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: CCTGGATTTGGATTAATTTCTC & 2015 The Author(s) Published by the Royal Society. All rights reserved.
analogue of kinship coefficient considering the phylogenetic

drial haplotypes did not cluster according to nuclear lineages

trimmed to 520 bp. We selected the GTR

neighbour-joining and maximum likelihood trees were obtained

analyses in MRBAYES v. 3.2 [8]. Branch support values for

and neighbour-joining analyses using MEGA5 [7], and Bayesian

logistic on genetic similarity between individuals

of mtDNA at the individual level, the effect of linear geographi-

neighbour-joining and maximum likelihood trees were obtained

by 1000 bootstrap pseudo-replicates. For Bayesian phylogenetic

ference, four Markov chains Monte Carlo runs were performed

4. Discussion

Our results reveal strong incongruences between mitochon-

drial and nuclear variations in the desert ant C. hispanica.

Contrasts with our expectation, mtDNA did not separate the

lineage colonies

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 ster-

eral F1 workers. By contrast, queens use parthenogenesis for the production of new

and ChF746: TTTATRATTACGTATTTRARG). The target frag-

ment was amplified following the TopTaq DNA polymerase (QIAGEN) manufacturer’s instructions with an annealing temp-

ature 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.

The analysis of a 520 bp portion of mitochondrial gene cox1 revealed 116 variable sites and 19 different haplotypes

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

not significantly different from zero (p = 0.989). Instead, the genetic similarity between haplotypes was significantly cor-

related 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].

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 mitochondri-
drial haplotypes did not cluster according to nuclear lineages and \( \Phi_{ST} \) index for differentiation between the two lineages was
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 Cataglyphis 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 Cataglyphis 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 Cataglyphis 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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References


