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Repeated evolution of queen parthenogenesis and social hybridogenesis in *Cataglyphis* desert ants

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Abstract

Over the last decade, genetic studies on social insects have revealed a remarkable diversity of unusual reproductive strategies, such as male clonality, female clonality, and social hybridogenesis. In this context, *Cataglyphis* desert ants are useful models because of their unique reproductive systems. In several species, queens conditionally use sexual reproduction and parthenogenesis to produce sterile workers and reproductive queens, respectively. In social hybridogenesis, two distinct genetic lineages coexist within a population, and workers result from mating between partners of different lineages; in contrast, queens and males are both produced asexually by parthenogenesis. Consequently, nonreproductive workers are all interlineage hybrids, whereas reproductives are all pure lineage individuals. Here, we characterized the reproductive systems of 11 species to investigate the distribution of the conditional use of sex and social hybridogenesis in *Cataglyphis*. We identified one new case in which sexual reproduction was conditionally used in the absence of dependent-lineage reproduction. We also discovered five new instances of social hybridogenesis. Based on our phylogenetic analyses, we inferred that both the conditional use of sex and social hybridogenesis independently evolved multiple times in the genus *Cataglyphis*.

KEYWORDS
ants, *Cataglyphis*, conditional use of sex, social hybridogenesis

1 | INTRODUCTION

How organisms switch from sexual to asexual reproduction is a queen problem in evolutionary biology. Hymenoptera with their haplo-diploid sex determination system represent unique systems to study the evolution of sex and asexuality. In most species, females are produced by sexual reproduction and are diploid, while males develop by arrhenotokous parthenogenesis and are haploid. Hymenoptera are, however, probably preadapted to the evolution of female (thelytokous) parthenogenesis since arrhenotokous parthenogenesis requires existing mechanisms for egg activation without fertilization (Rabeling & Kronauer, 2013). Consistent with this, female parthenogenesis through thelytokous parthenogenesis has been described in more than 586 species of Hymenoptera (van der Kooi, Matthey-Doret, & Schwander, 2017). In social Hymenoptera (ants, as well as social bees and wasps), the reproductive division of labour between reproductive queens and sterile workers may also drive the evolution of female parthenogenesis. Indeed, queens can take advantage of both sexual and asexual reproduction by conditionally using sex and parthenogenesis respectively for worker and queen production (Pearcy, Aron, Doums, & Keller, 2004): the use of thelytokous parthenogenesis for queen production allows queens to maximize their reproductive success, whereas sexual production of workers maintains genetic diversity within the colony. The conditional use of sex has been reported in six ant genera

Alexandre Kuhn and Hugo Darras contributed equally to the work.

so far: *Cataglyphis* (Pearcy, Clémencet, Chameron, Aron, & Doums, 2004), *Cardiocondyla* (Okita & Tsuchida, 2016), *Paratrechina* (Pearcy, Goodisman, & Keller, Goodisman & Keller, 2011), *Solenopsis* (Lacy et al., 2019), *Vollenhovia* (Kobayashi, Hasegawa, & Ohkawara, 2008), and *Wasmannia* (Fournier et al., 2005).

Sociality may also lead to the evolution of unusual reproductive strategies involving mating between partners from different species or lineages for the production of the sterile worker caste. Under these systems, caste determination, whether a diploid egg develops into a reproductive queen or a sterile worker, is genetically determined (Schwander, Lo, Beekman, Oldroyd, & Keller, 2010). Two genetic lineages coexist within populations. Workers develop from F1 hybrid crosses between lineages, whereas new reproductive queens develop from pure lineages eggs only. Pure lineage queens arise either from mating between partners from the same genetic lineage or from thelytokous parthenogenesis, while males are pure lineage sons produced by arrhenotokous parthenogenesis as is typical for hymenopterans. Interestingly, this system may allow colonies to benefit from superior hybrid workers due to hybrid vigour without the negative consequences of hybridization on reproduction as both queens and males are nonhybrids (Burke & Arnold, 2001; Umphrey, 2006). Several ant species have independently evolved such a mode of reproduction, called "social hybridogenesis". To date, social hybridogenesis has been reported in four ant genera; in two genera where queens arise from mating between same lineage partners, hereafter called "sexual social hybridogenesis"; *Messor* (Norman, Darras, Tranter, Aron, & Hughes, 2016; Romiguier, Fournier, Yek, & Keller, 2017) and *Pogonomyrmex* (Helms Cahan & Keller, 2003; Schwander, Helms Cahan, & Keller, 2007; Sirviö, Pamilo, Johnson, Page, & Gadau, 2011), in one genus where queens arise from thelytokous parthenogenesis called "clonal social hybridogenesis"; *Cataglyphis* (Eyer, Leniaud, Darras, & Aron, 2013; Leniaud, Darras, Boulay, & Aron, 2012), and in one genus where both sexual and clonal social hybridogenesis have been documented; *Solenopsis* (Helms Cahan & Vinson, 2003; Lacy et al., 2019).

Over the last few years, population genetic studies have revealed an amazing diversity of reproductive systems in *Cataglyphis* desert ants. This genus became a canonical model with which to study the evolution of unusual reproductive strategies in social Hymenoptera (Boulay et al., 2017). In some species, queens conditionally use asexual and sexual reproduction to generate the different female castes: new reproductive queens are produced asexually by thelytokous parthenogenesis, while workers arise from sexual reproduction (Pearcy, Aron, et al., 2004; Figure 1a). Other species display clonal social hybridogenesis, in which the conditional use of sex is combined with hybridization between distinct genetic lineages: workers arise from hybrid mating events by sexual reproduction, whereas daughter queens are produced asexually by thelytokous parthenogenesis (Darras, Leniaud, & Aron, 2014; Leniaud et al., 2012; Figure 1b). As a result, nonreproductive workers are all interlineage hybrids, while queens and males are all pure lineage individuals. Deviation from this caste-genotype association is extremely rare in natural colonies of hybridogenetic species of *Cataglyphis* (Darras Kuhn, & Aron, 2014, 2019; Eyer et al., 2013; Kuhn, Darras, & Aron, 2018).

The conditional use of sex appears to have a limited phylogenetic distribution. It has been documented in all the species of the *altisquamis* group that have been surveyed to date (*Cataglyphis hispanica*, *C. humeya*, *C. mauritanica*, and *C. velox*; Darras, Kuhn, & Aron, 2019; Eyer et al., 2013; Leniaud et al., 2012), as well as in one species of the *cursor* group (*C. piliscapa*, previously known as *C. cursor* var. *piliscapa*; Pearcy, Aron, et al., 2004; Table 1). As for clonal social hybridogenesis, it has been reported in three species of the *altisquamis* group thus far (*C. hispanica*, *C. mauritanica*, and *C. velox*; Eyer et al., 2013; Leniaud et al., 2012; Table 1).

It remains unknown whether the conditional use of sex and hybridogenesis are widespread reproductive systems in *Cataglyphis* and whether they arose from one or multiple independent evolutionary transitions. To address these questions, we carried out two research steps. First, we characterized the reproductive systems of

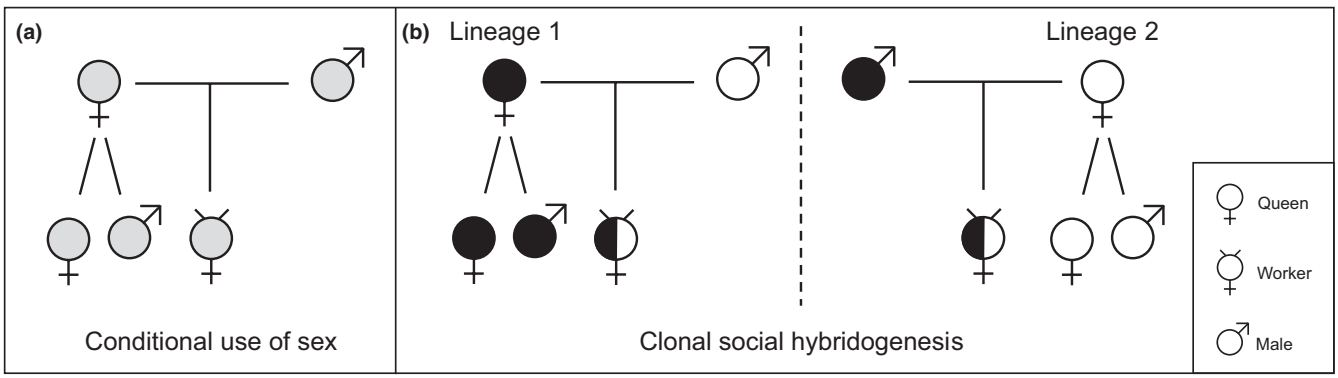


FIGURE 1 Conditional use of sex and clonal social hybridogenesis in *Cataglyphis* ants. (a) Under conditional use of sex, mother queens use sexual reproduction to produce workers and thelytokous parthenogenesis to produce daughter queens. The mother queens and their mate(s) belong to the same gene pool. (b) In clonal social hybridogenesis, two genetic lineages coexist in each population (represented in black and white; the labels indicate the maternal lineage of each colony). Workers are interlineage hybrids produced via sexual reproduction, whereas daughter queens are pure lineage individuals produced via thelytokous parthenogenesis.In both reproductive systems, males arise from arrhenotokous parthenogenesis, as is usually the case in Hymenoptera

TABLE 1 Breeding system and reproductive strategy of species in the *Cataglyphis altisquamis*, *C. cursor*, and *C. pallidus* species groups

Group	Species	Colony structure	Mating system	Reproductive system		No. colonies	No. populations	Sampling location	References
				Conditional use of sex	Clonal social hybridogenesis				
altisquamis	*C. altisquamis*	M	m-s	Yes	Yes	26	2	Israel	This study
	C. foreli	M	m	Yes	Yes	16	1	Iran	This study
	C. hispanica	M/P	s-d	Yes	Yes	68	14	Spain	Darras, Leniaud, et al. (2014)
	C. humeya	M	m	Yes	No	18	10	Spain	Darras, Leniaud, et al. (2014)
	C. kurdistanica	M	m	No	No	15	1	Iran	This study
	C. mauritanica	P	s-d	Yes	Yes	16	1	Morocco	Eyer et al. (2016)
	C. persica	M	s-m	No	No	11	1	Iran	This study
	C. velox	M/P	m-s	Yes	Yes	16	1	Spain	Eyer et al. (2016)
cursor	*C. aenescens*	M	m-s	Yes	No	12	2	Iran	This study
	C. cretica	M	s-d	Yes	Yes	33	2	Crete	This study
	C. hellenica	P	s-d	Yes	Yes	37	2	Greece	This study
	C. cursor	M	m	?	No	11	4	France	This study
	C. piliscapa	M	m	Yes	No	28	3	France	Pearcy, Clémencet, et al. (2004) and Doums et al. (2004)
	C. italica	P	s-d	Yes	Yes	31	2	Italy	This study
pallidus	*C. pallida*	M	m	?	No	9	1	Iran	This study
	C. takyrica	M	m	No	No	10	1	Iran	This study

Note: Colony structure, mating and reproductive system (i.e., presence of the conditional use of sex and clonal social hybridogenesis) as well as sampling information are provided for each species. The data for the 11 species examined in the study are highlighted in grey.
M, monogyny: >10% of colonies headed by a single queen; P, polygyny: >10% of colonies headed by >1 queen.
M, multiple mating: paternity frequency per queen is always ≥2 and is often ≥4; m-s, multiple-single mating: paternity frequency is usually >1, with a variable minority of queens being singly mated; s-d, single-double mating: paternity frequency is usually ≈1, with a minority of queens being doubly mated; s-m, single-multiple mating: paternity frequency is usually = 1, with a variable minority of queens mating ≥2 times; modified from Boomsma and Ratnieks (1996)

10 as yet unstudied species from the *altisquamis* group (four species), the *cursor* group (four species), and the *pallidus* group (two species). In addition, we conducted more detailed research into the reproductive system of one poorly described species of the *cursor* group. Second, we inferred the phylogenetic relationships among all the *Cataglyphis* species for which reproductive information was available, and reconstructed the evolution of reproductive systems within the genus.

2 | MATERIALS AND METHODS

2.1 | Sampling

The genus *Cataglyphis* consists of 92 species distributed across nine species groups (Agosti, 1990; Bolton, 2012). The reproductive systems of the species belonging to the groups *albicans*, *bicolor*, *bombycinus*, and *emmae* have been fairly well studied throughout the groups' ranges. In contrast, such information is available for only a small part of the ranges of the *altisquamis* and *cursor* groups (Figure 2), and no data have been obtained for any species in the *pallidus* group. To fill these gaps, we collected individuals belonging to 11 species of *Cataglyphis* ants in southern Europe and the Middle East between 2013 and 2016 (Figure 2 and Table 1). Four species of the *altisquamis* group were targeted (*C. altisquamis*, *C. foreli*, *C. kurdistanica*, and *C. persica* n.n.). Based on its morphological characteristics, *C. persica* n.n. was thought to be a subspecies of the *cursor* group when first described (Agosti, 1990; Paknia, Radchenko, Alipanah, & Pfeiffer, 2008). However, our genetic analyses have revealed that it actually belongs to the *altisquamis* group (see Results). We also collected individuals representing five species of the *cursor* group (*C. aenescens*, *C. cursor* [previously known as *C. cursor* var. *cursor*], *C. cretica*, *C. hellenica*, and *C. italica*; Table 1). Finally, we collected members of two species in the *pallidus* group (*C. pallida* and *C. takyrica*).

Colonies of each species were completely excavated, and adults (workers, reproductive mother queens, and, when present, alate daughter queens) were stored in absolute EtOH for later genetic analyses. In total, our data set contained information on 211 colonies representing 11 *Cataglyphis* species (range = 9–37 colonies per species, mean ± *SD* = 19.2 ± 10.5; Table 1).

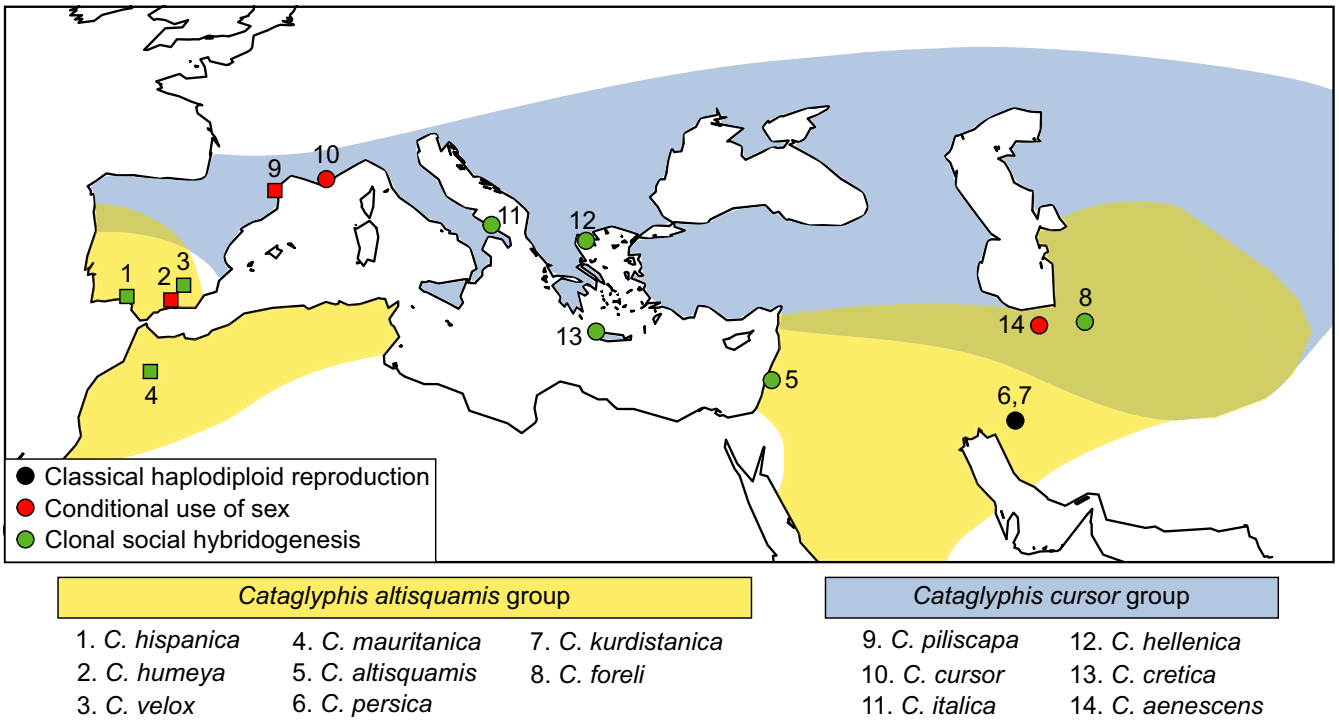


FIGURE 2 Distribution of different reproductive systems (classical haplodiploid reproduction, conditional use of sex, and clonal social hybridogenesis) in the *Cataglyphis altisquamis* and *Cataglyphis cursor* species groups. The distributions of both species groups are indicated in yellow and blue (modified from Radchenko, 2001). The sampling locations and reproductive systems for each of the 14 study species in the two species groups are indicated. For five species (squares), the reproductive system had been determined by previous research. In this study, we inferred the reproductive system of nine additional species (circles). The distribution of the *Cataglyphis cursor* group extends east to Mongolia and south to India (not shown) [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | DNA extraction and amplification

2.2.1 | Genotyping

For each colony sampled, all sampled queens (number of colonies within which mother queen[s] and/or alate daughter queens were sampled: 166/211 and 45/211, respectively; Table S6) and a sample of workers (mean sample size of workers per colony ± *SD* = 5.6 ± 1.9) were genotyped. The genotypes of the mother queens' mates were inferred from the genotypes of the queen(s) and workers. This process was straightforward because male ants are haploid. However, in hybridogenetic species, queens occasionally mate with males who do not father worker offspring (Darras, Kuhn, et al., 2014). To investigate the occurrence of such cryptic mating events, the contents of the queens' spermathecae of 382 queens (out of 415 collected) were genotyped (Table S6; see Kuhn, Bauman, Darras, & Aron, 2017 for a detailed description of the sperm collection procedure). DNA was extracted from the adult ants and the sperm samples using the Chelex 100 method (Kuhn et al., 2017; Walsh, Metzger, & Higuchi, 1991). A total of 22 microsatellite markers were used: *Cc11*, *Cc54*, *Cc76*, *Cc89*, *Cc93*, *Cc99*, *Ch01*, *Ch05*, *Ch06*, *Ch08*, *Ch11*, *Ch12*, *Ch22*, *Ch23*, *Ch25* (Darras, Leniaud, et al., 2014; Pearcy, Clémencet, et al., 2004), *Ch10*, *Ch19* (Darras et al., 2019), *Cm01*, *Cm03*, *Cm07*, *Cm11*, and *Cn03* (this study; Table S2). For each species, the adult ants and the sperm samples were genotyped at 7–10 microsatellite loci (Table S3). Multiplex PCR reactions were carried out using the Qiagen Type-it Microsatellite PCR Kit (10 µl reactions carried out following the manufacturer's instructions). The size of the different alleles was determined using the MapMarker Dy632 internal size standard (BioVentures Inc.) and PEAK SCANNER version 1.0 software (Applied Biosystems).

2.2.2 | Sequencing

Our phylogenetic analyses included representatives of 27 *Cataglyphis* species. There were 25 species whose reproductive systems were known: 14 had been previously described (Table S1) and 11 were analysed in the present study. We also included representatives of *C. cubica* and *C. emeryi*, whose reproductive systems remain unknown, to improve support for the phylogenetic positions of their species groups (*albicans* and *pallidus*, respectively). Finally, one Iranian *Proformica* species served as an outgroup. A single female (mother queen or worker) per species was used, except for hybridogenetic species, where queens from the two interdependent lineages were included (Table S1). Additional samples from geographically distant populations were also analysed whenever possible (Table S1).

We extracted DNA from the ants' legs using the Qiagen DNeasy Blood and Tissue Kit. For each specimen, we sequenced a portion of the mitochondrial gene *cytochrome oxydase I* (*cox1*) and of three

coding nuclear genes (*wingless*, *Wg*; *abdominal-A*, *Ab*; *longwave rhodopsin*, *Lr*). In addition, we used Exon-Primed-Intron-Crossing (EPIC) markers to further examine genetic variation among closely related species. Four EPIC primers designed for ants (*202*, *384*, *505* and *1281*; Ströher, Li, & Pie, 2013) were adapted for *Cataglyphis* using a draft genome of *C. hispanica* as a reference (Table S4). All primers used for sequencing are listed in Table S4. PCR reactions were carried out using MyTaq DNA polymerase (Bioline Reagents) in accordance with the manufacturer's instructions; a Tm of 56°C (nuclear DNA) or 45°C (mitochondrial DNA) was employed. DNA fragments were all sequenced in both forward and reverse directions.

Sequences from *C. cubica*, *C. livida*, *C. theryi*, *C. niger*, *C. savigny*, *C. viatica*, *C. bombycina*, *C. sabulosa*, *C. floricola*, *C. emmae*, and *C. piliscapa* were already available for *cox1*, *Ab*, *Lr*, and *Wg* (Aron, Mardulyn, & Leniaud, 2016). EPIC markers had not been sequenced for *C. livida*, *C. niger*, and *C. sabulosa*.

2.3 | Parthenogenetic production of queens

We assessed the mode by which alate daughter queens were produced (sexually or asexually) by comparing their genotypes with those of their mothers. For colonies headed by a single queen (i.e., monogyny), we compared the genotype of the queen with that of her daughter queens. For colonies with multiple mother queens (i.e., polygyny), we compared all queen genotypes (all mother and daughter queens). In *Cataglyphis*, nestmate mother queens are related and should have identical multilocus genotypes if the species uses parthenogenesis for queen production (Eyer et al., 2013; Kuhn et al., 2017). Queen parthenogenesis is achieved through automictic parthenogenesis with central fusion (Pearcy, Hardy, & Aron, 2006). This mode of parthenogenesis tends to increase homozygosity over generations as heterozygous loci that are far from the centromeres have a probability of up to 33% of becoming homozygous due to recombination (Pearcy et al., 2006). Therefore, a clonal daughter queen may have a multilocus genotype that is identical to that of her mother, or she may be homozygous at one or several loci for which her mother was heterozygous. A similar genetic outcome may arise if daughter queens were produced sexually by males sharing one allele with the mother queens at each locus. In monogynous colonies where the mother queen and its daughter(s) harboured similar genetic profile, we calculated the probability that this similarity resulted from the mother queen having mated with a male with no diagnostic allele. To this aim, we determined for each mother queen and at each locus the probability that a male would share one of the queen's alleles, following Pearcy, Aron, et al. (2004),

$$P_{\text{non-detection}} = (f_{1i} + f_{2i})$$

when the queen is heterozygous at the *i*th locus, where f_{1i} and f_{2i} are, respectively, the frequency of the first and the second allele at the ith locus in the studied population (and lineage for hybridogenetic species; see Results), and

$$P_{\text{non-detection}} = f_{1i}$$

when the queen is homozygous, that is, the population frequency of the allele (f_{1i}).

For each colony, the overall probability of absence of any diagnostic allele was obtained by multiplying the values for each of the loci. This approach could not be applied to species with multiple-queen colonies (i.e., *C. hellenica* and *C. italica*) because we could not identify the maternal genotype of the daughter queens.

To distinguish between clonal reproduction accompanied by loss of heterozygosity and sexual reproduction between allele-sharing partners, we compared the observed number of transitions to homozygosity to the number expected under conditions of sexual reproduction with Mendelian inheritance (Eyer et al., 2013). If daughter queens are produced by sexual reproduction from a father sharing one allele with the mother queens at all loci, they have a 50% probability of inheriting the same allele from their two parents at a given locus (i.e., displaying homozygosity) if the mother is heterozygous at this locus. This probability is 100% if the mother is homozygous at the locus. On the contrary, if daughter queens are produced by thelyoky with central fusion automixis, their probability to become homozygous at a locus for which the mother is heterozygous ranges from 0% to 33%, according to the recombination rate of the locus (see above; Pearcy et al., 2006). We performed a binomial test to determine whether the observed frequency of transitions to homozygosity in daughter queens was consistent with that expected under sexual reproduction with Mendelian inheritance (probability of 50%) or with that expected under automictic parthenogenesis with central fusion (probability of 0%–33%; Pearcy et al., 2006). We calculated the observed frequency of transitions to homozygosity across all markers as the number of observed transitions divided by the total number of opportunities to observe a transition to homozygosity among daughter queens (which is a function of the number of heterozygous loci in the mothers). As mentioned above, this approach was not applied to polygynous species (i.e., *C. hellenica* and *C. italica*), because we could not identify the maternal genotype for the daughter queens.

2.4 | Social hybridogenesis

In species or populations employing social hybridogenesis, workers are the F_1 hybrid offspring of parents belonging to two different gene pools or lineages (disassortative mating). In contrast, in nonhybridogenetic species or populations, workers are produced by sexual reproduction between partners belonging to the same gene pool (random mating). To determine whether or not workers resulted from hybrid matings, we analysed genetic variability among reproductive individuals and determined whether mating patterns were disassortative or random using the genotype webs approach (Darras et al., 2019). For each species, a Principal Coordinate Analysis (PCoA) based on the genotypes of the reproductives (the mother queens and their inferred male mates) was performed with GENALEX version 6.502 (Peakall & Smouse, 2006, 2012). The connections between the parental genotypes that co-occurred in the workers were then

plotted to reveal mating patterns (Figure 3). In hybridogenetic species, we expected to see two clusters and every parent to be connected to its mating partner in the other cluster and never within its own cluster. In nonhybridogenetic species, no clear pattern was expected because workers are produced by random mating between reproductives from the same gene pool (see Figure 3 in Darras et al., 2019 for an illustration of the expected results for hybridogenetic and nonhybridogenetic species).

Previous studies have shown that, in some hybridogenetic *Cataglyphis* ants, queens occasionally mate with males of the same lineage. Evidence of such intralineage mating was assessed by genotyping the content of queens' spermathecae and determining whether the sperm contained a diagnostic allele for the queen's lineage (Darras, Leniaud, et al., 2014).

2.5 | Evolution of reproductive strategies

2.5.1 | Phylogenetic analyses

Sequences were aligned with the Muscle algorithm (Edgar, 2004) implemented in ALIGNER version 3.7.1 (CodonCode Corporation). Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian inference (BI). ML analyses were conducted with RAXML version 8.1.2 (Stamatakis, 2014) implemented in RAXMLGUI version 1.5 (Silvestro & Michalak, 2012). RAXML analyses were conducted using a GTR + G model, 10 replicates, and bootstrap replicates (using the "thorough bootstrap" option) with automatic bootstopping according to a majority rule tree based criteria ("autoMR" option). BI analyses were performed using MRBAYES version 3.2.6 (Ronquist & Huelsenbeck, 2003); 24 different models were implemented using the corrected Akaike information criterion (AICc) approach (Akaike, 1974). JMODELTEST version 2.1.10 (Darriba, Taboada, Doallo, & Posada, 2012) was used to choose the best nucleotide substitution model for each marker among the models. Two runs implementing four MCMC chains (three hot, one cold; heat parameter set to 0.1) of one to two million steps each were conducted. The cold chains were sampled every 500 steps, and 25% of the samples were used as burnin. Convergence between the two independent runs was considered to be sufficient when the mean standard deviation of split frequencies was below 0.01. To verify that the sampling was adequate when estimating the posterior probability distributions, we checked that no trends were apparent when the trace of the log probability of the data was plotted against the generation of the run (ensuring stationarity) and that the effective sample sizes (ESSs) were sufficiently high; TRACER version 1.7 was used for these tasks (Rambaut, Drummond, Xie, Baele, & Suchard, 2018).

Analyses were first conducted on each locus separately to assess congruence among gene trees. As most of the supported relationships were congruent across loci, the eight sequences (for *202*, *384*, *505*, *1281*, *Ab*, *Lr*, *Wg*, and *cox1*) were concatenated. The best-fit partitioning scheme for the concatenated sequences was then identified using PARTITIONFINDER version 2.1.1 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016). In the program, we defined a total of 17 a priori partitions: a partition for each codon position for each of the coding genes (*cox1*, *Ab*, *Lr*, and *Wg*), a partition for each of the noncoding EPIC markers (*202*, *384*, *505*, and *1281*), and a partition for the portion of the *Lr* intron. The best-fit partitioning scheme identified by PARTITIONFINDER comprised 13 partitions. In general, in MRBAYES the values for all the other parameters were the same as in individual gene analysis. The exceptions were that the number of steps for the MCMC chains were 10 million, and the sample frequency was 5,000.

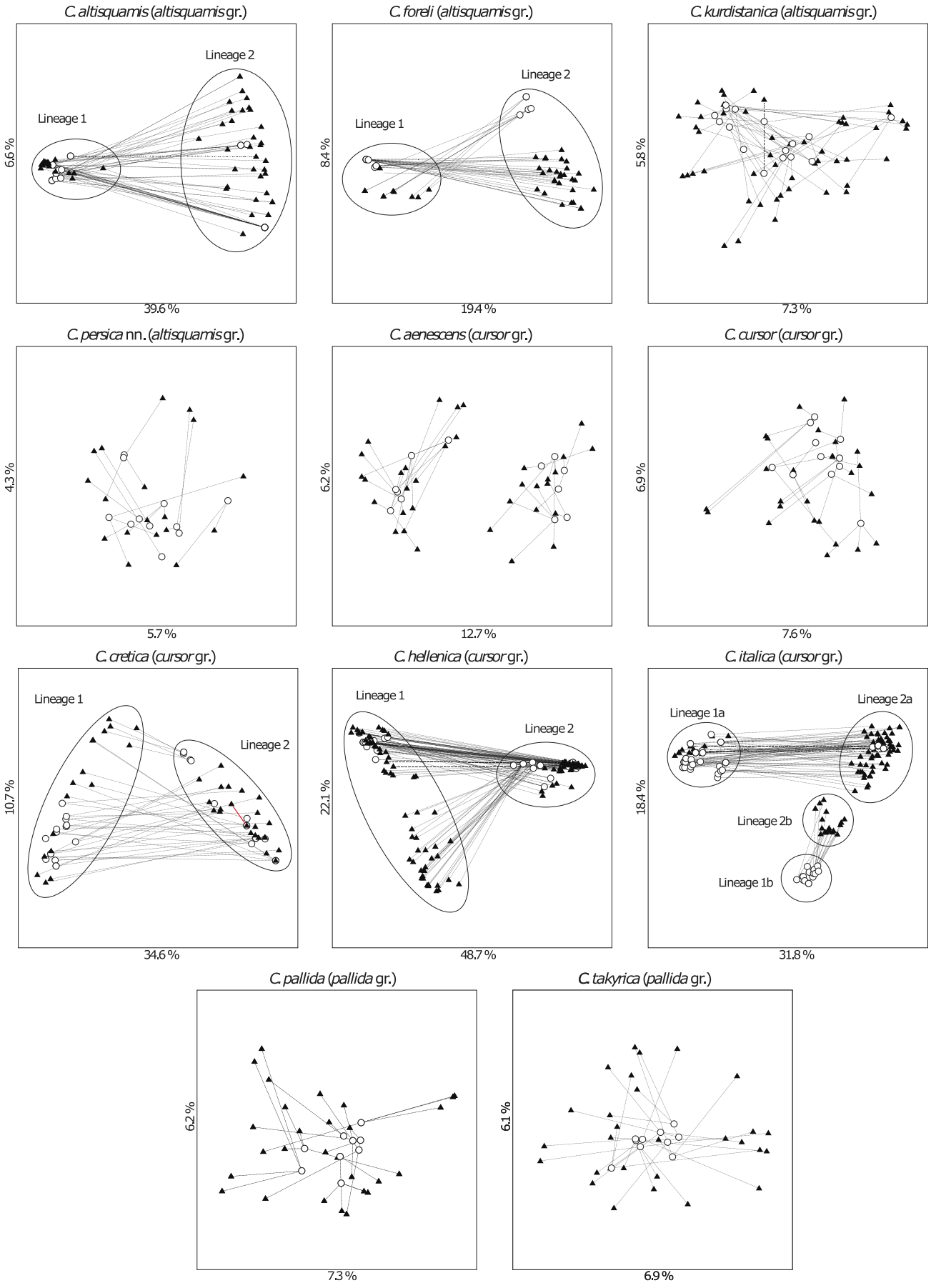
2.5.2 | Cytonuclear discordances in hybridogenetic species

In the two hybridogenetic species for which both nuclear and mitochondrial DNA were previously examined, namely *C. hispanica* and *C. velox*, marker variation revealed discordances: reciprocally monophyletic lineages were recovered when nuclear DNA was used, while individuals were clustered based on their geographical origin when mitochondrial DNA was used (Darras & Aron, 2015; Eyer, Leniaud, Tinaut, & Aron, 2016). To determine whether the same pattern occurred in other hybridogenetic species, we sequenced individuals from two geographically distant populations whenever possible. We then investigated whether mitochondrial DNA grouped individuals from different lineages based on their population of origin (cytonuclear discordance) or based on their lineage of origin.

2.5.3 | Stochastic character mapping of reproductive strategies

To determine whether the parthenogenetic production of queens and clonal social hybridogenesis evolved once or multiple times in the ant genus *Cataglyphis*, we proceeded as follows. Three reproductive systems were defined: classical reproduction (i.e., haplodiploid, sexual production of queens, and absence of dependent-lineage reproduction), conditional use of sex, and clonal social hybridogenesis. Each species was assigned to one of those categories based on the results of previous studies and of the present study (Table S1). Using the Bayesian consensus tree estimated from the concatenated sequences, we inferred the ancestral states of the reproductive systems via stochastic character mapping (Huelsenbeck, Nielsen, &

FIGURE 3 Genotype webs depicting mating patterns and genetic variation among reproductive individuals. For each species, a principal coordinate analysis (PCoA) was performed using the genotypes of mother queens (white circles) and their male mates (black triangles). The proportions of genetic variability explained by the first two principal components are indicated. Mating assortments are revealed by links connecting parental genotypes co-occurring in worker offspring. In hybridogenetic species, mating partners belong to distinct gene pools (delimited by ellipses); a single exception to this rule was observed in one colony of *Cataglyphis cretica*, where the parental genotypes belonged to the same genetic cluster (red connection in lineage 2). In nonhybridogenetic species, mating is random, and sexual partners belong to the same gene pool [Colour figure can be viewed at wileyonlinelibrary.com]


C. altisquamis (altisquamis gr.)
Lineage 1
Lineage 2
6.6 %
39.6 %
C. foreli (altisquamis gr.)
Lineage 1
Lineage 2
8.4 %
19.4 %
C. kurdistanica (altisquamis gr.)
5.8 %
7.3 %
C. persica nn. (altisquamis gr.)
4.3 %
5.7 %
C. aenescens (cursor gr.)
6.2 %
12.7 %
C. cursor (cursor gr.)
6.9 %
7.6 %
C. cretica (cursor gr.)
Lineage 1
Lineage 2
10.7 %
34.6 %
C. hellenica (cursor gr.)
Lineage 1
Lineage 2
22.1 %
48.7 %
C. italica (cursor gr.)
Lineage 1a
Lineage 2a
Lineage 2b
Lineage 1b
18.4 %
31.8 %
C. pallida (pallida gr.)
6.2 %
7.3 %
C. takyrica (pallida gr.)
6.1 %
6.9 %

Bollback, 2003). To do so, we used the make.simmap function implemented in the R package phytools (R Core Team, 2018; Revell, 2012). We employed the three available hierarchical models of transition between character states: equal rates (ER), symmetrical (SYM), and all rates different (ARD). In *Cataglyphis*, clonal social hybridogenesis always involves the conditional use of sex (Figure 1, Table 1) but in addition two genetic lineages coexist: workers are hybrids of the two lineages, whereas male and female reproductives are purebred of the maternal lineage. Consequently, we assigned an order to the character states given that the conditional use of sex is an obligatory transitional state between classical reproduction and clonal social hybridogenesis. As a precautionary measure, we also tested unordered models without constraints on the transitions between character states. To compare the fit of the six models (ER, SYM, ARD, each ordered and unordered) to the data, a likelihood ratio test based on log-likelihood scores was performed, and the AICc values (Hurvich & Tsai, 1989) of the models were examined. To account for the inherent stochasticity of the process, we performed 1,000 stochastic mapping replicates. Stochastic mapping can estimate ancestral states when the states at branch tips are unknown; it can also calculate posterior probabilities for the states of those unknown terminal taxa by defining a prior probability distribution for them. Here, we therefore assigned an equal prior probability for each state to *C. cubica* and *C. emeryi* because their reproductive systems were unknown. For *C. cursor* and *C. pallida*, the prior probabilities were equal for classical reproduction and the conditional use of sex (see Results). We also made the a priori assumption that the common ancestor of *Cataglyphis* and *Proformica* (the outgroup) had a classical haplodiploid reproductive system.

3 | RESULTS

Across the 11 species studied, we determined the genotypes of 508 queens (349 mother queens and 159 alate daughter queens, mean ± *SD* per species = 31.7 ± 36.1 and 14.4 ± 26.6, respectively) and 1,172 workers (106.5 ± 44.3 per species), using microsatellite markers (Table S6). We obtained the genotypes of 520 males based on the analyses of mother queen-worker genetic combinations (N = 211 colonies). In addition, the contents of 366 spermathecae (33.3 ± 60 per species) were genotyped to detect cryptic male mates (Table S6).

3.1 | Parthenogenetic production of queens

The field data and genetic data revealed that, for all the study species, colonies were typically headed by a single mother queen (i.e., displayed monogyny), with the exception of *C. hellenica* and *C. italica*, whose colonies were headed by multiple nestmate queens (i.e., displayed polygyny; Table 1). Pedigree comparisons showed that mother queens produced daughter queens by thelytokous parthenogenesis in six species: in *C. altisquamis* and *C. foreli* from the *altisquamis* group, as well as in *C. aenescens*, *C. cretica*, *C. hellenica*, and *C. italica* from the *cursor* group.

In the monogynous species *C. altisquamis*, *C. foreli*, *C. aenescens*, and *C. cretica*, the mother queen and her alate daughter queens had either identical genotypes at all loci (*C. foreli*: n = 8 queens [mothers + daughters] from three colonies; *C. cretica*: n = 6 queens [mother + daughters] from one colony) or exhibited differences at one locus that were best explained by recombination and loss of heterozygosity (i.e., resulting from automictic parthenogenesis with central fusion; *C. altisquamis*: n = 32 queens [mothers + daughters] from 12 colonies; *C. aenescens*: n = 7 queens [mother + daughters] from one colony). A deviation from this pattern was observed in *C. altisquamis* and *C. cretica*, where one daughter queen (from among the 21 and six individuals genotyped, respectively) had a genotype consistent with sexual reproduction. The hypothesis that the genetic similarity among the mother queen and her daughter queens resulted from sexual production of new queens by allele-sharing partners can be confidently excluded. First, the probability of queens sharing one allele with their male mate(s) at all loci was low for *C. altisquamis* ($P_{\text{non-detection}}$ ± *SD* = 0.03 ± 0.01, n = 9 colonies – data from population 1), *C. foreli* (0.007 ± 0.01, n = 3), *C. aenescens* (<0.001; n = 1) and *C. cretica* (<0.001; n = 1). Second, the results of the binomial tests indicated that transitions to homozygosity were significantly less frequent than expected under conditions of sexual reproduction in *C. aenescens* (number of observed transitions/total number of potential transitions to homozygosity among daughter queens: n = 1/36, p < .001), *C. altisquamis* (n = 1/63, p < .001), *C. cretica* (n = 0/24, p < .001), and *C. foreli* (n = 0/10, p < .001).

In the polygynous species *C. hellenica* and *C. italica*, as well as in two polygynous *C. cretica* colonies (Table S6), nestmate queens (mothers + alate daughters queens if any) had similar multilocus genotypes that reflected the occurrence of automictic parthenogenesis. However, while nestmate queens systematically belonged to a single clonal matriline in *C. hellenica* (n = 54 queens from 11 colonies) and *C. cretica* (n = 4 queens from 2 colonies), multiple clonal matrilines were found in 10 of the 26 (38%) colonies of *C. italica* (Table S6). In this species, there were an average of 1.8 matrilines per colony, and all the queens from a given matriline appeared to have been produced by automictic parthenogenesis (mean number of queens per matriline ± *SD* = 2.9 ± 1.9, n = 48 matrilines). It is unlikely that recent mutation events were responsible for the occurrence of multiple clonal matrilines within colonies, since the matrilines within a colony repeatedly displayed alleles differing by more than a single repetition of the microsatellite motifs, which is the typical mutation pattern of microsatellite markers (the stepwise mutation model; Ellegren, 2004). We were unable to assess the probability of queens sharing alleles with their male mate(s) nor to use the binomial test approach with the polygynous species *C. hellenica* and *C. italica* because we could not identify the maternal genotype for the daughter queens that we sampled. In these two species, a high proportion of queens (*C. hellenica*: 33% and *C. italica*: 24%) were homozygous at one or more loci for which nestmate queens were heterozygous.

Since no more than two alleles per locus were found within each matriline, this result was also best explained by loss of heterozygosity associated with automictic parthenogenesis with central fusion.

In the monogynous species *C. kurdistanica* (n = 1 daughter queen from one colony), *C. persica* (n = 87 daughter queens from eight colonies), and *C. takyrica* (n = 7 daughter queens from one colony), comparisons of mother and daughter queens' genotypes provided evidence that queens had been produced by sexual reproduction. In *C. persica*, female offspring had genotypes similar to those of their mothers in two colonies (PER1c08: four of 19 daughter queens and two of 17 workers; PER1c15A: three of four daughter queens). However, although the probability of queens mating with a male harbouring no diagnostic allele at any of the loci was low (mean $P_{\text{non-detection}} \pm SD = 0.02 \pm 0.007$, n = 2 colonies), the observed number of transitions to homozygosity was not significantly different from the expected value under conditions of sexual reproduction with Mendelian segregation (n = 10/18, p = .81, two-sided binomial test). We were unable to sample any daughter queens for *C. cursor* or *C. pallida*.

3.2 | Social hybridogenesis

As shown in Figure 3, the PCoA performed on the genotypes of the mother queens and their male mates revealed the presence of distinct genetic clusters in six species: *C. altisquamis* and *C. foreli* from the *altisquamis* group and *C. aenescens*, *C. cretica*, *C. hellenica*, and *C. italica* from the *cursor* group. In *C. altisquamis*, *C. foreli*, *C. aenescens*, *C. cretica*, and *C. hellenica*, two clusters could be distinguished along the first axes of the PCoA plots, which accounted for 13% to 49% of the total genetic variance. Four genetic clusters could be identified in *C. italica*. In both *C. aenescens* and *C. italica*, the genetic clusters corresponded to two geographically distinct populations: one cluster per population in *C. aenescens* and two clusters per population in *C. italica*.

The connections between the parents of each worker (i.e., the genotype webs) revealed interbreeding between genetic clusters from the same population in five species: *C. altisquamis*, *C. foreli*, *C. cretica*, *C. hellenica*, and *C. italica*. As expected under social hybridogenesis, all the workers were thus the product of hybrid mating between reproductive individuals originating from different gene pools. A single exception was observed in *C. cretica*, where the parents of one colony (CRE1c22) belonged to the same genetic cluster (red connection within lineage 2 in Figure 3). While colonies of this species typically contained several hundred workers (mean number of workers per colony ± *SD*: 472 ± 303, n = 28), this particular colony comprised only 41 workers. In line with these results, sperm genotyping indicated that most queens of a given lineage had mated with males from the alternative lineage, even though evidence of intralineage mating events was observed in *C. altisquamis* (six of 16 queens), *C. cretica* (four of 33 queens), *C. hellenica* (27 of 110 queens), and *C. italica* (54 of 186 queens; Table S6).

In *C. cursor*, *C. kurdistanica*, *C. pallida*, *C. persica*, and *C. takyrica*, the genotypes of reproductive individuals were scattered across the first two axes of the PCoA plots, and no clear genetic clusters could be identified. In these species, as well as in *C. aenescens*, mating appeared to be fairly random within each population/cluster (Figure 3).

3.3 | Evolution of reproductive strategies

3.3.1 | Phylogenetic analyses

Out of the 4,348 bp aligned sites from the concatenated sequences, 614 were variable and 352 were parsimony informative. The Bayesian and maximum-likelihood trees reconstructed from each locus separately and from the concatenated loci were consistent in their characterization of the phylogenetic relationships among species (Figure 4 and Figure S1), and their results matched those of a previously published phylogeny (Aron et al., 2016). All the species groups appeared to be reciprocally monophyletic and were supported by high Bayesian posterior probabilities and bootstrap values. Although the trees largely displayed consistent evolutionary relationships within species groups, conflicts were observed in two cases. In the *cursor* group, different levels of support were found for the positions of the hybridogenetic lineages of the closely related species *C. hellenica* and *C. italica*. The markers *505*, *1281*, *202*, *Ab*, and *cox1* all supported different relationships (Figure S1). In the *altisquamis* group, there were conflicts regarding the relationships of *C. velox* and *C. humeya* (*Lr*, *cox1*, *505*, and *1281*; Figure S1).

3.3.2 | Cytonuclear discordances in hybridogenetic species

As previously reported for the hybridogenetic species *C. hispanica* (Darras & Aron, 2015) and *C. velox* (Eyer et al., 2016), we found cytonuclear discordances in all hybridogenetic species for which two populations were sampled (*C. altisquamis*, *C. cretica*, *C. hellenica* and *C. italica*; Figures S2 and S3). In each species, the nuclear DNA clearly separated lineages but the mitochondrial DNA did not. Instead, the mitochondrial haplotypes clustered according to ant geographical origin (Figures S2 and S3).

3.3.3 | Stochastic character mapping of reproductive systems

Species displaying different reproductive systems—classical reproduction, conditional use of sex, and clonal social hybridogenesis—appear to be polyphyletic (Figure 4). Both the conditional use of sex and hybridogenesis were found in two distantly related clades: the *altisquamis* group and the *cursor* group. Within each, the distribution of these unusual reproductive systems was also polyphyletic or paraphyletic. We inferred the evolution of the conditional use of sex and clonal social hybridogenesis in the genus using stochastic character mapping. Comparisons of the log likelihoods and the AICc values of the six models tested (ordered and

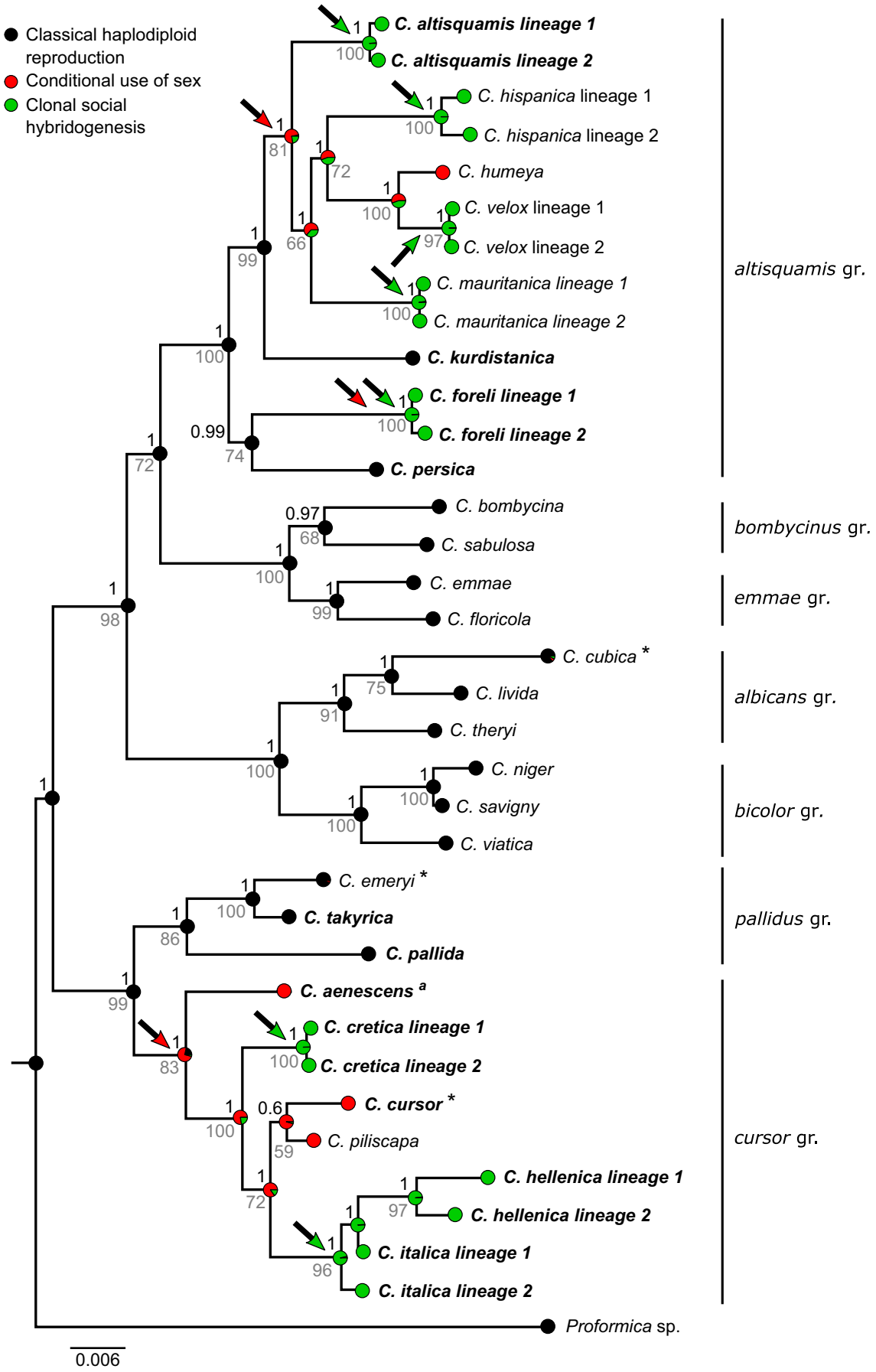

Classical haplodiploid reproduction
Conditional use of sex
Clonal social hybridogenesis
C. altisquamis lineage 1
C. altisquamis lineage 2
C. hispanica lineage 1
C. hispanica lineage 2
C. humeya
C. velox lineage 1
C. velox lineage 2
C. mauritanica lineage 1
C. mauritanica lineage 2
C. kurdistanica
C. foreli lineage 1
C. foreli lineage 2
C. persica
C. bombycina
C. sabulosa
C. emmae
C. floricola
C. cubica *
C. livida
C. theryi
C. niger
C. savigny
C. viatica
C. emeryi *
C. takyrica
C. pallida
C. aenescens a
C. cretica lineage 1
C. cretica lineage 2
C. cursor *
C. piliscapa
C. hellenica lineage 1
C. hellenica lineage 2
C. italica lineage 1
C. italica lineage 2
Proformica sp.
altisquamis gr.
bombycinus gr.
emmae gr.
albicans gr.
bicolor gr.
pallidus gr.
cursor gr.
0.006

FIGURE 4 Evolution of different reproductive systems in *Cataglyphis* ants. Bayesian consensus tree based on seven nuclear loci (*Ab*, *Lr*, *Wg*, *202*, *384*, *505*, *1281*) and one mitochondrial locus (*cox1*). The posterior probability values (in black) and maximum-likelihood bootstrap values (in grey) are reported near the nodes. The reproductive system used by each taxon is indicated: classical Hymenopteran haplodiploid reproduction (black circle), conditional use of sex (red circle), or clonal social hybridogenesis (green circle). The 11 species examined in this study appear in bold. [a]Note that in *C. aenescens*, both sexual populations and thelytokous populations have been reported (Mongolia, Cronin et al., 2016 and Iran, this study, respectively). The phylogeny refers to the Iranian thelytokous population. For each hybridogenetic species, the two interdependent lineages (lineage 1 and lineage 2) are represented. The posterior probabilities (PP) of the ancestral states for the reproductive systems, inferred using stochastic character mapping with an "all rates different" (ARD) model, are reported near the ancestral nodes in the form of a pie chart. The same symbol was used for species whose reproductive system was inferred through stochastic character mapping (denoted by an asterisk). Probable independent evolution of the conditional use of sex (red arrow) and of hybridogenesis (green arrow), again based on the ARD model (with PP > 50%), is indicated. The seven main *Cataglyphis* species groups are shown. The references used to assign the reproductive systems are listed in Table S1 [Colour figure can be viewed at wileyonlinelibrary.com]

unordered ER, SYM, and ARD; see Methods) indicated that they fit the data equally well (Table S5). The outputs of the six models were mainly consistent, and they all supported the idea that the common ancestor of *Cataglyphis* had a classical haplodiploid reproductive system (posterior probability, PP = 100%; Figure S4). Furthermore, they showed that the conditional use of sex and clonal social hybridogenesis evolved independently in the *cursor* and *altisquamis* species groups.

Since all models fit the data equally well, our assumption that the conditional use of sex is a prerequisite for clonal social hybridogenesis to evolve was retained and the ordered ARD model was selected as it allows each transition type to have a different rate, which probably better reflects real mechanisms. The results of this model are presented in Figure 4 (which is similar to Figure S4f). In the *cursor* group, the model supported the conditional use of sex as the most likely ancestral state (PP = 74.5%). The most probable scenario is that hybridogenesis evolved twice in this group: once in *C. cretica* and once in the *C. italica*–*C. hellenica* clade (PP of social hybridogenesis being the reproductive system of the last common ancestor between *C. cretica* and the *C. italica*–*C. hellenica* clade: 22.5%). In the *altisquamis* group, the model supported classical reproduction as the ancestral state (PP = 100%). Under such circumstances, queen production via parthenogenesis would have evolved independently twice: once in a common ancestor of the clade comprising *C. altisquamis*, *C. mauritanica*, *C. velox*, *C. humeya*, and *C. hispanica* (PP = 74.5%) and once along the branch leading to *C. foreli* because (a) the last common ancestor of *C. foreli* and its sister species *C. persica* probably displayed classical reproduction (PP = 100%) and (b) the parthenogenetic production of queens is a prerequisite for clonal social hybridogenesis. In this scenario, hybridogenesis in the *altisquamis* group would have independently emerged between two (in *C. foreli* and in the last common ancestor of the clade comprising *C. altisquamis*, *C. mauritanica*, *C. velox*, *C. humeya*, and *C. hispanica*) and five times (independent apparition in *C. foreli*, *C. altisquamis*, *C. mauritanica*, *C. velox*, and *C. hispanica*).

Stochastic mapping allowed us to infer the reproductive systems of species for which we had incomplete data. We discovered that the most likely reproductive system for *C. cubica*, *C. emeryi*, and *C. pallida* is classical reproduction (*cubica*: $PP_{classical}$ = 86.3%, $PP_{conditional\ use\ of\ sex}$ = 6.8%, $PP_{clonal\ social\ hybridogenesis}$ = 6.9%; *emeryi*: $PP_{classical}$ = 92.2%, $PP_{conditional\ use\ of\ sex}$ = 5%, $PP_{clonal\ social\ hybridogenesis}$ = 2.8%; *pallida*: $PP_{classical}$ = 91.3%, $PP_{conditional\ use\ of\ sex}$ = 8.7%); for *C. cursor*, it appears to be the conditional use of sex ($PP_{classical}$ = 0%, $PP_{conditional\ use\ of\ sex}$ = 100%).

4 | DISCUSSION

This study shows that both the conditional use of sex and clonal social hybridogenesis evolved repeatedly in the ant genus *Cataglyphis*. We discovered the occurrence of clonal social hybridogenesis in five new species: (a) *C. altisquamis* and *C. foreli*, members of the *altisquamis* species group, which contains all the previously identified hybridogenetic species (*C. hispanica*, *C. mauritanica*, and *C. velox*; Eyer et al., 2013; Leniaud et al., 2012) and (b) *C. cretica*, *C. hellenica*, and *C. italica*, which belong to the *cursor* species group. In addition, we discovered a new case of the conditional use of sex in the nonhybridogenetic species *C. aenescens*, a member of the *cursor* group. This result contrasts with the study of Cronin, Chifflet-Belle, Fédérici, and Doums (2016) on a Mongolian population of *C. aenescens*, showing that daughter queens arise from sexual reproduction. This species presents a large geographic distribution (Radchenko, 2001) and one may note exclude the possibility that variations in its reproductive mode occur across its range. Another explanation to account for this difference stems from the fact that the systematics of the *cursor* group is largely unresolved, so that the two populations studied could belong to different species. Overall, the present study shows that the conditional use of sex and clonal social hybridogenesis are widespread in both the *altisquamis* and the *cursor* species groups. In the former, only two of eight species studied so far appear to have maintained a classical reproductive system; five reproduce through social hybridogenesis in at least some part of their ranges, and one uses sex conditionally, but without dependent-lineage reproduction. Among the six species of the *cursor* species group, three reproduce through clonal social hybridogenesis and at least two use sex conditionally, also without dependent-lineage reproduction.

The five new cases of clonal hybridogenesis reported here share the main features of the reproductive system as originally described in *C. hispanica*, *C. mauritanica*, and *C. velox*. First, workers are produced by the hybridization of two sympatric yet divergent nuclear lineages. Consequently, virtually all workers were of hybrid origin. Purebred workers were detected in a single colony; however, this colony had a very low number of workers, which

suggests that such exceptions are probably a sign of maladaptation. A similar trend was reported in hybridogenetic *Pogonomyrmex* seed harvester ants, where colonies with purebred workers have a significantly lower productivity than colonies with interlineage workers (Helms Cahan et al., 2004). As a consequence, polygyny and/or polyandry may have been selected in hybridogenetic species to increase the probability that at least one mating between partners from alternative lineages occurs within colonies. Both polygyny and/or multiple mating were indeed documented in populations of all hybridogenetic *Cataglyphis* species investigated so far (Table 1). Second, interbreeding lineages are clearly divergent in the analyses employing nuclear DNA but are not recovered in the analyses employing mitochondrial DNA, which is consistent with gene flow between lineages. Such cytonuclear discordances have been previously reported in two hybridogenetic species of the *altisquamis* group (*C. hispanica* and *C. velox*; Darras & Aron, 2015; Eyer et al., 2016). Remarkably, this pattern was also evidenced in hybridogenetic species of the *cursor* group, indicating that the mechanism responsible for such cytonuclear discordances is closely associated to hybridogenesis in *Cataglyphis*. However, it remains unknown how hybridogenetic lineages exchange genetic material as reproductive individuals typically develop from pure lineage eggs preventing the introgression of genetic material from the alternative lineage. Two explanations, both relying on gene flow, were hypothesised by Darras and Aron (2015) to account for the cytonuclear discordances observed: (a) the sporadic production of hybrid queens and their recurrent backcross with males from their paternal lineage for new queen production would ultimately lead to offspring with introgressed mitochondria, and (b) the production of new queens by gynogenesis, providing opportunities for the leakage of paternal mitochondrial DNA. To date, however, none of these hypotheses have been explicitly tested. Third, reproductive queens of hybridogenetic species are typically produced asexually by thelytokous parthenogenesis, but they can also, on rare occasions, arise from sexual reproduction between partners of the same genetic lineage (intralineage mating). Indeed, we found alate daughter queens stemming from intralineage mating in both *C. altisquamis* and *C. cretica*. The multiple matrilines observed in *C. italica* nests may also have arisen from intralineage mating. However, we cannot exclude alternative hypotheses, including that there was cofoundation by different clones or adoption of foreign queens. The occasional production of new queens by intralineage mating has also been previously reported in *C. hispanica* and *C. mauritanica* kept under laboratory conditions (Darras, Kuhn, et al., 2014; Kuhn et al., 2018). This phenomenon is probably of great evolutionary significance for the maintenance of social hybridogenesis in *Cataglyphis*. If all queens were to be produced by parthenogenesis, males would only sire nonreproductive workers and thus fail to have any reproductive success. This issue could represent a threat for the long-term persistence of the system (Leniaud et al., 2012; Schwander & Keller, 2012). The sexual production of queens, although rare, may therefore provide males with non-null fitness expectancies. Furthermore, rare sexual events may allow queen lineages to avoid the long-term costs of asexuality (Schön, Van Dijk, & Martens, 2009).

Our phylogenetic analyses confirm the results of previous studies examining relationships among species groups (Aron et al., 2016). In particular, the *cursor* and *altisquamis* groups do not appear to be sister groups, despite their strong morphological convergence (Agosti, 1990; Knaden, Tinaut, Stökl, Cerdá, & Wehner, 2012; Radchenko, 2001) and similar reproductive strategies (this study). Furthermore, our results reveal that the *pallidus* group is closely related to the *cursor* group and that both diverged from other species groups early on in the genus' evolution. This finding contradicts past work suggesting that the *pallidus* group is related to the *emmae* and the *bombycinus* groups (Agosti, 1990; Radchenko, 2001). Although most of the phylogenetic relationships among species were consistent across the different single loci of nuclear DNA, some discordance was observed for certain species pairs. Discordances in phylogenetic trees can result from gene duplication, gene flow between species/lineages, or incomplete lineage sorting (Nakhleh, 2013). Such discordance was expected between *C. velox* and *C. humeya* which appear to have a reticulate evolutionary history (Darras et al., 2019; Eyer et al., 2016). Discordant relationships were also observed for lineages of *C. italica* and *C. hellenica*. Here, gene flow is doubtful because our study species had nonoverlapping ranges. Gene duplication also seems unlikely because the discordances were only observed for a few species and occurred for several genes. A more probable scenario is that ancestral polymorphism has been retained in recently isolated populations (i.e., incomplete lineage sorting), which is consistent with the taxonomic uncertainty associated with these groups.

The phylogenetic distribution of the conditional use of sex and clonal social hybridogenesis provides some clues regarding the conditions that may have favoured the evolution of these reproductive systems in *Cataglyphis*. The ability to produce females through thelytokous parthenogenesis is a labile character in Hymenoptera because the production of haploid males through arrhenotokous parthenogenesis serves as a form of preadaptation (Rabeling & Kronauer, 2013). In *Cataglyphis* ants, thelytokous parthenogenesis has evolved in at least four different phylogenetic groups (the *altisquamis*, *bombycinus*, *bicolor*, and *cursor* species groups), where it can be used by workers to produce daughter queens or other workers in orphaned colonies (Aron et al., 2016). In contrast, queens have been observed to use thelytokous parthenogenesis in only two of those groups: *altisquamis* and *cursor* (Aron et al., 2016). Here, we show that the parthenogenetic production of daughter queens by mother queens independently evolved at least two times: once in the last common ancestor of the *cursor* group and once in the *altisquamis* group (Figure 4). Stochastic character mapping suggested that the system emerged twice in the *altisquamis* group, but we cannot exclude the possibility that one of those events resulted from contagious parthenogenesis via interspecific introgression within the group (Simon, Delmotte, Rispe, & Crease, 2003). Under this hypothesis, incomplete reproductive isolation between sexual and parthenogenetic species would allow for a unidirectional, male-mediated, gene flow from the latter into the former. In this context, the introgression of

parthenogenesis inducing alleles may rapidly spread, converting sexual lineages into parthenogenetic ones (Simon et al., 2003). Queens that are the result of asexual reproduction are expected to have lower fitness than their sexually produced counterparts (Doums et al., 2013). This is because automictic parthenogenesis with central fusion increases homozygosity and results in the loss of intraindividual genetic diversity (Goudie et al., 2012; Pearcy et al., 2006). This loss may reduce survival and fertility, just like inbreeding depression (Charlesworth & Willis, 2009). Thelytokous parthenogenesis may also lead to the accumulation of mildly deleterious mutations (Muller, 1964). Interestingly, in both the *altisquamis* and *cursor* groups, new queens mate close to their natal nest and disperse on foot with nestmate workers to establish new colonies nearby. Having the help of workers during colony foundation greatly relaxes the external constraints faced by newly mated queens, and this system may have favoured the emergence of queen parthenogenesis as thelytokous queens with reduced fitness are thus assisted by genetically diverse, sexually produced workers during the most critical period of their reproductive lives (Pearcy, Aron, et al., 2004). In other *Cataglyphis* species groups, recently mated queens typically found new colonies via solitary dispersal, a stressful period that probably selects against low-fitness, clonal queens (*C. tartessica* is the only known species from these groups that disperses with workers; Amor et al., 2011). Indeed, queen parthenogenesis has never been observed in solitary founding queens in ants (Fournier et al., 2005; Okamoto, Kobayashi, Hasegawa, & Ohkawara, 2015; Pearcy et al., 2011; Peeters & Aron, 2017) or termites (Matsuura, 2010; Matsuura, 2017).

In hybridogenetic species of the ant genera *Messor* and *Pogonomyrmex*, queens are produced exclusively by intralineage sexual reproduction (Helms Cahan & Keller, 2003; Norman et al., 2016; Romiguier et al., 2017). Here, we showed that, in contrast, social hybridogenesis in *Cataglyphis* is strongly associated with queen parthenogenesis. We posit that the evolution of clonal social hybridogenesis follows the emergence of the conditional use of sex, although it is possible that the two strategies evolved simultaneously on the branch leading to *C. foreli* (Figure 4). The occurrence of queen parthenogenesis in *Cataglyphis* may have predisposed the genus to evolve clonal social hybridogenesis. One explanation is that social hybridogenesis was selected for in populations with clonal queens because of the fitness payoff of outbreeding in workers. Obligate crossing between divergent gene pools results in very high worker heterozygosity, which may improve colony fitness, possibly through heterosis (Burke & Arnold, 2001; Cahan, Julian, Schwander, & Keller, 2006; Umphrey, 2006). Species that utilise social hybridogenesis could therefore benefit from hybridization while minimizing the costs of hybrid sterility, as reproductive individuals (queens and males) develop from pure lineage eggs only and are therefore unaffected by the negative consequences of hybridization on reproductive potential (Burke & Arnold, 2001). Outbreeding may also have been selected for in populations with inbred clonal queens because it alleviates inbreeding depression in sexually produced workers and prevents the production of sterile diploid males that are homozygous at sex-determining locus/loci (Darras et al., 2019). Clonal social hybridogenesis could also reduce kin conflicts within colonies. As is usually the case within *Cataglyphis*, workers from hybridogenetic species are not sterile; they have kept ovaries and are amphitokous: they can lay both haploid and diploid eggs in the absence of the queen(s) under laboratory conditions (Aron et al., 2016). A kin conflict is predicted between the queens and their workers about the production of new queens. This is because mother queens are more closely related to their own thelytokous daughter queens (life-for-life relatedness, r = 1) than to the thelytokous daughters of their sexually produced worker offspring (i.e., their granddaughters; r = .5). In contrast, workers are more closely related to their own thelytokous daughters (worker-own daughters: r = 1; average in a colony headed by a single, once-mated queen: r = .75) than to the thelytokous daughters of the queen (r = .5). (Note that this logical holds for male production). Such reproductive conflict is evolutionary unstable and is predicted to result in the disappearance of thelytokous parthenogenesis in one or both castes (Goudie & Oldroyd, 2018). Interestingly, our data on *Cataglyphis* are consistent with this hypothesis. Though not sterile, workers from hybridogenetic species show low fertility and their offspring apparently suffer no viability. Experimental laboratory observations revealed that only a very small proportion of worker-laid haploid eggs and none of diploid eggs reach adulthood (Leniaud et al., 2012; Eyer et al., 2013; A. Kuhn and H. Darras, personal observation). In line with this, across all hybridogenetic *Cataglyphis* species studied so far, the hundreds of reproductive individuals collected from the field were of pure breeding lineages – no hybrid males or queens (except one; Darras et al., 2019) have been found, making very unlikely that worker-laid eggs (if any) achieve their development into viable and fertile hybrid sexuals. Altogether, these results suggest that obligate crossing may have lessened worker fertility, thereby reducing queen-worker conflict over male and female parentage within colonies.

Overall, this study shows that both the conditional use of sex (i.e., the parthenogenetic production of daughter queens) and clonal social hybridogenesis (i.e., the parthenogenetic production of daughter queens and the sexual production of hybrid workers from distinct gene pools) evolved at least twice in *Cataglyphis* ants. While thelytoky is probably an ancestral character in the genus (Aron et al., 2016), the origin of social hybridogenesis remains uncertain. In all the hybridogenetic *Cataglyphis* species studied to date (except for two: *C. italica* and *C. hellenica*), the interdependent lineages appear to be monophyletic and characterized by relatively low levels of divergence (Figure 4), which suggests they evolved recently in each species. Nevertheless, this interpretation does not consider the potential role of recombination between lineages. Even a low degree of gene flow would limit genetic divergence between lineages, making them appear to be more recent than they actually are. While evidence for gene flow between sympatric lineages has been observed (Darras & Aron, 2015; Darras et al., 2019), the extent to which such introgression reshuffles the genomes of hybridogenetic lineages over time remains unknown. Ultimately, such recombination could

lead to the speciation of new lineage pairs (Darras & Aron, 2015). It remains an open question as to whether all the instances of social hybridogenesis in *Cataglyphis* are recent dead ends that evolved independently or whether the system is ancient and has been maintained across speciation events in both the *altisquamis* and *cursor* species groups.

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AUTHOR CONTRIBUTIONS

A.K., H.D., and S.A. designed the project; A.K., H.D., and O.P. collected the samples; A.K. and H.D. performed the research and analysed the data; A.K., H.D., and S.A. wrote the paper.

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DATA AVAILABILITY STATEMENT

The microsatellite data set supporting this article are provided as part of the electronic supplementary material (Table S7). Sequence data were deposited in GenBank (accession numbers MK766470–MK766507 and MK810209–MK810441).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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