



Multiple mating and supercoloniality in *Cataglyphis* desert ants

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In social organisms, the breeding system corresponds to the number of breeders in a group, their genetic relationships, and the distribution of reproduction among them. Recent, genetically based studies suggest an amazing array of breeding system and reproductive strategies in desert ants of the genus *Cataglyphis*. Using highly polymorphic DNA microsatellites, we performed a detailed analysis of the breeding system and population genetic structure of two *Cataglyphis* species belonging to the same phylogenetic group: *C. niger* and *C. savignyi*. Our results show that both species present very different breeding systems. *C. savignyi* colonies are headed by a single queen and populations are multicolonial. Remarkably, queens show one of the highest mating frequency reported in ants ($M_p = 9.25$). Workers can reproduce by both arrhenotokous and thelytokous parthenogenesis. By contrast, colonies of *C. niger* are headed by several, multiply mated queens ($M_p = 5.17$), and they are organized in supercolonial populations made of numerous interconnected nests. Workers lay arrhenotokous eggs only. These results illustrate the high variability in the socio-genetic organization that evolved in desert ants of the genus *Cataglyphis*. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, 104, 866–876.

ADDITIONAL KEYWORDS: mating system – parthenogenesis – polyandry – polygyny – unicoloniality.

INTRODUCTION

The effect of the breeding system and dispersal behaviour on the distribution of genetic variation within and between populations is a major focus in molecular ecology (Clobert *et al.*, 2001). The distribution of the neutral genetic variation reflects not only past events of the species (e.g. migration, colonization, genetic bottleneck), but also their dispersal and breeding strategies. In social organisms, the breeding system corresponds to the number of breeders in a group, their genetic relationships, and the distribution of reproduction among them (Ross & Keller, 1995; Ross, 2001).

Eusocial Hymenoptera (ants, bees, wasps) show remarkable variation in breeding systems. Colonies typically consist of a single once-mated queen, and her sterile worker offspring. This basic family structure is thought to reflect the primitive state in the evolution of eusociality by kin selection (Hölldobler &

Wilson, 1990; Hughes *et al.*, 2008a; Boomsma, 2009). However, genetic studies have revealed many deviations from this archetypal colony kin-structure regarding both the number of reproductive queens per colony and their mating frequency (Bourke & Franks, 1995; Crozier & Pamilo, 1996a). Obligate multiple mating by queens has evolved repeatedly in bees, wasps, and ants (reviewed in Boomsma, Kronauer & Pedersen, 2009). Although facultative multiple mating commonly occurs across taxa, high levels of polyandry have been reported only in a few species with strictly single-queen societies (Boomsma *et al.*, 2009). In ants, queen mating frequency is usually lower than 2 (Crozier & Fjerdingstad, 2001; Strassmann, 2001), but high polyandry levels have evolved in the leaf cutter ants *Acromyrmex* and *Atta* (Schultz, Bekkevold & Boomsma, 1998; Boomsma, Fjerdingstad & Frydenberg, 1999; Murakami, Higashi & Windsor, 2000), the harvester ants *Pogonomyrmex* (Cole & Wiernasz, 2000; Rheindt *et al.*, 2004), and the army ants of the genera *Aenictus*, *Dorylus*, *Eciton*, and *Neivamyrmex* (Denny *et al.*, 2004; Kronauer

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et al., 2004; Kronauer, Schöning & Boomsma, 2006; Kronauer, Johnson & Boomsma, 2007).

Colony queen number may also vary greatly among species, among populations, and even among colonies within species. Colonies may be headed by a single queen (monogyny) or by tens to thousands of queens (polygyny). This social polymorphism is usually associated with profound changes in life-history strategies and dispersal behaviour (Bourke & Franks, 1995; Keller, 1995; Crozier & Pamilo, 1996b; Ross, 2001; Chapuisat, Bocherens & Rosset, 2004; Peeters & Molet, 2010). Monogyny is typically associated with long-range nuptial flights and independent colony foundation (without the help of workers). In some monogynous species, however, colony reproduction proceeds by fission: a mother colony first produces a batch of males and females, and then divides into two or a small number of monogynous daughter colonies that will be headed by a single queen. In contrast, under polygyny, mating occurs inside or in the vicinity of the natal nest, and colony reproduction proceeds by budding: a process whereby mated gynes leave their natal nest with a worker force to found a new colony containing a variable number of queens nearby. Dispersion by budding usually results in a pattern of genetic isolation-by-distance at the population level. In some ant species the daughter nests maintain exchanges of workers, brood, and food with the mother nest, leading to the formation of polydomous colonies, each composed of several nest units (Hölldobler & Wilson, 1990; Debout *et al.*, 2007). Multicolonial population structures comprising polydomous colonies have been described in several ant species [e.g. *Myrmica sulcinodis* (Pedersen & Boomsma, 1999); *M. ruginodis* (Walsh & Seppä, 2001); *Formica lugubris* (Gyllenstrand & Seppä, 2003); *F. truncorum* (Elias, Rosengren & Sundström, 2005); *Cataulacus mckeyi* (Debout *et al.*, 2003); *Plagiolepis pygmaea* (Thurin & Aron, 2008)]. In its most extreme form, polydomy can lead to the establishment of supercolonies consisting of several hundreds of nest units, with no aggression and free movement of workers among nests on a vast geographical scale (Pedersen *et al.*, 2006; Thomas *et al.*, 2006). Usually, within-population structuration cannot be detected, and relatedness among individuals within populations is extremely low to the point of occasionally being indistinguishable from zero (Pedersen *et al.*, 2006). Supercolonies can form unicolonial populations, a distinguishing trait of several invasive species (Passera, 1994; Helanterä *et al.*, 2009).

Cataglyphis ants are among the most characteristic and conspicuous insects of desert regions (Agosti, 1990). The genus has been extensively studied in many aspects, including orientation, systematics, and ecology (reviewed in Lenoir *et al.*, 2009). Recent,

genetically based studies revealed a dazzling array of breeding system and reproductive strategies. Whereas monogyny is the rule in some species, other species are strictly polygynous (Pearcy *et al.*, 2004a; Knaden & Wehner, 2006; Timmermans *et al.*, 2008, 2010). In contrast to the general link in ants between monogyny and long-range nuptial flights, young queens of the monogynous *C. cursor* and *C. floricola* mate close to their natal nest and colony reproduction proceeds by fission (Lenoir *et al.*, 1988; Hardy, Pearcy & Aron, 2008; Amor *et al.*, 2011; Cheron *et al.*, 2011). Second, in the *Cataglyphis* species studied so far queens are either facultatively or obligately multiply mated (Timmermans *et al.*, 2008, 2010; Pearcy *et al.*, 2009). Third, workers of all *Cataglyphis* species studied have retained ovaries and, hence, the ability to reproduce. Worker reproduction was reported in queenless colonies only. Workers can produce haploid, male eggs by arrhenothokous parthenogenesis (Cagniant, 2009; Timmermans *et al.*, 2010), but in some species, they are also able to produce diploid, female eggs through thelythokous parthenogenesis (Dartigues & Lenoir, 1990; Timmermans *et al.*, 2008; Cagniant, 2009). Finally, and most remarkably, queens of *C. cursor* use alternative modes of reproduction for the production of the queen and worker castes: new queens are produced asexually by thelytokous parthenogenesis, while workers are produced by normal sexual reproduction (Pearcy *et al.*, 2004a). Such a conditional use of sexual and asexual reproduction was not found in the other species of *Cataglyphis* investigated to date.

We performed a detailed analysis of the breeding system and population genetic structure of two *Cataglyphis* desert ants: *C. niger* and *C. savignyi*. Both species belong to the group *bicolor*, which is phylogenetically distant from the groups of either *cursor* (*C. cursor*), or *bombycinus* (*C. sabulosa*), or *albicans* (*C. livida*) or *altisquamis* (*C. mauritanica*) (Agosti, 1990), for which genetic data were previously reported (Knaden & Wehner, 2006; Timmermans *et al.*, 2008, 2010; Pearcy *et al.*, 2009). Using highly polymorphic DNA microsatellites, we investigated the population structure, number of queens in each colony, and queen mating frequency. We also examined whether workers in orphaned colonies have the ability to reproduce by either arrhenothokous or thelytokous parthenogenesis.

MATERIAL AND METHODS

COLLECTION AND SAMPLING

Samples of *Cataglyphis niger* and *Cataglyphis savignyi* were collected in Israel, at the end of February 2009. Twelve nests of *C. niger* were collected in

Ashqelon over an area of 4000 m², of which nine were excavated completely and three only partially. Distances between nests were carefully measured. The mean (\pm SD) distance between nests was 54.14 ± 31.53 m (range: 3.1–127.6 m). Colonies of *C. savignyi* were sampled in loess soil in Arad Park. The nests were widespread and distance between nests ranged from 5 to 200 m. All the nests found on a sampling area of 20 000 m² were collected. Thirteen nests were completely excavated and seven partially. For each species, a sample of workers from each nest was immediately stored in 99.8% ethanol for subsequent genetic analyses. The remaining part of the nest was brought to the laboratory and the number of workers and queens was counted. Colonies were maintained under standard conditions (28 ± 2 °C and natural photoperiod 12:12 h light/dark) and fed maggots and sugar water. The mean number of workers per nest is given in Table 1.

SAMPLE EXTRACTION AND MICROSATELLITE ANALYSIS

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 μ 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 μ L distilled water. We used six for *C. niger* and five for *C. savignyi* microsatellite loci previously described for *C. cursor* (Cc11, Cc26, Cc51, Cc54, Cc63a, Cc99 for *C. niger* and Cc11, Cc51, Cc76, Cc89, Cc99 for *C. savignyi*) (Pearcy *et al.*, 2004b). Loci were amplified as described previously (Timmermans *et al.*, 2008, 2010). Annealing temperature and magnesium concentration were optimized for each locus individually (available upon request). PCR products were genotyped using an automated ABI 3730 sequencer (Applied Biosystems, Foster city, CA, USA). The size of the different alleles was determined using the Peak Scanner version 1.0 analysis software (Applied Biosystems). Control for genotyping errors due to null alleles and allele drop-outs was performed with Micro-checker (Van Oosterhout *et al.*, 2004). Linkage disequilibrium and basic statistics were tested with GENEPOP ON THE WEB (Rousset, 2008).

SOCIAL STRUCTURE AND GENETIC RELATEDNESS

A total of 258 workers (mean \pm SE = 21.5 ± 0.9 , $N = 12$ nests) and 240 workers (12.0 ± 0.0 , $N = 20$ nests) were genotyped for *C. niger* and *C. savignyi*, respectively (Table 1). Descriptive statistics (i.e. the number of alleles, allele frequencies, observed heterozygosity, and expected heterozygosity), as well as tests for

Table 1. Number of nests sampled, mean number of workers per nest, mean number of workers (n_w) and pupae (n_p) genotyped, mean number of queens per nest, F_{ST} , F_{IT} , average relatedness among nestmate workers (r), absolute numbers of matings (M_p), and effective number of matings (M_{ep}) are given for each species

Species	No. of nests	No. of workers	n_w	n_p	No. of queens	F_{ST}	F_{IT}	r	M_p	M_{ep}
<i>C. savignyi</i>	20	536.2 ± 55.94 ($N = 13$)	12 ± 0 ($N = 20$)	23 ± 0.6 ($N = 8$)	1 ($N = 20$)	0.136 ± 0.008	0.021 ± 0.020	0.26 ± 0.03	9.25 ± 0.99	9.33 ± 1.84
<i>C. niger</i>	12	730.02 ± 243.34 ($N = 9$)	21.5 ± 0.9 ($N = 12$)	23.1 ± 0.4 ($N = 23$)	5.8 ± 1.8 ($N = 9$)	0.005 ± 0.003	-0.023 ± 0.008	0.020 ± 0.007	5.17 ± 0.57	4.28 ± 0.49

Means are shown \pm SE.

linkage disequilibrium and Wright's F -statistics were computed with FSTAT (Goudet, 1995) and GENEPOP ON THE WEB (Rousset, 2008). To determine if different nests belonged to the same genetic entity (i.e. polydomy), genotypic frequencies at all collection points were compared using a log-likelihood (G) based test of differentiation, from GENEPOP ON THE WEB (Rousset, 2008). Overall significance was determined using a Fisher's combined probability test; a Bonferroni correction was applied to account for multiple comparisons. Nests were considered as belonging to different colonies if genotypic differentiation was statistically significant ($\alpha < 0.0007$ and $\alpha < 0.0005$ after Bonferroni correction for *C. niger* and *C. savignyi*, respectively). Because of the lack of genetic differentiation between nests in *C. niger* (see Results), we tested for a possible presence of cryptic genetic clusters of nests by using the method implemented in the computer program Structure v.2 (Pritchard *et al.*, 2000). Individuals are assigned sequentially to clusters, or to two or more clusters if their genotypes indicate that they are admixed. We used the admixture model with a burn-in of 50 000 iterations and 100 000 iterations to estimate parameters. The whole procedure was repeated ten times for each value of k .

For *C. niger*, population structure was investigated by plotting $[F_{st}/(1 - F_{st})]$ coefficients between pairs of colonies against the \ln of the geographical distance (Slatkin, 1993). Significance of correlation coefficient between genetic differentiation and geographical distance was assessed with a Mantel test as implemented in GENEPOP ON THE WEB (Rousset, 2008). As mentioned above, the distances between nests of *C. savignyi* were too large to be measured and no isolation-by-distance could be analysed.

Intranest relatedness-coefficients r were estimated using the algorithm of Queller & Goodnight (1989) implemented in the program RELATEDNESS (version 5.0.8). Colonies were weighted equally and standard errors were obtained by jackknifing over colonies.

NUMBER OF MATRILINES PER COLONY

The minimum number of queens in each colony was determined from field observations. Queen genotypes were determined by direct genotyping. For the strictly monogynous *C. savignyi* (see Results), when the queen was not found during excavation the genotype of the presumed queen was reconstructed from workers offspring genotypes. Individuals were assigned as belonging to different matrilineal lines if they did not share an allele with the (presumed) queen at least in one locus. Assignment of individuals to matrilineal lines was confirmed with the maximum-likelihood methods implemented in the program COLONY 1.2 (Wang, 2004).

We examined the possibility that queens use thelytokous parthenogenesis for the production of new queens by comparing the mean observed heterozygosity H_o between queens and workers. Automictic parthenogenesis decreases heterozygosity at each generation (Pearcy, Hardy & Aron, 2006, 2011). Hence, production of queens by thelytoky and workers by sexual reproduction results in a significant decline in heterozygosity in queen lineages, but not in workers (Pearcy *et al.*, 2006, 2009).

QUEEN MATING FREQUENCY

From our field collection, we established 23 experimental nests of *C. niger* and eight experimental nests of *C. savignyi*, each containing a single queen and about 100 nestmate workers; all the brood was carefully removed. The nests were kept under standard rearing conditions (see above). A sample of 24 worker pupae (*C. niger*) or 12 worker pupae (*C. savignyi*) produced by each queen was removed from the nests and stored for genetic analyses. We estimated the minimum number of fathers contributing to the progeny of each queen (M_p) by reconstructing each paternal genotype from mother-offspring allele combinations with the maximum-likelihood methods implemented in the program COLONY 1.2 (Wang, 2004).

Because males may contribute unequally to offspring production, we estimated the effective mating frequency ($M_{e,p}$) following (Nielsen, Tarpy & Reeve, 2003):

$$M_{e,p} = \frac{(n-1)^2}{\sum_{i=1}^k p_i^2(n+1)(n-2) + 3 - n}$$

where n is the total number of offspring of a queen, k is the number of male mates and p is the proportional contribution to the brood of the i th male. This estimator has the advantage of being unbiased by the relative contribution of each male and gives a lower variance than other estimators. The effective number of patrilineal lines equals the absolute mating frequency when all males contribute equally.

We also calculated the probability of non-detection of additional patrilineal lines due to two fathers sharing the same alleles at all loci studied, using the equation:

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

where f_{ij} is the nest k level frequency of allele i at locus j , and n is the number of nests Boomsma & Ratnieks (1996).

WORKER REPRODUCTION

We tested potential worker reproduction by orphaning five nests of each species for at least 5 months.

Sex of worker-produced larvae was determined by flow cytometry (Aron, de Menten & Van Bockstaele, 2003). This method allows us to distinguish between haploid males (produced by arrenotokous parthenogenesis) and diploid females (produced by thelytokous parthenogenesis) on the basis of their DNA-nuclear content. Flow cytometric analyses were performed on a Partec PA (Germany) flow cytometer, after treatment of larvae with a diamidino-4',6-phenylindol-2-dichlorhydrate staining solution.

RESULTS

None of the microsatellite markers showed indication of null alleles; moreover, there was no evidence for linkage disequilibrium, consistent with independence of the marker loci. The number of alleles at the six microsatellite loci studied in *C. niger* ranged from four to 26, with a mean observed heterozygosity $H_O = 0.83$ (range: 0.64–0.95) and a mean expected heterozygosity $H_E = 0.81$ (range: 0.62–0.93). In *C. savignyi*, the number of alleles at the five microsatellite loci ranged from eight to 21, with a mean observed heterozygosity $H_O = 0.79$ (range: 0.65–0.94) and a mean expected heterozygosity $H_E = 0.67$ (range: 0.58–0.74).

Data from genetic analyses on population and colony structure and on the mating system for both species are summarized in Table 1.

SOCIAL STRUCTURE AND GENETIC RELATEDNESS

In *C. niger*, the fixation index F_{IT} was negative and slightly different from zero (mean \pm SE_{jackknife over loci} = -0.023 ± 0.008 , 95% CI: -0.04 to -0.01 , permutation test $P = 0.056$), suggesting a weak outbreeding effect. The F_{ST} estimate was very low, but significantly different from zero (0.005 ± 0.003 , 95% CI: -0.001 to 0.011 , permutation test $P = 0.022$), indicating a small genetic divergence between nests. However, the G -test showed that none of the 12 nests sampled could be genetically differentiated ($P > 0.0007$, CI: 0.07 – 0.91), i.e. they belonged to the same colony. Moreover, the absence of cryptic genetic clusters of nests in the population was supported by STRUCTURE, giving likelihood values that are equal across all values of k . Thus, the most parsimonious explanation to account for our data is $k = 1$. Genetic differentiation between pairs of colonies was significantly correlated with geographical distance, indicating that population genetic structure is characterized by a pattern of isolation-by-distance (Mantel test, $P < 0.0001$; Fig. 1). Together, these findings suggest that this *C. niger* population is unicolonial and that foundation of new nests occurs by budding.

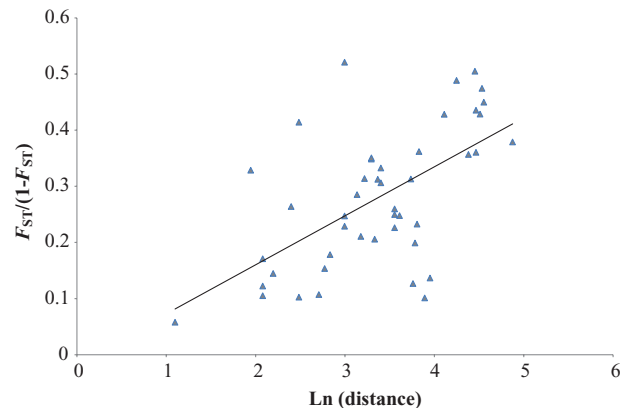


Figure 1. Analysis of isolation-by-distance in *Cataglyphis niger*. The relationship between pairwise estimates of $F_{ST}/(1 - F_{ST})$ and the logarithm of the geographical distance (m) is shown for paired nests ($r = 0.60$, Mantel test $P < 0.0001$).

In *C. savignyi*, the fixation index (F_{IT}) was not different from zero (mean \pm SE = 0.021 ± 0.020 ; 95% CI: -0.015 to 0.058 ; $N = 20$, permutation test $P = 0.113$). Both the F_{ST} estimate (0.14 ± 0.01 ; 95% CI: 0.12 – 0.15) and the G -test ($P < 0.0005$) indicated that the 13 nests sampled were genetically differentiated and belonged to distinct colonies.

The mean within-colony genetic relatedness r_{w-w} among nestmate workers was 0.020 (SE_{jackknife} = 0.007 , $N = 12$) in *C. niger* and 0.26 (SE_{jackknife} = 0.03 , $N = 20$) in *C. savignyi*. Both values were significantly lower than the 0.75 expected under monogyny, monoandry, and random mating in haplodiploid organisms (two-tailed t -tests, $P < 0.0001$ for the two species), but were still significantly different from zero ($P < 0.02$ and $P < 0.0001$ for *C. niger* and *C. savignyi*, respectively).

NUMBER OF MATRILINES PER COLONY

A total of 52 *C. niger* queens were collected from nine out of the 12 nests excavated; no queen was collected in the remaining three nests. The mean (\pm SE) number of queens per nest was 5.77 ± 1.18 and the mean relatedness between nestmate queens r_{q-q} was 0.1 (SE_{jackknife} = 0.03), significantly different from zero (two-tailed t -test, $t = 7.80$, $P = 0.001$).

In contrast, a single queen was excavated from each of eight colonies of *C. savignyi*; no queen was collected in the remaining five colonies. Genetic analyses allowed us to unambiguously assign all the workers sampled to the queen present in each nest. In the five colonies for which no queen was found, worker genotypes were compatible with single maternity and they allowed us to infer unequivocally the genotype of the queen. In line with these results, the average relatedness between the workers and the queen (collected

or inferred) r_{q-w} was 0.37 ($SE_{\text{jackknife}} = 0.12$), not significantly different from 0.5 expected under monogyny (two-tailed t -test, $t = 1.08$, $N = 13$, $P = 0.30$).

For both studied species, the mean observed heterozygosity H_0 in queens did not differ from the mean observed heterozygosity in workers, suggesting that queens are not produced by thelytokous parthenogenesis (*C. niger*: queens: $H_0 = 0.84$, range: 0.64–1, workers: $H_0 = 0.83$, 0.64–0.95; Wilcoxon matched paired test: $W = 0.1$, $P = 0.99$; *C. savignyi*: queens: $H_0 = 0.87$, 0.70–0.95, workers: $H_0 = 0.79$, 0.65–0.94; $W = 15$, $P = 0.07$).

QUEEN MATING FREQUENCY

Pedigree analyses from mother–offspring combinations were consistent with multiple mating in both *C. niger* and *C. savignyi* (Fig. 2). Queens of *C. niger* were mated with at least two to ten males, with a mean (\pm SE) absolute number of matings per queen $M_p = 5.17 \pm 0.57$, and a mean effective number of matings $M_{e,p} = 4.28 \pm 0.55$. In *C. savignyi*, queens had mated with 6–14 males. The number of patrines among offspring of a single queen was $M_p = 9.25 \pm 0.99$ and the effective number of matings reached $M_{e,p} = 9.33 \pm 1.84$. These results are robust, given that the non-detection error due to two males bearing the same alleles at all loci was very low for both species ($P_{\text{non-detection}} = 6 \times 10^{-16}$ and 0.00012, for *C. niger* and *C. savignyi* respectively). Moreover, the male mates of a queen were unrelated to each other (mean relatedness among the male mates of a single queen $r_{m-m} \pm SE_{\text{jackknife}} = 0.01 \pm 0.02$; 95% CI: -0.09 to 0.61 in *C. niger*; -0.007 ± 0.017 ; 95% CI: -0.071 to 0.076 in *C. savignyi*; both relatedness estimates are not significantly different from zero: two-tailed t -test: $t = 5.99$, $P > 0.6$ for both species).

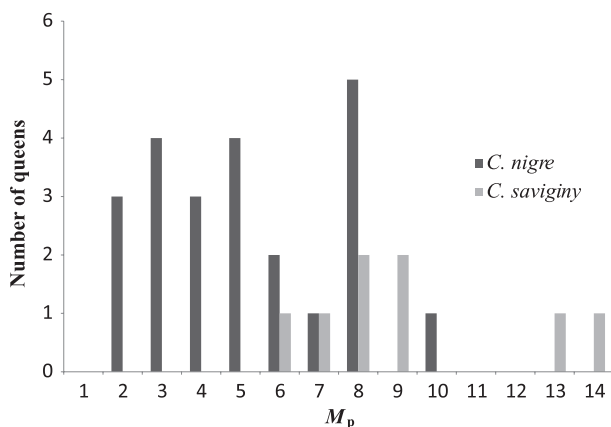


Figure 2. Distribution of the number of matings in *Cataglyphis niger* and *Cataglyphis savignyi*.

WORKER REPRODUCTION

Workers laid eggs in all orphaned colonies of *C. niger* and *C. savignyi*. Flow cytometry analysis of 50 developing larvae of *C. niger* showed that all were haploid, indicating that workers can produce males through arrhenotokous parthenogenesis. In contrast, analyses of 37 developing larvae of *C. savignyi* revealed that 22 were haploid and 15 diploid, showing that workers can produce both males by arrhenotokous parthenogenesis and females by thelytokous parthenogenesis.

DISCUSSION

Our genetic analyses revealed two novel features of the breeding system hitherto unknown within the genus *Cataglyphis*: a remarkably high level of queen mating frequency in *C. savignyi* and a supercolonial socio-genetic organization in *C. niger*.

MATING FREQUENCY

Multiple mating has been reported in all species of *Cataglyphis* studied so far: *C. sabulosa* (Timmermans *et al.*, 2008), *C. livida* (Timmermans *et al.*, 2010), and *C. cursor* (Pearcy *et al.*, 2009). Our data show that polyandry also occurs in *C. niger* ($M_p = 5.17$, range: 2–10) and *C. savignyi* ($M_p = 9.25$, range: 6–14). With a mean of more than nine mates per queen, *C. savignyi* is the most polyandrous *Cataglyphis* species reported to date. It also shows one of the highest mating frequencies found in ants, after the seed harvester ants (*Pogonomyrmex badius*, $M_p = 11$, Rheindt *et al.*, 2004), and the army ants (*Neivamyrmex nigricens*, $M_p = 14.9$, Kronauer *et al.*, 2007; *Eciton burchelli*, $M_p = 12.9$, Kronauer *et al.*, 2006; *Dorylus molestus*, $M_p = 17.8$, Kronauer *et al.*, 2004; *Aenictus laevipes*, $M_p = 17.8$, Kronauer *et al.*, 2007). Queens of *C. niger* and *C. savignyi* fall into the mating system class of obligate polyandry (*sensu* Boomsma *et al.*, 2009). Our results also constitute an exception to the documentation that obligate polyandry is characteristic for taxa with large colony size and monogyny as a rule (Kronauer *et al.*, 2007; Hughes, Ratnieks & Oldroyd, 2008b; Boomsma *et al.*, 2009). Colonies of *C. savignyi* have relatively small colony size (Table 1). Moreover, in *C. niger* both polygyny and obligate polyandry co-occur. The polygyny versus polyandry hypothesis (Keller & Reeve, 1994) predicts that multiple mating should be less common or lost when genetic diversity among workers is achieved through multiple queens per colony. Consistent with the theory, comparative investigation of the relationship between polyandry and polygyny for 241 species of ants, bees, and wasps showed a significant negative relationship between polyandry and polygyny (Hughes *et al.*, 2008b). The same trend was reported

from a direct comparison between closely related species of army ants: monogynous species are highly polyandrous, while the single species that is highly polygynous (i.e. *Neivamyrmex carolinensis*) is monandrous (Kronauer & Boomsma, 2007). Multiple mating seems to be the ancestral state in the genus *Cataglyphis* (L. Leniaud *et al.*, unpubl. data). Queen mating frequency in the polygynous *C. niger* is similar, or even higher, than in the monogynous *C. cursor*, *C. livida*, and *C. sabulosa* (see above). This pattern suggests no correlation between queen number and average number of matings within the genus *Cataglyphis*, but the sample size is too low for statistical confirmation.

Despite considerable empirical efforts, our understanding of the evolution of polyandry in the face of the costs of mating in social insects remains limited. Several genetic and non-genetic hypotheses have been proposed (Crozier & Fjerdingstad, 2001). Among these, both the 'reduction in within-colony conflicts' (Ratnieks & Boomsma, 1995) and the 'resistance to pathogens' (Sherman, Seeley & Reeve, 1988) hypotheses seem relevant to account for the evolution of multiple mating in *Cataglyphis*. Workers of all species possess functional ovaries and retain the capacity to lay unfertilized, male eggs. They may therefore compete with queens over male parentage. However, on relatedness grounds when mating frequency is greater than 2, workers should be selected to inhibit each other from reproducing because they are more closely related to the queen's sons than to other workers' sons (Ratnieks, 1988; Wenseleers & Ratnieks, 2006). The 'resistance to pathogens' hypothesis proposes that parasite pressure favours multiple mating by queens because individuals from distinct patriline in a colony can differ in their disease susceptibility. Thus, polyandry creates a larger set of potential resistance alleles in the colony and, by increasing genetic diversity between nestmate workers, it would reduce disease transmission efficiency. This hypothesis proves particularly relevant for scavenger ants such as *Cataglyphis*, where workers are potentially exposed to pathogens developing on dead arthropods.

SUPERCOLONIALITY IN *C. NIGER*

An interesting result of our work is that the nests of *C. niger* sampled over an area of 4000 m² were not genetically differentiated from each other. Together with the significant isolation-by-distance pattern, which is characteristic of a mode of dispersion by budding, this suggests a highly polydomous, supercolonial population structure. Polydomy has been described in the monogynous species *C. iberica* (Cerdá, Dahbi & Retana, 2002) and *C. bicolor* (Dillier

& Wehner, 2004). The mean number of nests per colony is 4.2 ± 0.5 for *C. iberica* and 3.2 ± 2.4 for *C. bicolor*, with an average distance between nests of 3.4–7.0 m and around 9.1 m, respectively (Cerdá *et al.*, 2002; Dillier & Wehner, 2004). By contrast, colonies of *C. niger* are polygynous, the 12 nests sampled belonged to the same colony, and the distance between nests reached up to 130 m. Consistent with a supercolonial structure, recognition experiments in *C. niger* suggested that workers from different nests widespread over hundreds of square metres were not aggressive to each other. By contrast, a high level of intraspecific aggression was observed between individuals from different colonies 15–50 km apart (Nowbahari, Feneron & Malherbe, 1999) (E. Nowbahari, pers. comm.). This is, to our knowledge, the first report of a supercolonial organization in the genus *Cataglyphis*. The results of our genetic analyses must be taken with some caution because, despite our sampling effort, our sample size was limited and all nests collected belonged to the same putative supercolony. This may have at least three consequences. First, we still have no indication over the actual size of the supercolony sampled; other nests located out of our collection area could have been part of the supercolony. Second, and more importantly, whether the population sampled is made of a single colony or several supercolonies remains unknown; it typically depends on the geographical scale considered (Pedersen *et al.*, 2006). In *C. niger*, the fixation index was marginally negative. This apparent outbreeding could stem from the low sample size (all nests belonging to a single colony) and from queens mating preferentially with males flying from other colonies. Third, relatedness estimates are probably biased because, by essence, supercolonies show no or minimal internal genetic differentiation between nests (Pedersen *et al.*, 2006; Helanterä *et al.*, 2009). The weak differentiation between nests translates into reduced estimates of F_{ST} and, hence, of genetic relatedness among nestmates (r). In fact, the genetic population structure is similar to that of a single colony. Further studies are required to clarify the social organization within colonies and the structure of colonies within populations and, more generally, how frequent is supercoloniality in *C. niger*.

Supercoloniality is a distinguishing trait of invasive ant species (Passera, 1994; Helanterä *et al.*, 2009), as documented in *Linepithema humile* (Giraud, Pedersen & Keller, 2002), *Solenopsis invicta* (Ross, Vargo & Keller, 1996), *Monomorium pharaonis* (Schmidt, d'Ettorre & Pedersen, 2010), *Pheidole megacephala* (Fournier, De Biseau & Aron, 2009), *Anoplolepis gracilipes* (Thomas *et al.*, 2010), and *Lasius neglectus* (Ugelvig *et al.*, 2008). Interestingly, supercoloniality was also reported in introduced

populations of the termite *Reticulitermes urbis* (Leniaud *et al.*, 2009). Nevertheless, unicolonial populations also occur in non-invasive species, such as the wood ants (*Formica truncorum*, Elias *et al.*, 2005; *Formica paralugubris*, Holzer, Keller & Chapuisat, 2009). The evolutionary pressures underlying supercoloniality have been extensively discussed (Holway *et al.*, 2002; Pedersen *et al.*, 2006; Helantera *et al.*, 2009). Supercoloniality avoids the costs of intra-specific competition at the local scale and, by bringing together the worker force of different nests, allows effective habitat monopolization (Holway, Suarez & Case, 1998; Holway & Suarez, 2004).

WORKER REPRODUCTION

In all *Cataglyphis* species studied, workers from queenless colonies were shown to produce haploid eggs by arrhenothoky (reviewed in Cagniant, 2009; Timmermans *et al.*, 2010). Production of diploid eggs through thelytoky was reported in the groups *altisquamis* (*C. mauritanica*, *C. velox*), *bombycina* (*C. bombycina*, *C. sabulosa*) and *cursor* (*C. cursor*) (Cagniant, 1973, 1979; Pearcy *et al.*, 2004a; Timmermans *et al.*, 2008; Cagniant, 2009; S. Aron & L. Leniaud, pers. observ.). Conversely, worker reproduction by thelytokous parthenogenesis was not detected in species belonging to the group *albicans* (*C. livida*, *C. diehli*, *C. otini*, *C. cubica*, *C. rubra*, *C. albicans*, and *C. theyri*) (Cagniant, 2009; Timmermans *et al.*, 2010). Our data show that, within the group *bicolor*, the ability of workers from queenless colonies to reproduce by arrhenothoky and/or thelytoky may vary greatly; while workers of *C. savignyi* (this study), *C. viatica*, and *C. bicolor* (Cagniant, 2009) lay both arrhenotokous and thelytokous eggs, workers of *C. niger* (this study) seem to reproduce through arrhenotokous parthenogenesis only. In *C. cursor*, the production of unfertilized, diploid eggs has been shown to result from automictic parthenogenesis with central fusion of polar nuclei at the end of the gametogenesis (Pearcy *et al.*, 2006). Whether the same mechanism underlies the parthenogenetic production of females by workers in the other species of *Cataglyphis* remains to be verified.

CONDITIONAL USE OF SEXUAL AND ASEXUAL REPRODUCTION BY QUEENS

Recent studies have shown that queens of social insects can circumvent the two-fold genetic cost of sex (Maynard Smith, 1978) by using alternative modes of reproduction for the production of the reproductive (queen) and non-reproductive (worker) female castes: new queens are produced asexually by thelytokous parthenogenesis, while workers are produced by normal sexual reproduction (Pearcy

et al., 2004a; Fournier *et al.*, 2005; Ohkawara *et al.*, 2006; Matsuura *et al.*, 2009). By selectively using asexual and sexual reproduction, mothers increase the transmission rate of their genes to their reproductive daughters, while maintaining genetic diversity in the worker force. Surprisingly, despite its evolutionary interest and the fact that this phenomenon was first discovered in the ant *C. cursor* (Pearcy *et al.*, 2004a), few studies investigated whether queens of other *Cataglyphis* species can benefit this reproductive strategy. This is partly due to the difficulty in collecting the queen(s) and her reproductive daughters in colonies of *Cataglyphis*, which often extend deeply in very rocky soil; moreover, in most species few colonies produce female sexuals and, in this situation, their number is very limited (Pearcy & Aron, 2006) (L. Leniaud & S. Aron, pers. obs.). Unlike *C. cursor*, queens of *C. sabulosa* and *C. livida* do not use such conditional mode of reproduction (Timmermans *et al.*, 2008, 2010). Similarly, our genetic analyses suggest that neither queens of *C. niger* nor queens of *C. savignyi* use thelytokous parthenogenesis for the production of new female sexuals. We found no excess of homozygosity in queens; the mean heterozygosity in queens and workers within each species were not different. Consistent with the absence of parthenogenetic production of reproductive females, nestmate queens in the polygynous species *C. niger* were all genetically different from each other. It should be stressed that our data do not allow us to examine properly the selective use of asexual reproduction for the production of new female sexuals. No virgin female was found at the period of collection (early spring) and none were produced under laboratory conditions, so that a direct test of the hypothesis based on the comparison of the genotypes among reproductive daughters, and between the queen and her reproductive daughters, could not be performed.

In short, *C. savignyi* and *C. niger* are two sister species of desert ants belonging to the same phylogenetic group (Agosti, 1990). However, our data highlight very different breeding systems and mating strategies. *Cataglyphis savignyi* colonies are strictly monogynous, populations are multicolonial, and queens show one of the highest mating frequencies ever reported in ants. Workers can reproduce through arrhenotokous and thelytokous parthenogenesis. By contrast, colonies of *C. niger* are polygynous and they are organized in supercolonial populations made of numerous interconnected nests. Queens are obligate multiply mated, which might challenge the polygyny versus polyandry hypothesis. Workers do reproduce by arrhenotokous parthenogenesis. These results illustrate the high variability in the socio-genetic organization that evolved in desert ants of the genus

Cataglyphis. More generally, they question why the breeding system varies so much, even among closely related species with apparently very similar ecology.

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