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ORIGINAL ARTICLE

There is more to the picture than meets the eye: adaptation for crypsis blurs phylogeographical structure in a lizard

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ABSTRACT

Aim We examined dorsal coloration in and genetic relationships among Iberian populations of the lizard *Psammodromus algirus* to determine the extent to which the current distribution of phenotypic variation is correlated with phylogeographical history or local environmental conditions.

Location Iberian Peninsula, western Palaearctic.

Methods We sequenced mitochondrial DNA (ND4 and adjacent tRNAs genes) in 36 populations, and seven microsatellite loci in eight representative populations. In 23 populations, lizards were classified according to the presence and intensity of a dorsal striped pattern, the heritability of which was estimated by means of mother–offspring regressions. To determine whether colour pattern is an adaptation for crypsis, we compared the time taken by humans to detect striped and unstriped lizards in different environments.

Results The analysis of mtDNA revealed an ancient split between a western clade, subdivided into south- and north-western haplogroups, and an eastern clade with central, south-eastern and eastern haplogroups. In contrast, nuclear markers showed a post-glacial admixture of central and western haplogroups, with the central haplogroup apparently isolated from the rest of its clade. This was consistent with variation in the dorsal striped pattern, a heritable phenotypic trait: central and western lizards were unstriped, whereas eastern lizards were striped. We then suggest that dorsal coloration promotes crypsis: in eastern locations detection times were longer for striped than for unstriped lizards, whereas the opposite was true in western and central locations.

Main conclusions Our results indicate that natural selection for crypsis may promote not only divergence within clades, as suggested by the apparent isolation between unstriped central lizards and striped members of eastern haplogroups, but also admixture between them. We conclude that ecologically driven selection is crucial for understanding the phylogeographical background of phenotypic variation, because recent adaptation to the environment can blur the effects of ancestral isolation.

Keywords

coloration, cytonuclear disequilibrium, heritability, Lacertidae, mtDNA polymorphism, predation, *Psammodromus*

INTRODUCTION

A central goal of evolutionary biology is to explain the morphological diversity observed within and across species. In fact, intraspecific variation, which is perhaps the most remarkable feature that is shared by all species, provides the raw material both for adaptation and speciation. For Darwin (1859), whose concept of species was largely morphological, the association between speciation and adaptation was straightforward: if speciation is defined by the accumulation

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of phenotypic differences between populations, then proof of speciation requires that such differences are demonstrably caused by natural selection (see review by Schluter, 2009). However, this link lost importance with the rise of the biological species concept (Mayr, 1942) and its emphasis on reproductive isolation rather than on adaptive differences. Nevertheless, recent decades have witnessed a revival of the idea that the macroevolutionary process of speciation results from ecologically based divergent selection, which is the kind of microevolutionary process that takes place at the level of intraspecific variation (Schluter, 2001, 2009; Rundle & Nosil, 2005; Rosenblum & Harmon, 2011). Intraspecific variation may arise for a number of reasons, such as geographical or genetic isolation, drift, mutation, migration, natural or sexual selection, phenotypic plasticity or a combination of any of these. As a consequence, the consistency between phenotypic differentiation among populations and underlying patterns of genetic variation can range between close match and outright disagreement. Depending on the amount of genetic structure, gene flow and the intensity of selection, populations of the same species adapted to different environments may cover the entire span between no apparent genetic structure and almost complete speciation (Rosenblum, 2006; Rosenblum & Harmon, 2011).

Here, we focus on the extent to which phenotypic variation is correlated with local environmental conditions versus phylogeographical history (Hoekstra *et al.*, 2005). Phylogeography has extended phylogenetic thinking to the intraspecific level (Avise *et al.*, 1987), allowed the geographical determinants of isolation to be tracked (Hewitt, 1996; Godinho *et al.*, 2008), and shed light on how demographic processes have shaped genetic and phenotypic variation at the interface between populations and species (Hickerson *et al.*, 2010). Within this framework, the Iberian Peninsula offers ample opportunities to compare the roles of phylogeographical history and environment in shaping phenotypic variation: it enjoys a variety of Mediterranean and Atlantic climates and has several east–west oriented mountain ranges that allow survival of species in the face of climate changes by altitudinal shifts (Hewitt, 1996). These characteristics, together with its large area, favoured the occurrence of multiple refugia during the Pleistocene glaciations (Gómez & Lunt, 2007). Multiple refugia are especially common for reptiles, whose low mobility and dependence on thermal conditions should have promoted fragmentation of populations in suitable habitats, facilitating the appearance of phylogeographical structure (Gómez & Lunt, 2007).

In this study, we analyse intraspecific differentiation in the large psammodromus *Psammodromus algirus* (Lacertidae; Linnaeus, 1758), a medium-sized (adult snout-vent length 60–90 mm) lizard which is widely distributed in shrub and woodland habitats of the Iberian Peninsula, south-eastern France and north-west Africa. This species consists of two reciprocally monophyletic mtDNA clades (Verdú-Ricoy *et al.*, 2010): an eastern clade confined to Iberia, and a western clade present in both Iberia and North Africa. Both clades diverged approximately between 3.6 and 3 Ma (Carranza *et al.*, 2006; Fitze *et al.*, 2011). We collected genetic and phenotypic data to set the phylogeographical background of phenotypic and current genetic variation. We characterized interpopulation differences in coloration according to the development of a dorsal striped pattern, a trait that has been regarded as an adaptation for crypsis in other taxa (Karpestam *et al.*, 2013). We also performed two experiments to test the effects of coloration on detectability by simulated (human) predators, and we measured habitat features potentially related to visibility for aerial predators. Finally, we estimated the heritability of the dorsal striped pattern by assessing its development in females and in their offspring. We used our data to test the following hypotheses: (1) If history is the only source of phenotypic differentiation, then the geographical distribution of the striped pattern should match that of the mtDNA clades; (2) alternatively, if local adaptation is strong enough to overcome the effects of history and gene flow, then no consistent relationship between the geographical distribution of the striped pattern and the mtDNA clades should be expected; and (3) if both local environmental conditions and phylogeographical history are important determinants of phenotypic variation, then the distribution of the striped pattern should match that of the mtDNA clades only in part. This latter hypothesis would also imply that recent adaptation to the environment can blur the effects of ancestral isolation, promoting either divergence within clades (if lizards of the same clade show different striped or unstriped patterns) or convergence between them (if lizards from different clades show the same patterns).

MATERIALS AND METHODS

Field sampling

We sampled 36 *P. algirus* populations (Table 1) for DNA extraction between April and June of 2006 and 2008–2012; 23 of these populations were also employed for phenotypic characterization of lizards, although not necessarily at the same time or using the same individuals in a given population. Lizards were noosed or captured by hand. When necessary (see below), lizards were transported to the laboratory and housed under the standard conditions described in Díaz *et al.* (2012). After laboratory work had finished, all animals were released at their site of capture.

Genetic variation

Mitochondrial DNA variation was studied on a sample of 319 individuals from 36 populations (Table 1). A fragment of the fourth subunit of the NADH dehydrogenase (ND4) and adjacent tRNAs (His, Ser and Leu) genes was amplified using primers ND4 and Leu (Arévalo *et al.*, 1994). Resultant sequences (length: 849 bp) were used to construct a haplotype network with gene genealogy software TCS 1.21

Table 1 Genetic and phenotypic characterization of 36 populations of the lizard *Psammodromus algirus* from the Iberian Peninsula. For each population, the following data are shown: sample size for mtDNA (N), haplotypes and haplogroups encountered (with the same codes as in Fig. 1a), number of unique haplotypes (N_U), haplotype diversity (H_D), nucleotide diversity (π, equal to the mean number of nucleotide changes among sequences), frequency of striped lizards (F_{STR}, with sample size in parenthesis) and frequency of striped lizards with a weak pattern of dorsal stripes ($F_{W\text{-}S}$). Population numbers as in Fig. 3(b).

No.	Population	Latitude (°N)	Longitude (°E)	N	Haplotypes	Haplogroup(s)	N_U	H_D	π	F_{STR}	$F_{W\text{-}S}$
1	Navacerrada*,†	40.726	−4.023	9	1–3	W2	2	0.417	0.44	0.17 (36)	0.83
2	El Pardo*,†	40.511	−3.755	9	1, 4–5	W2	2	0.556	1.39	0.20 (35)	1.00
3	Sotos de Sepúlveda	41.267	−3.567	8	1, 6–7	W2	2	0.714	0.86	–	–
4	Aldea del Fresno*,†	40.330	−4.244	8	1	W2	0	0.000	0.00	0.00 (8)	–
5	Cabañas de Tera*,†	42.011	−6.056	13	1, 8	W2	1	0.154	0.15	0.17 (23)	0.75
6	Pallarés*,†	38.057	−6.184	12	9–16	W1+W2	7	0.924	6.18	0.00 (23)	–
7	Helechosa de los Montes*	39.314	−4.900	5	11	W1	0	0.000	0.00	0.00 (5)	–
8	Villuercas*,†	39.468	−5.341	16	11, 42	W1+E3	0	0.525	24.68	0.20 (20)	1.00
9	Aranjuez*,†	40.016	−3.586	8	17–18	E2	1	0.250	0.25	1.00 (42)	0.29
10	Torrejoncillo del Rey*	40.035	−2.599	9	18	E2	0	0.000	0.00	0.95 (21)	0.15
11	Lerma	42.134	−3.650	30	18–20	E2	2	0.628	1.23	–	–
12	Hoces del Cabriel*	39.542	−1.518	15	18, 21–24	E2	3	0.562	0.65	1.00 (20)	0.15
13	Pioz	40.443	−3.161	7	18, 25	E2	1	0.286	0.29	–	–
14	San Martín de la Vega*	40.187	−3.544	7	18	E2	0	0.000	0.00	0.78 (9)	0.29
15	Villatobas*	39.839	−3.259	5	18	E2	0	0.000	0.00	1.00 (5)	0.00
16	Saelices	39.873	−2.788	4	18	E2	0	0.000	0.00	–	–
17	Villar de la Encina	39.627	−2.492	2	18, 26	E2	1	1.000	1.00	–	–
18	Vara de Rey	39.422	−2.320	4	18	E2	0	0.000	0.00	–	–
19	Villares del Saz*	39.856	−2.541	8	18, 27	E2	1	0.250	0.25	0.75 (8)	0.33
20	Vellisca*	40.139	−2.813	6	18, 28	E2	1	0.333	0.33	1.00 (6)	0.00
21	Honrubia	39.586	−2.220	4	18, 24	E2	0	0.500	0.50	–	–
22	Brihuega*	40.766	−2.930	10	18	E2	0	0.000	0.00	0.67 (12)	0.88
23	El Frasno	41.421	−1.489	12	18	E2	0	0.000	0.00	–	–
24	Abrucena*,†	37.119	−2.812	13	29–31	E1+E2	3	0.641	10.77	1.00 (21)	0.48
25	Morata	37.588	−1.555	9	18, 32–34	E1+E2	3	0.750	14.33	–	–
26	Valdepeñas	38.901	−3.477	10	35–38	E3	4	0.733	1.64	–	–
27	Despeñaperros	38.375	−3.519	7	39–42	E3	3	0.714	2.19	–	–
28	Pelahustán*,†	40.180	−4.607	10	42–43	E3	1	0.200	0.40	0.19 (31)	1.00
29	Monte de Batres*	40.224	−3.938	11	42, 44–45	E3	2	0.564	0.62	0.00 (11)	–
30	Talavera de la Reina*	39.936	−4.820	7	42, 46–49	E3	4	0.857	1.62	0.00 (7)	–
31	Villarejo de Montalbán*	39.760	−4.568	11	42, 50–52	E3	2	0.691	0.95	0.00 (11)	–
32	Malpica de Tajo*	39.912	−4.545	8	42, 50, 53–54	E3	2	0.750	1.89	0.00 (8)	–
33	San Román de los Montes	40.084	−4.730	3	42, 55–56	E3	2	1.000	2.00	–	–
34	Espinoso del Rey	39.652	−4.788	3	42	E3	0	0.000	0.00	–	–
35	Noez*	39.747	−4.197	10	42, 57–58	E3	2	0.644	0.73	0.00 (10)	–
36	Puerto Lápice*	39.328	−3.490	5	59–60	E3	2	0.600	1.20	0.40 (5)	0.50

*Populations with frequency data of striped lizards.

†Populations with microsatellite data.

(Clement *et al.*, 2000). The resulting network confirmed the existence of five haplogroups separated by more than 10 mutational steps and grouped into western and eastern clades with two (W1 and W2) and three (E1, E2 and E3) haplogroups respectively (see Results). Haplogroup identity was used as a categorical predictor in subsequent analyses.

In addition, seven microsatellite loci specifically designed for *P. algirus* (Bloor & Dávila, 2008) were amplified in eight populations (Fig. 1), which were chosen to represent localities either close to the borders of the distribution range or in the putative limit between eastern and western mitochondrial lineages (Verdú-Ricoy *et al.*, 2010). Microsatellite data were analysed with Peak Scanner™ 1.0 (Applied Biosystems, Carlsbad, California, USA). We used Arlequin 3.5.1 (Excoffier *et al.*, 2005) to test for deviations from Hardy–Weinberg equilibrium and for linkage disequilibria and to assess the extent of differentiation among mtDNA clades (estimated as the mean number of nucleotide changes). We used structure 2.3.3 to detect clusters that minimize Hardy–Weinberg and linkage disequilibria (Pritchard *et al.*, 2000). The number of ancestral clusters (K) was set to 4 after comparing log-likelihood ratios in multiple runs ($n = 20$ for each value of K between 1 and 12). This was the value that maximized mean log-likelihood ratio (Evanno *et al.*, 2005).

Finally, we combined mtDNA and microsatellite data to estimate migration rates between pairs of populations with IMa2, a coalescent-based method that uses loci from two or more populations and Markov chain Monte Carlo

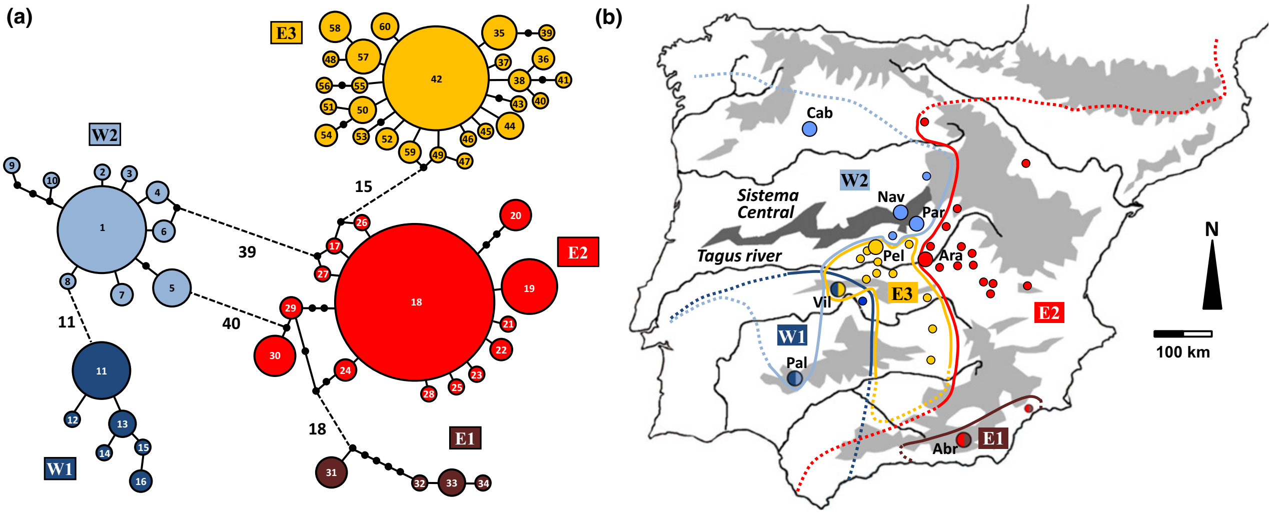

Figure 1 Phylogeography of the lacertid lizard *Psammodromus algirus* in Spain, western Palaearctic. (a) Haplotype network obtained from 319 sequences of an 849 bp mtDNA fragment (ND4 and associated tRNAs genes). Circle sizes are proportional to haplotype frequencies. Lines connecting circles represent single mutational steps, black dots represent missing intermediate haplotypes and dashed lines between circles represent larger number of mutational steps (as indicated with large, black numbers in the figure). Haplotypes numbers, shown inside the circles, correspond to those in Table 1. Different colours are used for each of the five haplogroups grouped into western (haplogroups W1 and W2) and eastern (haplogroups E1, E2 and E3) clades. (b) Physical map of the Iberian Peninsula showing the location of sampled populations, with population numbers as in Table 1; as well as the main mountain ranges. Populations with microsatellite data are shown with larger dots, and identified by acronyms as in Fig. 3. Lines show the putative distribution of the five haplogroups in the network, according to sampled populations (continuous lines) and to previous data by Carranza *et al.* (2006) and Verdú-Ricoy *et al.* (2010) (discontinuous lines). Populations with more than a single haplogroup (Pallarés, Villuercas and the two populations with E1 haplotypes) are indicated with double-coloured dots.

simulations of gene genealogies to estimate the posterior density of various parameters that are part of an 'isolation with migration' model (Hey, 2010).

A detailed explanation of all the genetic analyses performed can be found in Appendix S1.

Phenotypic variation and habitat characteristics

In a subsample of 23 populations (Table 1), lizards were assigned to a colour category according to the presence, length and intensity of a mid-dorsal dark stripe (see Appendix S1). In 14 of these populations, we had a large enough sample size ($n \geq 10$) to estimate the frequency of striped lizards with enough precision to model its dependence on landscape features. To assess vegetation cover at each of these 14 localities we walked a 1-km transect stopping every 10 m. At each stop, we placed a marked stick standing vertically on ground level to assess whether vegetation was contacted at 0, 10, 30, 50 or 100 cm height. If so, the type of the plant(s) contacted was noted (grass, deciduous shrub or perennial shrub). We also considered the presence or absence of leaf litter on the ground and of tree canopy over the vertical projection of the stick. Partial least squares regression (PLS), an extension of multiple regression explicitly designed to deal with numerous and highly collinear predictors (Carrascal *et al.*, 2009), was employed to model the relationship between the frequency of striped lizards and habitat variables.

Crypsis

We tested the influence of dorsal coloration on the detection times of lizards by humans, as a proxy for visually oriented natural predators. We chose two eastern (E2) and two western (W2) populations, and we captured three adult males at each of the four sites. All lizards from eastern populations were striped, and all lizards from western populations were unstriped. Pictures of lizards were taken at the four different capture sites by putting cold-anaesthetized lizards in randomly chosen realistic positions. Human detection times were tested by showing the images ($N = 48$, 3 lizards × 4 populations × 4 pictures) to a group of 12 researchers who were asked to find the location of the animal. Observers were questioned one by one, blindly with respect to others' performance. Upon detection, time was measured with a chronometer to the nearest 0.5 s, up to a maximum searching time of 1 min. Photographs were presented to subjects in a randomized order that was the same for all observers, thus leaving habitat, phenotype, habitat × phenotype interaction, observer (which was the only random factor) and error, as the only possible statistical sources of variation in detection times.

Because lizards belonging to the E3 haplogroup were genetically closer to the eastern striped lizards, but phenotypically closer to the western unstriped ones (see Results), we also compared the detectability of eastern unstriped (E3), western unstriped (W2) and eastern striped (E2) lizards at a single E3 location (Pelahustán) in a second experiment. Our aim was to verify if the colour pattern of E3 lizards would decrease their detectability in their own environment, and if their detection times would be more similar to those of W2 unstriped lizards than to those of striped lizards. We captured three adult males from the nearby populations of Aranjuez (E2, striped), El Pardo (W2, unstriped) and Pelahustán (E3, unstriped). Lizards were photographed, and the photographs examined by 14 researchers, following the aforementioned protocols.

Heritability

During the 2013 breeding season, we captured and transported 26 gravid female lizards to the laboratory from El Pardo, Pelahustán and Aranjuez, to assess the mother-to-offspring heritability of the dorsal coloration pattern, which is present from hatching. Female lizards were scored from 0 to 5 by five independent observers according to the presence, extension and intensity of the dorsal dark stripes (see Appendix S1). We checked gravid females daily to detect egg laying. After a female had laid a clutch, the eggs were removed and incubated until hatching. After hatching, juveniles were marked, weighed, measured and kept in individual terraria. When all hatchlings had emerged from the eggs, they were assigned a dorsal coloration score by the same observers. To assess whether interindividual variation in dorsal coloration is heritable, we tested the significance of the standardized regression coefficient of offspring mean scores on dam mean scores (which equals half the heritability, because only one parent's value is used). A detailed explanation of all the analyses performed can be found in Appendix S1.

RESULTS

Phylogeographical patterns based on mtDNA variation

We obtained 60 haplotypes from the analysis of 319 mtDNA sequences from 36 populations (Table 1). Of these, only six (haplotypes 1, 11, 18, 24, 42 and 50) were found in two or more populations; the remaining 54 haplotypes were unique to one population each, indicating strong genetic population structure. The resulting haplotype network had a strong phylogeographical signal (Fig. 1), with two main clades matching the eastern and western reciprocally monophyletic clades obtained by Carranza *et al.* (2006) and Verdú-Ricoy *et al.* (2010). The western clade had south-western (W1) and north-western (W2) haplogroups, whereas the eastern clade had a south-eastern haplogroup (E1), a widespread oriental haplogroup (E2) whose distribution range contained and surrounded that of E1, and a central haplogroup (E3) partly overlapping the range of the western clade (Fig. 1b). Interestingly, most populations had all haplotypes from a single haplogroup, the only exceptions being Abrucena and Morata (with E1 and E2 haplotypes), Pallarés (W1 and W2) and Villuercas (E3 and W1). Mean divergence between eastern and western clades was 5.73%; divergence between haplogroups was larger for the eastern clade (2.97%, 3.28% and 2.47% for E1-E2, E1-E3 and E2-E3 respectively) than for the western one (1.71% for W1-W2).

Visual inspection of the haplotype network revealed two different types of population histories. The topology showed by southern haplogroups (W1 and E1) suggested stable demographic histories, whereas W2, E2 and especially E3 showed star-shaped topologies typical of populations that have experienced a recent demographic expansion. This was supported by R_2 estimates (based on the difference between the number of singleton mutations and the average number of nucleotide differences; Ramos-Onsins & Rozas, 2002), which were low and significant for W2 ($R_2 = 0.048$, $P = 0.017$), E2 ($R_2 = 0.028$, $P = 0.006$) and E3 ($R_2 = 0.024$, $P < 0.001$), indicating ratios of singletons that are higher than expected under the assumption of constant population size and consistent with the hypothesis that rapid demographic growth has led to the accumulation of recent mutations in the outermost branches of the network. On the other hand, values for E1 ($R_2 = 0.246$, $P = 0.943$) and W1 ($R_2 = 0.105$, $P = 0.220$) did not depart significantly from the expectations of constant population size.

Latitude was negatively correlated with haplotype diversity ($r = -0.322$, $N = 36$, $P = 0.055$), total number of haplotypes ($r = -0.374$, $P = 0.025$), number of unique haplotypes ($r = -0.426$, $P = 0.010$) and nucleotide diversity ($r = -0.469$, $P = 0.004$), indicating a greater genetic diversity in southern populations.

Genetic structure according to microsatellites

All microsatellite loci were polymorphic for all populations under study except for locus Psal4 (it was monomorphic at Abrucena, Aranjuez, Cabañas de Tera and Pallarés). None of these loci deviated significantly from Hardy–Weinberg equilibrium after applying the sequential Bonferroni correction; 10 of 52 cases were significant at the unadjusted level. Twelve of 144 tests of linkage disequilibrium were nominally significant, but none of them remained significant after sequential Bonferroni adjustment, supporting the independent assortment of alleles at different loci.

Microsatellite-based Bayesian population assignment tests performed with STRUCTURE, after establishing the most likely number of ancestral clusters at $K = 4$, identified several groups of populations (Fig. 2): one formed by north-western lizards (Cabañas de Tera, from haplogroup W2), another one formed by the two southernmost populations sampled, including both western (Pallarés, with W1 and W2 haplotypes) and eastern (Abrucena, with E1 and E2 haplotypes) lizards, and two additional groups located south of the Sistema Central mountain ranges: a central-eastern group including a single population of striped lizards (Aranjuez, from haplogroup E2) and a central-western group

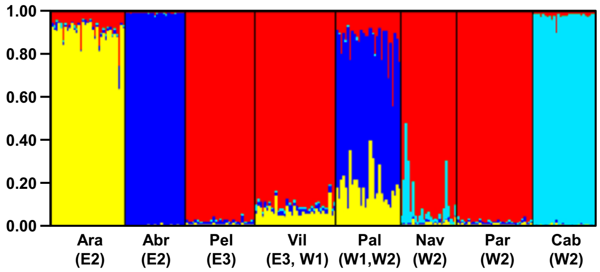

Figure 2 Genetic structure according to seven microsatellite loci of eight Iberian populations of the lizard *Psammodromus algirus*. Results of Bayesian assignment tests with the software STRUCTURE uncovered four distinct clusters represented with different colours. Each vertical bar represents a single individual (N = 33, 27, 31, 36, 29, 25, 34 and 29 for Aranjuez, Abrucena, Pelahustán, Villuercas, Pallarés, Navacerrada, El Pardo and Cabañas de Tera respectively; mtDNA haplogroups represented in each population are also indicated). Bar colours represent posterior probabilities of cluster membership. Note the similarity between the four central-western populations located south of the Sistema Central mountain range and assigned mainly to the red cluster.

comprising four populations of unstriped lizards with E3, W1 and W2 haplotypes (Villuercas, Pelahustán, El Pardo and Navacerrada). Thus, population structure depicted by microsatellites was inconsistent with the phylogeographical scenario deduced from mtDNA sequences. This was supported by the AMOVA results (Table 2), in which grouping of nuclear markers according to eastern versus western clades revealed no significant structure between groups (F_{ST} and $R_{\mathrm{ST}} = 0$, both $P > 0.75$).

Estimates of gene flow

Migration rates provided by IMa2 (Table 3) confirmed the pattern of genetic structure depicted by microsatellites. Most values of *m* significantly greater than zero (and all *m* values > 1) referred to pairs of populations within the central-western group defined by STRUCTURE (El Pardo, Navacerrada, Pelahustán and Villuercas). In contrast, no gene flow was detected between these populations and the nearby location of Aranjuez, despite its relatively short linear distance from El Pardo and Pelahustán.

Phenotypic variation and its association with habitat features

A one-way ANOVA comparing the frequency of striped lizards among the three haplogroups with enough data (E2, E3 and W2, with 8, 7 and 4 populations respectively) showed that E2

Table 2 AMOVAs with microsatellite data for the lizard *Psammodromus algirus* from the Iberian Peninsula.

	F-statistic	% var	*P*	*R*-statistic	% var	*P*
mtDNA clade						
Among groups	−0.004	−0.40	0.758	−0.030	−3.01	0.828
Among populations within groups	0.058	5.83	< 0.001	0.190	19.61	< 0.001
Within populations	0.054	94.57	< 0.001	0.166	83.40	< 0.001
Groups generated by STRUCTURE						
Among groups	0.042	4.25	< 0.001	0.132	13.17	< 0.001
Among populations within groups	0.017	1.65	< 0.001	0.060	5.21	< 0.001
Within populations	0.059	94.10	< 0.001	0.184	81.62	< 0.001

Note: data were classified according to (1) population of origin nested within mtDNA clade (populations with western haplotypes: Cabañas de Tera, El Pardo, Navacerrada and Pallarés; populations with eastern haplotypes: Abrucena, Aranjuez and Pelahustán); and (2) population of origin nested within groups generated by Structure (group 1: Cabañas de Tera; group 2: Abrucena and Pallarés; group 3: Aranjuez; group 4: El Pardo, Navacerrada, Pelahustán and Villuercas).

Table 3 Gene flow between pairs of populations of the lizard *Psammodromus algirus* on the Iberian Peninsula, based on IMa2 estimates.

	Aranjuez	Abrucena	Pelahustán	Villuercas	Pallarés	Navacerrada	El Pardo	Cabañas
Aranjuez	–	0.19	0.05	0.00	0.09*	0.00	0.01	0.00
Abrucena	0.02	–	0.06	0.00	0.00	0.00	0.00	0.00
Pelahustán	0.01	0.04	–	2.34*	0.05	0.98**	3.09*	0.00
Villuercas	0.00	0.01	0.17	–	0.02	0.14	0.24*	0.01
Pallarés	0.03	0.07	0.08	0.12	–	0.00	0.00	0.01
Navacerrada	0.10*	0.00	0.64*	0.81***	0.04	–	0.00	0.04
El Pardo	0.00	0.03	2.45*	1.23*	0.33*	2.00***	–	0.00
Cabañas	0.00	0.00	0.00	0.02	0.04	0.04	0.06*	–

Note: IMa2 estimates of mutation-scaled migration rate (*m*) between the donor (row) and recipient (column) population. Significance according to log-likelihood ratio tests: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

lizards were dominantly striped, whereas E3 and W2 ones were mostly unstriped (ANOVA with the data in Fig. 3, proportions arcsine-transformed: $F_{2,16} = 40.64$, $P < 0.001$; post hoc comparisons: $P < 0.001$ for E2 versus E3 and E2 versus W2, and $P = 0.664$ for E3 versus W2). Data from four additional populations (Helechosa, Pallarés, Abrucena and Villuercas; see Table 1) allowed us to tentatively extend this pattern to the remaining haplogroups, leading us to conclude that western and E3 lizards were mostly unstriped, whereas E1 and E2 lizards were striped (Fig. 3); overall, the average percentage of striped lizards was 90.5% for eastern populations (E1 and E2) and 9.5% for central and western ones (E3, W1 and W2).

Concerning habitat associations, a PLS analysis with frequency of striped lizards as the dependent variable yielded a single significant factor that explained 39.6% of the variance in the response variable ($F_{1,12} = 7.86$, $P = 0.016$). The predictors that contributed significantly to that factor (plant cover 1 m above ground level, tree cover and cover of deciduous shrubs) explained 84% of the variance in X-scores (the values that each population attains along the single factor derived from the PLS analysis; Fig. 4). In addition, X-scores were also positively correlated with longitude ($r = 0.592$, $N = 14$, $P = 0.026$), and they differed significantly among localities with western (W1 and W2), central (E3) and eastern (E1 and E2) haplogroups (Fig. 4: $F_{2,10} = 5.82$, $P = 0.021$), defining an east–west gradient of increasing vegetation cover.

Experiments on visual detectability of lizard phenotypes

Results of our first experiment showed that lizards were detected faster outside their natural environments: at eastern sites unstriped lizards were detected more rapidly than striped ones, and the opposite was true at western sites (site × phenotype interaction in mixed model ANOVA with phenotype as fixed factor and site and observer as random factors: $F_{3,33} = 92.43$, $P < 0.001$; Fig. 5). In our second experiment, detectability at Pelahustán differed significantly among lizards captured at Aranjuez (E2), El Pardo (W2) and native (E3) lizards (population effect in mixed model ANOVA with observer as a random factor: $F_{2,28} = 9.73$, $P = 0.0006$). Striped lizards from Aranjuez were detected faster (mean ± standard error = 19.2 ± 2.7 s) than unstriped lizards, either native (29.7 ± 3.1 s) or captured at El Pardo (39.2 ± 3.7 s; post hoc comparisons: Aranjuez versus Pelahustán $P = 0.031$, Aranjuez versus El Pardo $P = 0.001$ and Pelahustán versus El Pardo $P = 0.554$).

Heritability of dorsal coloration pattern

Overall, we obtained 27 effective clutches (with at least one viable egg) from 22 females: six from Aranjuez (from five females, one of which laid two clutches), 10 from El Pardo (one clutch per female) and 11 from Pelahustán (from seven females, five of which laid two clutches, including one whose first clutch was entirely composed of eggs that failed to hatch). Mean numbers of hatchlings per clutch (± SD) were 2.8 ± 1.9, 4.4 ± 1.1 and 5.5 ± 1.4 respectively. Mean colour scores of adults (± SD) at each of the three populations, pooling together males and females because sexual dimorphism was not significant, were 3.5 ± 1.3, 0.6 ± 0.5 and 0.9 ± 1.1 respectively ($F_{2,37} = 31.97$, $P < 0.001$).

The regression of mean offspring score on mean dam score was significant, with a standardized regression coefficient (± SE) of 0.74 ± 0.06 ($F_{1,119} = 148.38$, $P < 0.001$); that is, juvenile lizards had a dorsal pattern strongly similar to that of their mothers. Within-population regressions were significant for Aranjuez ($\beta = 0.82 \pm 0.15$, $F_{1,15} = 31.81$, $P < 0.001$) and Pelahustán ($\beta = 0.58 \pm 0.11$, $F_{1,58} = 29.53$, $P < 0.001$), but not for El Pardo ($\beta = 0.11 \pm 0.15$, $F_{1,42} = 0.54$, $P = 0.465$), probably due to the lack of variation in mean adult scores of this later population (Levene tests; Aranjuez versus El Pardo $P = 0.021$, Pelahustán versus El Pardo $P = 0.023$, Aranjuez versus Pelahustán: $P = 0.731$).

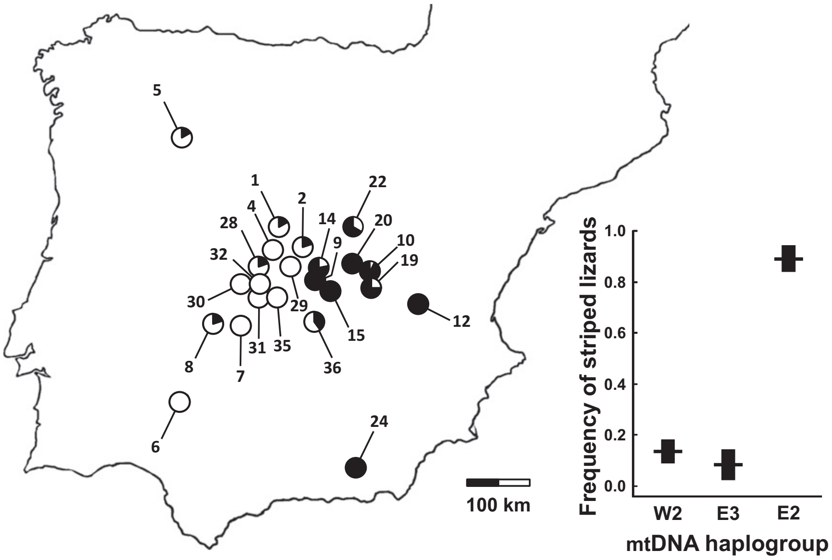

Figure 3 Left: frequencies of striped lizards (in black) in 23 Iberian populations of the lacertid lizard *Psammodromus algirus*, with population numbers as in Table 1. Right: haplogroup averages (± 1 SE) for W2 ($N = 4$ populations), E3 ($N = 7$) and E2 ($n = 8$). Whereas E2 lizards are mostly striped, E3 and W2 ones are mainly unstriped ($F_{2,16} = 40.64$, $P < 0.001$).

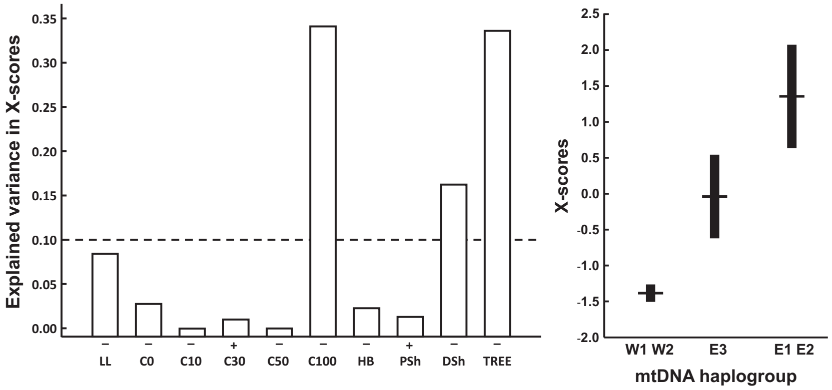

Figure 4 Habitat features associated with the frequency of striped individuals in Iberian populations of the lizard *Psammodromus algirus*: results of a partial least squares analysis (PLS) used to test for the relationship of the habitat variables considered (LL = leaf litter cover, C0–C100 = plant cover 0, 10, 30, 50 and 100 cm in height, HB = grass cover, PSh = cover of perennial shrubs, DSh = cover of deciduous shrubs and TREE = tree cover) with the frequency of striped lizards. Left: relative contribution of the habitat variables to the multivariate factor (X) predicting the frequency of striped lizards; variables with squared weights > 0.10 (dashed line) are significant. Right: mean X-scores (± 1SE) of western (W1 and W2), central (E3) and eastern (E1 and E2) populations. X-scores differ significantly among these groups ($F_{2,10}$ = 5.82, P = 0.021), defining an east–west gradient of increasing vegetation cover development.

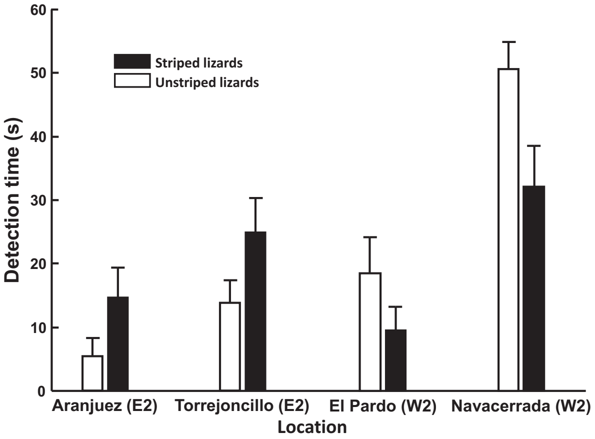

Figure 5 Detectability of two phenotypes (striped and unstriped; see Figure S1) of the lacertid lizard *Psammodromus algirus* in four populations located central Spain: detection times of striped and unstriped lizards (mean ± 1 SE) at two eastern localities of the E2 haplogroup where most lizards are striped (Aranjuez and Torrejoncillo) and two western localities of the W2 haplogroup where most lizards are unstriped (El Pardo and Navacerrada). The significant interaction between site and phenotype ($F_{3,33}$ = 92.43, P < 0.001) shows that lizards are detected faster outside their natural environments, and suggests that dorsal colour pattern is an adaptation for crypsis.

DISCUSSION

Phylogeographical structure and phenotypic variation

Our phylogeographical analyses of mtDNA confirmed the differentiation of eastern and western Iberian clades whose separation took place between 3 and 3.6 Ma, i.e. after the last opening of the Strait of Gibraltar and before the beginning of Pleistocene glaciations (Carranza *et al.*, 2006; Verdú-Ricoy *et al.*, 2010). Similar patterns of east–west differentiation have been described for other taxa, most noticeably for the related species *Psammodromus hispanicus* (Fitze *et al.*, 2011) and for perennial and deciduous oaks (*Quercus* spp.; Rodríguez-Sánchez *et al.*, 2010) that form the cleared forests where these lizards reach their highest population densities (western clade: Díaz & Carrascal, 1991; Díaz, 1997; eastern clade: Zamora-Camacho *et al.*, 2013). The similarity in the phylogeographical structure of the two *Psammodromus* species suggests a shared history of Plio–Pleistocene events, including long periods of isolation in similar glacial refugia and subsequent latitudinal and altitudinal expansions as the climate became warmer (from the coasts towards the central plateaux, or tracking up suitable microclimates along east–west oriented mountains).

As a result of these historical processes, *P. algirus* shows the typical pattern of latitudinal loss of genetic diversity caused by Quaternary glaciations (‘southern richness and northern purity’; Hewitt, 1999): the most basal haplotypes in both clades come from southern localities (Verdú-Ricoy *et al.*, 2010), and there are negative correlations between latitude and genetic diversity. Lower diversity at higher latitudes could reflect a rapid colonization of suitable habitat, with repeated founding events along the expansion edge leading to homozygosity and loss of alleles (Hewitt, 2000). However, an alternative explanation for the higher diversity of southern populations could be their secondary contact with anciently differentiated northern haplogroups that, after having survived the ice ages in valley bottoms or coastal areas (Gómez & Lunt, 2007), would have expanded their range in the current interglacial period. This second scenario is supported by the star-shaped topology of northern haplogroups in the haplotype network (W2, E2 and especially E3) and by their low and significant R_2 estimates (Ramos-Onsins & Rozas, 2002), which are typical of populations that have expanded recently and rapidly (Slatkin & Hudson, 1991; Avise, 2000). Such demographic expansions, which could have produced similar patterns in mitochondrial polymorphisms as those detected in our study, seem to have continued until significant geographic barriers have been encountered by dispersing individuals (e.g. no W1 haplotypes have been found north of the Tagus river, and no E3 haplotypes have been found north of the Sistema Central mountain ranges). This interpretation implies the existence of multiple Iberian refugia for *P. algirus*, and it is consistent with the role of glacial refugia in southern Europe not only as hotspots but also as melting pots of genetic diversity (Gómez & Lunt, 2007; Godinho *et al.*, 2008).

Within this context, the E3 haplogroup, which occupies an intermediate position between the western clade (W1+W2) and the remaining eastern haplogroups (E1+E2), deserves special attention because of its unstriped dorsal coloration, its genetic admixture with western lizards, and its transitional type of habitat (see below). The adjustment of mismatch distribution of pairwise differences, which in the case of E3 fits well to the Poisson distribution expected under a model of population expansion (see Appendix S2), further supports the recent spread of this group. Such expansion would have produced a secondary contact with western lizards, demonstrated by the mitochondrial polymorphism between W2 and E3 found at Villuercas, but not with eastern, unstriped lizards belonging to the E2 clade. In fact, E2-E3 is the only possible mtDNA polymorphism that has not been found in the southern half of Iberia. All this evidence, together with estimates of recent gene flow showed by the microsatellite data, suggests the contemporary existence of two separate gene pools in central Spain, one corresponding to striped lizards (E2) and the other to unstriped ones (E3, W1 and W2). Thus, phylogeographical structure revealed by the mtDNA data is unable to explain the current distribution of a heritable phenotypic trait that is functionally important and shows convergence between, and divergence within, different branches of the phylogeographical tree. In the following sections we try to shed light on these findings, which seem to support our third initial hypothesis, namely that recent adaptation to the environment by natural selection for crypsis has blurred the effects of ancestral phylogeographical structure.

Dorsal coloration as adaptation for crypsis

Results of our common-garden breeding experiment indicate that a substantial part of the interindividual variation in the pattern of dorsal stripes is heritable. Thus, dorsal coloration should be able to evolve in response to natural selection. Interestingly, the proportion of striped lizards with a weakly striped pattern was negatively correlated with the overall frequency of striped lizards ($r = -0.874$, $n = 15$, $P < 0.001$). In other words, populations tend to be composed either mainly of striped lizards with a marked pattern of dorsal stripes, or of unstriped lizards with a minority of weakly striped individuals. This is the sort of bimodal expression of a phenotypic trait which would be expected under two contrasting scenarios of strong directional selection (Pigliucci *et al.*, 2006), perhaps acting on a basis of underlying polygenic inheritance or indicating incomplete penetrance.

Concerning habitat associations, the variables included as significant predictors in the PLS factor that best explained geographical variation in the frequency of striped lizards were all related to forest development, giving more negative scores to habitats with lower visibility from above. Because PLS scores decreased in an east–west direction, visual opacity tended to be higher at more western locations, suggesting different selective pressures on lizard coloration and crypsis.

The most obvious link between these habitat features and dorsal coloration is camouflage, because disruptive colour patterns can render a target indistinguishable from its background (Cuthill *et al.*, 2005). Thus, when some of the contrasting colours in a striped pattern are coincident with the background, as is the case of dark and clear stripes in grassy habitats, they break up the animal's outline (Merilaita, 1998). The role of crypsis in our system was supported by the results of our two detection experiments, because detectability was always higher for animals with mismatched phenotype. Although strictly speaking our conclusions are restricted to human observers, previous studies have shown a remarkable similarity between humans and birds in the ability to discriminate between different colour patterns under laboratory conditions (Penney *et al.*, 2012), and simulations reveal that visual complexity of the background renders 'background matching' methods less reliable than those that address information processing by predators (Merilaita, 2003). Moreover, detection times measured by presenting images of prey on computer screens to humans were significantly correlated with colour contrast in RGB between potential preys and their background (Carrascal *et al.*, 2001), vigilance rates of potential preys (Carrascal *et al.*, 2001) and estimates of capture probabilities and survival of different colour morphs of free-ranging grasshoppers (Karpestam *et al.*, 2013).

The importance of visually oriented predation as a selective pressure for these lizards is consistent not only with the large number of avian species that prey on them (Salvador, 2014), but also with the influence of antipredator behaviour on other aspects of the ecology of these lizards: (1) thermoregulation, because the choice of compass directions around shrub patches allows basking lizards to remain within shorter reach from the security of shrubs than expected at random (Díaz, 1992); (2) escape tactics, as in deciduous forests lizards change their escape behaviour in response to changes in habitat structure (Martín & López, 1995) and (3) sexual selection, given that larger and more brightly coloured males are more active, overlap more females, and court them more frequently than smaller and less active ones, but at the cost of a higher risk of predation and increased mortality (Díaz, 1993).

Current genetic structure and maintenance of phenotypic differentiation

The existence of two separate gene pools in central Spain, one corresponding to striped lizards (E2 haplogroup) and the other one to unstriped lizards (E3, W1 and W2 haplogroups), was supported by nuclear markers, given that populations in the southern foothills of the central mountain ranges were pooled together in the same cluster. This gave rise to a typical pattern of mito-nuclear discordance (Toews & Brelsford, 2012), because the largest cluster defined by microsatellite alleles (red cluster in Fig. 2, including all central populations other than Aranjuez) grouped together two W2 populations, one E3 population and the only eastern-western (W1+E3) polymorphic population detected in our study. In all these populations, the unstriped colour pattern was dominant. IMa2 analyses were also indicative of high levels of gene flow within this cluster. In particular, the two populations acting as main allele donors, El Pardo and Pelahustán, had higher migration rates (Table 3: row averages of 0.86 and 0.93 respectively) than the remaining populations (row averages ≤ 0.23). Remarkably, these two locations are part of the almost continuous corridor of oak forests that cover the southern slopes of the central mountain ranges. We predict that more detailed sampling of central Iberia should provide more examples of mitochondrial admixing between eastern/central (E3) and western clades (W1 and W2), as it has been described for other Iberian lacertids (Godinho *et al.*, 2008; Miraldo *et al.*, 2011).

What factors could explain the maintenance of the current pattern of population differentiation? We hypothesize that ecological selection is driving both divergence between E2 (striped) and E3 (unstriped) lizards, and convergence between E3 and western lizards in central Spain. Three lines of evidence support this hypothesis. First, the distribution of the frequency of striped lizards in the 23 populations examined was clearly bimodal: the frequency of the commonest phenotype was smaller than 0.75 in only two of these populations (Fig. 3: localities 22, with 66% striped lizards, and 36, with 60% unstriped lizards), and these sites were both located close to the western and eastern edges, respectively, of the distribution ranges of their haplogroups (E2 and E3). Second, the estimated migration rate of microsatellite alleles to and from the Aranjuez population (E2) was extremely low, despite its short linear distance from the nearby populations of El Pardo (W2) and Pelahustán (E3). And thirdly, habitat differences between western (more forested) and eastern (less forested) habitats were significant, with central (E3) sites occupying an intermediate position. Such differences are consistent with the existence of an ecotone matching the division between eastern and western haplogroups of many Iberian taxa with similar phylogeographical patterns (Gómez & Lunt, 2007; Rodríguez-Sánchez *et al.*, 2010; Fitze *et al.*, 2011). In addition to the dataset analysed in this manuscript, supplementary information on differences in egg incubation times among egg-laying females belonging to different genetic lineages also reveal a close phenotypic link of the E3 haplogroup with the western clade (Díaz *et al.*, 2012, see Appendix S3).

Finally, we suggest that ecologically based divergent selection is causing the evolution of reproductive isolation, either complete or not (Nosil *et al.*, 2009), between striped and unstriped lizards. The selective advantage of the matching phenotypes in the habitats where they maximize crypsis can directly promote ecologically dependent reproductive isolation (Rundle & Nosil, 2005). Pre-zygotic isolation can arise if migrants suffer reduced survival because they are more easily detected by predators in non-native habitat, which could lower the rate of heterologous mating. In contrast, post-zygotic isolation can evolve if hybrid fitness is reduced due to an ecological mismatch between the phenotypes of hybrids and their environment (Rundle & Nosil, 2005). The bimodal distribution of the frequency of striped lizards at both sides of the ecotone, together with the negative correlation between the frequency of striped lizards and the proportion of them that show little contrast between stripes, suggest that 'pure' phenotypes have higher fitness than 'hybrid' ones. Second, it seems that the development of the sexual coloration of the head is more intense in western males (in which larger and older individuals have a brilliant red-orange patch on the sides of head, mental scutes and throat) than in eastern males (that show a less conspicuous orange coloration, usually restricted to labial scales) (J. A. Díaz, pers. obs.). Because head coloration is important for sexual selection in western males (Díaz, 1993; Salvador *et al.*, 1996; López *et al.*, 2003) and it may interfere with crypsis in eastern habitats if red-orange heads are more visible from above, such difference could promote assortative mating and therefore prezygotic isolation. However, chemosensory cues are also important for these lizards (López *et al.*, 2003), and we encourage future efforts to measure the composition of femoral secretions and the responses of both males and females from different populations along the ecotone.

To summarize our findings, our data show how an incipient process of allopatric divergence, defined by the ancient split of eastern and western mtDNA clades, may be blurred by ecological factors acting later on. Moreover, the same environmental factors that seem to be fostering the admixture of the western and E3 haplogroups, thereby promoting their phenotypic and genetic convergence, may also be causing the split of the E3 haplogroup from the rest of the eastern clade in an early process of ecological speciation. This is remarkable, because to our knowledge this is the first time that ecologically based divergent selection, which is the basic process involved in ecological speciation, is also documented to promote convergence between phylogeographical lineages. Such duality reinforces the view, emphasized in Futuyma's (1986) definition of evolutionary ecology, that we need ecological data to explain evolution as much as we need evolutionary processes to understand the ecology of extant organisms.

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SUPPORTING INFORMATION

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

Appendix S1 Extended material and methods.
Appendix S2 Mismatch distributions of E3 haplogroup.
Appendix S3 Differences in incubation times among haplogroups.

BIOSKETCHES

The authors are members and external collaborators of the Evolution and Conservation Biology Research Group of the Complutense University of Madrid (http://www.ucm.es/bcveng). Their research focuses on evolutionary biology, ecophysiology and the evolution of life histories along biogeographical gradients. To deal with the interesting questions that often arise in evolutionary ecology, lizards are used as study systems, bringing together biogeography, behavioural ecology, physiology, evolutionary ecology, population genetics and conservation biology in an integrative approach.

Author contributions: J.A.D., J.V.R. and A.S. conceived the study, performed data analyses and wrote the first version of the manuscript; J.V.R. performed genetic analyses. All authors contributed to data acquisition and manuscript writing.

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