



Research

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Population genetics

Introgression of mitochondrial DNA
among lineages in a hybridogenetic ant

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We report a remarkable pattern of incongruence between nuclear and mitochondrial variations in a social insect, the desert ant *Cataglyphis hispanica*. This species reproduces by social hybridogenesis. In all populations, two distinct genetic lineages coexist; non-reproductive workers develop from hybrid crosses between the lineages, whereas reproductive offspring (males and new queens) are typically produced asexually by parthenogenesis. Genetic analyses based on nuclear markers revealed that the two lineages remain highly differentiated despite constant hybridization for worker production. Here, we show that, in contrast with nuclear DNA, mitochondrial DNA (mtDNA) does not recover the two lineages as monophyletic. Rather, mitochondrial haplotypes cluster according to their geographical origin. We argue that this cytonuclear incongruence stems from introgression of mtDNA among lineages, and review the mechanisms likely to explain this pattern under social hybridogenesis.

1. Introduction

The desert ant *Cataglyphis hispanica* has evolved an unusual reproductive system. Two divergent genetic lineages co-occur as a complementary pair in all populations across the whole distribution range of the species [1,2]. Queens typically mate with a male originating from the alternative lineage and use its sperm to produce F₁ hybrid workers (figure 1). By contrast, new reproductive individuals (males and new queens) are produced asexually by parthenogenesis. As a consequence, the two lineages are maintained genetically distinct over generations. This reproductive system results from genetic determination of the caste fate: hybrid eggs are targeted to worker development, whereas pure-lineage, parthenogenetic eggs develop into new reproductive individuals [3]. Because reproductive offspring are produced by parthenogenesis in each of the two lineages, both nuclear and mitochondrial DNA (mtDNA) are expected to separate the two lineages. Contrary to this prediction, we show that mtDNA does not cluster according to lineages.

2. Material and methods

(a) Samples and sequencing

The ant *C. hispanica* inhabits the arid southwest of the Iberian Peninsula. To determine how mtDNA is partitioned within and between the two lineages, we sequenced a fragment of mitochondrial *cox1* gene from 23 reproductive individuals collected in 14 localities across the entire range of the species (figure 2a). These samples are a subset of those from a previous study by Darras *et al.* [1], which explored the phylogeographic structure of the species and showed that the two lineages carry highly divergent genotypes at 12 microsatellite markers. We selected nine individuals from the first genetic lineage *His1* and 14 from the alternative lineage *His2* (figure 2b). We designed a new primer pair for *cox1* by searching for conservative regions in the published sequences of *Cataglyphis* species (ChR58: CCTGGATTGGATTAATTCTC

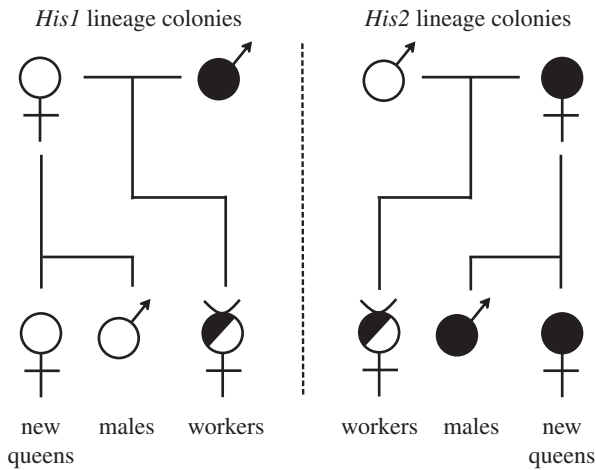


Figure 1. Reproductive system of *C. hispanica*. Two genetic lineages, *His1* and *His2*, coexist in each population. Queens mate with males originating from the alternative lineage to their own and use the sperm to produce sterile F_1 workers. By contrast, queens use parthenogenesis for the production of new queens and males. Black and white colours represent nuclear genomes from the two lineages. Adapted from Darras *et al.* [1].

and ChF746: TTTATRATTATTCGTTTRGARG). The target fragment was amplified following the TopTaq DNA polymerase (QIAGEN) manufacturer's instructions with an annealing temperature of 50°C. *Cataglyphis velox* was used as outgroup (accession: JN630796, [4]).

(b) Statistical analysis

Sequences were checked for quality using Codon Code ALIGNER v. 4.1 (Codon Code Corporation), aligned using MUSCLE [5] and trimmed to 520 bp. We selected the GTR + G + I model based on the corrected Akaike information criterion, as implemented in jMODELTEST v. 2.1.3 [6]. We performed maximum-likelihood and neighbour-joining analyses using MEGA5 [7], and Bayesian analyses in MrBAYES v. 3.2 [8]. Branch support values for neighbour-joining and maximum likelihood trees were obtained by 1000 bootstrap pseudo-replicates. For Bayesian phylogenetic inference, four Markov chains Monte Carlo runs were performed for 1 000 000 generations. Trees were sampled every 100 generations, and the initial 25% of trees were discarded as burn-in.

Two statistical analyses were performed to determine the pattern of genetic variation at mtDNA. First, we examined mtDNA differentiation among lineages. The toolbox SPADS [9] was used to estimate global Φ_{ST} [10] between haplotypes of the two lineages. Second, to examine the phylogeographic pattern of mtDNA at the individual level, the effect of linear geographical distance on genetic similarity between individuals N_{ij} (an analogue of kinship coefficient considering the phylogenetic distance between haplotypes) was tested using a Mantel test with 1000 permutations performed in SPAGeDi [11].

3. Results

The analysis of a 520 bp portion of mitochondrial gene *cox1* revealed 116 variable sites and 19 different haplotypes among 23 sequences. The same tree topology was obtained under maximum-likelihood, neighbour-joining and Bayesian analyses (see the electronic supplementary material, S1). In contrast with our expectation, mtDNA did not separate the two lineages (figure 2b, black and white discs): the mitochondrial haplotypes did not cluster according to nuclear lineages and Φ_{ST} index for differentiation between the two lineages was

not significantly different from zero ($p = 0.989$). Instead, the genetic similarity between haplotypes was significantly correlated with their geographical distance (slope = -0.32 ; Mantel test, $R^2 = 0.49$; $p < 0.001$). In four populations, the two lineages even shared the same haplotype (populations 25, 37, 38/39 and 47).

These results are unlikely to be explained by NUMTs contaminations (non-functional copies of mtDNA inside the nuclear genome) as NUMTs are expected to evolve in a nuclear-specific manner and, thus, be lineage-specific [12].

4. Discussion

Our results reveal strong incongruences between mitochondrial and nuclear variations in the desert ant *C. hispanica*. Whereas the species consists of two highly divergent nuclear lineages that co-occur as a complementary pair in all populations [1], there is no clustering of mtDNA variants into the two lineages. Rather, mitochondrial haplotypes of reproductive individuals of both sexes cluster according to their geographical origin, independently of their nuclear lineage (figure 2b).

Discordances between genetic markers are generally explained by differential dispersal of males and females, incomplete lineage sorting or introgression [13]. Differential dispersal of sexes may lead to discrepancies between maternally (mtDNA) and biparentally (nuclear DNA) inherited markers: when males are the dispersing sex, biparentally inherited markers are expected to show weaker population subdivision than maternally inherited markers. In the incomplete lineage sorting, lineage divergence is too recent to achieve complete reciprocal monophyly, and some gene trees reflect unsorted ancestral variation [14]. Neither of these two explanations may account for the pattern of mtDNA found in this study, which is characterized by both a strong geographical structure and unsorted variations among lineages. In the absence of gene flow, mitochondrial genomes from the two lineages are expected to accumulate significant genetic differences, especially since lineages have diverged from each other a long time ago [1].

Rather, the sharing of mitochondrial haplotypes between sympatric, but genetically divergent lineages is the signature of mitochondrial introgression, i.e. the movement of mtDNA from one lineage into the gene pool of another through hybridization [15]. Introgressive hybridizations between different species have been shown to generate similar biogeographic patterns in the army ant genus *Dorylus* [16] as well as several other organisms [13]. In *C. hispanica*, introgression among lineages is surprising, because reproductive individuals develop from pure-lineage eggs. Therefore, the consequences of hybridization are expected to be restricted to non-reproductive F_1 workers. In fact, three alternative mechanisms may explain introgression in *C. hispanica*. (i) Although female caste determination has been shown to be under genetic control [3], one may not completely exclude the possibility that hybrid (worker-destined) eggs sometimes develop into new reproductive queens. Because mitochondria are usually inherited from the mother, recurrent backcrossing of hybrid queens with males from their paternal lineage will ultimately lead to offspring with introgressed mitochondria (mtDNA from the maternal lineage and nuclear DNA from the paternal lineage). No hybrid queens have been

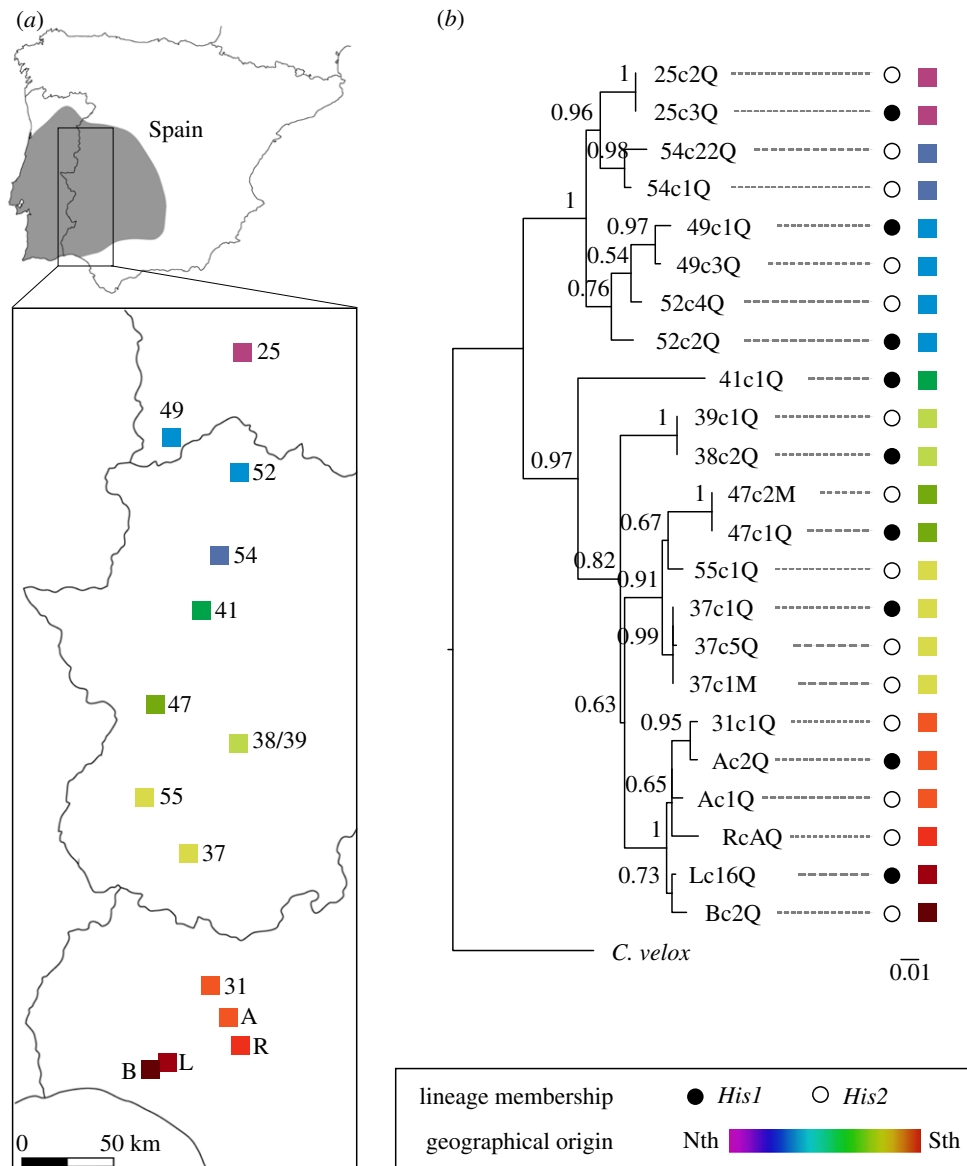


Figure 2. Mitochondrial DNA variation among reproductive individuals of *Cataglyphis hispanica*. (a) Sampling locations. Fourteen populations were sampled along a 400 km transect across the species distribution range (grey colour). (b) Maximum-likelihood tree inferred from a portion of *cox1* gene. Numbers at nodes indicate bootstrap values for 1000 replicates. Mitochondrial haplotypes cluster according to their geographical origin (coloured squares) independently of their nuclear lineage membership (black and white discs). Specimen name gives population, colony number and sex (Q, queen; M, male).

reported so far in our study species [1–3]. However, previous studies on *Pogonomyrmex* harvester ants where caste is also genetically determined have shown that hybrid queens occur at low frequency and require large sampling effort to be detected [17]. (ii) In orphaned colonies, workers of *C. hispanica* have the ability to lay arrhenotokous eggs, which develop into hybrid males (H. Darras and S. Aron 2014, unpublished data). Such males could father new hybrid queens, a situation that may lead ultimately to offspring with introgressed mitochondria. Both hybrid queens and hybrid males occur in *Pogonomyrmex* ants [18]. Nevertheless, interlineage hybrid queens of *Pogonomyrmex* are known to have reduced fitness, if any [17]. Accordingly, analyses of both nuclear and mtDNA have failed to provide any evidence of gene flow among lineages [19]. (iii) Alternatively, the introgression of mtDNA among lineages may stem from gynogenesis, which is an incomplete form of parthenogenesis whereby a sperm cell comes into contact with the egg, but does not transmit

its nucleus to the zygote [20]. This results in the production of a parthenogenetic progeny containing nuclear material derived primarily from the mother. However, as the egg is physically poked by a sperm cell, occasional transmission of sperm DNA into the egg can happen. In *C. hispanica*, gynogenesis can be an accidental phenomenon resulting in interlineage mitochondrial introgression over time. Although animals' mitochondria are generally transmitted from the mother to the offspring without any contribution from the father, there is increasing evidence that sperm mitochondria may sometimes leak into the zygote [21]. Unfortunately, distinguishing between the occurrence of hybrid sexuals or gynogenesis is challenging. Colonies of *C. hispanica* produce only a few sexuals, and a low frequency of hybrid reproductives could have remained undetected in our sampling. On the other hand, parthenogenetic eggs are produced during a very short period of the year, making their sampling for cytological studies convoluted.

While previous genetic analyses based on microsatellite markers indicated that no current gene flow occurs between the two lineages of *C. hispanica* [1,2], this study reveals mitochondrial introgression among lineages. This shows that the two lineages are not completely isolated as previously believed, and that part of their genomes may recombine from time to time. Rare recombination events could play a key role in the stability and evolution of social hybridogenesis in *Cataglyphis*. First, occasional recombination would prevent lineages from becoming too divergent from each other, so that a viable hybrid genome is maintained for the production of the worker caste. In particular, mitochondrial introgression would enable the coordinate evolution of nuclear and mitochondrial genomes to maintain optimal functions in hybrids as cytonuclear incompatibilities can result in decreased hybrid fitness [22]. Second, recombination is the most likely

mechanism to explain the evolution of new lineage pairs and the occurrence of social hybridogenesis in the two sister species of *C. hispanica*, *C. velox* and *C. mauritanica* [23].

Data accessibility. DNA sequences: GenBank accessions: KP420153–KP420175.

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Authors' contributions. H.D. and S.A. designed the study. H.D. carried out the molecular laboratory work and the statistical analyses. H.D. and S.A. wrote the paper.

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Competing interests. We have no competing interests.

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