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A geographical mosaic of coevolution in a slave-making host–parasite system

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Rossomyrmex minuchae;
tolerance.

Abstract

Three different isolated populations of the slave-making ant *Rossomyrmex minuchae*, sympatric with its obligate host *Proformica longiseta*, are known from the high mountains of southern Spain. To test the prediction that the slave-maker and its host represent a coevolutionary geographical mosaic, we studied the variation in the cuticular hydrocarbons (CHCs) as the trait most likely to show the selection mosaic, plus trait remixing by the gene flow in the populations of each species by means of microsatellites. We found within populations, host and parasite had more similar CHC profiles than between the populations or between parasites and allopatric hosts. The differences between the CHC profiles of the host and parasite, which may be responsible for the level of tolerance towards the parasite, varied between the populations suggesting the existence of a selection mosaic of coevolution. Furthermore, *P. longiseta* showed higher gene flow than *R. minuchae*, which would allow local variation in the coevolution of the host and parasite while allowing some trait remixing.

Introduction

Over the last 20 years, a number of convincing examples of coevolution between parasites and their hosts have demonstrated the importance of coevolution as an evolutionary process, shaping the organization of communities and influencing the diversification of life (Thompson, 1999a, 2005). According to the geographical mosaic theory of coevolution (GMTC), the outcome of interactions and the degree of specialization vary between physical and biotic environments and depend on the community context in which the interaction takes place (Thompson, 1994, 1999b).

In this work, we studied a social host–parasite system, in which the slave-maker ant *Rossomyrmex minuchae* is endemic to only three known populations in southern Spain (Fig. 1), of which two were recently found (Ruano *et al.*, 2007), separated on three different high mountains. These *R. minuchae* populations, located at 1850–2200 m a.s.l., have been isolated at least since the intervening habitat became inhospitable because of warming during some of the last Pleistocene interglacials (Sanllorente *et al.*, 2010). Its obligate host *Proformica longiseta* is also endemic to the high mountains of southern Spain, showing a discontinuous distribution among mountains but a continuous distribution between 1650 and 2800 m a.s.l., depending on the altitude of the different sierras (Ruano *et al.*, 2007). *Rossomyrmex minuchae* is a scarce species and difficult to locate because of its low rate of daily and seasonal activity but extensively studied in the Sierra Nevada (SN) population in the last years. This parasite, with small nests (122.2 slave-makers per nest), performs a low number of raids each season (2.2 raids per season) (Ruano & Tinaut, 1999). During raids in which host nests are intensively exploited, the parasite steals the complete host brood for 2 or 3 days (Ruano & Tinaut, 1999).

Nestmate recognition is a key element in the organization of social insects. It is the ability to distinguish between classes of individuals, which is essential to avoid competition, predation and parasitism (Wilson, 1971). The maintenance of societies is achieved via a recognition system based on chemical odours on the cuticle. Hydrocarbons are known to play a crucial role in nestmate

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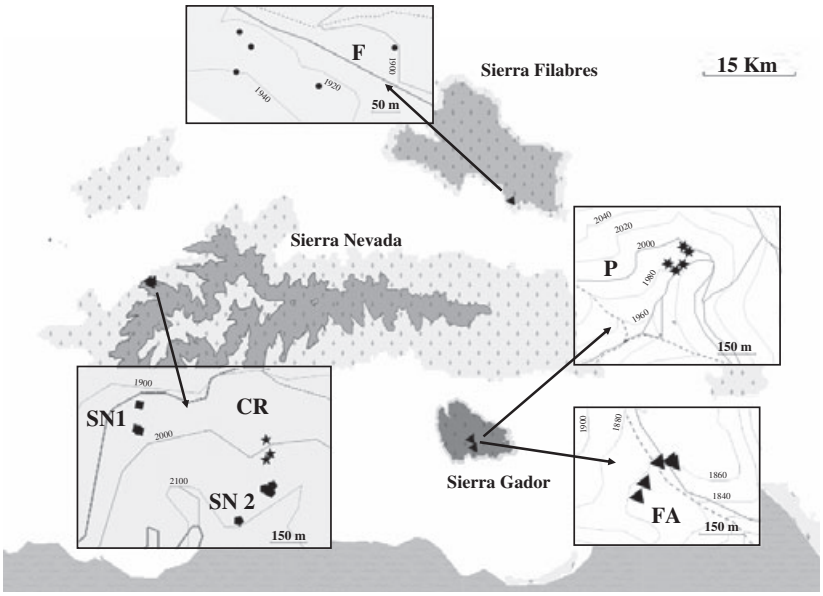

Fig. 1 Location of the three studied populations and six collecting sites of *Rossomyrmex minuchae*. In dark grey is the continuous distribution of *Proformica longiseta* in the three high mountains. Different black shapes indicate *R. minuchae* sampled nests at different collecting sites (F) Filabres [Fuente Alta (FA) and Pinos (P)] Gador, and [Sierra Nevada (SN) 1, SN2, and Casillas Rojas (CR)] SN.

recognition (Lenoir *et al.*, 1999; D'Ettorre *et al.*, 2002; Howard & Blomquist, 2005; Ozaki *et al.*, 2005; Hefetz, 2007; D'Ettorre & Lenoir, 2010) and constitute a species-specific chemical signal for some social insects (Copren *et al.*, 2005; Martin *et al.*, 2008). In SN population, the reaction of *P. longiseta* during raids is not aggressive, not showing resistance (Ruano & Tinaut, 1999; Zamora-Muñoz *et al.*, 2003; Errard *et al.*, 2006). The aggression rate is positively correlated with differences in cuticular hydrocarbons (CHCs). In fact, sympatric free-living *P. longiseta* showed a higher cuticular profile similarity with the parasite than allopatric populations (Errard *et al.*, 2006), and sympatric *P. longiseta* showed a lower-than-expected aggression to its parasite, compared with allopatric *P. longiseta*, in laboratory confrontations. Moreover, the parasite never injured sympatric *P. longiseta* workers during laboratory confrontations (0% of killed sympatric ants), but when confronted with allopatric *P. longiseta*, 56% of host ants were killed or injured (Zamora-Muñoz *et al.*, 2003). This result points out to the evolution of a reduced aggression or nestmate discrimination in the sympatric *P. longiseta* population of SN by means of increased CHCs similarity, as the only chance of a raided host nest to survive (58% of raided nests survive in SN, F. Ruano & A. Tinaut, unpublished).

This scenario for SN population could be different in each of the other isolated populations. The GMTC predicts differences in the outcome or trajectory of the coevolutionary process among the local communities owing to their composition and strength of ecological selection pressures through competition and resource availability. Gomulkiewicz *et al.* (2007) proposed some ideas to test the GMTC, to detect (i) coevolutionary hot and cold spots; (ii) a selection mosaic, showing spatial variation in fitness functions describing reciprocal selection; and (iii) trait remixing carried out by gene flow.

1 Coevolutionary hot spots (zones of sympatry) and cold spots (zones of allopatry): The GMTC assumes that fitness interactions among species vary geographically in intensity, with regions in which reciprocal selection occurs (hot spots) and other regions in which a species is completely unaffected by the other (cold spots). In obligate host–parasite systems, reciprocal selection is maximal owing to the fact that diffuse coevolution is reduced and interactions are very important and reciprocal, affecting fitness.
2 A selection mosaic refers to spatial variation in the interspecific frequency-dependent fitness functions describing reciprocal selection among the interacting species. Among the strategies employed by social parasites to integrate into their host colony are the existence and/or acquisition of similar chemical profiles between the social parasites and their hosts (see Lenoir *et al.*, 2001; Akino, 2008). One of the ways for hosts to evolve resistance is to avoid being parasitized in the first place (Brandt *et al.*, 2005). This can be achieved by reliable enemy recognition, even if this is not completely effective, or even slave rebellion (Achenbach & Foitzik, 2009; Achenbach *et al.*, 2010), thus reducing the overall success of raids. Otherwise, reduction in aggression (Zamora-Muñoz *et al.*, 2003) or tolerance (Svensson & Råberg, 2010) is another possible outcome in these host–parasite systems.

Chemical distances between CHC profiles are good indices to evaluate recognition ability and potential aggression between host and parasites, because differences in CHCs are positively correlated with geographical distances and aggression (Zamora-Muñoz *et al.*, 2003; Errard *et al.*, 2006; Nash *et al.*, 2008; Ugelvig *et al.*, 2008). In this case, we tried to investigate whether a selection mosaic exists in nestmate recognition in the three different sympatric and isolated populations of *P. longiseta–R. minuchae* host–parasite system interaction, as an indicator of different coevolutionary outcomes. In this study, we test whether the results obtained for the first population (SN, Zamora-Muñoz *et al.*, 2003) are common to the two new populations or whether the differences in the CHCs distances between free-living hosts and parasite are different in each population. These differences could be attributable to different selection pressures acting since Pleistocene separation in the three populations (Sanllorente *et al.*, 2010).

3 Trait remixing (Thompson, 2005; Gomulkiewicz *et al.*, 2007): The pattern of this mosaic changes locally with time when the interacting populations change, especially when migration occurs. Some previous studies have pointed out that gene flow is very important for local coevolution as a main source of genetic variation. However, researchers do not agree on the effect of migration on coevolution: some observed that gene flow enables beneficial adaptations at a particular time and space (Forde *et al.*, 2004; Morgan *et al.*, 2005). In patches where the parasite can migrate at a higher rate than the host, the parasite will be locally adapted to its host, and in patches where the host can migrate at a higher rate than the parasite, the host will be locally adapted, whereas if both the species have similar migration rates, no local adaptation is expected (Gandon *et al.*, 1996). Other studies point out that the absence of, or restricted, gene flow is essential for local coevolution, given that migration homogenizes the populations. Thus, selection may be weak in patches where host and parasite overlap, and the widespread species may not be adapted to the other (Nuismer *et al.*, 2003; Nash *et al.*, 2008). We studied whether gene flow exists among *R. minuchae* and *P. longiseta* populations using microsatellite markers.

In this work, we tested the GMTC for the three currently known, isolated *R. minuchae* populations (Ruano *et al.*, 2007) and its host *P. longiseta*, using both chemical (CHCs) and genetic traits (microsatellites). We predicted that the CHC profiles (cuticular distances) of both the host and parasite are population specific, showing a selection mosaic for this trait, with different degrees of mimicry as evidence for a local mosaic of coevolution taking place in three different areas independently. The outcomes of these interspecific interactions could differ between populations because of different selection pressures (Thompson, 2005) expected after isolation. To determine whether trait remixing for CHCs has taken place, the gene flow (inferred using microsatellites) among the populations for both the species was compared. An asymmetry in the gene flow between the species can elucidate the current situation of the geographical mosaic of coevolution.

Materials and methods

Study area and field work

Live ants were collected with an aspirator during summer 2006. Only three populations of this species are known to date: the first one in SN [SN1, SN2 and Casillas Rojas (CR) collecting sites], the second one in Sierra de Gador [Fuente Alta (FA) and Pinos (P) collecting sites] and the last one in Sierra de los Filabres (F). For the *R. minuchae* nests, we recorded Universal Terminal Mercator (UTM) coordinates using a global positioning system (GPS) (Garmin) and calculated the straight-line distances between the nests by trigonometry. For *P. longiseta*, a single UTM coordinate per collecting site was recorded, because some inter-nest distances were shorter than the GPS sensitivity (5-m accuracy). The distance between the collecting sites varied from 190 m to 61 km (Table 1). In all these localities, for chemical analyses, we sampled *R. minuchae* nests with their corresponding *P. longiseta* slaves and free-living *P. longiseta* nests. A total of 26 *R. minuchae* mixed nests and 31 free-living *P. longiseta* were sampled (Table 2). For genetic analyses, up to five workers per nest were used (23 nests for the parasite: three from SN1, three from SN2, three from CR, four from F, five from FA and five from P collecting sites; and 31 for the host (free-living nests): three from SN1, five from SN2, three from CR, 10 from F, five from FA and five from P).

Chemical and genetic analyses

Individual ants were frozen at −18 °C for 1 h and then immersed for 10 min in 1 mL of pentane. The extracts were stored at −18 °C until the analyses were carried out. Cuticular content was identified using pools of at least 20 workers, by combined gas chromatography/mass spectrometry (operating at 70 eV, Turbomass system;

Table 1 Geographical distances in metres between the populations and subpopulations studied: Sierra Nevada (SN1, SN2 and CR), Sierra de Gador (FA and P) and Sierra de Filabres (F).

	FA	P	F	SN1	SN2	CR
FA	0					
P	415	0				
F	39 559	39 146	0			
SN1	61 351	61 177	60 797	0		
SN2	60 734	60 557	60 315	620	0	
CR	60 815	60 636	60 271	564	190	0

CR, Casillas Rojas; FA, Fuente Alta; P, Pinos.

Table 2 Nest codes and total number of chromatograms (in brackets) used for chemical analyses.

Population	Locality	*Rossomyrmex* nests (R)	Enslaved *Proformica* nests (E)	Free-living *Proformica* nests (FL)
Sierra Nevada	SN1	14-17-30 (11)	14-17-30 (15)	11-12-13 (15)
Sierra Nevada	SN2	56-64-65-68 (20)	56-64-65-68 (20)	11-12-13-14-15 (25)
Sierra Nevada	CR	1-2-3 (15)	1-2-3 (15)	1-2-3 (15)
Filabres	F	2-3-4-5-6 (25)	2-3-4-5-6 (25)	11-12-13-14-15-16-17-18-19-20 (50)
Gador	FA	14-15-17-22-23-24 (30)	14-15-17-22-23 (22)	11-13-14-15-16 (21)
Gador	P	13-21-24-27-28 (25)	13-21-24-27-28 (21)	11-13-14-15-16 (25)

CA, Casillas Rojas; FA, Fuente Alta; P, Pinos.

Perkin-Elmer, Norwalk, CT, USA) using a nonpolar DB-5 fused silica capillary column. The samples were run using a temperature programme from 150 °C (2 min of initial hold) to 300 °C at 5°C min^{-1}, with 10 min of final hold. Quantification was by gas chromatography using the same gas chromatograph and temperature programme, but with flame ionization detection. Where possible, five extracts from individual ants were used for each colony. When the individual extracts were not sufficiently concentrated, two or, in some cases, three extracts were pooled. As intracolonial variation was very low, we used the mean percentage of peaks for each colony. Identification of substances was made based on the 23 compounds previously published for *R. minuchae* and its host in SN (Errard *et al.*, 2006), plus a detailed analysis of additional small peaks.

The total DNA was extracted from the workers using a Puregene DNA Isolation Kit (Gentra Systems). *Rossomyrmex* workers were genotyped at 11 microsatellite loci: Ccurll, Ccur46, Ccur63b, Ccur76, Ccur89, Ccur99, FEll, FE19, FE21, FE37 and FE51 (Gyllenstrand *et al.*, 2002; Pearcy *et al.*, *2004*). *Proformica* workers were genotyped at seven loci: Ccurl1, Ccur26, Ccur63, Ccur76, FE19, FE37 and FL12 (Chapuisat, 1996). Microsatellites were amplified by PCR in a 10-μL reaction containing 2.5 ng of DNA, 0.2 nM of each primer, 0.25 nM of each dNTP, Ix MBL^{TM} buffer, 2.5 mM of of $MgCl_2$ (overall), 0.5 μL of MBL^{TM} Taq DNA polymerase and 7 μL of distilled water. PCR conditions were 94 °C for 3 min to denature the samples, followed by 11 touch-down temperature cycles of denaturing at 94 °C for 15 s, annealing at 55–45 °C for 30 s and extension at 72 °C for 30 s, followed by 30 cycles at 94 °C for 15 s, 50 °C for 15 s and 72 °C for 30 s. The PCR products were genotyped at Unidad de Genómica. SCAI (University of Córdoba) using an ABI3lO sequencer. We scored the genotypes using GENESCAN software v 3.7 (Applied Biosystems, Carlsbad, CA, USA).

Statistics

As there were great qualitative and quantitative differences in the chemical profiles (too many zeros or very low values), a discriminant analysis was not applicable, and therefore, we performed cluster analysis with Euclidean distances and Ward method on the percentages of all the peaks (see Elmes *et al.*, 2002). As it was not possible to normalize the data, differences in the hydrocarbon composition for free-living hosts and parasites were tested using Kruskal–Wallis analysis. We also performed a principal component analysis (PCA) to assess which hydrocarbons were responsible for group differentiation. The analyses were carried out with STATISTICA 7 for Windows (StatSoft, Tulsa, OK, USA).

Pairwise genetic differentiation (F_{ST}) between *R. minuchae* nests were calculated with FSTAT 2.9.3.2. (University of Lausanne, Lausanne, Switzerland), and partial Mantel tests (10 000 permutations) were used to correlate geographical, genetic (F_{ST}/l − F_{ST}) and chemical distances.

The differences in the hydrocarbon composition between the colonies and between the populations were quantified using a modification of the standard genetic distance of Nei, as described by Dronnet *et al.* (2006). Differences in the chemical distance between populations of sympatric free-living *P. longiseta* and *R. minuchae* were used as an index of the variation in the current outcome of coevolution from resistance to tolerance (i.e. the selection mosaic in this system).

Rates of gene flow (numbers of dispersers per generation) among the three populations were estimated according to Barton & Slatkin (1986), based on the distribution of rare alleles, using GENEPOP software (University of Montpellier, Montpellier, France; Raymond & Rousset, 1995) in its Web implementation. The estimate was corrected for size from the closest regression line (see Barton & Slatkin, 1986) provided by the program.

Results

Hot and cold spots

The current distribution of both the species was confirmed by exhaustive sampling around the known populations and in other potential biotopes across the Iberian Peninsula. The host was found to be widely distributed between 1650 and 2900 m a.s.l., but absent below 1650 m, whereas the parasite was confined to 1850–2200 m a.s.l. We consider these areas of overlap as coevolutionary hot spots with strong reciprocal selection

because of continuous interaction, whereas areas where the potential host occurs but that are empty of parasites are cold spots (Fig. 1).

Selection mosaic

From gas chromatography-mass spectrometry (GC-MS), a total of 46 peaks were identified, including a series of *n*-alkanes (C_{23}–C_{34}) and mono-, di- and trimethyl alkanes (Table S1, Fig. S1). For the SN population, these data confirmed the previous findings of Errard *et al.* (2006): the major peaks for *R. minuchae* were nonacosane (nC_{29}, peak 23), 3-MeC_{29} (peak 28), 8- + 10-MeC_{30} (peak 30), 8,12- + 8,14- $DiMeC_{30}$ (peak 31), 4,8- + 4,10$diMeC_{30}$ (peak 35), 4,10, 12-$TriMeC_{30}$ (peak 37) and 8,14 + 8,16$DiMeC_{32}$ (peak 42). In addition, two alkenes were also found ($C_{24:1}$ and $C_{26:1}$) in small quantities. The major peaks (> 5%) were identical in Gador, but completely different in Filabres. In SN and Gador, all the major peaks had more than 28 carbon atoms, whereas in Filabres, some major peaks were also found in the C_{24}–C_{28} region. The total percentage composition of peaks in the C_{24}–C_{28} range was 44.86% (±SD 8.65) in Filabres, whereas it was 14.65% (±6.15) and 23.31% (±8.26) for SN and Gador, respectively (Kruskal–Wallis $H = 14.34$, $P < 0.0001$; SN vs. Filabres, $P = 0.0005$; Gador vs. Filabres, $P = 0.06$; Gador vs. SN, $P = 0.18$).

Cluster analysis indicated that the three chemotypes of *R. minuchae* populations appeared clearly separated (Fig. 2). The Filabres chemotype was well separated from Gador and SN mainly owing to the absence of peaks 28 ($3C_{29}$), 31 (8,12- + 8,14$diMeC_{30}$) and 35 (4,8- + 4,10-diMeCO) and the predominance of short-chain hydrocarbons (Fig. S2). Furthermore, Gador and SN chemotypes were also differentiated, but only quantitatively.

Exactly, the same groupings were observed when the chemotypes of slaves were added to the cluster analysis, except FA-E27, which was misclassified in the SN group (Fig. 3). This confirms the similarity of the profiles between the slaves and parasites in each of these groups. However, within the same colony, the slave-makers and slaves rarely grouped together in the analysis (only one case, SNl-30) (Fig. 3). This indicates that, in mixed nests, the CHC profiles of slaves and parasites retain some quantitative differences and that CHC profiles of host ants altered after enslaved, as previously documented (Errard *et al.*, 2006).

The CHCs of the potential hosts (free-living nests scattered in the hot spots) clustered into three groups: Filabres were again completely separated, and Gador and SN shared the other two groups (Fig. 4).

The CHC differences between the parasite and free-living hosts (mean cuticular differences, Nei index), an indicator of the host–parasite recognition ability and of the current outcome of coevolution, were significantly lower in the SN population than in Gador and Filabres, which were similar to each other (Kruskal–Wallis $H = 9.9$, $P = 0.007$, Least Significant Difference (LSD) *post hoc* comparisons) (Fig. 5).

We found a strong positive correlation between chemical and geographical distances across all populations (Mantel test, $r = 0.56$; $P = 0.01$). However, when analysed separately, there was no correlation within Filabres and Gador populations (Mantel test: $P = 0.44$, $P = 0.90$ respectively) and a negative correlation within

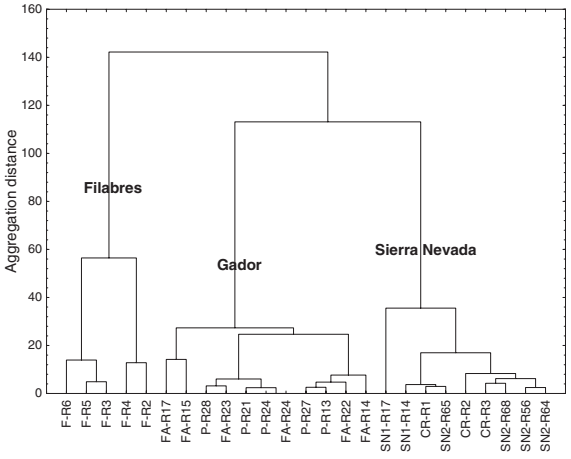

Fig. 2 Cluster analysis (Ward's method using Euclidian distances) based on the cuticular hydrocarbon profiles of *Rossomyrmex minuchae* (R) in the three isolated populations (F) Filabres [Fuente Alta (FA) and Pinos (P)] Gador, and [Sierra Nevada (SN) 1, SN2, and Casillas Rojas (CR)] SN. Numbers are colony codes.

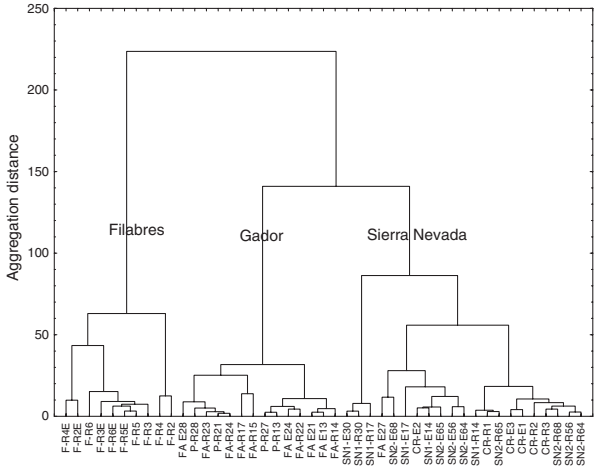

Fig. 3 Cluster analysis (Ward's method using Euclidian distances) based on the cuticular hydrocarbon profiles of *Rossomyrmex minuchae* (R) and the slaves (Enslaved *Proformica longiseta*) (E) in the three isolated populations, (F) Filabres [Fuente Alta (FA) and Pinos (P)] Gador, and [Sierra Nevada (SN) 1, SN2, and Casillas Rojas (CR)] SN. Numbers are colony codes.

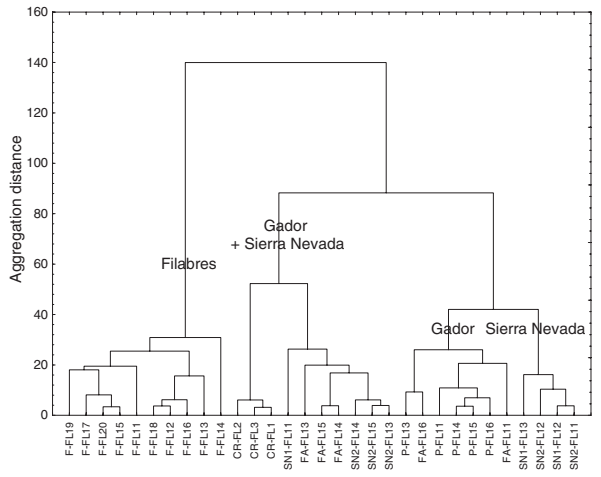

Fig. 4 Cluster analysis (Ward's method using Euclidian distances) based on the cuticular hydrocarbon profiles of free-living (FL) *Proformica longiseta* from (F) Filabres [Fuente Alta (FA) and Pinos (P)] Gador, and [Sierra Nevada (SN) 1, SN2, and Casillas Rojas (CR)] SN. Numbers are colony codes.

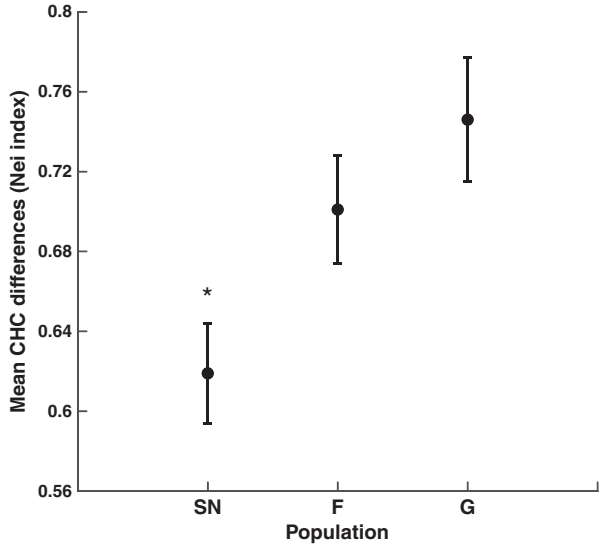

Fig. 5 Mean (±SE) cuticular hydrocarbon differences (Nei Index) between free-living host and parasite nests, compared between populations. The asterisk indicates a significant difference between Sierra Nevada and F (Filabres) + G (Gador) (Kruskal–Wallis test, LSD *post hoc* comparisons).

SN population ($r = -0.5$, Mantel test, $P < 0.01$) because of high distances between collecting sites.

Trait remixing

The estimated number of *R. minuchae* migrants between populations per generation after correction for size was 0.076, whereas this value for *P. longiseta* was 1.538. Although Whitlock & McCauley (1998) warn about the inaccuracy of gene-flow estimates based on indirect measures, such as microsatellite data, they also considered that these estimates are likely to be accurate within certain limits. Considering that the estimated differences in gene flow between *R. minuchae* and *P. longiseta* are very high, there is a good probability that they indicate differences in the migration rates between the species.

Across the parasite populations, there were strong positive correlations between CHC profiles and genetic differences (Mantel test, $r = 0.52$; $P = 0.01$), as well as between genetic and geographical distances (Mantel test, $r = 0.88$; $P = 0.01$). However, we found no or less correlation when comparing these variables within populations (chemical and genetic distances: Mantel test, SN $P = 0.16$, Filabres $P = 0.69$, Gador $r = 0.38$, $P = 0.02$; genetic and geographical distances: SN $P = 0.35$, Filabres $P = 0.27$, Gador $r = 0.36$, $P = 0.02$), at least for our sample sizes. Nevertheless, population differentiation between the two sample sites in Gador resulted in a low value of differentiation and nonsignificance ($F_{st} = 0.085$, $P = 0.06$). Thus, *R. minuchae* populations were chemically and genetically differentiated, with population-specific CHC profiles. In contrast, the nests within each sierra were found to be very similar for both the traits.

Discussion

Hot and cold spots

The three known populations (SN, Sierra de Gador and Sierra de Filabres) have probably been isolated since the intervening habitat became inhospitable because of a warming period during one of the last Pleistocene interglacial periods (Sanllorente *et al.*, 2010). According to the current distribution of the genus (Tinaut *et al.*, 2008), *Rossomyrmex* and its *Proformica* host could have arrived on the Iberian Peninsula during the Pliocene, coinciding with any of the reported invasions of vertebrates from Central Asia (Arribas *et al.*, 2001). It is possible that in the course of the climatic fluctuations of the Pleistocene, their populations migrated up and down the mountains, coming into contact at low altitude during some of the glacial maxima. The degree of differentiation found in our study suggests that the three populations were isolated much earlier than the time since the last glacial maximum (younger Dryas), which was reported to be warmer than the previous ones (Lomolino *et al.*, 2005) and might not have been cold enough to reunite the *Rossomyrmex* populations. Population differentiation in the Iberian Peninsula during the Pleistocene has also been reported in some species of birds, such as the azure-winged magpie (Fok *et al.*, 2002) and stonechats (Illera *et al.*, 2008) as well as

among other insects (Ribera & Vogler, *2004*; Cánovas *et al.*, 2008).

All these, together with the obligatory parasitism of *R. minuchae* and the important effect of its raids on host nests that caused the evolution of tolerance in SN population, lead us to assume that sympatric areas are hot spots.

Selection mosaic

The three *R. minuchae* populations appeared well differentiated in their hydrocarbon profiles, especially the Filabres population. Furthermore, the CHC distances (Nei distances) between the slave-makers and potential slaves (free-living workers) were significantly different in one population (SN) compared to the other two (Gador and Filabres). The highest similarity in the CHC profiles that we found between the parasite and its sympatric host population in SN may explain the behaviour of sympatric *P. longiseta*, which are less aggressive towards *R. minuchae* than allopatric individuals, sampled from an uninfected population in SN separated 5 km apart (Zamora-Muñoz *et al.*, 2003). Reduced aggression or tolerance (Svensson & Råberg, 2010) towards the parasite may be advantageous for *P. longiseta*, because *P. longiseta* workers die with high probability when fighting with *R. minuchae* (Zamora-Muñoz *et al.*, 2003), so that high host aggression could represent a large cost when the nest is under attack. However, when *P. longiseta* workers did not fight with the parasite, Zamora-Muñoz *et al.* (2003) found that *R. minuchae* became less aggressive during raids, allowing the survival of a fragment of the colony, normally including the queen.

Differences in Nei distances between Gador–Filabres to SN may indicate a selection mosaic for CHCs, which may be expressed as different ability of the host to detect and be aggressive against slave-maker invasions in each population, as has been observed in other cases or species (Nash *et al.*, 2008; Ugelvig *et al.*, 2008). Such different host–parasite aggression patterns have been shown between different host species and among populations of the same host species in other host–parasite systems (Bauer *et al.*, 2009).

The hydrocarbon profiles of slave-makers and slaves clustered together in the same population, but rarely in the same colony. This result confirms our previous data for the SN population showing CHC similarity between the host and parasite, increasing after enslavement, but not an exact congruence (Errard *et al.*, 2006), as each species retains small quantitative differences. This supports the observation that in mixed colonies, the host and the parasite are able to differentiate each other inside the colony.

The hydrocarbon profiles of free-living hosts clustered together for SN and Gador populations with respect to Filabres, appearing clearly separated. This probably occurs as a result of highest gene flow between these geographically closest populations, which were isolated after Filabres.

Therefore, host–parasite CHCs in each population are closer together than among populations of the same species, allowing to assume the existence of a unique coevolutionary process in each population.

Trait remixing

Estimated gene flow was an order of magnitude higher in the host species (*P. longiseta*) than in the parasite (*R. minuchae*). The number of migrants of *P. longiseta* may be somewhat overestimated, because females are wingless, and hence dispersal is carried out mainly by haploid males (Berg *et al.*, 1998). However, gene flow should not be much lower, because this finding is supported by previous studies showing weak genetic differentiation among its populations and the absence of isolation by distance within populations (Fernández-Escudero *et al.*, 2001). In the case of the parasite, males and females are winged and dispersal has been reported for both the sexes (Ruano & Tinaut, 2005), so estimates of gene flow may be more accurate than those for the host. Nevertheless, the great differences in estimates obtained for both the species suggest strong migration restriction for *R. minuchae*, when compared with *P. longiseta*.

To determine whether *R. minuchae* or *P. longiseta* have adapted their CHCs to each other, the gene flow and the previous results comparing the profiles of allopatric and sympatric host SN populations are crucial. We consider that the strongest selective pressure for *P. longiseta* must be the presence and host exploitation of the parasite, as environmental conditions are found to be basically the same in all the populations. However, selective environmental conditions may also have played a role in the differentiation of the host–parasite cuticular profiles in each population. An interesting point in this interaction is the isolation of *R. minuchae* populations when compared with *P. longiseta*. The main source of variation for the parasite should be genetic drift and/or mutation, whereas gene flow seems to be almost nonexistent. On the other hand, the host species is expected to be more influenced by migration owing to its large distribution range. Thus, our results are in agreement with Gandon *et al.* (1996), because the species with the highest migration rate (*P. longiseta*) appears to be locally adapted to the other, at least in SN. This conclusion is also in accordance with the difference in the genetic diversity found in both the species, being lower in the parasite (Sanllorente *et al.*, 2010) than in the host (Fernández-Escudero *et al.*, 2001; O. Sanllorente, F. Ruano & A. Tinaut, unpublished), probably leading to a better adaptation ability in *P. longiseta*.

Thus, assuming the existence of (i) hot and cold spots, (ii) a selection mosaic in CHC and aggressiveness among the populations and (iii) higher gene flow in *P. longiseta* than in *R. minuchae*, reflecting a probable

better adaptation ability of the host than the parasite, we find evidence to support the hypothesis of a geographical mosaic of coevolution in this host–parasite system. According to this, each host–parasite population is in a different coevolutionary time, as evidenced by the different CHC distances between parasites and hosts in each sierra, which probably produce different host strategies to minimize the effects of parasitism on fitness: from resistance to tolerance.

Therefore, we predict different host–parasite aggression patterns in different populations of the *P. longiseta*–*R. minuchae* system, ranging from some kind of resistance or avoidance of parasitism in Gador and Filabres *P. longiseta* populations to reduction in aggression or tolerance in SN (Zamora-Muñoz *et al.*, 2003), although this needs further experimental assessment.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Cuticular hydrocarbons composition (mean % ± SD) in each population of *Rossomyrmex minuchae* (R), *Proformica longiseta* slaves (E), and free-living *P. longiseta* (FL) from Sierra Nevada (SN), Filabres (F), and Gador (G).

Figure S1 Gas chromatograms of total body washes of 20 pooled workers of *Rossomyrmex minuchae* from Filabres (a), Gador (b), and Sierra Nevada (c). C is squalene contaminant.

Figure S2 Principal component analysis for cuticular hydrocarbon differences on the relative proportions of the 46 peaks identified for *Rossomyrmex minuchae* from three different populations (Sierra Nevada, Gador, and Filabres).

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