid male load hypotheses. *Insect. Soc.* **56**: 94-102
- Queller D.C. and Goodnight K.F. 1989. Estimating relatedness using genetic markers. *Evolution* **43**: 258-275
- Ross K.G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Mol. Ecol.* **10**: 265-284
- Rousset F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219-1228
- Ratnieks F.L.W. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**: 217-236
- Ravary F. and Jaisson P. 2004. Absence of individual sterility in thelytokous colonies of the ant *Cerapachis biroi* Forel (Formicidae, Cerapachyinae). *Insect. Soc.* **51**: 67-73
- Sommer S., von Beerent C. and Wehner R. 2008. Multiroute memories in desert ants. *Proc. Natl. Acad. Sci. U.S.A.* **105**: 317-322
- Starr C.K. 1984. Sperm competition, kinship, and sociality in the aculeate Hymenoptera. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Smith R.L., Ed), Academic Press, Orlando, Florida, pp 427-464
- Steck K., Hansson B.S. and Knaden M. 2009. Smells like home: desert ants, *Cataglyphis fortis*, use olfactory landmarks to pinpoint the nest. *Front. Zool.* **6**: 5
- Strassmann J. 2001. The rarity of multiple mating by females in the social Hymenoptera. *Insect. Soc.* **48**: 1-13
- Timmermans I., Hefetz A., Fournier D. and Aron S. 2008. Population genetic structure, worker reproduction and thelytokous parthenogenesis in the desert ant *Cataglyphis sabulosa*. *Heredity* **101**: 490-498
- Trontti K., Thurin N., Sundstrom L. and Aron S. 2007. Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea*. *Behav. Ecol.* **18**: 298-303
- Tsuji K. and Yamauchi K. 1995. Production of females by parthenogenesis in the ant *Cerapachys biroi*. *Insect. Soc.* **42**: 333-336.
- Van Oosterhout C., Hutchinson W.F., Wills D.P.M. and 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. and Menzel R. 1969. Homing in the ant *Cataglyphis bicolor*. *Science* **164**: 192-194
- Wehner R., Marsh A.C. and Wehner S. 1992. Desert ants on a thermal tightrope. *Nature* **357**: 586-587
- Wehner R., Boyer M., Loertscher F., Sommer S. and Menzi U. 2006. Ant navigation: One-way routes rather than maps. *Curr. Biol.* **16**: 75-79
- Wenseleers T., Helanterä H., Hart A.G. and Ratnieks F.L.W. 2004. Worker reproduction and policing in insect societies. An ESS analysis. *J. Evol. Biol.* **17**: 1035-1047
- Wohlgemuth S., Ronacher B. and Wehner R. 2001. Ant odometry in the third dimension. *Nature* **411**: 795-798
- Wolf H. and Wehner R. 2005. Desert ants compensate for navigation uncertainty. *J. Exp. Biol.* **208**: 4223-4230
- Ziegler P.E. and Wehner R. 1997. Time-courses of memory decay in vector-based and landmark-based systems of navigation in desert ants, *Cataglyphis fortis*. *J. Comp. Physiol.* **181**: 13-20