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, colonisation, 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 *et al.*, 2008).
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 polymorphic colonies, each composed of several nest units (Hölldobler & Wilson, 1990; Debout et al., 2007). Multicolonial population structures comprising polymorphic colonies have been described in several ant species [e.g. Myrmica sulcinodis (Pedersen & Boomsma, 1999); M. ruginodis (Walin & Seppä, 2001); Formica lugubris (Gyllenstrand & Seppä, 2003); F. truncorum (Elias, Rosengren & Sundström, 2005); Cataulacus meckeyi (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 struc- turation 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; Helantera 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 arrenothokous 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 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 arrenothokous or thely- tokous 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 (±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 µg 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 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 ± 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...
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 $\left[F_{st}/(1 - F_{st})\right]$ 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 matrilines if they did not share an allele with the (presumed) queen at least in one locus. Assignment of individuals to matrilines 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 $H_0$ 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 patrilines equals the absolute mating frequency when all males contribute equally.

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

$$P_{\text{non-detection}} = \prod_i \sum_j f_{ij}^2$$

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 ± SEjackknife 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: \(-0.01\) 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 uniclonial and that foundation of new nests occurs by budding.

In C. savignyi, the fixation index (\( F_{IT} \)) was not different from zero (mean ± 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 \( r_{ww} \) among nestmate workers was 0.020 (SEjackknife = 0.007, \( N = 12\)) in C. niger and 0.26 (SEjackknife = 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 (±SE) number of queens per nest was 5.77 ± 1.18 and the mean relatedness between nestmate queens \( r_{qq} \) was 0.1 (SEjackknife = 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 (SEjackknife = 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: $H_0 = 0.93$, 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 (±SE) absolute number of matings per queen $M_q = 5.17 ± 0.57$, and a mean effective number of matings $M_{eq} = 4.28 ± 0.55$. In C. savignyi, queens had mated with 6–14 males. The number of patrilines among offspring of a single queen was $M_q = 9.25 ± 0.99$ and the effective number of matings reached $M_{eq} = 9.33 ± 1.84$. 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 ($P_{non-detection} = 6 × 10^{-16}$ 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 $r_{s-mean} ± SEjackknife = 0.01 ± 0.02$; 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).

**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 ($M_q = 5.17$, range: 2–10) and C. savignyi ($M_q = 9.25$, 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, $M_q = 11$, Rheindt et al., 2004), and the army ants (Neivamyrmex nigriiceps, $M_q = 14.9$, Kronauer et al., 2007; Eciton burchelli, $M_q = 12.9$, Kronauer et al., 2006; Dorylus molestus, $M_q = 17.8$, Kronauer et al., 2004; Aenictus laevilipes, $M_q = 17.8$, 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.

![Figure 2. Distribution of the number of matings in Cataglyphis niger and Cataglyphis savignyi.](https://academic.oup.com/biolinnean/article-abstract/104/4/866/2452569)
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 monogynous (Kronauer & Boomsma, 2007). Multiple 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; Helantera 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; Helantera 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 arrenothoky (reviewed in Cagniant, 2009; Timmermans et al., 2010). Production of diploid eggs through thelytoky was reported in the groups *altisquammis* (*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. theyi*) (Cagniant, 2009; Timmermans et al., 2010). Our data show that, within the group *bicolor*, the ability of workers from queenless colonies to reproduce by arrhenotoky 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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