

# Hybridogenesis through thelytokous parthenogenesis in two *Cataglyphis* desert ants

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## Abstract

Hybridogenesis is a sexual reproductive system, whereby parents from different genetic origin hybridize. Both the maternal and paternal genomes are expressed in somatic tissues, but the paternal genome is systematically excluded from the germ line, which is therefore purely maternal. Recently, a unique case of hybridogenesis at a social level was reported in the desert ant *Cataglyphis hispanica*. All workers are sexually produced hybridogens, whereas sexual forms (new queens and males) are produced by queens through parthenogenesis. Thus, only maternal genes are perpetuated across generations. Here, we show that such an unusual reproductive strategy also evolved in two other species of *Cataglyphis* belonging to the same phylogenetic group, *Cataglyphis velox* and *Cataglyphis mauritanica*. In both species, queens mate exclusively with males originating from a different genetic lineage than their own to produce hybrid workers, while they use parthenogenesis to produce the male and female reproductive castes. In contrast to single-queen colonies of *C. hispanica*, colonies of *C. velox* and *C. mauritanica* are headed by several queens. Most queens within colonies share the same multilocus genotype and never transmit their mates' alleles to the reproductive castes. Social hybridogenesis in the desert ants has direct consequences on the genetic variability of populations and on caste determination. We also discuss the maintenance of this reproductive strategy within the genus *Cataglyphis*.

**Keywords:** ants, genetic caste determination, genetic lineages, parthenogenesis, social hybridogenesis

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## Introduction

In hybridogenetic species, parents belonging to different species or genetic lineages hybridize. Their hybrid daughters will reproduce by backcrossing with a male of the paternal species/lineage and utilize his sperm to fertilize their eggs. However, young female experience abnormal meiosis during oogenesis: the chromosome sets of the mother and the father fail to recombine and the paternal chromosomes are selectively discarded in the germinal cell line. Females therefore only transmit the genetic material of their mother. As a consequence, both the maternal and paternal genomes participate in the formation of the somatic cell line, while the germ line is purely maternal (Schultz 1969; Avise 2008).

A social version of hybridogenesis was recently reported in ants. Like other social insects, the major organizing principle of ant societies is reproductive division of labour, whereby one or a few individuals (the queens) specialize in reproduction, whereas the others (the workers) usually forego their own reproduction to participate in cooperative tasks such as building the nest, collecting food, rearing the young and defending the colony (Hölldobler & Wilson 1990). In hybridogenetic ant species, workers arise from systematic hybridization events between genetically distinct lineages, while pure-lineage transmission is preserved in the reproductive caste. So far, social hybridogenesis has been reported in three ant taxa: *Pogonomyrmex* seed harvester ants (Helms Cahan *et al.* 2002; Helms Cahan & Keller 2003), *Solenopsis* fire ants (Helms Cahan & Vinson 2003) and the desert ant *Cataglyphis hispanica* (Leniaud *et al.* 2012). In the *Pogonomyrmex barbatus/rugosus* species

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complex, at least eight divergent lineages co-occur in specific lineage pairs (Helms Cahan *et al.* 2006; Schwander *et al.* 2006, 2007). Colonies of each lineage are headed by a single queen mated with several males; interlineage hybrids develop into workers, whereas purebred females become new reproductive queens (Helms Cahan & Keller 2003; Helms Cahan *et al.* 2006; Schwander *et al.* 2007). A similar reproductive system has evolved in a hybrid zone between two fire ant species, *Solenopsis geminata* and *Solenopsis xyloni*. Colonies of *S. xyloni* contain multiple queens, each mated with a single male (Helms Cahan & Vinson 2003). Queens mated with a conspecific *S. xyloni* male produce only reproductive females, whereas those mated with a heterospecific *S. geminata* male produce only sterile workers. In both *Pogonomyrmex* and *Solenopsis* hybrid systems, caste determination (i.e. whether a female larva develops into a reproductive queen or a worker) is therefore genetically influenced and permits pure-lineage transmission across generations through intra-lineage mating. However, hybridogenesis in these species differs from 'classical', nonsocial hybridogenesis, because both the maternal and the paternal intra-lineage genomes are transmitted to the next generation of reproductive queens.

Recently, a new system of social hybridogenesis was reported in the ant *Cataglyphis hispanica* where only maternal genes are transmitted across generations (Leniaud *et al.* 2012). In this species, two genetic lineages co-occur in pairs across all populations studied. Colonies are headed by a single queen mated with a single male originating from the alternative lineage than their own. Remarkably, queens use sexual reproduction to produce interlineage hybrid workers, but reproduce asexually through thelytokous parthenogenesis to produce new queens. Males develop from unfertilized eggs by arrhenotokous parthenogenesis, as is usually the case in Hymenoptera. Thus, the sexual forms belong all to the queen lineage. As a consequence, both the maternal and paternal genomes are expressed in the hybrid worker force, whereas the germ line is purely maternal in *C. hispanica*.

We report two new cases of social hybridogenesis, in which queens are produced asexually *via* parthenogenesis, whereas workers develop from hybrid crosses between genetically divergent lineages, in the *Cataglyphis* desert ants *Cataglyphis velox* and *Cataglyphis mauritanica*. These species belong to the same phylogenetic group as *C. hispanica* (group *altisquamis*; Agosti 1990; Knaden *et al.* 2012). In contrast to *C. hispanica*, genetic analyses show that colonies of *C. velox* and *C. mauritanica* are headed by several queens and that the vast majority of nestmate queens share the same multilocus genotype. As is the case in nonsocial hybridogenesis,

only the maternal genome is transmitted from one generation to another. These results indicate that such an unorthodox system of reproduction is more common in ants than has been realized. We discuss the impact of social hybridogenesis on the genetic variability of populations and on caste determination, as well as the conditions for maintenance of this reproductive strategy within the genus *Cataglyphis*.

## Materials and methods

### Field collection and sampling

Sixteen colonies of *Cataglyphis velox* and 16 colonies of *Cataglyphis mauritanica* were excavated, respectively, in the Sierra Nevada (Spain) in May 2009 and in Azrou (Morocco) in May 2010. Care was taken not to miss any room or gallery that might contain individuals. All adult ants (queens, workers and sexual offspring if any) as well as brood at various stages (eggs, larvae, and pupae) were collected. In the Sierra Nevada, *Cataglyphis velox* colonies usually produce sexuals later in the season (July), and no sexual offspring were found across the 16 colonies sampled. By contrast, new (winged) queens and males were collected from *C. mauritanica* field colonies. A sample of workers from each nest, as well as all new queens and males, was directly stored in 98% ethanol for subsequent genetic analyses. Colonies were brought to the laboratory; they were maintained under standard conditions (28 °C ± 2 °C and natural photoperiod 12:12-h light/dark) and fed on cockroaches and sugar water.

### Genetic analyses

Genotypes of ants were determined at five statistically independent microsatellite loci for *C. velox* (Cc26, Cc63a, Cc96, Cc99 and Cc100) and seven loci for *C. mauritanica* (Cc11, Cc26, Cc60, Cc54, Cc63a, Cc63b and Cc80) previously developed for the species *Cataglyphis cursor* (Percy *et al.* 2004). Individual ant DNA was isolated by Chelex-extraction (Walsh *et al.* 1991). Four ant legs were digested in 100 µL of Chelex 5% for 2 h at 85 °C. After 3-min centrifugation at 20 000 g, 75 µL of the supernatant was taken and stored at 4 °C. PCR was performed with a TProfessional thermocycler (Biometra). Annealing temperature and magnesium concentration were optimized for each locus (available upon request). The amplified products were separated on ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA, U.S.A.) and sized against Rox 350HD (*C. velox*) and MapMarker 400 (*C. mauritanica*) standards sizing using the PEAK SCANNER version 1.1 analysis software (Applied Biosystems). Controls for genotyping

errors due to null alleles were analysed following the Expectation Maximization algorithm of Dempster *et al.* (1977) implemented in the FREE NA software (Chapuis & Estoup 2007). Additional tests of heterozygote deficiency and estimation of linkage disequilibrium were performed in GENEPOP on the Web (Rousset 2008).

#### *Social structure, genetic relatedness and queen-mating frequency*

Sixteen workers were genotyped from each colony ( $N = 16$ ). Allele frequencies, measures of observed and expected heterozygosity and  $F$ -statistics were estimated using FSTAT (Goudet 1995). To determine whether different nests belonged to the same genetic entity, genotypic frequencies at all collection points were compared using a log-likelihood (G)-based test of differentiation, from GENEPOP on the Web. 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 ( $\alpha < 0.0006$  and  $\alpha < 0.0004$  after Bonferroni correction for *C. velox* and *C. mauritanica*, respectively). The minimum number of queens in each colony was determined from field observations.

Relatedness coefficients ( $r$ ) were estimated using the program RELATEDNESS (version 5.0.8), according to the algorithm described by Queller & Goodnight (1989). Colonies were weighted equally and standard errors (SE) were obtained by jackknifing over colonies.

#### *Mating frequency*

Queen-mating frequency was estimated from mother-offspring genetic analyses. In the laboratory, multiple-queen colonies were split into experimental nests containing one queen and an efficient worker force (50–300 workers); a total of 19 and 21 experimental nests were set up for *C. velox* and *C. mauritanica*, respectively. They were examined twice a week, and the worker pupae were removed and stored at  $-80^\circ\text{C}$  for microsatellite analyses. The absolute number of mating per queen ( $M_p$ ; minimum number of queens' mates inferred from worker genotypes) was estimated from pedigree analyses from mother-offspring genetic combinations. Overall, 440 pupae were genotyped for *C. velox* ( $x \pm \text{SE} = 23.2 \pm 0.54$ ,  $N = 19$  nests) and 505 pupae for *C. mauritanica* ( $x \pm \text{SE} = 24.05 \pm 0.90$ ,  $N = 21$ ). Male genotypes were inferred from the queen and worker's genotypes. Each worker was assigned to a given patriline with the maximum-likelihood method implemented in the software COLONY 1.2 (Wang 2004).

Because males may contribute unequally to offspring production, we also estimated the effective mating

frequency ( $M_{e,p}$ ) following (Nielsen *et al.* 2003) (Equ. 16). 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 patrines equals the absolute mating frequency when all males contribute equally. We calculated the probability of nondetection of additional patrines 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 & Ratnieks 1996).

#### *Parthenogenetic production of queens*

The mode of production (sexual or asexual) of new queens was assessed in two ways. First, we examined the possibility that queens use thelytokous parthenogenesis for the production of new queens by comparing the mean observed heterozygosity ( $H_o$ ) between the queens and the workers. Previous studies showed that thelytokous parthenogenesis proceeds by automixis with central fusion in other *Cataglyphis* species (Pearcy *et al.* 2006). Because this mode of parthenogenesis decreases heterozygosity at each generation (Pearcy *et al.* 2006, 2011a), production of new queens by thelytoky is expected to result in a significant decline in heterozygosity in queen lineages. Such a decrease is not expected for workers, which are produced by sexual reproduction (Pearcy *et al.* 2006, 2009). Second, given that new queens were produced in *C. mauritanica*, we were able to compare their genotypes with that of the mother queens. We tested the hypothesis that new queens were produced sexually by males sharing one allele with the mother queens at all loci by comparing the observed number of transitions to homozygosity to the expected value under Mendelian segregation. If new queens were fathered by males sharing one allele with the mother queens at all loci, they are expected to inherit the same allele from their father and their mother (i.e. being homozygous) with a 50% probability at each heterozygous locus in the mother. Thus, new queens should display a 50% decrease in heterozygosity when compared with the colony queens.

#### *Sexual production of hybrid workers from two separated gene pools*

In both *C. velox* and *C. mauritanica*, we found an extremely high level of heterozygosity among workers at all loci studied (see Results), indicating that alleles harboured by the queens and their mate(s) were almost never identical. This may stem from sexual partners

belonging to different gene pools while workers arise from the interbreeding between them as reported in the sister species *C. hispanica* (Leniaud *et al.* 2012). We tested the existence of different genetic groups among sexual partners (i.e. 24 mother queens and their 47 inferred mates for *C. velox*; 111 mother queens, 22 new queens and 24 inferred males for *C. mauritanica*), using Bayesian clustering method implemented in the population assignment program STRUCTURE V.2.3.3 (Pritchard *et al.* 2000). This software estimates the number of distinct genetic groups ( $K$ ) structuring the population. We ran a series of models with  $K$  ranging from 1 to 10, using all loci; we fixed the burn-in period to 25 000 and the running length to 50 000 under an admixture ancestry model to give consistent results over runs. The most probable value of  $K$  was estimated using the *ad hoc* statistic  $\Delta K$  (Evanno *et al.* 2005). For each individual sampled (mother queens, their inferred mates and worker offspring), the software assigns a probability of belonging to each of these groups. The belonging of queens and their mates to a genetic lineage was then confirmed by differences in allelic frequencies for each locus.

## Results

### *Social structure, genetic relatedness and queen-mating frequency*

Field collections showed that nests of *Cataglyphis velox* and *Cataglyphis mauritanica* were headed by several queens (i.e. polygyny). In *C. velox*, 10 of 16 nests were polygynous (queen number per colony:  $x \pm SE = 2.85 \pm 0.56$ ; range: 2–9); a single queen was found in three nests and no queen was found in the three remaining. In *C. mauritanica*, all 16 nests were polygynous with a number of queens per colony ranging from 2 to 19 ( $x \pm SE = 6.94 \pm 1.03$ ).  $G$ -tests indicated that the nests sampled were genetically differentiated and belonged to distinct colonies ( $P < 0.0006$  and  $P < 0.0004$  for *C. velox* and *C. mauritanica*, respectively). For both species,  $F_{ST}$  estimates were significantly different from zero ( $x \pm SE = 0.106 \pm 0.019$  for *C. velox* and  $0.154 \pm 0.023$  for *C. mauritanica*; permutation tests,  $P < 0.001$ ), indicating genetic differentiation between colonies.

Mother-offspring genetic analyses were consistent with multi-mated queens in *C. velox*: 17 of 19 queens were found mated with two to five males; two queens mated once. The mean ( $\pm SE$ ) absolute number of mating per queen was  $M_p = 2.37 \pm 0.22$ , and the mean effective number of mating per queen was  $M_{e,p} = 1.84 \pm 0.18$ . In *C. mauritanica*, 18 of 21 queens were singly mated and three were doubly mated, giving  $M_p = 1.14 \pm 0.08$  and  $M_{e,p} = 1.07 \pm 0.04$ . Nondetection error due to two males

sharing the same alleles at all loci was very low for both species ( $P_{\text{nondetection}} = 0.00034$  for *C. velox* and  $< 0.0001$  for *C. mauritanica*).

### *Parthenogenetic production of queens and males*

In *C. velox*, the mean observed heterozygosity ( $H_o$ ) was significantly lower among queens than among workers for all loci surveyed ( $x \pm SE = 0.565 \pm 0.143$  and  $0.976 \pm 0.013$ , respectively;  $G$ -total across loci = 702.4; d.f. = 4,  $P < 0.0001$ ). The mean number of alleles per locus was also lower among the queen caste ( $x \pm SE = 2.4 \pm 0.24$  and  $11.6 \pm 1.12$  alleles per locus in queens and workers, respectively;  $t$ -test,  $P < 0.001$ ). Within the 10 polygynous colonies, genetic analyses showed that 86% of nestmate mother queens ( $n = 37$ ) had identical genotype at all five loci sampled. The 14% remaining mother queens were homozygous at one or several loci for which the other queens were heterozygous; in this case, they were always homozygous for one of the two alleles found in their nestmates queens. Thus, 14% of the mother queens experienced an increased in homozygosity supporting that they were produced by automictic parthenogenesis. In line with this, the mean genetic relatedness among nestmate queens was extremely high ( $r_{qq} \pm SE_{\text{jackknife}} = 0.92 \pm 0.13$ ), and significantly superior to 0.75 expected if they were full sisters produced by sexual reproduction ( $t$ -test,  $P < 0.004$ ).

Like in *C. velox*, the mean observed heterozygosity in *C. mauritanica* was significantly lower among queens than among workers ( $x \pm SE = 0.544 \pm 0.144$  and  $0.903 \pm 0.056$ , respectively;  $G$ -total = 1314.8; d.f. = 6,  $P < 0.0001$ ). The mean number of alleles per locus was also significantly lower among the queen caste than among the worker caste ( $x \pm SE = 3.0 \pm 0.75$  alleles per locus in queen of the lineage A and  $1.7 \pm 0.18$  in queens of the lineage B while  $6.3 \pm 1.50$  in worker caste;  $t$ -test, All  $P < 0.001$ ). Genetic analyses indicated that 95% of nestmate mother queens ( $n = 111$ ) had identical multilocus genotype. The 5% remaining queens were homozygous at at least one locus for which nestmate queens were heterozygous. Consistent with these results, we found a strong average relatedness among queens within colonies ( $r_{qq} \pm SE_{\text{jackknife}} = 0.98 \pm 0.06$ ). Twenty-two new queens from five colonies ( $x \pm SE = 4.40 \pm 2.27$ ) were collected in *C. mauritanica*. All were strictly genetically identical to the mother queens of their natal nest; none carried alleles from their mothers' mate. Mother queens harboured four or five heterozygous loci, leading to 104 independent opportunities to observe potential transitions to homozygosity among new queens. Yet, not a single transition to homozygosity occurred in the new queens. The complete absence of transition to homozygosity is signifi-

cantly lower than expected (52 of 104) if new queens were fathered by males sharing one allele with the mother queens under Mendelian segregation (Binomial test,  $P < 0.001$ ). This gives strong support to the hypothesis that new queens are produced by thelytokous parthenogenesis. The 14 males collected on the field were haploid; all carried alleles from the colony queens, indicating that they were produced by arrhenotokous parthenogenesis.

Altogether, these results indicate that queens are produced by thelytokous parthenogenesis in *C. velox* and *C. mauritanica*.

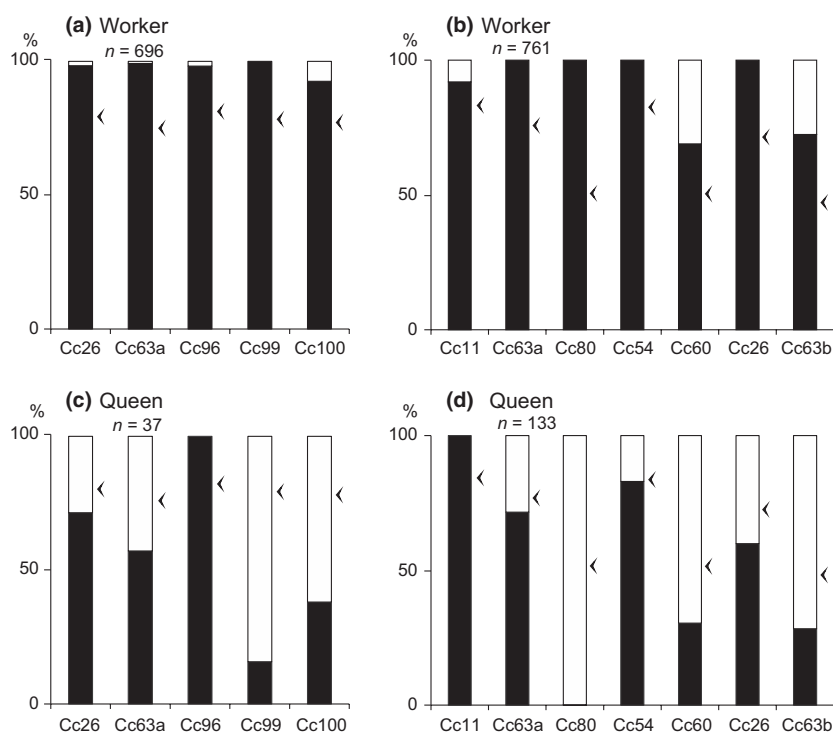
#### Sexual production of hybrid workers from two separated gene pools

All workers genotyped for *C. velox* ( $n = 696$  workers,  $N = 16$  colonies) and *C. mauritanica* ( $n = 761$ ,  $N = 16$ ) carried at several loci alleles that were not harboured by the queens, indicating that they were produced by classical sexual reproduction. Remarkably, in both species observed heterozygosity by loci among workers was extremely high and significantly different than expected under random mating at the population scale (*C. velox*:  $x \pm SE = 0.976 \pm 0.013$ , range across loci:  $[0.925 - 1]$ ;  $G\text{-total} = 545.5$ ;  $d.f. = 4$ ,  $P < 0.0001$ ; *C. mauritanica*:  $x \pm SE = 0.903 \pm 0.056$ , range across loci:  $[0.689 - 1]$ ;  $G\text{-total} = 932.1$ ;  $d.f. = 6$ ,  $P < 0.0001$ ) (Fig. 1). Population assignment analyses showed that the queens

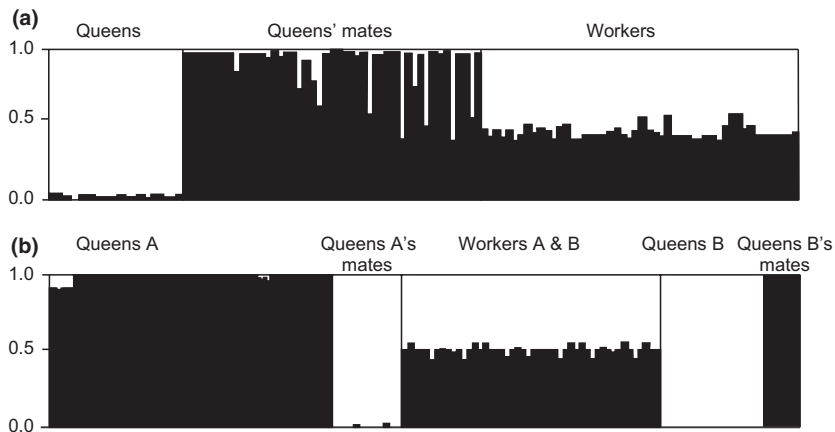
and their mates clustered into two genetically distinct groups (Fig. 2). In our sample of *C. velox*, all queens belonged to the same genetic group and were mated with males from the other, co-occurring group (Fig. 2a). By contrast, in *C. mauritanica* queens of either gene pool were sampled (Fig. 2b). However, all were mated with males originating from the alternative genetic group. Thus, two distinct gene pools coexist and interbreed to produce workers in the studied populations of *C. velox* and *C. mauritanica*. Consistent with these results, in both species we found strong differences in allelic frequencies between sexual partners, for each locus surveyed (Fisher's exact tests,  $P < 0.001$  for all loci; Fig. S1, Supporting Information). Moreover, sexual partners shared few alleles; most alleles are diagnostic for each genetic lineage.

#### Discussion

Our results reveal two new cases of social hybridogenesis in the ant genus *Cataglyphis*. In both *Cataglyphis velox* and *Cataglyphis mauritanica*, queens are produced asexually through thelytokous parthenogenesis, whereas workers develop from hybrid crosses between genetically different lineages. Males are produced by arrhenotokous parthenogenesis, as is typical in Hymenoptera. Hence, both the maternal and paternal genomes are expressed in the worker caste, while only maternal genome spreads across generations through parthenoge-



**Fig. 1** Proportion of heterozygous (black) and homozygous (grey) in workers (a and b) and queens (c and d) population for each locus surveyed, in *Cataglyphis velox* (a and c) and *Cataglyphis mauritanica* (b and d). The mean expected heterozygosity by locus, assuming that female offspring (both workers and queens) are produced by sexual reproduction with random mating within each population, is indicated by arrowheads.



**Fig. 2** Graphical representation of STRUCTURE results for  $K = 2$  genetic groups for the queens, their inferred mates and a sample of 50 randomly chosen workers in *Cataglyphis velox* (a) and *Cataglyphis mauritanica* (b). Each group is characterized by a colour and each individual is represented by a vertical bar representing its probability of being associated to each group. Queens belonging to a single genetic lineage were sampled for *C. velox* (Fig. 2a), whereas queens from both genetic lineages were sampled for *C. mauritanica* (Fig. 2b).

netic production of pure-lineage reproductives. Social hybridogenesis in these species appears identical to that first described in the closely related species *Cataglyphis hispanica* (Leniaud *et al.* 2012). The finding of such a reproductive system in two additional *Cataglyphis* species suggests that social hybridogenesis is not a local adaptation of recent origin. Evolution of social hybridogenesis in *Cataglyphis* requires two features: parthenogenesis for the production of queens and inter-lineage hybrids developing in the worker caste. Parthenogenetic production of reproductive females occurs in several distantly related species of *Cataglyphis*, supporting that it could be ancestral in the genus (Schwander & Keller 2012). As for hybridization, it may have evolved in a common ancestor before the species diverged. *C. velox* and *C. hispanica* are considered to have colonized Spain from North Africa during desiccation period of the Mediterranean Sea (Wehner *et al.* 1994). Therefore, both these species must have diverged from the African *C. mauritanica* at least 5.3 Myr ago when Mediterranean Sea was refilled. Altogether, these arguments plead in favour of an ancient origin of social hybridogenesis in these species. Our results also indicate that this unusual reproductive system is not linked with the social structure; it occurs both in monogynous species (*C. hispanica*) and polygynous species (*C. velox* and *C. mauritanica*). A direct consequence of social hybridogenesis in polygynous species is that all nestmate queens are highly closely related.

Parthenogenetic production of male and female sexuals by queens in hybridogenetic *Cataglyphis* desert ants has two major outcomes: it aborts genetic mixing in queen populations and negates male fitness. First, asexual production of new reproductives by queens affects the apportionment of genetic variability within each lineage. Queen genomes are transmitted through automictic parthenogenesis, what may result in an increase in the level of homozygosity at each generation (Pearcy

*et al.* 2006). Our study shows that 14% of the mother queens in *C. velox* and 5% in *C. mauritanica* experienced an increase in homozygosity compared with nestmate queens. Such a transition to homozygosity was not found in new queens of *C. mauritanica*; this is probably due to the reduced frequency of transitions in this species (5%) and our limited sample size ( $n = 22$  new queens). Increased homozygosity may affect several fitness components leading to inbreeding depression (Keller & Waller 2002), such as reduced defences against pathogens (Hamilton 1987; Sherman *et al.* 1988; Schmid-Hempel 1998), or increased production of sterile diploid males instead of diploid females due to homozygosity at the sex-determining locus (or loci) (Cook & Crozier 1995; Pearcy *et al.* 2009). The second consequence of parthenogenetic production of male and female sexuals by queens in hybridogenetic *Cataglyphis* desert ants is the apparent absence of male fitness. By contrast with the *Pogonomyrmex* and *Solenopsis* hybrid complex, where males father new queens *via* intra-lineage mating (Helms Cahan & Keller 2003; Helms Cahan & Vinson 2003), males of hybridogenetic *Cataglyphis* species benefit no direct fitness as they sire only nonreproductive workers. Consistent with this, our genetic analyses show that not a single queen was produced by sexual reproduction in *C. velox* and *C. mauritanica*; similar results were found in *C. hispanica* (Leniaud *et al.* 2012). Males presumably are under selection to achieve reproductive success. In the ant species *Wasmannia auropunctata* (Fournier *et al.* 2005), *Vollenhovia emeryi* (Kobayashi *et al.* 2008) and *Paratrechina longicornis* (Pearcy *et al.* 2011b), males have evolved a mechanism in response to parthenogenetic production of new queens: their sperm most likely eliminates the maternal chromosomes of the eggs upon fertilization, resulting in the production of haploid sons that are genetically identical to their father. Clonal reproduction therefore occurs for both sexes: new queens are clones of their mother, while males are clones of their father. This results

in the formation of two 'species', one consisting exclusively of males and the other of queens. Such a reproductive strategy has not been observed in *Cataglyphis*, to date. Moreover, because males do not contribute genes to the next generation, their production represents an unnecessary cost for colonies. Selection should favour a strong reduction in male allocation and sex forsaken at the expense of a completely asexual reproductive system where both queens and workers arise from parthenogenesis. To date, the factors maintaining male function in hybridogenetic *Cataglyphis* species remain unknown.

Evolution of social hybridogenesis in the desert ants is rooted in the maintenance of genetically differentiated female lineages, in spite of their systematic hybridization in the worker force. The lack of gene flow between female lineages stems from that queens develop exclusively from pure-lineage eggs produced by parthenogenesis. In theory, the segregation between female lineages can be broken down by production of new queens carrying both paternal and maternal alleles (Curry *et al.* 2010; Leniaud *et al.* 2012). This could be achieved in two ways: (i) through production of hybrid queens by workers and/or (ii) through production of hybrid queens by classical sexual reproduction. (i) In several species of *Cataglyphis*, workers have retained ovaries and can produce males by arrhenotokous parthenogenesis but also female offspring by thelytokous parthenogenesis (reviewed in Lenoir *et al.* 2009; Leniaud *et al.* 2011). Worker reproduction was reported in queenless colonies only. Orphaned workers of *C. velox* and *C. mauritanica* indeed lay both haploid and diploid eggs under laboratory conditions (unpublished data). However, whether such diploid eggs achieve their development into viable and fertile hybrid new queens on the field and cause some gene flow between lineages is unknown. (ii) Concerning reproductive queens, our data show that none developed from sexual reproduction in hybridogenetic *Cataglyphis* ants (this study and Leniaud *et al.* 2012). Nevertheless, one may not completely exclude the possibility that some queens may develop sporadically from fertilized eggs; interlineage hybrids indeed occur in other species where genetic lineages coexist (see above, Foucaud *et al.* 2010; Helms Cahan & Keller 2003; Percy *et al.* 2011b). Occasional development of interlineage hybrids into queens could potentially lead to the emergence of new lineage-pairs, as previously reported in New Caledonian populations of the little fire ant *W. auropunctata* (Foucaud *et al.* 2006).

A direct consequence of the interdependent genetic lineage system found in *Cataglyphis* is the existence of a strong caste-genotype association. In *C. velox*, *C. mauritanica* and *C. hispanica*, the queen caste always develop from pure-lineage eggs while workers arise from

hybridization of two genetically distinct lineages. In social insects, the system of caste determination varies along a continuum from strict environmental caste determination (ECD) to strict genetic caste determination (GCD) (Anderson *et al.* 2008; Schwander *et al.* 2010). Strong genetic caste determination has been reported in five ants only: *Pogonomyrmex* (Helms Cahan & Keller 2003), *Solenopsis xyloni* (Helms Cahan & Vinson 2003), *Wasmania auropunctata* (Fournier *et al.* 2005), *Vollenhovia emeryi* (Ohkawara *et al.* 2006) and *Paratrechina longicornis* (Percy *et al.* 2011b). In these species, the phenotype-genotype association appears quasi-perfect, with all workers being interlineage hybrids and all queens being pure-lineage individuals. Nevertheless, rare exceptions still persist. In clonal populations of *W. auropunctata*, about 2% of new queens arise from sexual reproduction and a similar proportion of workers from parthenogenesis (Foucaud *et al.* 2010). The same figure occurs in natural populations of the harvester ant, where 0–9% of adult females present a mismatch between the genotype and expected phenotype (Schwander *et al.* 2007; Sirvio *et al.* 2011). For example, about 1.9% of interlineage hybrids develop into queens in populations with very unequal lineage frequencies (Helms Cahan & Keller 2003; Schwander *et al.* 2007). Likewise, a few workers are produced by parthenogenesis in *P. longicornis* and *V. emeryi* (0.04% and up to 5%, respectively; Percy *et al.* 2011b; Ohkawara *et al.* 2006). Our results show that in both *C. velox* and *C. mauritanica* all the workers arise from sexual reproduction ( $n = 696$  and  $n = 761$ , respectively), while all the queens sampled are produced by parthenogenesis ( $n = 37$  and  $n = 133$ ). Identical results were reported in *C. hispanica*, where all workers ( $n = 820$ ) developed from fertilized eggs and all queens ( $n = 24$ ) from parthenogenesis (Leniaud *et al.* 2012). To our knowledge, hybridogenetic *Cataglyphis* species illustrate the most genetically 'hard-wired' caste determination system (*sensu* Schwander *et al.* 2010) ever reported in social insects.

So far, social hybridogenesis in *Cataglyphis* desert ants has been found in the three species *C. velox*, *C. mauritanica* and *C. hispanica*. These species belong to the same phylogenetic group *altisquammmis*. A single population of *C. velox* and *C. mauritanica*, and four spatially close populations of *C. hispanica* have been studied. Our results show that, in each population, all the queens are produced asexually *via* parthenogenesis, whereas all the workers develop from hybrid crosses between genetically divergent lineages. Furthermore, a single pair of genetic lineages characterizes each species. Whether social hybridogenesis occurs in other populations of these species and whether several genetic lineages evolved across populations within each species remains unknown. In addition, whether this unique reproduc-

tive strategy evolved once or several times in the *altisquamis* group and, more generally, within the ant genus *Cataglyphis*, clearly merits further investigation.

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### Data accessibility

Sampling locations and microsatellite genotype data for all individuals are available in Table S1 (Supporting information).

### Supporting information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Allele frequencies for queens and males in *C. velox* and *C. mauritanica*.

**Table S1** Sampling locations of *Cataglyphis velox* and *C. mauritanica*, and microsatellite genotype data for all individuals.