

Sociogenetic organisation of two desert ants

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Abstract Desert ants of the genus *Cataglyphis* evolved a remarkable diversity in their reproductive strategies. In *Cataglyphis* species where social organisation was described so far, colonies are headed by one or multiple queens, queens being singly or multiply mated, and workers and/or queens possess the ability to reproduce asexually via thelytokous parthenogenesis. Here, we investigate the social organisation of *C. bombycina* (group *bombycinus*) and *C. theryi* (group *albicans*) using highly polymorphic microsatellite markers. Our results show that both species are characterized by monogynous colonies and multiply mated queens, supporting the idea that polyandry is an ancestral trait of the genus. No evidence for parthenogenetic reproduction by queens was found. One distinctive feature of the species *C. bombycina* among the genus is the presence of a morphologically distinct soldier caste, with highly developed scythe blades jaws. In the only colony where a significant number of soldiers have been sampled, the distribution of patriline is fundamentally different between the soldier and the worker caste. This result suggests a genetic contribution to worker caste determination in this species, and certainly awaits further investigation.

Keywords *Cataglyphis* · Reproductive strategies · Polyandry · Social organisation · Caste determinism

Introduction

The ant genus *Cataglyphis* is one of the most conspicuous ants in desert habitats around the Mediterranean regions. Recently, both field observations and genetic analyses have revealed that *Cataglyphis* desert ants have evolved a remarkable diversity in their sociogenetic organisation that affects both their life history and population kin structure. First, species differ greatly in the number of breeding females per colony; some are strictly monogynous (single-queen colonies; *C. cursor*, *C. sabulosa*, *C. livida*, *C. savigny*, *C. hispanica*, *C. emmae*), whereas others are highly polygynous (multiple-queen colonies; *C. niger*, *C. mauritanica* (Pearcy et al., 2004a; Knaden and Wehner, 2006; Timmermans et al., 2008, 2010; Leniaud et al., 2011, 2012; Eyer et al., 2013, Jowers et al., submitted). Second, they exhibit a wide range of mating system classes, from obligate monoandry (*C. hispanica*) to obligate polyandry (almost always ≥ 2 matings per queen; *C. cursor*, *C. livida*, *C. sabulosa*, *C. niger*, *C. savigny*, *C. emmae*) (Timmermans et al., 2008, 2010; Pearcy et al., 2009; Leniaud et al., 2011, 2012, Jowers et al., submitted). Third, workers of several species have retained their reproductive potential and do reproduce in the absence of the queen. They possess the ability to produce haploid males by arrhenotokous parthenogenesis (*C. livida*, *C. holgerseni*, *C. diehli*, *C. otini*, *C. cubica*, *C. rubra*, *C. albicans*, *C. bombycina*, *C. theryi*), but also, in some cases, diploid female offspring by thelytokous parthenogenesis (*C. cursor*, *C. viatica*, *C. mauritanica*, *C. sabulosa*, *C. savignyi*) (Cagniant 1973, 2009; Dartigues and Lenoir 1990; Timmermans et al., 2008, 2010; Leniaud et al., 2011). Fourth, some *Cataglyphis* ants have evolved the remarkable ability to selectively use alternative modes of reproduction for the production of reproductive and non-reproductive offspring: workers are produced by normal

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sexual reproduction from fertilised eggs, whereas new queens are almost exclusively produced by thelytokous parthenogenesis (*C. cursor*, *C. hispanica*, *C. mauritanica*, *C. velox*) (Pearcy et al., 2004a; Leniaud et al., 2012; Eyer et al., 2013). This strategy allows queens to increase the transmission rate of their genes to their reproductive female offspring, while maintaining genetic diversity in the worker force. However, a major consequence of parthenogenetic production of female sexuals by queens is the apparent absence of male fitness, since males of parthenogenetic *Cataglyphis* species father only non-reproductive workers. In *C. cursor*, queens optimise their fitness by regulating the proportion of parthenogenetic and fertilised eggs laid over time (Aron et al., 2011). Thelytokous eggs are laid in early spring when workers raise the diploid brood into female sexuals, afterwards, the proportion of parthenogenetic eggs laid decreases rapidly and all the eggs arise from sexual reproduction and give rise to workers (Aron et al., 2011). Fifth, in species belonging to the phylogenetic group *Altisquamis*, it was recently shown that both male and female sexuals are produced asexually through parthenogenesis, while workers arise from sexual reproduction from hybrid crosses between genetically distinct lineages (*C. hispanica*, *C. velox*, *C. mauritanica*) (Leniaud et al., 2012; Eyer et al., 2013). This exceptional mode of reproduction corresponds to a social version of hybridogenesis (Avisé, 2008; Schwander and Keller, 2012), whereby both the maternal and paternal genomes are expressed in the worker caste, while only the maternal genome is perpetuated across generations. Finally, dispersal and mode of colony foundation may greatly vary among species. In ants, dispersal and foundation of new nests are generally associated with the colony social organisation (Keller, 1991). Under polygyny, mating occurs in the vicinity of the natal nest, colony reproduction proceeds by fission and populations are genetically structured (*C. niger*, *C. mauritanica*), while monogyny is characterized by long-range nuptial flights, independent colony foundation and no isolation by distance pattern (*C. sabulosa*, *C. livida*, *C. savigny*, *C. hispanica*, *C. bicolor*, *C. emmae*) (Knaden and Wehner, 2006; Timmermans et al., 2008, 2010; Leniaud et al., 2011, Jowers et al., submitted). Consistent with this, kinship-distance relationships show a strong local population genetic structure associated with a sex-biased gene flow with reduced female dispersal (Hardy et al., 2008). Yet, recent works have shown a lack of association between the mode of colony founding and the social structure (monogynous or polygynous) of the colonies in several species. For example, although colonies of *C. cursor* and *C. floricola* are strictly monogynous, young queens mate close to their natal nest and colony reproduction proceeds close by (Lenoir et al., 1988; Clémencet et al., 2005; Amor et al., 2011; Cheron et al., 2011). This indicates that there is no causal association between the founding strategy

and the number of colony queens in these species and that both factors may evolve independently (Cronin et al., 2013).

Given this multiplicity of strategies, an extensive study of the sociogenetic organisation across several species of *Cataglyphis* desert ants are much in need to understand the evolution of reproductive systems within the genus. In this study, we infer the breeding system and population genetic structure in two *Cataglyphis* species, *C. bombycina* and *C. theryi*, for which the sociogenetic organisation remains completely unknown. These species belong to two distinct phylogenetic groups, *bombycinus* and *albicans*, respectively (Agosti, 1990; Cagniant, 2009; Knaden et al., 2012). Like all *Cataglyphis* species, there is a marked size polymorphism among the worker caste (Cerdá, 2001). In the silver ant *C. bombycina*, a distinct, physical caste of large-headed soldiers with highly developed scythe blades jaws has been described (Délye, 1957; Maichier, 2012). Here, we use highly variable microsatellite loci to analyse the population structure, the number of queens in each colony and their mating frequencies. We also test whether queens conditionally use sexual reproduction and parthenogenesis for the production of workers and new daughter queens, respectively.

Materials and methods

Collection and sampling

Samples of *Cataglyphis bombycina* and *C. theryi* were collected in Morocco, in April 2011 and May 2010, respectively. Ten colonies of *C. bombycina* were excavated in Amerzgane; the mean distance between nests \pm SD was 114.40 ± 62.60 m (range 28.44–201 m.). Twelve colonies of *C. theryi* were sampled around Azrou; except for one nest (T2colA) located 19 km apart from the others, the mean distance between nests \pm SD was 54.14 ± 32.99 m (range 8.49–134.85 m). A sample of workers from each colony was immediately stored in 99.8 % ethanol for subsequent genetic analyses. Colonies were brought to the laboratory and the number of queens was counted; they were maintained under standard conditions (28 ± 2 °C and natural photoperiod 12:12 light:dark) and fed mealworms and sugar water.

Genetic analysis

Genotype of ants was determined at eight microsatellite loci for *C. bombycina* (Ccur11, Ccur80, Ccur60, Ccur63b, Ccur65, Ccur76, Ccur63a, Cc89) and five for *C. theryi* (Ccur11, Ccur58, Ccur65, Ccur51, Ccur60) previously developed for the species *C. cursor* (Pearcy et al., 2004b). Individual ant DNA was extracted using Chelex extractions

(Walsh et al., 1991). Ant legs were crushed and incubated for 1h 30 min at 85 °C in 100 µl of 5 % Chelex with constant agitation. After a 3 min centrifugation at 12,000 rpm, 80 µl of the supernatant was transferred into a 1.5-ml tube. PCR were performed as described previously (Timmermans et al., 2008). Annealing temperature and magnesium concentration were optimised for each locus individually (available upon request). PCR products were genotyped using an automated Applied Biosystems ABI 3730 Sequencer (Applied Biosystems, Foster city, CA, USA). The size of the different alleles was determined using Peak Scanner version 1.0 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). This method is reliable when populations are at Hardy–Weinberg equilibrium, which was indeed the case in our study (see Results). Linkage disequilibrium and basic statistics were tested with Genepop on the Web (Rousset, 2008).

Social structure and genetic relatedness

A total of 240 workers ($x \pm SE = 24 \pm 0$, $N = 10$ colonies) and 184 workers ($x \pm SE = 15.42 \pm 5.45$, $N = 12$) were genotyped for *C. bombycina* and *C. theryi*, respectively. Descriptive statistics (i.e. the number of alleles, allele frequencies, observed 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). In each population, we assessed isolation by distance by contrasting microsatellite F_{ST} values of each pair of colonies against their geographic distance. We plotted ($F_{ST}/(1 - F_{ST})$) 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 a Mantel test as implemented in Genepop on the Web (Rousset, 2008). Within-colony relatedness coefficients (r) were estimated using the algorithm of Queller and Goodnight (1989) implemented in the software Relatedness (version 5.0.8). Colonies were weighted equally and standard errors were obtained by jackknifing over colonies.

Number of matriline per colony

The minimum number of queens in each colony was determined from field observations. For *C. theryi*, the pedigree of the queens was determined by direct genotyping. Because no queen was found during excavation in *C. bombycina*, the genotype of the presumed queen(s) was reconstructed from workers genotypes. This revealed to be relatively easy, since the species appears to be strictly

monogynous; the genotype of the single queen could be reconstructed without ambiguity (see Results). Assignment of individuals to matriline was confirmed with the maximum-likelihood methods implemented in the program Colony 1.2 (Wang, 2004).

Queen mating frequency

Queen mating frequency was estimated from mother–offspring genetic analyses. From our field collection, we settled 12 experimental nests of *C. theryi* each containing a single queen and about 100 nestmate workers; all the brood was carefully removed. After 2 months, a sample of 12 worker pupae ($x \pm SE = 12 \pm 0$, $N = 12$) produced by each queen was collected from the nests for genetic analyses. Mating frequency in *C. bombycina* was estimated from the genotype of field workers. 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. In addition, because males may contribute unequally to offspring production, we estimated the effective mating frequency ($M_{e,p}$) with the method of Nielsen et al. (2003; Eq. 16). We also calculated the probability of non-detection of additional patriline 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 frequency of the allele i at locus j (Boomsma and Ratnieks, 1996).

Parthenogenetic production of queens

To examine whether new queens arise from sexual reproduction or parthenogenesis, we compared the pedigree of the queens with that of their sexual daughters. New queens harbouring only alleles identical to those of their mother at each locus were considered parthenogenetically produced; the probability that they were fathered by a male with no diagnostic allele (no allele distinct from those of the queen) at any of the loci was indeed low, ranging from 0.002 to 0.036 across colonies (estimated following Pearcy et al., 2004a).

Results

In overall, no linkage disequilibrium was detected between the loci, and none of the microsatellite markers showed indication of null alleles. In *C. bombycina*, the number of alleles at the eight microsatellite loci studied ranged from 2 to 6, with a mean observed heterozygosity $H_0 = 0.46$ (range 0.19–0.82) matching the mean expected heterozygosity $H_E = 0.44$ (range 0.19–0.77). In *C. theryi*, the number of

alleles at the five microsatellite loci ranged from 2 to 15, with a mean observed heterozygosity $H_0 = 0.68$ (range 0.21–0.94) slightly higher than the expected heterozygosity $H_E = 0.49$ (range 0.22–0.90). No deviation from Hardy–Weinberg equilibrium was detected for any specific locus.

Data from genetic analyses for both species are given in Table 1.

Social structure and genetic relatedness

Both *C. bombycina* and *C. theryi* are characterized by random mating. The fixation index F_{IT} was not different from zero in either species (permutation tests, $P = 0.34$ and $P = 0.44$, respectively). In the two species, F_{ST} estimates were significantly different from zero indicating a genetic divergence between nests (permutation tests, $P = 0$ for both species). Genetic differentiation between pairs of colonies was not correlated with the geographical distance, suggesting that population genetic structure is not characterized by a pattern of isolation by distance (Mantel test, $P = 0.18$ and $P = 0.42$, respectively).

For both species, the mean within-colony genetic relatedness r_w among nestmate workers was 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 still significantly higher than zero ($P < 0.0001$).

Number of matriline per colony

A single queen was found in each of the 12 colonies of *C. theryi*. Genetic analyses allowed assigning unambiguously all the workers sampled to the queen present in each nest. By contrast, colonies of *C. bombycina* are spread out and no queen was collected during excavation. However, worker genotypes were compatible with offspring of a single queen in all colonies. This allowed us to infer the genotype of each colony queen unequivocally. Consistent with monogyny in *C. bombycina* and *C. theryi*, the average relatedness between the workers and the queen (collected or inferred) r_{q-w} was not significantly different from 0.5 expected in colonies headed by a single queen (two-tailed t test, $t \leq 1.40$, $P > 0.19$ for both species).

Queen mating frequency

The distribution of genotypes in mother–offspring combinations and genotypes of field workers were consistent with queens mating multiply in the two *Cataglyphis* species. The average absolute mating frequency was $M_p = 5.70$ (range 3–8 males) in *C. bombycina* and 2.5 (range 2–4 males) in *C. theryi*. The average effective number of fathers $M_{e,p}$ was 5.08 and 2.29, respectively. These results are robust; the non-detection error due to two males bearing the same alleles at all loci was indeed low for both species ($P_{\text{non-detection}} = 0.0029$ and 0.00012 for *C. bombycina* and *C. theryi*, respectively). We used the reconstructed paternal genotypes to estimate relatedness of the parental individuals. Queens were on average not related to their mates (*C. bombycina* $r_{q-m} = -0.09 \pm 0.10$; $P = 0.38$; *C. theryi* $r_{q-m} = 0.10 \pm 0.10$; $P = 0.33$), neither were the males that had mated with the same queen related to each other in *C. bombycina* ($r_{mm} = 0.03 \pm 0.03$; $P > 0.05$). In *C. theryi*, the mates of a queen were slightly related ($r_{m-m} = 0.15 \pm 0.06$; $P = 0.03$) (Table 1).

In *C. bombycina*, we examined whether offspring from different patriline within a colony differ in their propensity to develop into worker and soldier castes. Few soldiers (1–4) were found in all colonies sampled; however, they were collected in sufficient number in only one colony. We determined the genotype of 24 workers and 22 soldiers at 8 microsatellites loci. The distribution of the inferred patriline among the worker and soldier castes revealed to be significantly different ($\chi^2 = 34.77$, $df = 3$, $P < 0.000001$). Among the workers, the distribution of the patriline did not differ with the theoretical distribution of an equal share between all queen's mates ($\chi^2 = 5.66$, $df = 3$, $P = 0.13$),

Table 1 Population-wide inbreeding coefficient among workers (F_{IT}), genetic differentiation between colonies (F_{ST}), mean within-colony relatedness among workers (r_w), average relatedness between the workers and the colony queen (r_{q-w}), and between sexual females (new queens) and the colony queen (r_{q-g}), estimated from field colonies in *Cataglyphis bombycina* and *C. theryi*

	<i>C. bombycina</i>	<i>C. theryi</i>
# Workers typed (# colonies)	240 (10)	184 (12)
$F_{IT} \pm SE$	-0.02 ± 0.03	0.008 ± 0.07
$F_{ST} \pm SE$	0.17 ± 0.02	0.28 ± 0.03
$r_w \pm SE$	0.31 ± 0.05	0.56 ± 0.06
$r_{q-w} \pm SE$	0.43 ± 0.05	0.50 ± 0.04
$r_{q-g} \pm SE$	0.40 ± 0.15	0.45 ± 0.10
# Workers typed (# colonies)	238 (10)	144 (12)
$M_p \pm SE$	5.70 ± 1.50	2.50 ± 0.67
$M_{e,p} \pm SE$	5.08 ± 2.07	2.29 ± 0.79
$r_{q-m} \pm SE$	-0.09 ± 0.10	0.10 ± 0.10
$r_{m-m} \pm SE$	0.03 ± 0.03	0.15 ± 0.06

Paternal genotypes were reconstructed to estimate the relatedness between the queen and her mates (r_{m-q}), and among the male mates of a same queen (r_{m-m})

Data are given with SE jackknife over loci

Estimates of the absolute (M_p) and effective ($M_{e,p}$) mating frequencies were obtained from mother–offspring genetic combinations under laboratory conditions in *C. theryi* and from field workers in *C. bombycina*

whereas patriline were unequally distributed among the soldiers ($\chi^2 = 14$, $df = 3$, $P = 0.003$).

Parthenogenetic production of queens

Fifty-one new queens ($x \pm SE = 17 \pm 5.2$; $N = 3$) of *C. bombycina* and 28 new queens ($x \pm SE = 5.6 \pm 1.94$; $N = 5$) of *C. theryi* were collected on the field. In all colonies, the genotype of the new queens differed from that of the mother queen at least two loci, indicating that they were not produced by thelytokous parthenogenesis. In line with this, the average relatedness between the new queens and the mother queen (r_{q-g}) was not different from the average relatedness between the workers and the colony queen (r_{q-w}) (paired t test: $t = 2.86$, $N = 10$, $P = 0.850$ for *C. bombycina* and $t = 0.464$, $N = 12$, $P = 0.648$ for *C. theryi*). In both species, all the patriline found in reproductive daughter queens occurred among the workers.

Discussion

The present results indicate monogyny, polyandry, random mating and production of new reproductive queens through sexual reproduction (rather than parthenogenesis) in *C. bombycina* and *C. theryi*. In addition, no viscosity was found in the study populations suggesting nuptial flights and independent colony foundation, as is typically the case in monogynous ants. This result must be taken with some caution, however, because biparentally inherited nuclear genes may be unaffected by genetic viscosity as males can be efficient dispersers and may counteract the effect of restricted queen dispersal (Cronin et al., 2013). All queens were found multiply mated, indicating that both species belong to the class of obligately polyandrous ants (sensu Boomsma et al., 2009). The observed level of polyandry is, however, different to that reported earlier in other species of the group *bombycinus* (e.g. *C. sabulosa*) and *albicans* (e.g. *C. livida*). In our study population of *C. bombycina*, the queens (inferred) had mated with 3–8 males and the mean effective mating frequency $M_{e,p} = 5.08$. This was higher than reported for *C. sabulosa* (1–5 males, $M_{e,p} = 2.3$; Timmermans et al., 2008). Conversely, queens of *C. theryi* had mated with 2–4 males and $M_{e,p} = 2.29$, which is marginally lower than that found in the sister species *C. livida* (2–8 males, $M_{e,p} = 3.39$; Timmermans et al., 2010). Polyandry is most likely an ancestral feature of *Cataglyphis* ants as it is present in all species studied so far, except some species of the groups *altisquamis* (Leniaud et al., 2012; Eyer et al., 2013) where a reversion to single mating seems to have occurred secondarily. Why females mate with multiple males remains a controversial issue in evolutionary biology (Boomsma and Ratnieks, 1996; Arnqvist and

Nilsson, 2000; Jennions and Petrie, 2000; Slatyer et al., 2012a, b). Polyandry costs females energy, it increases the risk of predation, disease transmission and exposure to deleterious seminal products, and it induces immunity costs (Chapman et al., 1995; Jennions and Petrie, 2000; Baer et al., 2006; den Boer et al., 2010). Furthermore, in social insects it reduces within-colony relatedness, thereby decreasing the inclusive fitness for the workers who rear the queen's brood (Crozier and Pamilo, 1996). The occurrence of multiple mating in many insect colonies, therefore, calls for some explanation. Both genetic and non-genetic benefits have been proposed to select for polyandry by queens (reviewed by Ratnieks and Boomsma, 1995; Crozier and Fjerdingstad, 2001; Brown and Schmid-Hempel, 2003; Mattila and Seeley, 2007; Boomsma et al., 2009; Fjerdingstad, 2012). To date, three explanations based on the benefits of increased genetic diversity in the offspring seem particularly relevant in desert ants (see Timmermans et al., 2010 for a detailed discussion on the origin of multiple mating in *Cataglyphis*). First, mating with different males could allow a more efficient division of labour by increasing the production of genetically diverse workers varying in their inclination to perform different tasks (Crozier and Page, 1985; Robinson and Page, 1988; Jones et al., 2004; Mattila and Seeley, 2007). Empirical studies indeed showed that task preference is at least partly genetically influenced in *C. cursor*, with workers from different patriline differing in their propensity to perform a given task in the colony (Eyer et al., 2013). Second, increased genetic diversity within colonies would favour social harmony due to reduction of conflicts between the queen and the workers over the maternity of males (Trivers and Hare, 1976; Ratnieks, 1989). When the effective number of matings by queens exceeds two, kin selection theory predicts worker policing (workers prevent male production by worker nest mates) and self-restraint (workers do not even attempt to reproduce when policing is effective) (Ratnieks, 1988; Ratnieks et al., 2006; Wenseleers and Ratnieks, 2006). Workers of most *Cataglyphis* ants are capable of producing haploid males from unfertilized eggs. However, obligate polyandry in most species results that workers are on average more closely related to brothers than to nephews (sons of other workers). On relatedness grounds, they are therefore selected to rear the queens' sons instead of the workers' sons. Third, genetic diversity would improve colony-level resistance to pathogens (Sherman et al., 1988; Schmid-Hempel, 1998; Baer and Schmid-Hempel, 1999; Seeley and Tarpay, 2007). This may be particularly significant for *Cataglyphis* ants, where the vast majority of species is scavengers and workers are likely to be contaminated with various pathogens growing on dead arthropods.

To date, the occurrence of a distinct soldier caste has been described in two *Cataglyphis* species: *C. kurdistanica*

(Pisarski, 1965) and *C. bombycina* (Délye, 1957). Our genetic analyses show that patriline are differently represented in the two subcastes of foragers and soldiers in the silver ant. This suggests a genetic contribution in worker caste determination. However, this result calls for some caution because of our small sample size (22 soldiers from a single colony). To date, a genetic component to worker caste determination has been reported in a handful of ant species. In the polygynous ant *Formica selysi* (Schwander et al., 2005) and *Camponotus consobrinus* (Fraser et al., 2000), workers belonging to different matriline differ significantly in size. Likewise, in the monogynous species *Acromyrmex echinator* (Hughes et al., 2003), *Atta colombica* (Evison and Hughes, 2011; Holman et al., 2011), *Pogonomyrmex badius* (Rheindt et al., 2005) and the army ant *Eciton burchellii* (Jaffé et al., 2007), workers from different patriline have different propensities to develop into major or minor worker castes. The occurrence of a genetic component to the development of polymorphism in the worker caste has direct implications for our understanding of the evolution of polyandry in ants because task allocation is often associated with morphological adaptations (Hölldobler and Wilson, 1990; Hughes et al., 2003). Hence, multiple mating may improve the division of labour through intracolony genetic polyethism. In the silver ant, the exact role of soldiers remains unclear. This worker subcaste may benefit the colony by increasing the efficiency of key tasks, such as the transport of large particles of sand during nest construction (Délye, 1956), the storage of liquid food in the gaster (i.e. repletes) (Maichier, 2012) and, as is usually the case in other species, the defence of the colony against intruders (Hölldobler and Wilson, 1990). Clearly, the species offers an intriguing situation for further studies on a genetic component to worker caste polymorphism.

Neither queens of *C. bombycina* nor queens of *C. theryi* use thelytokous parthenogenesis for the production of new female sexuals. Pedigree analyses indeed show that the genotype of new female sexuals differs from that of the colony queen at several loci, indicating that they arise from sexual production. Consistent with this, queens are equally related to their sexual daughters and their workers (Table 1). Similar results were documented in *C. sabulosa* (Timmermans et al., 2008), *C. livida* (Timmermans et al., 2010), *C. savigny* and *C. niger* (Leniaud et al., 2011). This suggests that parthenogenetic production of new female sexuals by queens did not evolve in the phylogenetic groups *bombycinus* (*C. bombycina*, *C. sabulosa*), *albicans* (*C. theryi*, *C. livida*) and *bicolor* (*C. savigny*, *C. niger*). To date, the ability of queens to use asexual and sexual reproduction, respectively, for the production of reproductive and non-reproductive offspring has been reported in four *Cataglyphis* species belonging to the groups *cursor* (*C. cursor*) (Pearcy et al., 2004b) and *altisquamis* (*C. hispanica*, *C.*

velox, *C. mauritanica*) (Leniaud et al., 2012; Eyer et al., 2013). Phylogenetic studies suggest that these groups are closely related (Knaden et al., 2012), supporting a single transition to parthenogenetic production of new queens in the genus.

In short, this study shows that *C. bombycina* and *C. theryi* are very similar in their sociogenetic organisation. In both species, colonies are headed by a single queen, queens are polyandrous, there is random mating, and reproductive and non-reproductive daughters arise from classical sexual reproduction. Though preliminary, our results also indicate a possible genetic influence on worker caste polymorphism in the silver ant *C. bombycina*. However, this possibility requires additional studies. The reproductive system reported in this study seems typical in the ant genus *Cataglyphis*. However, as mentioned above (see Introduction), derived breeding systems have evolved in many species. Further works are needed to infer whether patterns, such as polygyny, polyandry and the conditional use of parthenogenesis for queen production have evolved once or several times independently, and to assess where on the phylogeny reproductive system transitions have taken place. A molecular phylogeny of the genus *Cataglyphis* based on mitochondrial DNA was recently proposed by Knaden et al. (2012). Unfortunately, this phylogeny does not include the model species studied in the present work. A new molecular phylogeny including all species for which reproductive strategies have been studied so far, based on a combination of nuclear and mitochondrial markers, is currently in progress.

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