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RESEARCH ARTICLE

The spatial distribution does not affect host–parasite coevolution in *Rossomyrmex* ants

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Abstract Host and parasite distributions are crucial to understand the coevolutionary outcomes of their relationships. This comes from the fact that the distribution of a species (fragmented vs. continuous habitats) influences its dispersal opportunities. In this work, we studied the effect of the spatial distribution on dispersal and coevolution between three species of social parasite ants of the genus *Rossomyrmex* (one distributed in high mountains in Spain and two distributed in extended plains in Turkey and Kazakhstan) and their ant hosts *Proformica*. We analysed the variation at the mitochondrial gene cytochrome *c* oxidase (COI) to infer female dispersal for parasites as well as the cuticular hydrocarbons (CHCs) of parasites and hosts to study their coevolutionary process, given that CHCs are involved in nestmate recognition. Our genetic results revealed a surprising scarce variation at COI for the three parasite species, suggesting selective forces that prevent from mutation fixation. Therefore, COI appeared to be a poor tool to study dispersal. Furthermore, chemical results showed population differentiation for all host–parasite systems, pointing that coevolution would take place at a local scale regardless of the spatial distribution or dispersal opportunities of the counterparts.

Keywords COI · Coevolution · Cuticular hydrocarbons · Spatial distribution · *Rossomyrmex* · Slave-making ants

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Introduction

Host–parasite coevolution is an intriguing issue to study due to its numerous implications in evolutionary biology, ecology and the diversification of life (Thompson, 1999). The distribution of both host and parasite is crucial as the size of populations and their inter-connexion would determine the coevolutionary outcomes (Avise, 1994; Brandt et al., 2007). In addition, parasites and hosts usually do not show complete overlap in their spatial distributions but coevolutionary hot and cold spots. Several studies indicate that dispersal to some extent increases local adaptation by the introduction of genes potentially beneficial (Gandon et al., 1996; Gandon and Michalakis, 2002; Forde et al., 2004; Morgan et al., 2005), although natural selection should also be strong enough to permit this local adaptation otherwise it would not occur (Frankham et al., 2002). As parasite fitness depends almost completely on successful host exploitation, natural selection should act stronger on them than on hosts. As a consequence, parasites have usually been predicted to have an evolutionary advantage (thus leading the coevolutionary process) in the form of larger population sizes, shorter generation times or higher mutation and migration rates (see Brandt et al., 2007). However, some examples have been found of parasite maladaptation (Gandon and Nuismer, 2009; Adiba et al., 2010).

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Several theoretical and experimental studies have shown that the species (host or parasite) dispersing at the highest rate would be locally adapted to the antagonist, whereas if both species have similar dispersal rates, local adaptation would not be expected (Gandon et al., 1996; Lively, 1999; Ruano et al., 2011). In this sense, dispersal is a crucial life-history trait determining the distribution of genetic variability and sometimes the survival of populations depends completely on it (Clobert et al., 2001). In continuous habitats individuals can disperse as far as their physical condition permit it in order to mate or find a new territory. However, in fragmented habitats, dispersal is restricted given the reduced suitable patches of habitat and the presence of geographical barriers, which would lead to a decrease in population sizes and genetic differentiation among populations (Frankham et al., 2002). In fact, population isolation and restricted dispersal have also been pointed by some authors to be a necessary condition for local adaptation (Nuismer et al., 2003; Nash et al., 2008). Therefore, it seems clear that the spatial distribution could play an important role in the interaction between parasites and hosts for its consequences on dispersal, genetic diversity and host–parasite coevolution.

Social insects, such as ants, are more prone to population declines and extinction than non-socials due to two main factors (Pamilo and Crozier, 1997; Chapman and Bourke, 2001): (1) their effective population sizes are not proportional to their biomass because only few individuals will reproduce, (2) ants are haplodiploid so that males are haploid, thus reducing genetic variation and favouring the effects of genetic drift (Hedrick and Parker, 1997; Frankham et al., 2002). One way to avoid this situation is to disperse from the natal nest in order to enhance the opportunities to mate with a non-relative. The typical rule for ants is that both sexes are winged and perform nuptial flights (Bourke and Franks, 1995). Because mitochondrial DNA is matrilineal inherited, it can be used to infer female dispersal rates (Prugnolle and de Meeus, 2002; Cooper et al., 2010).

In addition, within ants there are about a 3% of species that are social parasites, which are ant societies living at the expense of another ant society of a different species (Hölldobler and Wilson, 1990). Social parasites are usually close relatives to their hosts (Emery, 1909; Wilson, 1971) and show similar generation times, what make them react to each other at a comparable evolutionary speed. However, geographical differences in the host–parasite coevolutionary process can arise as a consequence of local adaptive strategies, a scenario known as the geographical mosaic of coevolution (Thompson, 1994). A trait playing a key role in the interactions between hosts and parasites in ants is cuticular hydrocarbons (CHCs) given that they are involved in nestmate recognition (Lenoir et al., 2001; Ozaki et al., 2005; Hefetz, 2007; Akino, 2008) and are usually species-specific (or even nest-specific, e.g. Nowbahari et al., 1990; Copren et al., 2005; Martin et al., 2008; d'Ettorre and Lenoir, 2010). In this sense, congruency of CHCs is the basis for the tolerance shown by the hosts towards their social parasites; in several cases, this chemical congruency has been demonstrated to occur by not only natural selection (Brandt et al., 2005; Errard et al., 2006) but also chemical acquisition (e.g. parasite camouflage; see Lenoir et al., 2001; d'Ettorre et al., 2002).

Rossomyrmex is a genus of slave-making ants inhabiting steppe habitats in the Paleartic. Its host species belong to the genus *Proformica*. The most studied species, *Rossomyrmex minuchae*, inhabits three high mountains (above 2,000 m a.s.l.; see Ruano et al., 2007) of the South East of Spain and is classified as vulnerable by the IUCN Red List of Threatened Species and as endangered in the Red Book of Spanish Invertebrates (Martínez-Ibáñez et al., 2006). In contrast, its host is present at higher densities and altitudinal ranges within the same mountains, thus inhabiting wide areas free of parasitism or cold spots (Ruano et al., 2011). A recent study on *R. minuchae* using microsatellites (Sanllorente et al., 2010) showed that the three parasite populations are genetically very different and are geographically isolated (no dispersal would occur among mountains). Also, previous studies on CHCs in this species demonstrated that chemical profiles of sympatric hosts are more similar to that of their parasites than to other allopatric populations of the same host species; this would be a result of natural selection on hosts favouring the survival of raided nests by avoiding fights between species provided that hosts always lose against the slave-makers and subsequently die (Zamora-Muñoz et al., 2003; Errard et al., 2006). Furthermore, a geographical mosaic of coevolution has also been evidenced in the three isolated populations with each host–parasite population at a different coevolutionary trajectory (Ruano et al., 2011). On the contrary, little is known of the other species of *Rossomyrmex* and their hosts, all of them living in extended plains in Asia without remarkable geographical barriers (Marikovsky, 1974; Tinaut et al., 2010), so with a supposed more continuous distribution. Population genetics theory states that genetic diversity is positively correlated with population size and this, in turn, is reduced as a consequence of the habitat fragmentation (Frankham et al., 2002). In agreement with this, *R. anatolicus* (from Turkey) shows higher levels of microsatellite variation than *R. minuchae* but little genetic differentiation between its two analysed populations (425 km distant). In contrast, for *R. quandratinodum* (native of Southeastern Kazakhstan and Northwestern China), no significant differences in microsatellite variation were found compared to *R. minuchae* (Sanllorente et al., 2010).

In this work, we compared two spatial distributions (rather continuous in extended plains or fragmented in

isolated mountains) and their effect on dispersal opportunities in three slave-making ants of the genus *Rossomyrmex* measured as the variation of the mitochondrial gene cytochrome c oxidase (COI), because this is important for the adaptive potential of a species. The mitochondrial gene COI has been widely used in phylogeny and phylogeography studies from all the animal kingdom and it has been considered even as an animal barcoding tool (Hebert et al., 2003, 2004; Smith et al., 2005). We predicted that the Asian species *R. anatolicus* and *R. quandratinodum* (both distributed in extended plains) should exhibit higher levels of mitochondrial genetic variation than *R. minuchae* (distributed in isolated high mountains), thus a similar result to that found for microsatellites. We also studied the effect of the spatial distribution (continuous or fragmented) in the coevolution of these slave-making ants and their hosts. To do so, we analysed the cuticular hydrocarbons of the three *Rossomyrmex* species as well as those of their host species. We predicted that the host–parasite systems continuously distributed should be less congruent in their CHCs than the fragmented one, given that in the Asian plains parasites and hosts would show similar rates of dispersal. As the three slave-making species studied present monogynous nests and similar mating and raiding behaviours (Marikovsky, 1974; Ruano and Tinaut, 1999, 2005; Tinaut et al., 2010), differences in their population structure and colony odour should not be expected because of different life-history traits.

Materials and methods

Sampling and laboratory analyses

For the genetic analysis ants were collected from 2004 to 2007 and preserved in absolute ethanol. We analysed one worker per nest, having a total of 74 sampled nests (Table 1) corresponding to three populations of *Rossomyrmex minuchae* separated by about 60 km (Sierra Nevada, Gador and Filabres, named SN, G and F, respectively; see Ruano et al., 2007), two populations of *R. anatolicus* from Turkey separated by around 500 km (Belembaçi Beli in Konya province and Ziyaret Tepesi in Sivas province, named BB and ZT, respectively) and one population of *R. quandratinodum* from South Eastern Kazakhstan (near Charyn Canyon, named CC). Despite an intensive field work, we could not collect as many nests as desired due to the difficulty to find parasitized nests; however, we still consider the comparison of the distributions interesting, specially from a coevolutionary point of view. DNA was extracted using the Puregene DNA Isolation Kit (Gentra Systems). A 576-bp fragment of the mitochondrial COI was amplified using primers developed for *F. exsecta*: COI exsecta 2F (GGATCNCCAGANATNGCTTANCCTCG) and COI exsecta 2R (TAATNGCAAAAACNGCTCCTA) designed by B. Holzer (University of Lausanne). PCR amplifications were carried out in a 10-μL reaction containing 2.5 ng of DNA, 0.2 nM of each primer, 0.25 nM of each dNTP, 2.5 mM of $MgCl_2$ (overall) and 0.5 U of *Taq* DNA Polymerase. PCR products were purified with the AccuPrep PCR Purification Kit (Bioneer) and sequenced in an ABI Prism 310.

To extract the cuticular hydrocarbons, 5 frozen (at −18°C) or live ants per nest were immersed for 10 min in 1 mL of pentane. Hydrocarbons were identified by combined gas chromatography/mass spectrography (Turbomass system, Perkin-Elmer, Norwalk, CT, USA, operating at 70 eV) using a non-polar DB-5 fused silica capillary column. Samples were run using a temperature program from 150°C (2 min of initial hold) to 300°C at 5°C min^{-1}, with 10 min of final hold. Quantification was achieved by gas chromatography using the same Perkin-Elmer FID-GC with the same column and temperature program. When possible, five individual extracts were used for each colony. When the individual extracts were not sufficiently concentrated, they were pooled by two or three. For the identification of the chemical components, previous data on *R. minuchae* and *Proformica longiseta* were used (Errard et al., 2006, Ruano et al., 2011). In order to study the host–parasite coevolution, the host species (enslaved and free-living nests) were also sampled from the same populations: *P. longiseta* in Spain (SN, G and F), *P. korbi* in Turkey (BB and ZT) and *P. sp* in Kazakhstan (CC). In total, we analysed 26 mixed nests of *R. minuchae*, 6 of *R. anatolicus* and 3 of *R. quandratinodum*; as for the unparasitized host nests we analysed 31 of *P. longiseta*, 6 of *P. korbi* and 4 of *P. sp*.

Statistical procedures

Chromatograms of the COI gene were first checked by eye for base call accuracy using the program Chromas Lite 2.01 (Technelysium Pty Ltd). Alignment of the genetic sequences was conducted with ClustalW using BioEdit version 5.0.6 (Hall, 1999). Haplotypes were identified with the DNASP v.5 software (Librado and Rozas, 2009). The resulting mitochondrial DNA haplotypes were analysed for sequence variation using the software ARLEQUIN 2.000 (Schneider et al., 2000). A minimum spanning tree for haplotypes was constructed using pairwise differences. We also tested for purifying selection on COI region by comparing synonymous and non-synonymous substitutions per site using MEGA v.5 (Tamura et al., 2011). Analyses were conducted using the Nei-Gojobori method (Nei and Gojobori, 1986) and bootstrapping 500 replicates.

Genetic structuring in *R. minuchae* was assessed by conducting an analysis of molecular variance (AMOVA, Excoffier et al., 1992) and significance was obtained by

Table 1 Number of sampled nests (N), number of haplotypes (nh), haplotype diversity (hd), nucleotide diversity (π) of COI in each population and GenBank accession numbers for the mitochondrial sequences

Species	Population	*N*	nh	hd	π (%)	GenBank accession nos.
R. minuchae	G	30	3	0.315	0.065	GU147935[a], GU147936[a], GU181201[a]
	F	8	2	0.25	0.043	GU181202[a], GU181203[a]
	SN	26	2	0.077	0.013	GU147937[a], GU181204[a]
R. anatolicus	BB	4	1	0	0	HQ585985
	ZT	3	1	0	0	HQ585985
R. quandratinodum	CC	3	2	0.667	0.116	HQ585986, HQ585987

[a] Sequences originally published in Sanllorente et al. (2010)

performing 1,000 permutations of data. The overall as well as the pairwise differentiation between populations (Φ_{ST}) was estimated by permuting haplotypes between populations. Mantel tests with 10,000 permutations were performed for *R. minuchae* populations to investigate for local isolation by distance (a matrix of geographical distances against a matrix of pairwise genetic distances) with the FSTAT 2.9.3.2 software (Goudet, 2002). Given the low number of sampled nests for the Asian species, these analyses were not performed for them.

For the CHCs analyses we used the mean data for each colony (3–5 samples). We performed cluster analysis with Euclidean distances and Ward method on the percentages of all the peaks (see Elmes et al., 2002) as there were great qualitative and quantitative differences in the chemical profiles (too many zeros or very low values). We also calculated the differences in hydrocarbon composition among colonies modifying the standard genetic distance of Nei (see Dronnet et al., 2006). The chemical distances between the parasites and their free-living hosts were considered as an indicator of the coevolutionary process for each case.

Results

Mitochondrial variation

All COI sequences were translated into amino acids according to the invertebrate mitochondrial genetic code and no stop codons were detected. The number of variable sites was 69, whereas the number of parsimony informative polymorphic sites was 63. No indels were found. Base composition was A–T biased (65.4%) with the highest proportion at the third codon position (80.7%). The codon-based test of selection showed a significant deviation of the strict neutrality hypothesis in favour of purifying selection (*Z* test, $z = 2.502$; $P = 0.007$) for which nucleotide mutations would be purged.

For *R. minuchae* we analysed the seven haplotypes previously described by Sanllorente et al. (2010). In this species, haplotypes were population specific (ranging 1–3), with a most frequent one and another extremely rare (Table 1; Fig. 1). Haplotype differences between populations of *R. minuchae* were highly significant (Table 2). The AMOVA analysis confirmed that 98.86% of these differences occurred among populations ($P < 0.01$) while only a 1.14% was due to intrapopulation variation. The minimum spanning network also grouped haplotypes into the three populations considered, being F the population most similar to the Asian species (Fig. 1). Mantel tests did not show evidences for isolation by distance in any of the populations (all $P > 0.05$), so that geographical distance was not proportional to mitochondrial genetic differences. In *R. anatolicus*, all the samples shared the same haplotype independently of the sampling site, whereas two haplotypes were found for *R. quandratinodum* (Table 1).

Host–parasite coevolution

The three *Rossomyrmex* species analysed and their *Proformica* slaves had the same hydrocarbon substances, but differed in relative quantities (Fig. S1; Table S1). When one

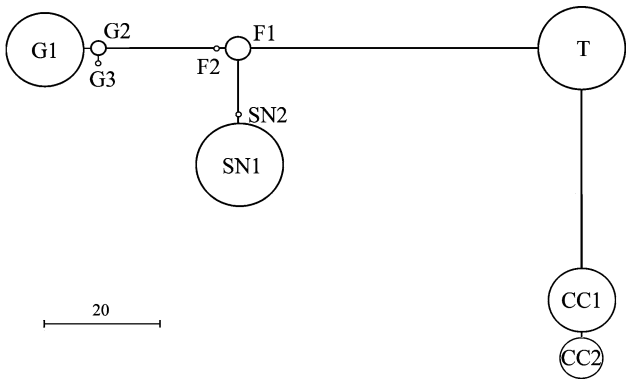

Fig. 1 Minimum spanning network of haplotypes. Length is proportional to the number of substitutions and areas of circles reflect the relative frequency of each haplotype. *R. minuchae* populations correspond to G, F and SN haplotypes, *R. anatolicus* to T haplotype and *R. quandratinodum* to CC haplotypes

Table 2 Pairwise Φ_{ST} (below diagonal) and their significance (above diagonal; $*P < 0.05$) for the three *R. minuchae* populations

	G	F	SN
G		*	*
F	0.984		*
SN	0.990	0.986	

peak is marked as zero, it is generally present as traces. *R. quandratinodum* had more saturated alkanes (50%) than the other species (maximum 26%). Cluster analysis for the slave-making genus alone (Fig. 2) showed that the Turkish ZT population clustered closer to the Kazak CC than Turkish BB, which indicates that *R. anatolicus* presents at least two chemotypes. Our results on the Spanish populations confirmed that each of the three populations had its own chemotype (see Ruano et al., 2011). Moreover, the Spanish F population was more similar to the Asian species than to the other two Spanish populations.

Considering this surprising result and that the relationship between hosts and parasites is already known for the Spanish populations (see Ruano et al., 2011), we added the *Proformica* hosts (free-living and enslaved) to the analysis for F and the Asian lineages (Fig. 3). As expected, we found the slaves, free-living and parasites from the same population associated, except for the Kazak host–parasite system, for which the hosts were heterogeneous (CC-FL in ellipse in Fig. 3). In accordance with Fig. 2, the Spanish F population clustered separately from the Asian ones. The Turkish population of ZT clustered with the Kazak CC, while the other Turkish population (BB) clustered separately.

The differences in CHC between parasites and their potential hosts (measured as the modified Nei index) were

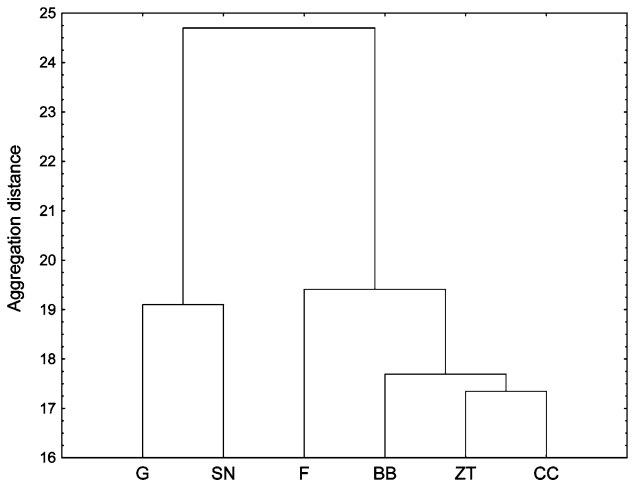

Fig. 2 Dendrogram (Ward method, Euclidian distances) of the cuticular hydrocarbon profiles in *Rossomyrmex minuchae* (G, F and SN populations), *R. anatolicus* (BB and ZT populations) and *R. quandratinodum* (CC population). (Elaborated using STATISTICA)

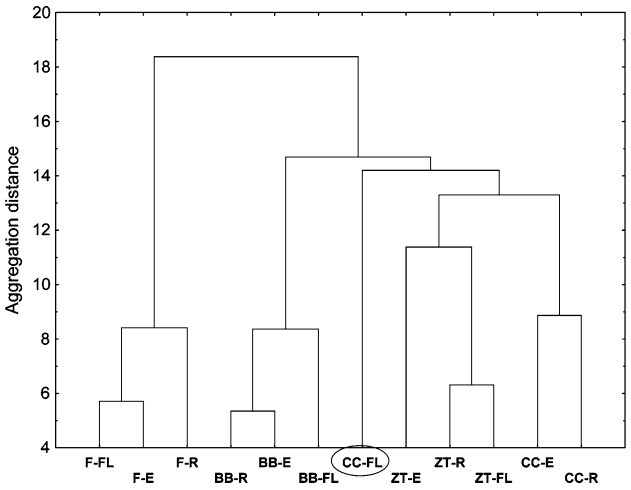

Fig. 3 Dendrogam (Ward method, Euclidian distances) of the cuticular hydrocarbons in *Rossomyrmex* (R) and their *Proformica* host species (E for the enslaved and FL for the free-living). *R. minuchae* is represented by F population, *R. anatolicus* by BB and ZT populations and *R. quandratinoudm* by CC population. CC-FL is in ellipse to highlight that it is separated from the enslaved (CC-E) and the parasites (CC-R). (Elaborated using STATISTICA)

significantly lower in the Turkish population of ZT than in F and CC, while no differences were found with BB, the other Turkish population (Kruskal–Wallis test $H = 9.36$, $P = 0.025$; LSD post hoc comparisons).

Discussion

Mitochondrial variation

In this study, we observed that the isolation of the Spanish *R. minuchae* populations has led to a greater population differentiation than in the Asian species. Mitochondrial genetic analyses reflected significant differences among *R. minuchae* populations; however, within populations differences were very low and not significant; each population has its private haplotypes (two or three) but in all cases there is one haplotype very frequent and the rest are very rare. This suggests that populations may have gone through a continued scenario of small population size and genetic drift that led to the loss of less frequent haplotypes, a similar conclusion to that obtained from microsatellites (Sanllorente et al., 2010). Therefore, genetic analyses (from microsatellites and mitochondrial COI) support that the Spanish *R. minuchae* presents a fragmented distribution, what is in accordance with its spatial distribution in high mountains separated by great deep valleys.

Furthermore, at a local scale, mitochondrial genetic analyses of isolation by distance failed to find genetic viscosity within the Spanish *R. minuchae* populations, which could be interpreted as not restricted female dispersal within

populations, especially in G and F, with higher values of haplotype and nucleotide diversities (Table 1); however, this result could also be explained by a lack of power of the method given the low number of haplotypes per population. In fact, Sanllorente et al. (2010) found evidences for isolation by distance in G population using microsatellites (more variable than COI). Restricted female dispersal has been documented from other monogynous ants (and even in slave makers) but usually in these cases genetic differentiation at short distances was evident (Foitzik and Herbers, 2001; Clémencet et al., 2005). Short range dispersal could be a restriction imposed by the slave making condition of *R. minuchae*: females should find a mature *Proformica* nest to initiate their own and flying too far would be disadvantageous if females land on areas where there are no host nests or their density is low, thus the possibilities of successfully invading a host nest would not be high.

For the Asian species one would expect higher levels of mitochondrial DNA variation given that these species inhabit extended plains. In agreement with this, we found a proportionally higher mitochondrial variation within population in the Kazak *R. quandratinodum* than in the Spanish *R. minuchae* if we consider its smaller sample size (two haplotypes in 3 nests compared to the two haplotypes from 26 nests in SN; see Table 1). In contrast, we found a surprising lack of haplotype variation in the Turkish *R. anatolicus*, with the same single haplotype (Fig. 1) in both populations analysed despite being separated by 425 km. Although there was no a priori evidence for a lack of variation in COI for this species, this may indicate that the present wide area of distribution of *R. anatolicus* would have been colonized from a single ancestral population; nevertheless, previous results with microsatellites showed that this species had a genetic diversity significantly higher than the Spanish *R. minuchae* (Sanllorente et al., 2010). This lack of genetic variation at COI could be explained by some selective forces preventing from mutation fixation, what is in agreement with the purifying selection detected for this gene. The mitochondrial gene COI is highly constrained by natural selection as it is a coding sequence, whereas microsatellites are not, thus the latter are, in general, more variable (Hartl and Clark, 1997). Furthermore, the fact that the same haplotype is present in both Turkish populations and the lack of significant population differentiation according to microsatellites (Sanllorente et al., 2010) points that they should be interconnected to some extent, thus revealing that *R. anatolicus* would be continuously distributed. In fact between both populations analysed (BB and ZT) it spreads the great Anatolian plateau without any relevant geographical barriers, allowing dispersal within it.

What seems clear is that the mitochondrial gene COI is scarcely variable independently of the habitat and thus a poor genetic tool to study dispersal in the *Rossomyrmex* ants compared to microsatellites.

Host–parasite coevolution

Chemical profiles of the Asian *Rossomyrmex* suggest local differentiation despite their continuous distribution, as reflected in the clear separation of both Turkish populations (Fig. 2). Interestingly, one of these populations (BB) clusters separately from the other Asian lineages. The Spanish F population appears closer to the Asian lineages than the other Spanish populations, what is in accordance with the minimum spanning network (Fig. 1), in which F would show the most conservative haplotypes of *R. minuchae*. This suggests that from the three Spanish populations, F would have maintained the most ancestral traits for both COI and CHCs. A similar situation was found in the ant *Cataglyphis cursor*, for which populations geographically close showed higher differences at CHC profiles than in other more distant populations (Nowbahari et al., 1990).

The differences in CHC between parasites and their host species (here estimated as Nei distances) indicate differences in their relationship as a result of different life histories and coevolutionary processes, as was demonstrated for the Spanish *R. minuchae* populations (Ruano et al., 2011). In contrary to our predictions, our results suggest that the Turkish parasite–host system seems to be the most similar chemically in both populations analysed and according to Zamora-Muñoz et al. (2003) this host species should be the least aggressive. Therefore, our results support that population isolation is not strictly necessary for coevolution. Indeed, dispersal may favour local coevolution in species broadly distributed, as it has been shown in other slave-making systems (see Fischer and Foitzik, 2004; Brandt et al., 2007). In contrast, as the Kazak parasites and their enslaved hosts appeared clustered together but not the free-living hosts, they would be expected to be the most aggressive host–parasite system (the Spanish *R. minuchae* would be at an intermediate level with complete local clustering but higher differences in CHCs than the Turkish *R. anatolicus*). The observed chemical differences between Kazak parasites and hosts suggest that raids should be very aggressive on both parts and few hosts would survive. This is consistent with our observations on a parasitized nest in the lab, in which hosts seemed to avoid any contact with parasites so that coexistence would be difficult. In addition, the fact that the Kazak *R. quandratinodum* nests present a significantly lower proportion of hosts than the other *Rossomyrmex* ants (Tinaut et al., 2010) may indicate that many hosts would not be successful at avoiding attacks from their parasites and be killed. On the other hand, this result would not mean that host and parasite are not adapted to each other to some extent (given the cluster between

parasites and their enslaved hosts), but that it seems to exist a wider range of variation for this trait in the Kazak *P. sp* host than in the other two host species studied.

To the light of our results we can conclude that cuticular hydrocarbons are a useful trait to study local host–parasite coevolution given that they do not seem to be influenced by the spatial distribution or the dispersal opportunities of the counterparts. This comes from the fact that in our analyses, parasites and hosts always appeared clustered locally regardless of being distributed in mountains or plains.

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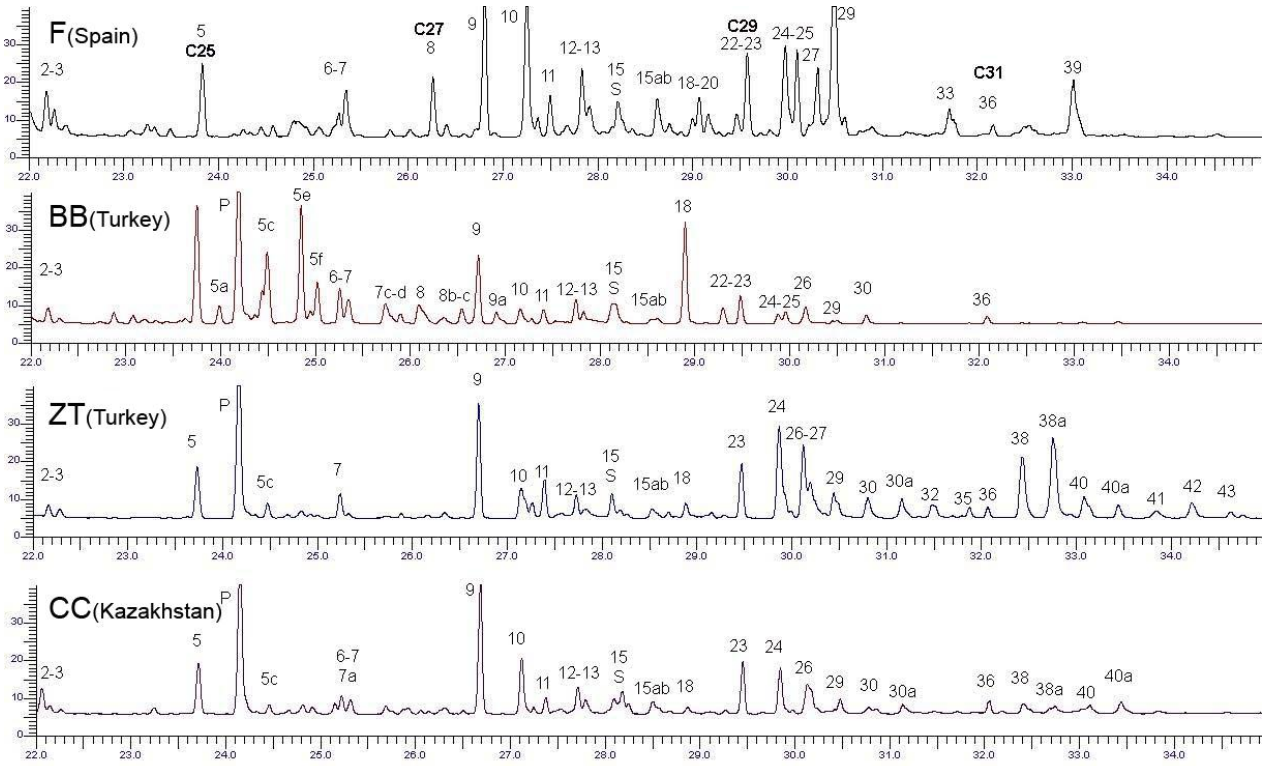
766 Table S1. Hydrocarbon composition of *Rossomyrmex* (R) workers from Sierra Nevada (SN), Filabres (F),
767 Gador (G), Ziyaret Tepesi (ZT), Belembaçi Beli (TBB) and Charyn Canyon (CC). sd = Standard
768 deviation.

N°	Name	SN-R	F-R	G-R	ZT-R	ZT-R sd	BB-R	BB-R sd	CC-R	CC-R sd
2	C24:1	1,9	3,3	3,2	0,2	0,2	0,1	0,1	2,9	0,9
3	C24	1,3	1,7	1,7	0,9	0,2	1,2	0,2	1,3	0,5
4	6C24	0,5	0,6	0,7	0,8	0,1	0,3	0,1	0,7	0,1
5	C25	2,0	4,8	3,1	3,9	0,2	9,9	5,2	5,4	3,5
5a	4,8,12TMC24	0,0	0,0	0,0	0,0	0,0	0,9	0,8	0,0	0,0
5b	7C25	0,0	0,0	0,0	0,2	0,0	0,2	0,1	0,2	0,2
5c	5C25	0,0	0,0	0,0	0,9	0,1	6,1	4,7	0,9	0,2
5d	9,13+9,15C25	0,0	0,0	0,0	0,3	0,0	0,2	0,1	0,5	0,2
5e	3C25	0,0	0,0	0,0	0,6	0,1	6,9	4,4	1,5	0,3
5f	5,9+5,13+5,17C25	0,0	0,0	0,0	0,3	0,2	3,0	2,0	0,7	0,2
6	C26:1	1,1	1,7	1,9	1,6	0,4	4,0	3,1	0,9	0,3
7	C26	1,8	3,5	2,6	0,3	0,2	1,7	1,2	2,3	1,1
7a	8+10C26	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,7
7b	11,13,15+13,15,17TriMeC25	0,0	0,0	0,0	0,1	0,1	0,7	0,1	1,0	0,3
7c	8,12C26	0,0	0,0	0,0	0,1	0,0	1,4	0,9	0,0	0,0
7d	6C26	0,0	0,0	0,0	0,2	0,0	0,7	0,5	0,4	0,4
8	4C26	0,4	2,1	0,7	0,0	0,0	3,9	4,3	0,3	0,2
8a	8,xC26	0,0	0,0	0,0	0,2	0,1	0,2	0,4	0,3	0,1
8b	C27:1	0,0	0,0	0,0	0,6	0,1	0,8	0,2	0,8	0,2
8c	4,8+4,10C26	0,0	0,0	0,0	0,1	0,0	1,0	0,8	0,2	0,2
9	C27	2,3	8,4	2,8	5,8	1,0	5,9	0,7	11,8	5,9
9a	4,8,12TM C26	0,0	0,0	0,0	0,0	0,0	1,0	0,8	0,1	0,1
10	11+13C27	0,3	9,1	1,3	4,5	2,8	3,1	2,9	3,6	0,9
10a	7C27	0,0	0,0	0,0	1,1	0,2	1,1	1,0	0,5	0,2
11	5C27	0,1	1,9	1,2	2,0	0,1	1,6	0,7	1,3	0,6
11a	9,13+11,13C27	0,0	0,0	0,0	0,5	0,1	1,1	1,4	0,8	0,3
12	3C27	0,8	3,4	1,3	1,3	0,2	3,6	2,9	2,4	0,7
13	5,15+5,17C27	0,7	1,6	1,5	1,1	0,3	1,5	0,8	1,9	0,5
14	C28 + squalene	1,4	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0
15	8+10+12+14C28	0,4	2,6	1,0	1,6	0,5	5,4	3,9	2,1	1,4
15a	13,15,17TriMeC27	0,0	0,0	0,0	0,4	0,1	0,2	0,2	2,4	0,9
15b	8,12+8,14+10,12C28	0,0	0,0	0,0	1,0	0,1	1,1	0,2	1,4	0,4
16	7C28	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0
17	6C28	0,2	0,0	0,9	0,4	0,1	0,0	0,1	0,3	0,1
18	4C28	0,6	0,4	1,3	0,9	0,3	6,5	5,1	0,7	0,3
19	10,12+10,14C28	0,3	1,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0
20	7,11,19C27	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0
21	6,10+6,12+6,14C28	0,7	0,0	0,5	0,6	0,1	0,4	0,4	0,4	0,1
22	4,8+4,10+4,12C28	2,1	1,2	1,5	0,2	0,0	1,1	0,8	0,4	0,2
23	C29	5,3	2,6	2,8	12,7	6,6	2,8	0,9	23,8	3,1
23a	4,8,12TriMeC28	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1
24	11+13+15C29	1,2	6,7	2,0	13,8	6,8	0,8	0,2	3,9	1,3
25	7C29	0,2	4,9	2,0	0,0	0,0	1,1	0,3	0,3	0,2
26	5C29	0,7	2,4	1,3	7,2	1,4	2,7	1,9	4,7	1,6
27	9,13+11,15+13,17C29	0,0	11,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0
28	3C29	5,1	0,0	5,2	0,0	0,0	0,5	0,6	0,6	0,5
29	5,9+7,17C29	0,0	10,2	0,0	1,7	0,4	2,0	2,7	1,2	0,8
29a	C30	0,0	0,0	0,0	1,3	0,1	0,5	0,7	0,9	0,4
30	8+10C30	7,0	0,9	14,9	0,3	0,3	0,6	0,3	0,6	0,5
30a	11,13,15TriMeC29	0,0	0,0	0,0	1,6	0,1	0,6	0,8	1,0	0,6

31	8,12+8,14C30	11,9	0,2	6,4	0,1	0,0	0,0	0,0	0,1	0,2
32	4C30	0,7	0,0	1,1	1,5	0,2	0,3	0,4	0,4	0,3
33	9,11,16C29	0,0	1,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0
34	6,10+6,14C30	1,5	0,0	3,1	0,2	0,0	0,1	0,2	0,3	0,2
35	4,8+4,10C30	9,0	0,0	9,0	0,7	0,1	0,1	0,1	0,2	0,1
36	C31	0,0	0,5	0,0	0,6	0,1	0,9	0,4	1,5	0,6
37	4,10,12TriMeC30	13,4	1,4	1,6	0,0	0,0	0,0	0,0	0,3	0,3
38	9+11+13+15C31	0,7	0,5	1,9	5,0	0,5	2,4	4,0	1,5	0,6
38a	11,15+13,15C31	0,0	0,0	0,0	7,6	1,2	1,3	2,1	1,1	0,7
39	9,19C31	0,4	4,7	2,6	0,2	0,1	0,4	0,5	0,3	0,2
40	5,17C31	1,2	2,0	3,0	1,9	0,1	0,9	1,1	0,7	0,7
40a	C32	0,0	0,0	0,0	0,9	0,1	0,5	0,3	1,7	0,7
40b	5,10,14TriMeC31	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
41	8+10+11+12+14C30	3,4	0,0	7,3	0,9	0,1	0,3	0,5	0,5	0,2
42	8,14+8,16C32	14,5	0,0	6,2	1,3	0,1	0,1	0,2	0,0	0,0
43	6,10+6,14C32	0,2	0,0	0,5	0,3	0,1	0,0	0,0	0,2	0,2
43a	C33	0,0	0,0	0,0	0,1	0,0	0,2	0,1	0,4	0,1
44	11+13+15+17C33	0,3	0,0	0,5	1,5	0,3	0,9	1,5	0,8	0,3
44a	11,15+13,15C33	0,0	0,0	0,0	4,1	0,6	1,2	2,2	0,5	0,2
45	9,17C33	3,8	0,0	0,1	0,1	0,2	0,7	0,9	0,0	0,0
45a	5,10+5,12C33	0,0	0,0	0,0	1,3	0,2	0,6	0,9	0,5	0,3
45b	C34	0,0	0,0	0,0	0,3	0,2	0,2	0,2	0,2	0,2
46	10,14+10,16C34	0,8	0,0	0,0	0,1	0,0	0,0	0,0	0,1	0,1
	Total	100,0	100,0	100,0	100,0		100,0		100,0	
	Total alkanes	14,1	21,5	14,3	26,8	5,4	24,0	2,0	49,2	4,7
	Total methyl alkanes	22,1	33,8	43,6	43,1	5,1	43,8	3,9	27,2	5,4
	Total dimethyl alkanes	47,3	35,3	35,4	23,8	4,0	22,1	6,7	12,8	3,5
	Total trimethyl alkanes	13,4	4,4	1,6	2,6	1,1	4,6	1,7	5,7	2,0
	Total alkenes	3,0	5,0	5,1	2,4	2,4	4,9	3,1	4,6	0,9
	Total < 28C	14,6	42,1	23,3	27,7		62,3		43,8	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