

Can scent-mediated female mate preference explain an abrupt mtDNA cline in *Lacerta schreiberi*?

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Summary

Divergence in female mate preferences can strongly influence the structure and dynamics of hybrid zones. We examined the potential role of female mate preferences in maintaining an abrupt west-east mtDNA cline between two deeply divergent genetic lineages of *Lacerta schreiberi*, a lizard endemic to the Iberian Peninsula. The lineages are largely morphologically cryptic but with respect to the mtDNA cline, western males tend to be less parasitized, in better body condition and more intensely coloured than eastern males, a pattern that cannot be explained by environmental variation alone. The lineages may also differ in unmeasured aspects such as physiology, behaviour and olfactory signals, which may influence mate choice. As female mate attraction has been found to vary with olfactory cues in lacertid lizards, we experimentally tested whether females were differentially attracted to femoral pore secretions of males from the two genetic backgrounds. Females did not prefer scents of 'higher quality' western males, nor did they prefer the scents of males belonging to their own genetic

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background. This suggests the abrupt mtDNA cline is unlikely to be explained by assortative mating of matrilineages that distinguish male genetic background based on scent.

Keywords: hybrid zone, secondary contact, mate choice, pre-zygotic barrier, olfactory, scent, lizard.

Introduction

Pre-copulatory sexual selection is a key process influencing the dynamics of hybrid zones. Female mate choice, for example, has been implicated in both the rapid movement of hybrid zones (reviewed in Buggs, 2007) and asymmetric introgression of morphological traits (e.g., Parsons et al., 1993; Stein & Uy, 2006). Female choice may increase hybridisation through preference for heterospecific males (Veen et al., 2001) or reduce hybridisation and ultimately lead to reproductive isolation if reinforcing selection favours assortative preferences (reviewed in Servedio & Noor, 2003). Assessing female mate preferences is, therefore, crucial for predicting the evolutionary consequences of hybridisation.

The Iberian endemic lizard, *Lacerta schreiberi*, provides an opportunity to examine the potential role of sexual selection in structuring a secondary contact zone. Two divergent lineages of *L. schreiberi* form a hybrid zone in the mountains of the Iberian Central System (Godinho et al., 2006, 2008). Genetic data indicate that the two lineages of *L. schreiberi* probably diverged as early as the Pliocene and persisted in separate glacial refugia, one in north-western Iberia and the other in the Spanish Central System (Paulo et al., 2001; Godinho et al., 2008). At a broad spatial scale, nuclear data have revealed evidence for repeated historical admixture between the two lineages (Godinho et al., 2008). However, within the Central System mountain range, phylogeographic studies have identified a sharp cline in mitochondrial DNA, which is coincident with clines in nuclear markers (both proteins and microsatellites, Godinho et al., 2003, 2006, 2008). These studies have also identified localities where lizards of both mitochondrial lineages are found (Godinho et al., 2003, 2006, 2008). The admixture zone is centred on a watershed, which potentially represents an environmental barrier to gene flow as *L. schreiberi* is largely restricted to riparian habitats (Brito et al., 1998). Assessing the influence of prezygotic barriers and fitness differentials across

this contact would allow deeper insight into the post-glacial evolutionary dynamics of this system.

The *L. schreiberi* system is one of few examples of a well-characterised animal hybrid zone between genetically divergent yet apparently morphologically cryptic lineages (but see Phillips et al., 2004). However, there are subtle morphological differences between males to the west and east of the mtDNA cline (Stuart-Fox et al., data not shown). Compared to eastern males, western males have fewer ticks and blood parasites (haemogregarines), are in better body condition and are more intensely coloured, traits which are potentially associated with male quality. Although environmental variation across the contact could explain the differences in parasite load and body condition, these are uncorrelated with color expression, suggesting that the differences in coloration are due to differences in the two genetic backgrounds (Stuart-Fox et al., data not shown). Moreover, the lineages potentially differ in many other unmeasured aspects such as physiology, behavior and olfactory signals. Assessing the mechanism behind restricted gene flow in this cryptic hybrid zone, therefore, requires direct experimental tests. In terms of mate preferences, we predict that females should either show assortative preferences for males with the same genetic background to their own or alternatively, females from both genetic backgrounds should prefer the more intensely colored, less parasitized western males.

In lacertid lizards, female mate preference or association behaviour is based primarily on olfactory cues and there is strong evidence that females are able to assess aspects of male quality (e.g., body condition, fluctuating asymmetry, parasite load, dominance) and genetic relatedness based on their scents (Martin & Lopez, 2000, 2006a,c; Lopez et al., 2003; Olsson et al., 2003; Martin et al., 2007). If females use these cues to preferentially mate with males from their own genetic background, this would maintain an association between preference genes and matrilineally inherited markers such as the mitochondrion, resulting in an abrupt, stable mtDNA cline. Alternatively, if females used these cues to preferentially mate with higher quality western males, the hybrid zone would be expected to shift east. To distinguish these scenarios, we tested whether females consistently prefer the scents of males (1) with a genetic background the same as (or different to) their own or (2) belonging to the 'higher quality' western populations, irrespective of the female's genetic background.

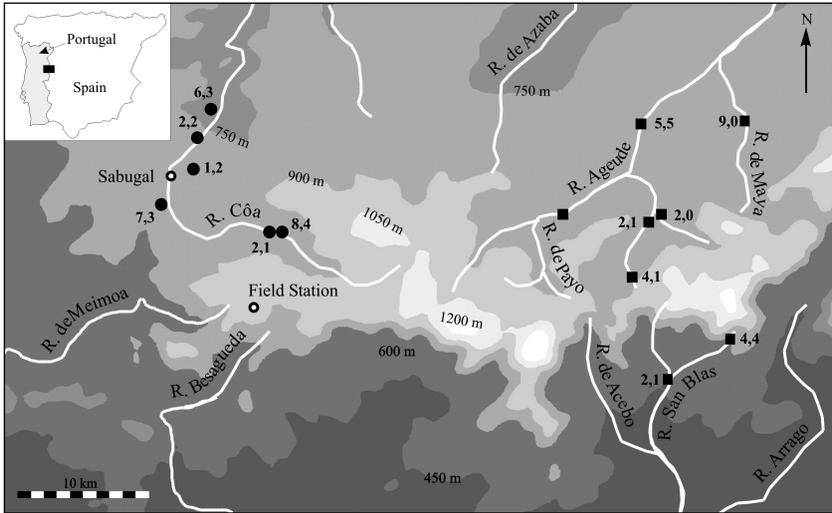


Figure 1. Elevation map of sampling localities with sample sizes for males and females (in that order) for each locality. Filled circles and squares represent sites with mtDNA of the western and eastern lineages respectively. The inset shows the position of the study area (black rectangle) on the Iberian Peninsula. Sampling localities are located along rivers (white lines) as the species is almost exclusively riparian.

Materials and methods

We studied populations of *L. schreiberi* in the Central System mountains of the Iberian Peninsula (40.213–40.399°N, 6.572–7.108°W, approx. 800 m altitude) during early Spring (April 20–May 15, 2006). At this elevation, lizards had only recently emerged from winter and likely to be actively searching for mates as females were not yet obviously gravid. We caught sexually mature adult lizards, returned them to the field station (Malcata Nature Reserve), conducted behavioural trials and released them (within 5 days) at their precise site of capture (Figure 1). At the field station, lizards were housed temporarily in enclosures approx. 40 × 25 × 30 cm high containing bark substrate, a leafy branch and concave ceramic tile for shelter and water.

Genetic backgrounds

We sampled individuals from localities between 15 and 25 km west, and between 10 and 25 km east of the previously identified mtDNA transition (Godinho et al., 2006, 2008, Figure 1). We verified that our sampling localities fell on either side of the transition by sequencing 850 base pairs of the

mitochondrial cytochrome *b* gene for each individual. Protocols follow those in Godinho et al. (2006). The sampled frequency of introgressed mtDNA was 0/43, and 0/41 in western and eastern localities respectively, confirming that they bracket the mtDNA cline.

Behavioural trials

We used 16 western and 12 eastern females in at most two trials each ($N = 47$ trials in total). Females were presented with a pair of scents from a western and eastern male. Each male scent (27 western and 29 eastern) was used in at most two trials. The average size of males used in trials was 92.3 ± 9.0 mm SD and pairs of males used for scent trials were size-matched to within $3.9 \pm 5.3\%$ SD of SVL.

We extracted male scents by gently squeezing male femoral pores with forceps and stored the waxy plugs in air-tight tubes at -20°C . In addition, we swabbed the males' ventral region, cloaca and femoral pores with a cotton applicator dipped in a hexane solvent, which quickly evaporates, leaving the male odour compounds impregnated in the swab. The scent choice trials were conducted in an enclosure (50 cm L, 30 cm W and 30 cm D) lined with a double layer of clean butcher's paper extending 5–10 cm up each side. The enclosure size was chosen to maximise the likelihood of females sampling both scents and to ensure tongue flicks could be clearly seen in the video footage. In each enclosure we placed two clean shelters (concave terracotta tiles $16 \times 8 \times 4$ cm). For each trial, we placed one cotton swab at the entrance of the concave ceramic tile shelter and smeared a small amount of femoral pore wax (approx. three 2×1 mm plugs) next to it. Trials were conducted outdoors under sunny conditions during the lizards' natural activity period. Each trial was video-taped and lasted approximately one hour. Between trials, both the butcher's paper and tile shelters were changed. All tiles were cleaned thoroughly before re-use following Downes and Shine (1998).

From the videos, we scored two measures of preference: (1) the number of directed tongue flicks to the swab and femoral pore secretions (defined as occurring within a 5 cm radius of the swab) and (2) time spent in or basking on each ceramic shelter. Each measure was standardised for total trial time. We tested for an effect of male genetic background on female scent preference using a two-way repeated measures ANOVA (PROC MIXED, SAS 9.1) with male genetic background (W or E) as the repeated (within subject) factor and female ID as the subject. Male and female genetic background and

the interaction between them were fixed effects. Power analyses were done with the SAS macro `fpower 1.2` (Friendly, 2006).

Results

There was no effect of male genetic background on the number of directed tongue flicks per minute ($F_{1,40} = 0.06$, $p = 0.81$, Figure 2a) or time spent in or on shelters ($F_{1,40} = 0.09$, $p = 0.77$, Figure 2b). Thus, females did not consistently prefer males from either the western or eastern genetic backgrounds. Given our sample size, we had power of 0.92 to detect an effect size of $\delta \geq 0.5$, which is small to moderate (Cohen, 1988). The interaction between male genetic background and female genetic background was not significant in either model. Thus, females showed no preference for the scents of males belonging to either the same or different genetic background to their own based on the number of directed tongue flicks ($F_{1,40} = 0.64$, $p = 0.43$, Figure 2a) or time spent in or on shelters ($F_{1,40} = 0.44$, $p = 0.51$, Figure 2b). Given our sample size, we had power of 0.91 to detect a significant interaction with an effect size of $\delta \geq 1.1$, which is moderate to large (Cohen, 1988).

Discussion

We found no evidence that female *L. schreiberi* discriminate male scents based on genetic background in this system, although they clearly directed

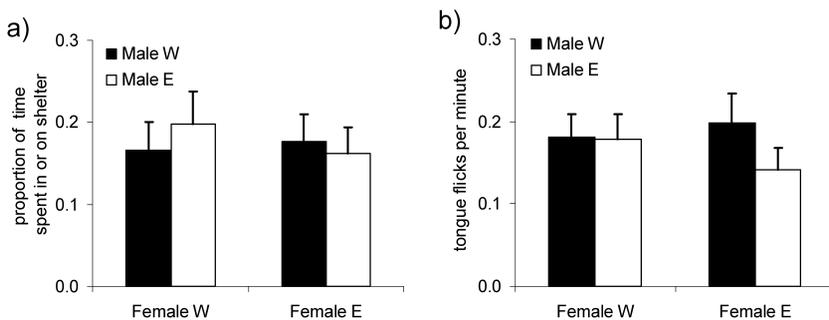


Figure 2. Female preference for males from each genetic background measured as (a) proportion of time spent in or on the ceramic shelters and (b) number of tongue flicks per minute directed by females towards swabs and femoral pore secretions.

tongue flicks to the swabs and secretions in the trials. We found a lack of discrimination despite using larger quantities of femoral pore secretion than in similar scent choice trials on other lacertid lizards (e.g., Olsson et al., 2003; Lopez & Martin, 2005; Martin et al., 2007). Moreover, our sample sizes provided sufficient power to detect the moderate to large effect sizes found by similar studies, which generally had smaller sample sizes (e.g., Lopez et al., 2003; Olsson et al., 2003; Martin & Lopez, 2006a).

In other closely related lacertid lizards, females discriminate chemical compounds in male femoral pore secretions, based on male health state (Martin et al., 2007), symmetry (Martin & Lopez, 2000, 2006a), age (Lopez et al., 2003), T-cell mediated immune response (Lopez & Martin, 2005; Martin & Lopez, 2006a) and similarity of MHC loci (Olsson et al., 2003). However, female *L. schreiberi* did not show a consistent preference for males from the west, which are less parasitised, in better body condition and more intensely coloured. There are two potential explanations for the lack of female discrimination of the scents of males from the two genetic backgrounds in *L. schreiberi*. First, females may base preferences on male traits other than parasite load and coloration, such as body size or dominance, or they may rely on visual rather than olfactory cues for mate choice. For example, assortative mating based on body size has been demonstrated in *Lacerta monticola* and one of the mechanisms generating this pattern is active female rejection of copulation attempts by smaller males (Lopez et al., 2003). Even if females rely on olfactory cues for mate choice, they may choose mates based on traits such as MHC similarity (e.g., Olsson et al., 2003), which may not differ consistently between males from the two genetic backgrounds. The second possible explanation is that male scent in this species does not covary with the traits that differentiate the eastern and western lineages, namely parasite load, body condition and coloration, although there is empirical evidence that females can discriminate the scent of more versus less parasitised males in the lacertid *Psammmodromus algirus* (Martin et al., 2007).

We also found no evidence for assortative female preferences; females did not prefer the scents of males with either the same or opposite genetic background to their own. Similarly, females of the lacertid lizard, *Podarcis hispanica*, do not discriminate the scents of males belonging to morphologically and genetically divergent populations at a contact zone (Martin & Lopez, 2006b). In several species, including lizards, females prefer the scents

of novel males or prefer the scents of males that are more genetically dissimilar (e.g., Olsson et al., 2003; Parrott et al., 2007). However, pairs that are too genetically dissimilar may show some degree of genetic incompatibility (Mays & Hill, 2004). If there is a fitness cost of hybridisation, female mate preferences will be under reinforcing selection (Servedio & Noor, 2003). For a system at equilibrium, female preferences should reflect a balance between the fitness benefits of outbreeding and the costs of genetic incompatibility (Mays & Hill, 2004). The lack of strong preferences in *L. schreiberi* suggests first that pre-copulatory female choice is unlikely to play a strong role in any potential fitness differential across the mtDNA transition if mate choice is mediated by olfactory cues in lacertid lizards (Lopez et al., 2003; Olsson et al., 2003; Martin & Lopez, 2006a). Second, it suggests that the abrupt mtDNA cline is unlikely to be explained by assortative mating of matrilineages that distinguish male genetic backgrounds based on their scent.

The order of magnitude of mate preference that would be necessary to maintain a cline as abrupt as that observed at the *L. schreiberi* mitochondrial locus can be gauged by considering the difference s^* in mean fitness between populations at the centre and the edge of a contact zone (Barton & Gale, 1993). For selection against the formation of hybrids due to female positive assortative mating, the fitness differential s^* relates to the reduction in mean fitness at the centre of the zone due to females encountering their disfavoured mating type. The strength of selection can be estimated as $C(1/w)^2$, where w is the width of the cline, measured in units of σ , the standard deviation of the distance between parent and offspring, that is, the scale of inter-generational (female) dispersal, and C is a constant in the range 3–4, which depends on the detailed nature of the selection mechanism. A cline width of the same order of magnitude as the scale of dispersal could, therefore, only be maintained by very strong effects of female preference. In *L. schreiberi*, the frequency of western lineage mitochondria changes from zero to one in localities separated by as little as 500 m. This suggests that either the scale of female dispersal is less than one hundred metres per generation, or the effects of female choice would have to be very strong to maintain the observed clines ($s^* > 0.1$). While extreme female philopatry is possible, it is inconsistent with the recent history of *L. schreiberi*: the upland corridor where the contact occurs must have been (re)-colonised post-glacially (Godinho et al., 2008). Based on a generation time of three years for this species (Ferrand de Almeida et al., 2001) and colonisation distance of at least 50 km

for each lineage, female dispersal of less than 100 m per generation is vanishingly improbable. This suggests that very strong female choice would be required to maintain mitochondrial frequency changes from zero to one in localities separated by as little as 500 m (even if the watershed were acting as an environmental barrier to gene flow, producing a local reduction in σ), and we should easily have been able to detect such strong preferences in our experiments.

This interpretation, however, relies on a link between female scent preferences and precopulatory mate choice. Our experiments assessed both female scent sampling behaviour (directed tongue flicks) as well as female spatial association with male scents (time spent in or on shelters), which have been widely assumed to indicate mate preferences in lacertids (e.g., Martin & Lopez, 2000, 2006a; Lopez et al., 2002, 2003; Lopez & Martin, 2005). However, the link between female scent choice, acceptance or rejection of male courtship and copulation and male reproductive success in these species has yet to be conclusively established. Moreover, in Swedish sand lizards, *Lacerta agilis*, females appear to mate indiscriminately when receptive and primarily exhibit postcopulatory rather than precopulatory mate choice (reviewed in Olsson & Madsen, 2001). It is, therefore, possible that precopulatory female mate choice in *L. schreiberi* is unrelated to scent preferences or that the mechanism of inter-sexual selection is primarily postcopulatory.

Studies comparing the rate at which prezygotic versus postzygotic isolation arises in different large groupings of species have been identified as one of the most effective recent approaches to understanding speciation (Orr et al., 2007): equal rates found in allopatric species of *Drosophila* (Coyne & Orr, 1989, 1997) are in contrast to higher prezygotic rates in birds (Price & Bouvier, 2002). To our knowledge no such study has been carried out for lizards, although based on phylogenetic proximity, they may be more similar to birds than to flies. The second approach identified as contributing to recent understanding of speciation is the meticulous study of species pairs, partitioning the contribution of various forms of reproductive isolation (Orr et al., 2007). Secondary contact provides an obvious natural laboratory in which to study barriers to gene flow between taxon pairs, and the current study assesses the pre-mating contribution to the barrier in *L. schreiberi* due to olfactory cues, important in closely related lacertids. Additional fine scale studies across the contact zone using multiple nuclear loci are required in

order to assess the post-mating contribution to barriers to gene flow in *L. schreiberi*.

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