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Evolution of hybridogenetic lineages in *Cataglyphis* ants

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Abstract

In most social Hymenoptera, a diploid egg develops into either a queen or a worker depending on environmental conditions. Hybridogenetic *Cataglyphis* ants display a bizarre genetic system, where queen-worker caste determination is primarily determined by genetic factors. In hybridogenetic populations, all workers are F1 hybrids of two distinct lineages, whereas new queens are nearly always pure-lineage individuals produced by clonal reproduction. The distribution and evolutionary history of these hybridogenetic populations have not yet been thoroughly analysed. Here, we studied the phylogeographic distribution of hybridogenetic populations in two closely related Spanish species: *Cataglyphis humeya* and *Cataglyphis velox*. Hybridogenesis has been previously documented in a locality of *C. velox*, but whether this system occurs elsewhere within the range of the two species was yet unknown. Queens and workers from 66 localities sampled across the range of the species were genotyped at 18 microsatellite markers to determine whether queens were produced by parthenogenesis and whether workers were hybrids of divergent lineages. Populations with F1 hybrid workers were identified by combining genetic, geographical and mating assortments data. In most populations of *C. velox*, workers were found to be hybrids of two divergent lineages. Workers were however produced via random mating in two marginal populations of *C. velox*, and in all populations studied of its sister species *C. humeya*. High-throughput sequencing data were obtained to confirm inferences based on microsatellites and to characterize relationships between populations. Our results revealed a complicated history of reticulate evolution that may account for the origin of hybridogenetic lineages in *Cataglyphis*.

KEYWORDS
breeding systems, hybridization, insects, phylogeography

1 | INTRODUCTION

Females of social Hymenoptera (ants, social bees and wasps) usually develop into either reproductive queens or nonreproductive workers depending on environmental factors or social cues (Schwander, Lo, Beekman, Oldroyd, & Keller, 2010). However, four ant taxa have independently evolved an unusual genetic system called "social hybridogenesis," wherein queen-worker caste determination is primarily shaped by genetic factors (Cahan et al., 2002; Cahan & Vinson, 2003; Julian, Fewell, Gadau, Johnson, & Larrabee, 2002; Leniaud, Darras, Boulay, & Aron, 2012; Norman, Darras, Tranter, Aron, & Hughes, 2016; Romiguier, Fournier, Yek, & Keller, 2017). In these taxa, 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 from mating between partners from the same genetic lineage (in hybridogenetic populations of the ant genera *Messor*, *Pogonomyrmex* and *Solenopsis*; Romiguier et al., 2017) or from

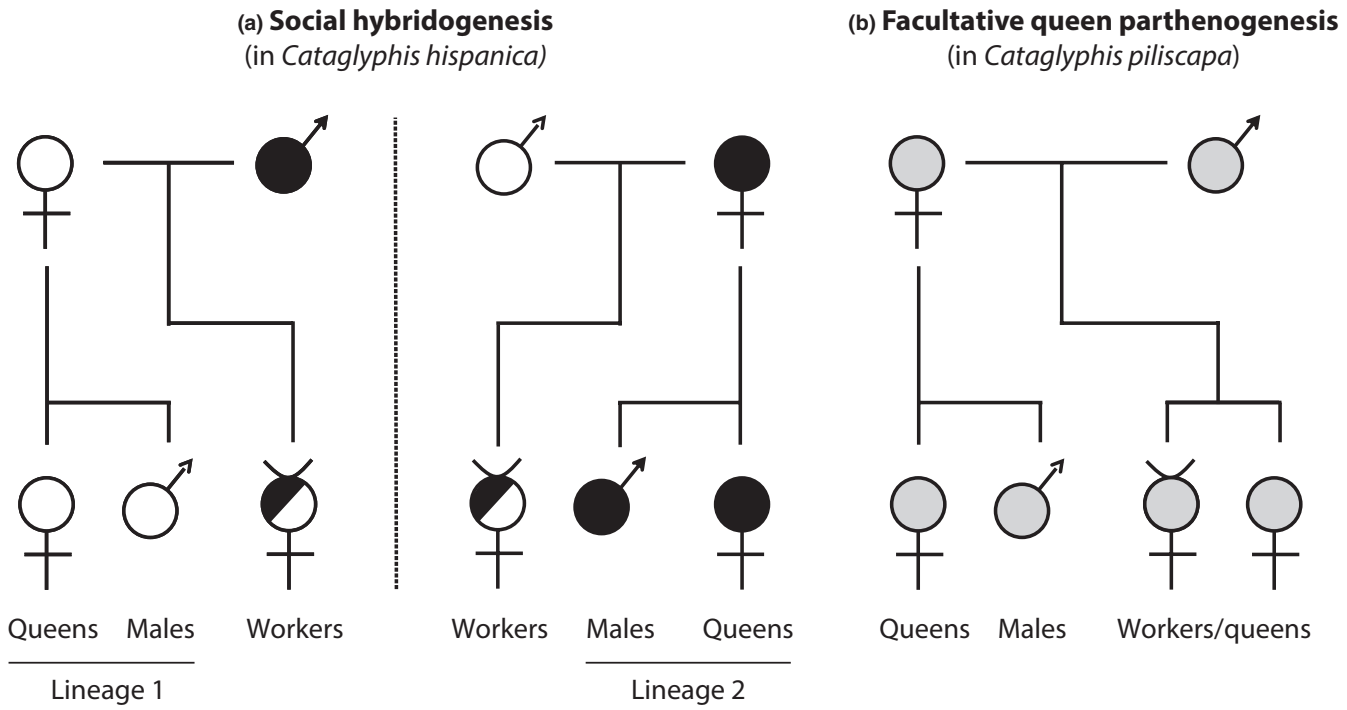


FIGURE 1 Two unusual reproductive systems are found in *Cataglyphis* ants. (a) Under social hybrodgenesis, two lineages coexist in a population. Queens mate with males originating from the alternative lineage to their own and use the sperm to produce sterile F1 workers. By contrast, queens use parthenogenesis for the production of new queens and males. This mode of reproduction has been documented in *Cataglyphis hispanica* and *C. mauritanica*. Our data demonstrate that it also occurs across a large part of *Cataglyphis velox*'s range (*vel1*–*vel2* population). (b) Under facultative queen parthenogenesis, queens may be produced by either thelytokous parthenogenesis or sexual reproduction, males are produced by arrhenotokous parthenogenesis, and workers are produced by sexual reproduction with random matings. This mode of reproduction was originally described in *Cataglyphis piliscapa* (previously known as *C. cursor*). Our results show that *Cataglyphis humeya* and two populations of *Cataglyphis velox* (*velA* and *velB*) have similar reproductive systems

thelytokous parthenogenesis (in *Cataglyphis* ants; Aron, Marduly, & Leniaud, 2016). In all species, males are pure-lineage sons produced by arrhenotokous parthenogenesis, as is typical for hymenopterans.

The form of social hybridogenesis observed in *Cataglyphis* ants is arguably the most bizarre one. In this genus, males are necessary for the production of hybrid workers. However, males never transmit their genes to the next generation since reproductive individuals arise from parthenogenetic eggs only (Leniaud et al., 2012; Figure 1a). This genetic system is expected to be short-lived because selection should act on lineages to stop the production of males (Schwander & Keller, 2012). Against all odds, however, social hybridogenesis is found in at least three sister species of the genus: *Cataglyphis hispanica* (Leniaud et al., 2012), *Catagylphis velox* and *Cataglyphis mauritanica* (Eyer, Leniaud, Darras, & Aron, 2013). The ability to produce females from unfertilized eggs seems an ancestral character of *Cataglyphis* (Aron et al., 2016) and may have facilitated the evolution of social hybridogenesis in these species (Schwander & Keller, 2012). How hybridogenetic lineages of *Catagylphis* emerge and evolve however remains obscure. In particular, whether hybridogenetic lineages are widespread within and across species is still poorly known (Eyer, Leniaud, Tinaut, & Aron, 2016).

The Iberian Peninsula is home to three closely related species: *C. hispanica*, *C. velox*, and *C. humeya* (Figure 2). We have previously shown that two hybridogenetic lineages coexist across a large part of *C. hispanica*'s range (Darras & Aron, 2015; Darras, Leniaud, & Aron, 2014; Leniaud et al., 2012). In *C. velox*, social hybridogenesis has only been documented at a single locality (Eyer et al., 2013). It is unknown whether this reproductive system occurs elsewhere within the species' range (Eyer et al., 2016). For *C. humeya*, no information on the mode of reproduction is currently available. Here, we analysed the distribution of social hybridogenesis in the species *C. velox* and *C. humeya*. To determine whether populations of these species employ social hybridogenesis, we genotyped queens and workers at 18 microsatellite markers and searched for evidence of two of the system's key characteristics: the parthenogenetic production of new queens and mating between divergent lineages for worker production. No strong genetic structure was found using microsatellites. However, a "genotype web" approach combining genetic, geographical and mating assortments information allowed us to detect hybridogenetic populations in which the parents of workers originate from different gene pools. We next used SNP markers to examine the relationship between hybridogenetic and nonhybridogenetic populations.

2 | MATERIALS AND METHODS

2.1 | Focal species and sampling method

Catagylphis velox and *Cataglyphis humeya* are two closely related sister species that are endemic to the southern Iberian Peninsula (Tinaut, 1990a, 1990b). The distribution ranges of the two species

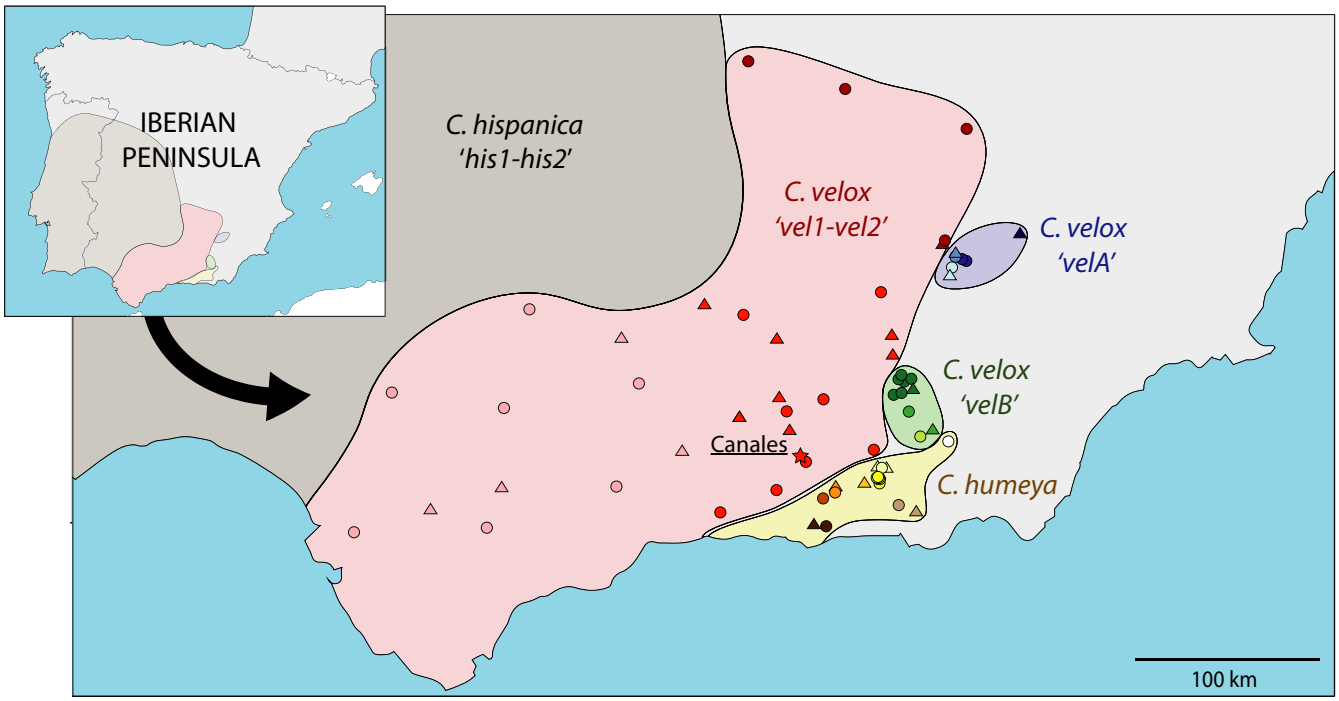


FIGURE 2 Distribution areas of *Cataglyphis hispanica* (grey), *Cataglyphis velox* (red, blue and green) and *Cataglyphis humeya* (yellow) based on our field surveys of species presence conducted between 2012 and 2016 and the research of Tinaut (1990a, 1990b). In *C. hispanica*, two interdependent hybridogenetic lineages (*his1* and *his2*) coexist across the whole distribution range of the species (Darras et al., 2014). In *C. velox*, different reproductive systems occur in three part of the species' range. Two interdependent lineages, *vel1* and *vel2*, are found in most of the species' distribution range (red; this study and Eyer et al., 2013). By contrast, reproductive individuals do not belong to interdependent lineages in the two marginal mountainous populations, *velA* (blue) and *velB* (green) (this study). In *C. humeya* (yellow), no population appear to reproduce by social hybridogenesis (this study). The collection sites of *C. velox* and *C. humeya* surveyed are shown: circles indicate localities where queens and workers were excavated (*N* = 42 localities); triangles indicate localities where only workers were sampled (*N* = 24 localities); the star indicates the population of *C. velox* from Canales previously studied by Eyer et al. (2013). Colours of symbols distinguish different geographical regions, defined to ease interpretation in Figure 3

are separated by the Sierra Nevada mountain and do not overlap. In the easternmost part of its distribution, *C. velox* is found in two marginal mountainous areas (*velA* and *velB* on Figure 2; see also Figure S1). No colonies were found beyond these areas. A total of 91 colonies were sampled from 66 different localities across the full distribution ranges of the two species (Figure 2). We collected whole colonies including queens and workers at 31 localities for *C. velox* (*N* = 48 colonies) and at 11 localities for *C. humeya* (*N* = 16 colonies; Table 1). *C. velox* had been shown to reproduce by social hybridogenesis in one of these localities (Canales; Eyer et al., 2013). We obtained new genetic data for four colonies originating from this population. We also obtained worker genotypes from 18 localities for *C. velox* (*N* = 39 workers from 21 colonies) and six localities for *C. humeya* (*N* = 10 workers from six colonies).

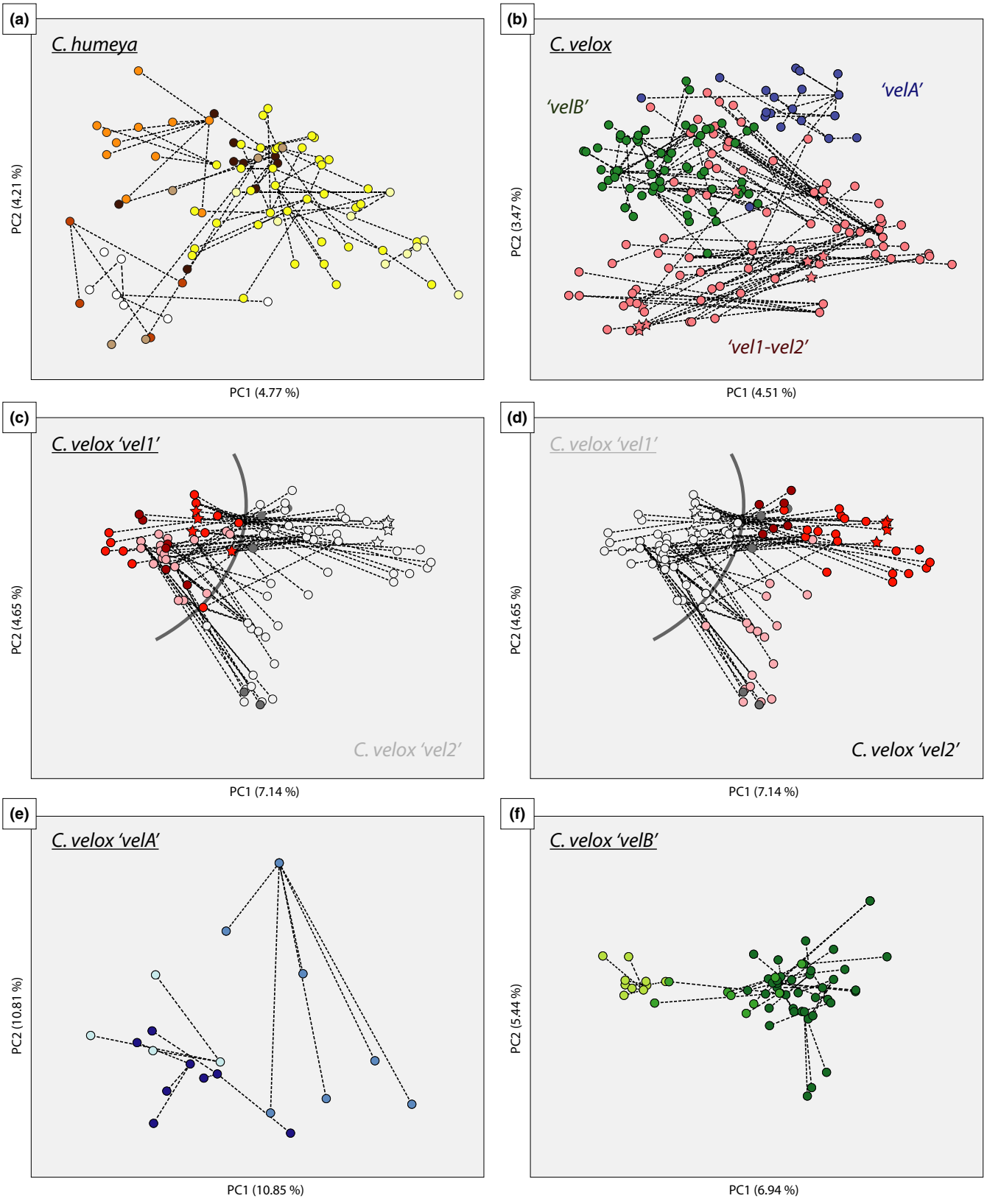
2.2 | Microsatellite genotyping

DNA was extracted from adult ants (Darras et al., 2014). For each colony, we genotyped all reproductive queens, new virgin queens and males (when present), as well as a group of workers (mean ± *SD* = 4.6 ± 2.0 workers per complete colonies; two workers per colonies were taken from the others). Samples were typed at 18 microsatellite loci: *Cc11*, *Cc26*, *Cc54*, *Cc61*, *Cc63a*, *Cc93*, *Cc99*, and *Cc100* (Pearcy, Clemencet, Chameron, Aron, & Doums, 2004); *Ch01*, *Ch05*, *Ch06*, *Ch08*, *Ch11*, *Ch22*, and *Ch23* (Darras et al., 2014); *Ch10* ($[TCG]_n$, F: TCGAATCTAGCGGAATGTCA and R: TGTCCCGTTACACCACGATA), *Ch19* ($[TC]_n$, F: CTGCTTTTCTAATCACGCAAAC and R: GAAGATGC CTTTCTGAGGGC), and *Cn03* ($[CT]_n$, F: CAGCCTGCACCAGATGATT and R: CAAGGGTAGCGAAAGACGAG). The last three markers were designed for this study using available libraries (Darras et al., 2014; Saar, Leniaud, Aron, & Hefetz, 2014). Loci were amplified in four multiplex mixes: MP1 = *Cc54*, *Cc93*, *Ch01*, *Ch06*, *Ch08*, *Ch11*, *Ch23* (Ta = 58°C); MP2 = *Cc11*, *Ch05*, *Ch10*, *Ch19*, *Ch22*, *Cn03* (Ta = 58°C); MP3 = *Cc26*, *Cc100*, *Cc63a* (Ta = 62°C); MP4 = *Cc61*, *Cc99* (Ta = 58°C). All the loci were highly polymorphic, displaying 16 to 47 alleles (mean number of alleles per locus ± *SD*=26.7 ± 9.3). Haploid paternal genotypes were reconstructed from queen-worker allele combinations.

2.3 | Social hybridogenesis

2.3.1 | Queen parthenogenesis

We investigated whether queens used sexual reproduction or parthenogenesis to produce new female reproductives by comparing



the genotypes of mother queens with that of new daughter queens, when the latter were available. We also compared the genotypes of mother queens in colonies headed by multiple queens (polygyny). In *Cataglyphis*, polygyne colonies are headed by related queens remaining in their natal nest after mating (Aron et al., 2016). One can therefore infer their reproductive origin via genotypic analyses. Strictly identical queen genotypes were seen as evidence of thelytokous parthenogenesis (Eyer et al., 2013).

FIGURE 3 Microsatellite analyses of genetic diversity in reproductive individuals by means of principal coordinates analyses (PCoA) enhanced using a "genotype web" approach. Mating assortments are represented with dashed lines connecting parental genotypes found co-occurring in workers. Colours stand for different geographical regions (Figure 2). The percentage of variation explained by each axis is indicated. (a) Genetic variation across the range of *C. humeya* inferred from 84 queens and males (*N* = 11 localities). *C. humeya* does not reproduce by social hybridogenesis. Reproductive individuals from the same geographical area tend to cluster on the PCoA plot. (b) Genetic variation across the range of *C. velox* (red: *vel1-vel2* population, blue: *velA* population, green: *velB* population) inferred from 170 queens and males (*N* = 31 localities). Stars indicate samples of *C. velox* from the Canales population previously studied by Eyer et al. (2013). To ease interpretation of the information uncovered by this plot, we considered several data subsets (c–f). (c and d) Genetic variation across the hybridogenetic *vel1-vel2* population of *C. velox* (*N* = 20 localities). The two plots are based on the same PCoA, but highlights different genetic lineages (*vel1* on plot C and *vel2* on plot d). The bold grey line separates the two lineages on each plot. Queens of each lineage produce workers with sperm from the alternative lineage than their own (dashed lines). Reproductive individuals inferred by STRUCTURE as being hybrids are shown in grey colour. (e) Genetic variation across the panmictic *velA* population of *C. velox* (*N* = 4 localities). (f) Genetic variation across the panmictic *velB* population of *C. velox* (*N* = 7 localities)

2.3.2 | Phylogeographical analyses

To determine whether workers were produced by sexual reproduction between partners from the same lineage (random mating) or from different lineages (disassortative mating), we first identified whether divergent genetic groups were present across the distribution ranges of *C. velox* and *C. humeya*. In hybridogenetic populations, sterile workers display F1 hybrid genetic combinations that are not passed on to subsequent generations. Consequently, using these genotypes in phylogeographical analyses can yield misleading results. Since we started out with no information on the reproductive system present at each locality, we made our initial phylogeographical inferences based on queen and their male mates genotypes alone. Once reproductive systems had been identified, workers were added to the analyses to further refine the results. To avoid frequency biases, a single queen per colony was used when nestmate queens had identical multilocus genotypes. Haploid male genotypes were encoded as diploid by doubling their alleles to analyse queen and male data sets together.

Three widely used methods to study population genetic structure were tested to determine the number of genetic clusters across the distribution ranges of *C. velox* and *C. humeya*: principal coordinates analyses (PCoA), STRUCTURE (Pritchard, Stephens, & Donnelly, 2000) and DAPC (Jombart, Devillard, & Balloux, 2010). None of these performed very well on our data (see Supporting Information). In particular, these analyses did not reveal any clear evidence of large-scale lineage pairing in *C. velox*, although at least one population of *C. velox* was reported to have two hybridogenetic lineages (Eyer et al., 2013). The failure of these analyses to properly detect population subdivision might be explained by the coexistence of both hybridogenetic and nonhybridogenetic populations across the range of *C. velox*. As classical methods failed to delineate lineages in *C. velox*, we took advantage of the additional information contained in worker genotypes to increase our power to detect potential cryptic lineages. Mating patterns inferred from worker genotypes indeed provided valuable clues as to the distribution of hybridogenetic lineages. In a hybridogenetic population, the parents of workers originate from two divergent gene pools. In contrast, nonhybridogenetic workers are produced by sexual reproduction between partners from the same gene pool. To visualize mating patterns, we first constructed PCoA plots based on pairwise genetic distances between queen genotypes and male genotypes (inferred from queen-worker genotype comparisons) with GENALEX v6.5 (Peakall & Smouse, 2012). These plots were then bolstered with connections between parental genotypes that co-occurred in each worker. Individuals originating from different regions were depicted in different colours to facilitate interpretation (Figure 2). This "genotype web" approach allowed us to convey genetic information (PCoA coordinates), geographical information (colours) and information on mating assortments (connections between parental genotypes) using a single figure. This method aims at identifying areas of a PCoA plot that may correspond to divergent populations when no distinct clusters of points can be distinguished. We compared the results obtained using different geographical partitions and picked out the partition that best explained the observed mating assortments. Two specific emergent patterns were looked after: (a) In hybridogenetic populations, reproductives belong to two divergent lineages that are expected to be found in two different areas of the PCoA plot. Because workers are all interlineage hybrids, connections between workers' parental genotypes must be found among areas only. Geographical structure, if any, would be observed within lineages (Darras et al., 2014). (b) In nonhybridogenetic populations, reproductives belong to the same gene pool and should not cluster into different areas of the PCoA plot. Rather, they are expected to cluster according to their geographical origin. Panmixia would be reflected by random connections between reproductives from the same geographical region.

In an hybridogenetic population, workers carry F1 genotypes deviating from Hardy-Weinberg equilibrium (Leniaud et al., 2012). To determine whether workers were more heterozygous than expected under Hardy-Weinberg equilibrium, heterozygote excesses were tested for each locus with GENEPOP version 4.2. (Rousset, 2008). Because multiple genotypes from one colony are not independent, a resampling procedure was performed to test for deviations from Hardy-Weinberg equilibrium (Vargo, 2003). A single worker was selected at random for every colony and the procedure was repeated 10 times to generate 10 data sets. Bonferroni adjustment for multiple comparisons was applied to account for this resampling strategy.

Our "genotype web" analyses suggested that the two hybridogenetic lineages previously described in one locality of *C. velox* were

TABLE 1 Collection localities, species, number of colonies sampled, number of queens per colony (all queens found in a colony were genotyped), number of workers genotyped per colony, number of male patrilines inferred from workers per colony, and number of new sexuals genotyped (Q, new queens; M, new males). Localities where colonies were completely excavated are highlighted in grey. Only workers were sampled in other localities

	ID	Locality	Species (population)	Colonies	Queens	Workers	Patrilines	Sexuals
1	s96	Alcaraz	*velox* (*vel1-vel2*)	1	1	4	4	–
2	a04[d]	Alfacar	*velox* (*vel1-vel2*)	1	–	2	–	–
3	s73	Antequera	*velox* (*vel1-vel2*)	1	1	4	4	–
4	a01[d]	Arjona	*velox* (*vel1-vel2*)	1	–	2	–	–
5	s82	Bogarre	*velox* (*vel1-vel2*)	1	7	4	3	–
6	s10	Cabra	*velox* (*vel1-vel2*)	1	1	4	1	–
7	can[a]	Canales	*velox* (*vel1-vel2*)	4	1, 1, 1, 1[a]	1, 1, 1, 1	1, 1, 1, 1	–, –, –, –
8	caz[b]	Cazorla	*velox* (*vel1-vel2*)	5	1, 1, 1, 1, 1	1, 1, 1, 1, 1	1, 1, 1, 1, 1	–, –, –, –, –
9	s62	Ciudad Real	*velox* (*vel1-vel2*)	1	1	4	2	–
10	s70	Cozvijar	*velox* (*vel1-vel2*)	1	1	4	1	–
11	s98	Cuevas del Campo	*velox* (*vel1-vel2*)	1	–	2	–	–
12	s71	Deifontes	*velox* (*vel1-vel2*)	1	3	4	2	–
13	a09[d]	Espejo	*velox* (*vel1-vel2*)	1	–	2	–	–
14	s61	Jaén	*velox* (*vel1-vel2*)	1	1	4	1	–
15	s57	Jerez de la Frontera	*velox* (*vel1-vel2*)	2	2, 1	4, 5	3, 4	–, –
16	s66	La Calahorra	*velox* (*vel1-vel2*)	1	1	3	1	2Q
17	a03[d]	La Cerradura	*velox* (*vel1-vel2*)	1	–	2	–	–
18	s58	La Lantejuela	*velox* (*vel1-vel2*)	1	3	6	5	–
19	s12[b]	Loja	*velox* (*vel1-vel2*)	1	–	2	–	–
20	s74	Montecorto	*velox* (*vel1-vel2*)	1	2	4	5	–
21	s60	Posadas	*velox* (*vel1-vel2*)	1	3	4	3	–
22	s97	Poyotello	*velox* (*vel1-vel2*)	1	1	3	2	–
23	a14[d]	Poyotello	*velox* (*vel1-vel2*)	1	–	2	–	–
24	s90	Pozo Alcón	*velox* (*vel1-vel2*)	1	–	1	–	–
25	rE[c]	Pruna	*velox* (*vel1-vel2*)	1	–	1	–	–
26	a07[d]	Puerto del Zegri	*velox* (*vel1-vel2*)	1	–	2	–	–
27	a11[d]	Puerto Lope	*velox* (*vel1-vel2*)	1	–	2	–	–
28	s63	San Carlos del Valle	*velox* (*vel1-vel2*)	1	1	5[f]	1	–
29	sev[c]	Sevilla	*velox* (*vel1-vel2*)	1	1	2	2	–
30	s83	Sierra de Tejeda	*velox* (*vel1-vel2*)	1	1	8[e]	–	–
31	s81	Sierra Nevada	*velox* (*vel1-vel2*)	2	3, 1	4, 4	4, 3	–, –
32	rD[c]	Villamartín	*velox* (*vel1-vel2*)	1	–	1	–	–
33	a15[d]	Calar de la Santa	*velox* (*velA*)	1	–	2	–	–
34	s91	Cortijos Nuevos	*velox* (*velA*)	1	–	2	–	–
35	s92	Cortijos Nuevos	*velox* (*velA*)	1	3	4	3	–
36	s64	Santiago de la Espada	*velox* (*velA*)	2	1, 1	6, 3	4, 2	–, –
37	s93	Santiago de la Espada	*velox* (*velA*)	2	–,–	2, 2	–,–	–, –
38	s94	Santiago de la Espada	*velox* (*velA*)	1	1	4	3	–
39	s95	Santiago de la Espada	*velox* (*velA*)	1	1	4	3	–

(Continues)

TABLE 1 (Continued)

	ID	Locality	Species (population)	Colonies	Queens	Workers	Patrilines	Sexuals
40	s88	Baúl	*velox* (*velB*)	2	–,–	2, 2	–,–	–, –
41	s89	Baúl	*velox* (*velB*)	1	1	4	1	–
42	s16[b]	Baza	*velox* (*velB*)	1	–	2	–	–
43	s65	Freila	*velox* (*velB*)	3	1, 1, 1	4, 6, 6	3, 5, 3	–, –, –
44	s84	Freila	*velox* (*velB*)	2	1, 1	4, 4	4, 4	–, –
45	s85	Freila	*velox* (*velB*)	2	1, 1	4, 4	4, 2	–, –
46	s80	Sierra de Baza	*velox* (*velB*)	2	1, 1	4, 3	4, 4	–, –
47	s87	Sierra de Baza	*velox* (*velB*)	2	1, 1	4, 4	3, 4	–, –
48	a17/18[d]	Sierra de Baza	*velox* (*velB*)	2	–, –	2,2	–, –	–, –
49	s86	Zújar	*velox* (*velB*)	2	1, 1	4, 4	2, 4	–, –
50	s75	Capilerilla	*humeya*	1	1	4	3	–
51	s77	Castala	*humeya*	1	1	4	4	–
52	s67[b]	Laroles	*humeya*	2	1, 1	8, 7	4, 4	8M, –
53	s67[c]	Laroles	*humeya*	2	1, 1	6, 7	4, 6	–, –
54	s67[d]	Laroles	*humeya*	1	1	8	4	–
55	s67[e]	Laroles	*humeya*	1	1	6	5	–
56	s67[f]	Laroles	*humeya*	1	1	8	4	4Q + 3M
57	s68	Los Caballeros	*humeya*	2	1, 1	6, 8	3, 7	8M, –
58	a36[d]	Lújar	*humeya*	1	–	1	–	–
59	s78	Puerto De La Ragua	*humeya*	2	2, 1	4, 4	3, 4	–, –
60	a19[d]	Puerto De La Ragua	*humeya*	1	–	2	–	–
61	a20[d]	Puerto De La Ragua	*humeya*	1	–	1	–	–
62	s69	Rubite	*humeya*	1	1	9	8	–
63	a16[d]	Sierra de Gádor	*humeya*	1	–	2	–	–
64	s79	Sierra de Los Filabres	*humeya*	2	1, 1	4, 12[e]	2, 2	–, –
65	s14[b]	Trevélez	*humeya*	1	–	2	–	–
66	s76	Válor	*humeya*	1	–	2	–	–

[a]Locality previously studied by Eyer et al. (2013), only one queen per colony was genotyped in this locality.
[b]Leg PA. Eyer.
[c]Leg R. Boulay.
[d]Leg A. Tinaut.
[e]Workers belong to two matrilines.
[f]Worker count includes three drifters.

widespread across a large part of its distribution range (see Results). To assign reproductive individuals to lineages and to identify individuals of potentially mixed ancestry, we used the Bayesian assignment method implemented in STRUCTURE v. 2.3.4 (Pritchard et al., 2000) with a reduced data set of loci that had distinctly different allele size distributions among lineages in the reference hybridogenetic locality (Canales). Clustering assignment were based on 20 independent runs of STRUCTURE, each with 200,000 MCMC iterations, a burnin period of 100,000 and an admixture model with independent allele frequencies. Similarity coefficients between pairs of runs and the assignment values for each individual resulting from averaging among runs were calculated using the R-script structure-sum-2009 (Ehrich et al., 2007).

Previous studies suggested that hybridogenetic lineages of *Cataglyphis* may recombine from time to time (Darras & Aron, 2015; Eyer et al., 2016). To test for gene flow between hybridogenetic lineages, we estimated the kinship coefficient *Rij* (Streiff et al., 1998) between sympatric reproductive individuals originating from different genetic lineages and compared its value to those obtained after 10,000 random permutations of individuals among localities. A significantly larger value than expected would suggest ongoing gene flow among lineages (i.e., sympatric individuals tends to be more closely related than allopatric individuals). Permutation tests were performed with SPAGEDI v1.5 (Hardy & Vekemans, 2002).

2.4 | High-throughput sequencing

"Genotype webs" supported the recognition of a single genetic group in *C. humeya* and four genetic groups in *C. velox* (see Results).

To confirm inferences based on microsatellites and to characterize phylogenetic relationships between groups, high-throughput sequencing data were obtained for four individuals of *C. humeya* and 14 individuals of *C. velox* (3–4 per group) sampled evenly across the distribution ranges of the species. All but one of the selected samples were reproductive individuals; one sample was a worker from a nonhybridogenetic population. Fifteen individuals (three per genetic group) were used in restriction-site associated DNA sequencing. DNA samples were digested with *EcoRI*, subjected to adapter ligation, and RAD-sequenced on an Illumina HiSeq 4000 (PE150; ~3× genome-wide depth). We also performed low-coverage whole genome sequencing for three other individuals – two *C. velox* and on *C. humeya* – on a HiSeq 2000 (PE100; ~27× genome-wide depth).

One lineage of *C. hispanica* (*Chis*1) was used as an outgroup and reference for analyses. Phylogenetic analyses performed with more outgroups (the two lineages of *C. hispanica* and the two lineages of *C. mauritanica*) yielded similar results as the ones presented in the paper (data not shown). The genome of a *C. hispanica* male was sequenced on a HiSeq 2000 (PE100; ~42× genome-wide depth). The short-read library was assembled into a 205 Mb draft genome using SPADES v3.13 (Bankevich et al., 2012). BUSCO analysis (Simão, Waterhouse, Ioannidis, Kriventseva, & Zdobnov, 2015) revealed that 79% of the single-copy orthologs specific to Hymenoptera were complete in the assembly suggesting that a reasonable coverage of the genome was obtained. Reads from the *C. hispanica*, *C. humeya* and *C. velox* libraries were mapped to the *C. hispanica* assembly using the bwa-mem algorithm (Li & Durbin, 2009) and multi-sample variant calling was performed with FreeBayes (Garrison & Marth, 2012). Variants were filtered using VCFTOOLS (Danecek et al., 2011) to extract high-quality biallelic SNPs (parameters: DP > 4 and QUAL > 20) with no missing data and <50% of heterozygosity. In total, we obtained 166,072 polymorphic SNPs, among which 134,840 were polymorphic in *C. velox* or *C. humeya*.

The glPca function of the R package ADEGENET v1.4-2 (Jombart, 2008; Jombart & Ahmed, 2011) was used to carry out principal component analysis (PCA) of *C. velox* and *C. humeya* samples. The *p*-distances between individuals were obtained using a custom script (Bongaerts, 2017) and pairwise weighted *F*st values among the five genetic groups were estimated using the Weir and Cockerham estimator implemented in VCFTOOLS (Danecek et al., 2011).

Phylogenetic relationships were first inferred using the maximum likelihood method RAXML v8.2.10 (Stamatakis, 2014). We applied Lewis's ascertainment bias correction and performed 100 bootstrapping replicates to evaluate branch supports. Three complementary approaches were used to determine whether reticulation occurred in the studied groups. A neighbour-net network was constructed with SPLITSTREE v5.0.3 (Huson & Bryant, 2006) to identify conflicting signals in the data. SPLITSTREE outputs a network where incompatible pairs of splits are represented by box-like structures. Patterns of population splits and mixtures were then inferred using TREEMIX v1.13 (Pickrell & Pritchard, 2012). TREEMIX models rely on the assumptions that mixture events are discrete and that the history of populations is largely tree-like. Five models with 0 to 4 migration events were considered. The percentage of variance explained by each model was calculated with the RADPIPE package for R (Card, 2015). In addition, admixture tests based on D-statistics were performed with ADMIXTOOLS v5.1 (Patterson et al., 2012). The *D*-statistic test compares the proportions of ABBA and BABA SNP patterns in a set of four populations to determine whether there is an excess of shared derived variants between two populations. The number of standard errors that D is from zero forms a *Z*-score. Given a topology (((P1, P2), P3), P4), a positive *Z*-score indicates that gene flow occurred between P1 and P3 (or P2 and P4), while a negative *Z*-score indicates that gene flow occurred between P1 and P4 (or P2 and P3). Four-population comparisons with an absolute *Z*-score higher than 3 were considered to exhibit significant admixture.

3 | RESULTS

From the 48 whole colonies of *C. velox* sampled, 36 were headed by one queen and 12 by several queens (Figure S1). In contrast, all but one of the 16 colonies of *C. humeya* were headed by a single queen. For both species, the results of genotypic analyses were consistent with workers having been sexually produced (N = 269 genotyped workers). Comparisons of queen and worker genotypes revealed the presence of multiple worker patrilines in 20 of the 25 single-queen *C. velox* colonies for which at least four workers had been genotyped and in all 15 single-queen *C. humeya* colonies (Table 1). These findings indicate that queens of these species typically mate with multiple males. Across all excavated colonies, the queen-worker genotype comparisons allowed us to infer the genotypes of 122 male mates for 47 *C. velox* queens and 67 male mates for 16 *C. humeya* queens.

3.1 | Different reproductive systems in *Cataglyphis velox*

Our data set included, as reference points, the genotypes of four queens and four males of *C. velox* belonging to the two hybridogenetic lineages previously observed in Canales (Eyer et al., 2013). We expected the reproductives of *C. velox* to group into two divergent genetic lineages in both PCoA and STRUCTURE analyses as it was previously documented in *C. hispanica* (Darras et al., 2014) and other hybridogenetic ants (Norman et al., 2016). Contrary to our expectations, no apparent structuring in two lineages was found (see Supporting Information). The two genetic lineages observed in Canales were located in different areas of PCoA plots but did not belong to two distinct clusters (Figure 3b,d).

To check for the presence of potentially cryptic lineages, we used a "genotype web" approach to determine whether workers from different localities were produced via random mating or disassortative mating (as in Canales). Adding connections between parental genotypes co-occurring in workers to the PCoA plot revealed some noticeable patterns. About half of the connections occurred along the first PC axis and took place between reproductive partners originating from two different areas of the plot: one individual was typically

situated on the left side of the plot, while its mate was found on the bottom-right side of the plot (Figure 3b; red points). These results suggested that disassortative mating for worker production was a common phenomenon. The remaining half of the connections were generally shorter and appeared to have random directions. Three quarter of these occupied the top-left area of the plot (Figure 3b; green points), while the rest were found within the top-right area of the plot (Figure 3b; blue points). We assessed the geographical distribution of these three matting patterns and found that they corresponded to three geographically distinct regions. These populations were named *vel1-vel2*, *velA*, and *velB* (Figures 2 and 3c–f). Below, we explain how these populations differ from each other.

3.1.1 | *vel1-vel2*: a large hybridogenetic *C. velox* population

The *vel1-vel2* population was spread across most of *C. velox*'s distribution range (32 localities), including Canales where two hybridogenetic lineages, *vel1* and *vel2*, were previously identified (Figure 2). In all colonies sampled (N = 41), workers were highly heterozygous consistent with interlineage hybrids (mean heterozygosity per locus ± SD = 0.88 ± 0.10; N = 114 workers). Two loci, Ch19 and Ch23, showed a significant excess of heterozygotes in comparison to Hardy–Weinberg expectations in most of the 10 resampled worker data sets (p < 0.05 after Bonferoni correction for 10/10 data tests and 7/10 data sets, respectively). In line with this, mating assortments revealed by the "genotype web" approach showed that workers were the progeny of disassortative matings between reproductive individuals originating from two distinct, yet adjacent areas of the PCoA plot (Figure 3c,d). These results suggested that the two hybridogenetic lineages described in Canales were widespread in this population. Within each lineage, reproductives from the same geographical area tended to cluster together on the PCoA plot.

Reproductive individuals were assigned to each of the two lineages using the program STRUCTURE. This analysis was performed with a reduced data set of eight loci that had different allele size distributions among lineages in the reference Canales population (*Cc26*, *Cn3*, *Ch08*, *Ch11*, *Ch19*, *Ch23*, *Cc93* and *Ch99*). Similarity among runs exceeded 98% suggesting that the clustering assignments were rather robust. Of 89 genotypes, 83 were inferred to have pure ancestry (>85% assignment to a single lineage), three were inferred to have low levels of admixture (19%, 21% and 24%, respectively) and three appeared to be interlineage F1 hybrids (one queen and two males) (Table S1). All pure lineage males but one were found mated with queens of the alternative lineage than their own.

The lineages *vel1* and *vel2* had similar allele size ranges at most of the 18 markers (Figure S2). Nonetheless, allele frequencies differed markedly at a few markers (e.g., *Cn03*, *Cc26*, and *Cc54*), and two markers showed highly lineage-specific patterns (*Ch19* and *Ch23*). At marker *Ch19*, *vel1* reproductive individuals were generally homozygous for allele size 293 bp. The sole exception came from colony s58c1, where *vel1* queens were heterozygous with allele sizes 293 bp and allele 299 bp. In contrast, all *vel2* individuals had allele sizes that were greater than 293 bp. At marker *Ch23*, a bimodal distribution of allele sizes corresponding to the two lineages was observed: *vel2* individuals had allele sizes ranging from 84 bp to 93 bp (91% of the individuals had allele sizes smaller than 89 bp), whereas *vel1* individuals had allele sizes ranging from 89 bp to 117 bp (94.5% of the individuals had allele sizes larger than 93 bp). At 12 of the 32 localities, only workers were sampled and thus inference of parental genotypes was not possible. However, the worker genotypes were all consistent with those expected from crosses between *vel1* and *vel2* reproductive individuals (N = 20 workers). All workers were heterozygous at marker *Ch19* (with allele 293 bp and an allele larger than 293 bp) and at marker *Ch23* (with an allele smaller than 95 bp and an allele larger or equal to 95 bp).

One colony was headed by an F1 hybrid queen (s83c1). Eight workers were genotyped in this colony. Four were the queen's offspring. The other four had F1 hybrid genotypes that were nearly identical to the one of the present queen, suggesting they were produced by a former queen. The F1 hybrid queen may thus have been produced by either sexual reproduction or worker parthenogenesis (Chéron, Monin, Fédérici, & Doums, 2011). The occurrence of rare hybrid queens may facilitate gene flow among lineages. To examine whether introgression had occurred between *vel1* and *vel2*, we estimated pairwise *Rij* values between reproductive individuals from the two lineages (the above-mentioned hybrid queen was not considered for this analysis). The observed mean *Rij* value between sympatric individuals belonging to different lineages was larger than expected (*Rij* = 0.0121, mean expected value after permutation ± SD = −0.0911 ± 0.030, $P_{obs > exp}$ = 0.0008). In other words, *vel1* and *vel2* individuals that were close geographically tended to be more genetically similar. This suggests that recent gene flow had occurred among lineages. Below, we further explore this issue using high-throughput sequencing data.

In the seven multiple-queen colonies sampled in the *vel1-vel2* population, all nestmate queens had the same multilocus genotype (N = 23 queens from seven different localities). In addition, two genetically identical virgin queens were found in colony s66c1. These results were indicative of parthenogenetic queen production as expected under social hybridogenesis.

3.1.2 | *velA* and *velB*: two marginal nonhybridogenetic *Cataglyphis velox* populations

Two marginal populations situated at the eastern edge of *C. velox*'s distribution range were identified using our "genotype web" approach: *velA* (present at seven localities) and *velB* (present at 10 localities) (Figure 2). The *velA* and *velB* populations occurred in two mountainous regions separated by nearly 50 km. No colonies of *C. velox* could be found in this interval suggesting that the populations were not connected (Figure 2). The inferred ranges of *velA* and *velB* stood alongside the one of the *vel1-vel2* population, but these populations appeared to be adapted to different environments. Field

observations indeed showed that habitats change rather abruptly as one moves from typical lowland habitats of the *vel1-vel2* population to the highland habitats of *velA* and *velB*. Our sampling however did not allow us to exclude the possibly that some geographical overlap exists between the *vel1-vel2* and the *velA*/*velB* populations.

The *velA* and *velB* populations were found in two different areas of the PCoA plot (Figure 3b). The segregation of these two populations of *C. velox* was largely supported by STRUCTURE analyses (Figure S2b). In contrast to what was observed in the *vel1-vel2* population, reproductive individuals from the same geographical area tended to group together on PCoA plots, and mating assortments appeared to be random within geographical regions (Figure 3e,f). In accordance with these results, workers were not as heterozygous as those of the hybrid workers of the *vel1-vel2* population (mean heterozygosity per locus ± *SD* = 0.75 ± 0.15, *N* = 29 for *velA* and 0.79 ± 0.12, *N* = 69 for *velB*). Only two markers in *velA* workers and three markers in *velB* workers had mean heterozygosity levels above 90%. This result contrasts with what was observed in the *vel1-vel2* population, where heterozygosity levels in workers exceeded 90% at 9 of 18 markers. No locus showed significant deviations from Hardy-Weinberg equilibrium in any resampled worker data sets ($p > 0.05$ after Bonferoni correction in all 10 data tests of *velA* and *velB*). Furthermore, workers did not display hybrid allele combinations at any marker, including *Ch19* and *Ch23* that were diagnostic of the *vel1* and *vel2* lineages.

In both *velA* and *velB* populations, queens were able to produce new queens via thelytokous parthenogenesis. In the *velA* population, we observed three genetically identical nestmate queens at locality s92 and two genetically identical neighbouring mother queens at locality s64. In the *velB* population, neighbouring queens with extremely similar genotypes were found at two localities. At locality s65, three queens had nearly identical genotypes at all loci; the sole difference resulted from two single-repeat mutations. At locality s80, two queens were identical at 15 markers; genotypes at two markers differed due to one repeat mutation, and one marker was homozygous in one queen but heterozygous in the other. Overall, these results are consistent with the hypothesis that queens belonged to clonal lineages, with small genetic differences at loci that had experienced recent stepwise mutation events or that had recombined and lost heterozygosity (Pearcy, Hardy, & Aron, 2011).

3.2 | *Cataglyphis humeya* does not reproduce by social hybridogenesis

Cataglyphis humeya workers did not appear to be hybrids arising from disassortative mating between hybridogenetic lineages. Workers had only moderate levels of heterozygosity (mean heterozygosity per locus ± *SD* = 0.76 ± 0.12; *N* = 115 workers from 17 localities) and no locus showed significant deviation from Hardy-Weinberg equilibrium in any resampled worker data sets ($p > 0.05$ after Bonferoni correction in all 10 data tests). In line with this, our "genotype web" approach indicates a single, homogeneous genetic group within the species. Reproductive individuals tended to cluster according to their geographical origin on the PCoA plot and the mating assortments inferred from the worker genotypes were fairly random within geographical regions (Figure 3a).

Two pieces of evidence support the conclusion that *C. humeya* queens can produce new queens by parthenogenesis. First, in the only colony headed by two mother queens (s78c1), the two queens had identical genotypes at all 18 loci typed. Second, four virgin queens were found in one colony (s67Fc1); they all had the same multilocus genotype as their mother. Twenty males were found in three colonies; all were haploid and carried the alleles of the mother queen, which is consistent with arrhenotokous parthenogenesis.

3.3 | Phylogenetic relationships among hybridogenetic and nonhybridogenetic populations

"Genotype webs" supported the recognition of three nonhybridogenetic populations (*C. velox velA*, *C. velox velB* and *C. humeya*), as well as of one hybridogenetic population consisting in two lineages (*C. velox vel1* and *C. velox vel2*). This approach was, however, not suitable for the examination of genetic differentiation as population differentiation was low and only a small number of microsatellite markers were genotyped.

Nineteen representative samples including a *C. hispanica* outgroup were sequenced to assess level of genetic differentiation among populations and to characterize phylogenetic relationships among hybridogenetic and nonhybridogenetic populations (Figure 4a). SNP-based analyses confirmed that the groups identified with microsatellite markers were well differentiated. Between-group F_{ST} values ranged from 0.23 to 0.48 (Table S2) and the groups formed five tight clusters on PCA plots (Figure 4b; see also Figure S3). The tree topology estimated with RAxML was also well supported (Figure 4c). All the genetic groups previously identified were reciprocally monophyletic. However, *C. velox* appeared to be paraphyletic, with *C. humeya* nested in the clade. In addition, the hybridogenetic lineages *vel1* and *vel2* were not sister lineages. Instead, *C. humeya* and *vel1* were sister taxa, while *vel2*, *velA* and *velB* formed another phylogenetic group.

Phylogenetic networks were estimated to gain insight into non-tree-like evolutionary processes. The neighbour-net split analysis showed conflicting signals at the core of the network suggesting that the evolutionary history of *C. humeya* and *C. velox* was reticulated (Figure 4d). Therefore, we used TreeMix to reconstruct phylogenetic relationships while taking into account discrete reticulation (migration) events. We acknowledge that the history of the studied populations may violate some of the assumptions of TreeMix (see Methods) and recommend that these results are treated with caution. The tree topology inferred from TreeMix when no migratory events were allowed differed from the one estimated with RAxML regarding the position of *C. humeya*. On this tree accounting for 99.19% of the total variance, *C. humeya* and *C. velox* formed two reciprocally monophyletic groups (Figure S4). The underlying binary tree topologies for

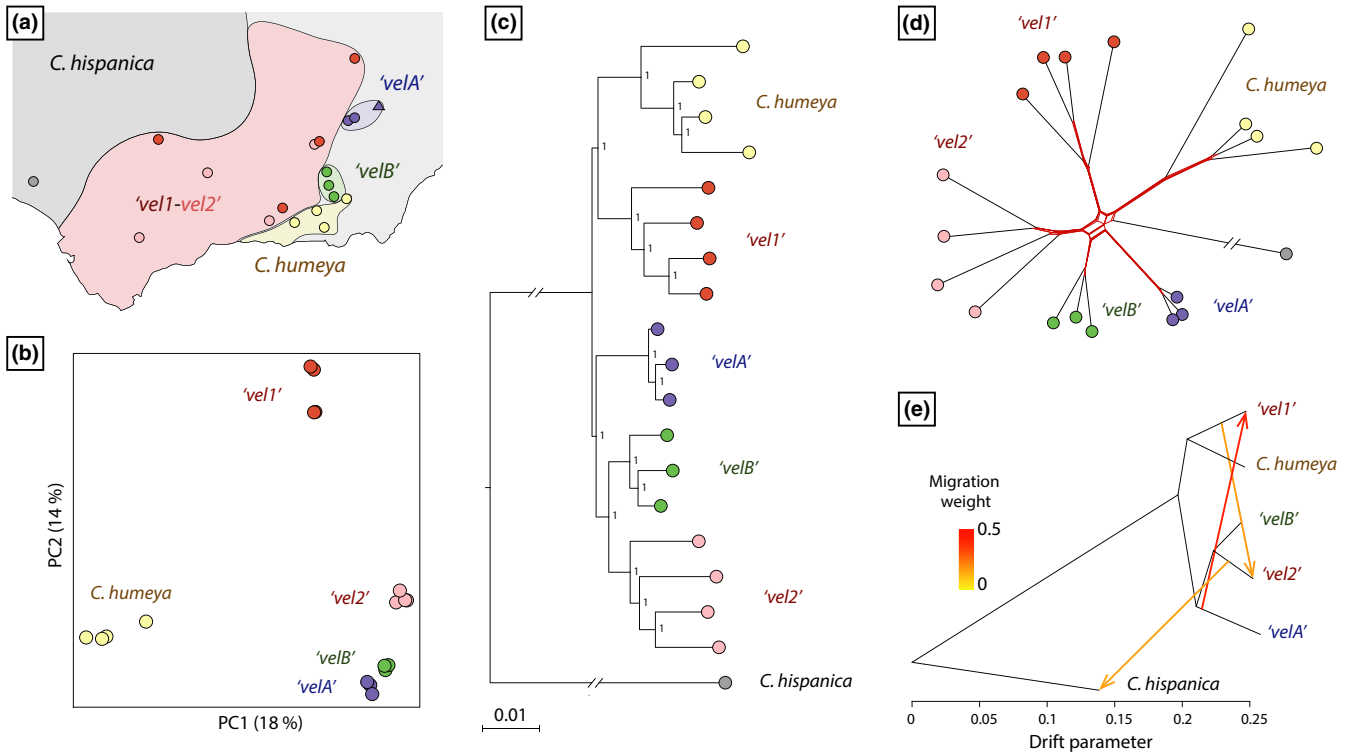


FIGURE 4 Genetic variation across the range of *Cataglyphis velox* and *Cataglyphis humeya* based on SNP markers. (a) Location of the 19 representative samples used for sequencing. Four samples were used for *C. humeya* (yellow). Fourteen samples were used for *C. velox*: three samples from the *velA* population (blue), three from the *velB* population (green) and eight from the *vel1-vel2* population (four for each of the two hybridogenetic lineages; light red and dark red). One sample of *C. hispanica* was used as a reference for analyses. (b) Principal component analysis based on 134,840 SNPs. The two first PC axes are shown and the percentage of variation explained by each axis is indicated (see also Figure S3). (c) Maximum likelihood tree based on 88,801 homozygous SNPs. To root our phylogenetic analyses, we used a queen of *Cataglyphis hispanica* as an outgroup. Bootstrap values are indicated. Branch lengths indicate the estimated number of substitutions per variable site. (d) Neighbour-net network constructed using SPLITSTREE v5.0.3 tree based on 88,801 homozygous SNPs. Box-like structures (in red) represent incompatible pairs of splits. (e) TreeMix graph with three migration events based on 166,072 SNPs. Migration arrows are coloured according to their weight. TreeMix graphs with 0 to 2 migration events and residual fits from the analyses are shown in Figures S4–S5

models allowing migratory events were however similar to the topology estimated with RAxML. Four migration events were added to the tree sequentially. The first three models had migration edges with significant *p*-values ($p < 0.0005$). The first TreeMix model with admixture picked up migration from *vel1* to *vel2* and accounted for 99.65% of the total variance. The second model added gene flow from *velA* to *vel1* and explained 99.91% of the total variance. The third model added migration from *vel2* to *C. hispanica* (Figure 4e). This model explained virtually 100% of the total variance.

Gene flow from *vel1* to *vel2* was further supported by the observation of genetic distances. The *vel1* population was less differentiated from the *vel2* population than from any other population (Weir and Cockerham weighted *Fst*: $Fst_{vel1\text{-}vel2} = 0.317$, $Fst_{vel1\text{-}velA} = 0.464$, $Fst_{vel1\text{-}velB} = 0.383$ and $Fst_{vel1\text{-}hum} = 0.388$; Table S2), although it clustered with *C. humeya* on the maximum likelihood tree. Similarly, pairwise *p*-distances between *vel1* and *vel2* individuals were always lower than between *vel1* and *C. humeya* individuals (Table S3). Pairwise *p*-distances were also consistent with the occurrence of recent gene flow among lineages. Two interlineage pairs of queens from neighbouring localities were sequenced in the *vel1-vel2* population: 81c1 (*vel1*) and 70c2 (*vel2*) were located 21 km apart, while CAZ10 (*vel1*) and CAZ11 (*vel2*) were sympatric. Each queen of these pairs was more similar to the other one of its pair than to the geographically distant queens of the alternative lineage (Table S4). To test for signatures of admixture in four-population trees, we calculated *D*-statistics. A test was considered significant if the resulting *Z*-score (the number of standard errors that *D* is from zero) had an absolute value higher than three. Most comparisons had significant Z-scores suggesting that the histories of the studied species were complex. Particularly high *Z*-scores were obtained for four-population comparisons with P1 = *vel1*, P2 = *C. humeya*, P3 = {*vel2*, *velA* or *velB*} and P4 = *C. hispanica* suggesting gene flow between *vel1* and the other *C. velox* populations (Table S5). The highest *Z*-score was obtained using P3 = *vel2* consistent with gene flow between the *vel1* and *vel2* branches.

Altogether, these analyses revealed that *C. velox* and *C. humeya* display a complex reticulate evolutionary history with recent gene flow between the hybridogenetic lineages *vel1* and *vel2*, and probable historical admixture events between populations that are now geographically isolated from each other.

4 | DISCUSSION

Two unusual modes of reproduction have been documented in *Cataglyphis* ants: conditional use of sex and social hybridogenesis. Under conditional use of sex, queens facultatively use parthenogenesis and sexual reproduction to produce new queens, but always employ sexual reproduction for the production of workers (Figure 1b). This mode of reproduction is found in several populations of *C. piliscapa*, a species of the *Cataglyphis cursor* species group (Doums, Cronin, et al., 2013; Pearcy, Aron, Doums, & Keller, 2004). Under social hybridogenesis, queens use parthenogenesis to produce new queens and mate with males originating from another lineage than their own to produce worker offspring (Figure 1a). So far, social hybridogenesis has been reported in three species of the *Cataglyphis altiquamis* group: in all 14 surveyed populations of *C. hispanica* (Darras et al., 2014; Leniaud et al., 2012), in one population of *C. velox* and in one population of *C. mauritanica* (Eyer et al., 2013). In a recent study, (Eyer et al., 2016) found no clear evidence for widespread social hybridogenesis in *C. velox*. These results were based on sequences of slowly evolving genes (four nuclear genes) obtained from workers. Hybridogenetic lineages are, however, most easily identified from reproductive individuals using either a large number of markers (e.g., SNPs; Romiguier et al., 2017) or rapidly evolving markers (e.g., microsatellite sequences; Darras et al., 2014). Here, to investigate the presence of cryptic hybridogenetic lineages in *C. velox* and its sister species *C. humeya*, we obtained queen and their male partner genotypes at 18 polymorphic microsatellite markers. Genetic structure across these microsatellites appeared rather weak. This result may stem from recent divergence and gene flow homogenising populations. To detect the presence of cryptic patterns, we used a "genotype web" approach combining information on microsatellite genotypes, geographical distributions, and mating assortments in diagnostic plots. We show that the two unusual reproductive strategies previously described in *Cataglyphis* co-occur across the distribution range of *C. velox*, while all *C. humeya* populations studied appear to use conditional use of sex.

In *C. velox*, reproduction proceeds via social hybridogenesis in the large *vel1-vel2* population. In this population, reproductives from two sympatric lineages (*vel1* and *vel2*) coexist and interbreed at each locality. Consequently, workers are all interlineage hybrids resulting from disassortative sexual reproduction, whereas queens and males are pure lineage individuals produced by parthenogenesis. All nestmate queens of *C. velox* had the same multilocus genotype, indicating that they result exclusively from clonal reproduction. Similarly, not a single sexually produced queen was identified in hybridogenetic populations of *C. hispanica* (Darras et al., 2014; Leniaud et al., 2012). In contrast, in the two marginal *C. velox* populations *velA* and *velB* as well as in all *C. humeya* populations studied, workers were produced via random mating. Evidence of asexual queen production was detected in these populations, indicating that queens use thelytokous parthenogenesis for the production of daughter queens, as shown in *C. piliscapa*. Although we found no direct evidence for facultative queen production by sexual reproduction, we could not completely exclude that sexual reproduction occurred in some colonies of these populations. In *C. piliscapa*, 4% to 50% of the queens were found to be produced by sexual reproduction (Cronin, Fédérici, Doums, & Monnin, 2012; Doums, Cronin, et al., 2013; Pearcy, Aron, et al., 2004). If sexually-produced queens occur at similar frequencies in the *C. velox* and *C. humeya* populations as in *C. piliscapa*, it is likely that they have gone unnoticed in our study due to small sample sizes.

The *vel1-vel2* population was distributed across 48,000 km^2, an area that covers most of the range of *C. velox*. Likewise, large distribution ranges were observed for hybridogenetic populations of *Pogonomyrmex barbatus* × *rugosus* (Anderson et al., 2006; Mott, Gadau, & Anderson, 2015; Schwander, Cahan, & Keller, 2007), *C. hispanica* (Darras et al., 2014) and *Messor barbarus* (Norman et al., 2016; Romiguier et al., 2017). Interestingly, while *vel1-vel2* occupies a large arid lowland area typical of *Cataglyphis* ants, the nonhybridogenetic populations *velA*, *velB*, and *C. humeya* are all found in small mountainous regions situated at the margins of the *vel1-vel2* range. A tentative explanation could be that the *vel1-vel2* population outcompete nonhybridogenetic populations in favourable, low-elevation regions due to, for example, worker heterosis (Cahan, Julian, Schwander, & Keller, 2006). In suboptimal mountainous habitats, panmixia may be selected for due to the difficulty of finding a mate originating from a different gene pool when colony density is low and habitat is fragmented.

High-throughput sequencing data confirmed that the *vel1*, *vel2*, *velA*, *velB* and *C. humeya* genetic groups identified with our "genotype web" approach were biologically meaningful. The groups were all monophyletic and well supported. Our phylogenetic analysis however recovered *C. velox* as paraphyletic with respect to *C. humeya*. The paraphyly was caused by the *vel1-vel2* population, in which interbreeding occurs between *vel1* individuals related to *C. humeya* and *vel2* individuals related to *C. velox*. As this hybridogenetic population defies common species concepts (Dubois, 2011), we choose not to synonymize *C. humeya* with *C. velox* at this point.

Our data provided evidence for local gene flow between the *vel1* and *vel2* lineages. Gene flow among hybridogenetic lineages is likely to be of evolutionary significance for the maintenance of social hybridogenesis in *Cataglyphis* since it may counteract the accumulation of deleterious mutations in each lineage and prevent these from becoming too divergent from each other which could lead to genetic incompatibilities in the hybrid workers (Darras & Aron, 2015; Romiguier et al., 2017). The occurrence of gene flow between the *vel1* and *vel2* lineages is further backed up by the analysis of mitochondrial DNA. In *C. velox*, mitochondrial variation closely reflected geographical origin rather than maternal lineage (Eyer et al., 2016). The two lineages identified here were not recovered as monophyletic by this previous study. A similar cytonuclear incongruence was described in the hybridogenetic species *C. hispanica*, where it was interpreted as an evidence for recurrent mitochondrial introgression among lineages (Darras & Aron, 2015). While genetic exchanges may be facilitated by the great closeness of the hybridogenetic lineages, it is yet unclear how these occur. One potential path could be the occasional production of hybrid queens by sexual reproduction. We

found one *vel1/vel2* hybrid queen in our sampling. This queen was able to produce workers, but whether it could produce new sexuals is unknown. In hybridogenetic *Pogonomyrmex*, interlineage hybrid queens display impaired reproductive success (Schwander, Keller, & Cahan, 2007). Darras and Aron (2015) argued that gene flow among lineages could also occur if queens were produced by gynogenesis, an incomplete form of parthenogenesis involving sperm. Gynogenesis has also been suggested in the ant *Myrmecia impaternata* (Taylor, 2015). Interestingly, two microsatellites out of 18 had nonoverlapping allele size distributions in the *vel1* and *vel2* lineages suggesting that they were situated in genomic regions resistant to gene flow. Such regions may be associated with genes involved in interlineage mate recognition or genes for which allelic interdependency among lineages is required to produce viable F1 worker offspring.

Admixture analyses indicate that *C. velox* and *C. humeya* have reticulate evolutionary histories with probable historical introgression events between populations that are now geographically isolated (i.e., *velA* and *vel1*, and *vel2* and *C. hispanica*). Our results suggest that the *vel1* and *vel2* lineages have evolved from two divergent nonhybridogenetic populations: *vel1* appears to have originated from a population related to *C. humeya*, while *vel2* may have originated from a population related to *velA*. We hypothesize that these two putative ancestral populations may have diverged in allopatry and became interdependent upon secondary contact. Hereafter, we explain why this may have happened. Our data indicate that new queens are typically produced by parthenogenesis in all populations of *C. velox* and *C. humeya*. It is likely that queens were also produced by parthenogenesis in the ancestral populations that gave rise to *vel1* and *vel2*. In *Cataglyphis*, queen parthenogenesis proceeds by automixis with central fusion (Pearcy, Hardy, & Aron, 2006). This mode of reproduction increases inbreeding over time in recombination-prone genomic regions (Doums, Cronin, et al., 2013; Pearcy et al., 2011) and, simultaneously, may result in the accumulation of recessive deleterious mutations in nonrecombining genomic regions. Both phenomena are expected to have deleterious effects on the fitness of offspring (Charlesworth & Willis, 2009). An additional cost of inbreeding in Hymenoptera stems from the production of sterile diploid males that are homozygous at the complementary sex determination locus/loci (Doums, Ruel, et al., 2013; Heimpel & de Boer, 2008; Pearcy, Timmermans, Allard, & Aron, 2008). Production of sterile diploid males in *Cataglyphis* with parthenogenetic queens may therefore stem from two different sources: matched mating resulting in fertilized eggs developing into diploid males rather than workers, and thelytokous parthenogenesis with loss of heterozygosity resulting in production of diploid males rather than queens. In species with parthenogenetic queen production, outbreeding through disassortative mating may alleviate inbreeding depression in the sexually produced workers and may preclude the production of homozygous diploid males. Having a hybrid worker force may also be beneficial if hybrids perform better than purebred workers due to heterosis (Burke & Arnold, 2001; Umphrey, 2006). Remarkably, reproductive individuals would not be affected by the potential negative consequences of hybridization on reproductive potential as both queens and males develop from pure-lineage unfertilized eggs in these populations (Seifert, 1999). It is therefore possible that interlineage mating has been selected in a historical secondary contact zone between two nonhybridogenetic populations and gave rise to the *vel1-vel2* lineage pair. The discovery that two parental nonhybridogenetic populations may be at the origin of social hybridogenesis in *C. velox* is reminiscent of previous findings in other hybridogenetic ants. In *Pogonomyrmex*, two hybridogenetic lineage pairs related to the nonhybridogenetic species *Pogonomyrmex barbatus* and *Pogonomyrmex rugosus* have been identified (Anderson et al., 2006; Helms Cahan, Cahan, & Keller, 2003; Schwander, Cahan, et al., 2007). Within each pair, one lineage seems to derive from *P. barbatus*, while the other lineage appears to derive from *P. rugosus* (Sirviö, Pamilo, Johnson, Page, & Gadau, 2011). In *Solenopsis*, a hybridogenetic population consisting of individuals related to the nonhybridogenetic species *Solenopsis geminata* and *Solenopsis xyloni* has also been described (Helms Cahan & Vinson, 2003). Several widely divergent opinions have been discussed regarding the exact role of hybridization in the origin of social hybridogenesis in these taxa (Anderson et al., 2006; Cahan et al., 2002; Helms Cahan et al., 2003; Linksvayer, Busch, & Smith, 2013; Linksvayer, Wade, & Gordon, 2006; Umphrey, 2006).

Current data do not, however, provide definitive support for the hypothesis that the last common ancestor of *C. velox* and *C. humeya* was characterized by a panmictic breeding system and that the current hybridogenetic lineages vel1 and vel2 are of recent origin. An alternative scenario would be that the origin of hybridogenetic lineages preceded the splitting of the *velA*, *velB* and *C. humeya* lineages. This would conform to our early thesis that hybridogenetic lineage pairs of *C. hispanica*, *C. velox* and *C. mauritanica* all derived from a common ancestral pair of hybridogenetic lineages (Darras et al., 2014). The phylogenetic pattern found in the present study would therefore be the result of hybridogenetic lineages reverting back to panmixia; *C. humeya* would be a panmictic population derived from the *vel1* lineage, whereas the *velA* and *velB* populations would have derived from the *vel2* lineage. This second scenario appears however less parsimonious than the previous one, as it would require multiple reversions from a system where queen and worker traits were under selection in different genotypes (pure-lineage and hybrid, respectively) to a system where all castes now belong to the same lineage.

Our study introduces a method called “genotype web” for visualizing mating assortment patterns on multivariate representation methods such as PCA or PCoA. This approach is inspired by the “haploweb” method, which consists in drawing connections between haplotype sequences found co-occurring in heterozygous individuals to detect "fields for recombination" on a graph (Flot, Couloux, & Tillier, 2010). The “haploweb” method has proven to be useful to delineate species in unusual organisms such as rotifers (Debortoli et al., 2016) or corals (Flot et al., 2011; Terraneo, Benzoni, Arrigoni, & Berumen, 2016). We believed that the “genotype-web” method could present a similar interest for the analysis of genotype data in other unusual organisms.

Our analyses yield new insight into the phylogeographical distribution of social hybridogenesis in *Cataglyphis* ants, but did not provide a definitive answer to the debated question of how social

hybridogenesis evolved in ants (Gordon & Friedman, 2017). The lack of information on the breeding systems used by common ancestors and the genetic mechanisms underlying social hybridogenesis prevented us from reaching unambiguous conclusions. Yet, the occurrence of gene flow among lineages and the evidence that both hybridogenetic and nonhybridogenetic populations co-occur in the species *C. velox* seems to indicate that social hybridogenesis in *Cataglyphis* is not as strict in the long-term as previously hypothesized by Darras et al. (2014). Difficulties in inferring the evolutionary pathways leading to social hybridogenesis were also encountered by researchers working on *Pogonomyrmex barbatus* × *rugosus* and *Messor* ssp. (Romiguier et al., 2017; Sirviö et al., 2011). Although phylogeographical and phylogenetic studies are still struggling to clarify the evolution of hybridogenetic systems in ants, we expect these data to be much useful once the genetic mechanisms underlying these unusual systems start to be identified.

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AUTHOR CONTRIBUTIONS

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

DATA ACCESSIBILITY

The microsatellite data set supporting this article are provided as part of the electronic supplementary material (genotypes.txt). Sequence reads were deposited in the NCBI Short Read Archive (SRA accession numbers SRR8539897-SRR8539900 and SRR8544297-SRR8544311). The draft assembly of *Cataglyphis hispanica* and the variant call file have been made available on figshare (https://doi.org/10.6084/m9.figshare.7694480.v1 and https://doi.org/10.6084/m9.figshare.7689161.v1).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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