Low genome-wide divergence between two lizard populations with high adaptive phenotypic differentiation

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Abstract

Usually, adaptive phenotypic differentiation is paralleled by genetic divergence between locally adapted populations. However, adaptation can also happen in a scenario of non-significant genetic divergence due to intense gene flow and/or recent differentiation. While this phenomenon is rarely published, findings on incipient ecologically-driven divergence or isolation by adaptation are relatively common, which could confound our understanding about the frequency at which they actually occur in nature. Here, we explore genome-wide traces of divergence between two populations of the lacertid lizard Psammodromus algirus separated by a 600 m elevational gradient. These populations seem to be differentially adapted to their environments despite showing low levels of genetic differentiation (according to previously studies of mtDNA and microsatellite data). We performed a search for outliers (i.e. loci subject to selection) trying to identify specific loci with FST statistics significantly higher than those expected on the basis of overall, genome-wide estimates of genetic divergence. We find that local phenotypic adaptation (in terms of a wide diversity of characters) was not accompanied by genome-wide differentiation, even when we maximized the chances of unveiling such differentiation at particular loci with FST-based outlier detection tests. Instead, our analyses confirmed the lack of differentiation on the basis of more than 70,000 SNPs, which is concordant with a scenario of local adaptation without any degree of isolation by environment. Our results add evidence to previous studies in which local adaptation does not lead to any kind of isolation (or early stages of ecological speciation), but maintains phenotypic divergence despite the lack of a differentiated genomic background.

Introduction

Overall, the genotypes and phenotypes of populations undergoing local adaptation are expected to be spatially congruent, i.e. genetic diversity is expected to follow similar differentiation patterns as adaptive phenotypic diversity (Coop et al. 2010, Tigano and Friesen 2016, Fris et al. 2018, Simmonds et al. 2020). This positive correlation is commonly known as 'isolation by adaptation', and it is usually studied by estimating ecologically adaptive among-populations divergence as a proxy of the divergent patterns of selection that cause local adaptation (Schluter 2000, Nosil et al. 2008, Funk et al. 2011). In theory, isolation by adaptation should be theoretically detectable in the form of divergence peaks among locally selected loci (or among loci genetically correlated with them), but if populations remain isolated long enough, selectively neutral loci can also become differentiated, which makes the detection of outlier loci empirically challenging (Krohn et al. 2018, Llanos-Garrido et al. 2019). However, discordances may also appear, whereby two populations may be genetically undifferentiated while showing evident phenotypic divergence (Moody et al. 2015, Palmer and Kronforst 2015, Shaner et al. 2015). This can happen, despite the existence of processes that blur overall genomic divergence (e.g. gene flow, recent divergence), only when natural (or sexual; Yang et al. 2018) selection is strong enough to overcome such processes, favoring the presence of locally divergent regions with variants under selection within an otherwise undifferentiated overall genomic background (Burri 2017, Wang et al. 2019).

While numerous studies have dealt with locally adapted populations occupying different environments or

isolated by ecological barriers (Rosenblum 2006, Orsini et al 2013, Sexton et al. 2014, Zhao et al. 2020), only a few have focused on the lack of correlation between isolation and adaptation (Feder et al. 2013). Moreover, it has been suggested that there is a significant publication bias against such studies (Krohn et al. 2018). This possible under-representation of apparent negative results may lead to biased estimates of how frequently local adaptation occurs without isolation, and this is precisely the reason why replicated studies on species or populations with different degrees of isolation are needed (Feder et al. 2013, Talla et al. 2017, Sendell-Price et al. 2020). In fact, if studies that do not find any effect of environmental gradients on genetic differentiation are rarely published, examples of incipient ecological speciation and/or isolation by environment may artificially be deemed frequent or even widespread (Hendry 2009, Shafer and Wolf 2013, Sexton et al. 2014).

The aim of this study is to elucidate the patterns of genetic differentiation that underlie phenotypic divergence between two populations of the lacertid lizard *Psammodromus algirus* separated by a 600-700 m altitudinal gradient. This gradient is associated with a large number of habitat differences, including forest type (deciduous vs. perennial) or average annual rainfall (1170 vs. 438 mm; see the Methods section for a detailed explanation of these habitat differences). The analysis of mitochondrial DNA sequences has shown that these populations present very little genetic differentiation (Verdú-Ricoy et al. 2010, Díaz et al. 2017), even though they differ in a wide variety of adaptive phenotypic characteristics, including many life history traits (Iraeta et al. 2006, 2010, 2011, and 2013, Llanos-Garrido et al. 2017; Table 1). The evidence for such adaptations is based on previous studies that have shown, through reciprocal transplant and common garden experiments, that these adaptive phenotypic differences are not sustained solely by environmental effects (Iraeta et al. 2006, 2013). Therefore, there must be a genetic basis to determine such phenotypic differences between these apparently undifferentiated populations, even if such genetic basis does not lead to an environmentally based isolation. To define the degree of genetic differentiation between the two populations, we used a GBS genomic scan based on 73,291 SNPs that allowed us to analyze the genetic structure and distance between them. In addition, we used a Bayesian method of detection of SNPs with genetic distances between populations greater than expected given the degree of background genomic differentiation (i.e. F_{ST} based outlier test; Bayescan: Foll and Gaggiotti 2008). With this approach, we tried to define polymorphisms possibly associated with the patterns of divergent selection that promote the observed adaptive differences (Bonhomme et al 2010). Thus, if local adaptation has led to isolated populations, we expect to find a genome-wide differentiation standard that could hamper the detection of divergence peaks at adaptive loci. Alternatively, we might find an undifferentiated genomic background where such peaks should be easy to detect, at least in theory.

We used an approach in which all genetic variants are used to infer the basal level of genomic differentiation, defining outliers as SNPs with a greater degree of divergence and with allelic frequencies deviated from the expected under neutral selection (Lewontin and Krakauer 1975). The reason why comparing differentiation peaks with adjacent regions should facilitate this task is that the degree of differentiation is heterogeneously distributed throughout the genome (Campagna et al 2015). Therefore, detecting a divergence peak in a specially conserved region is challenging using delocalized genetic variation, which may find such degree of divergence even below the background genomic differentiation, but which may actually be very divergent within its genomic location (Lawson y Petren, 2017). This scenario is especially common in the coding regions where the genetic basis of phenotypic diversity is located, so that approaches with delocalized SNPs do not usually respond to what is the genetic basis of a given phenotype, but describe the general patterns of genetic differentiation that lay behind the process of local adaptation (e.g. Tigano et al. 2017, Llanos-Garrido et al. 2019). Thus, while uncovering the specific genetic basis behind already published adaptations between these populations would be specially challenging given the methodology we used, it is still possible to elucidate whether such local adaptation is accompanied by any degree of isolation (or genome-wide differentiation) or not (Krohn et al. 2018).

Material and methods

Study system

Psammodromus algirus (Linneus, 1758) is the most abundant lacertid lizard in the Mediterranean scrubland

and forest habitats of the Iberian Peninsula (it is only absent from the northern Eurosiberian area). It is a medium sized species (snout-vent length: 60-90 mm; body mass: 6-16 g) in which variation in body size is an important indicator of fitness, as it determines survival, male attractiveness, and female fecundity (Díaz 1993, Díaz et al. 2005, Martın and Forsman 1997, Iraeta et al. 2013). *P. algirus* males present a reddish nuptial coloration during the months of highest activity (April-June) that can range from small sublabial marks in subdominant males to occupy practically the entire head in the largest males. This visual signaling is complemented by the existence of femoral pores as a means of chemical signaling. The number of femoral pores is higher in males and their activity increases as the breeding season advances in response to increased blood androgen levels (Chiu and Maderson 1975, Cole 1966, Diaz et al. 1994).

The populations in this study are separated by an altitudinal gradient of 600-700 m and differ in mean annual temperature, precipitation and habitat structure. Previous studies on these populations revealed very little genetic differentiation inferred with mitochondrial DNA (Verdú-Ricoy et al. 2010), despite the fact that a wide number of adaptive phenotypic variables are markedly different (Table 1).

The montane population is located at Navacerrada (Cerro de la Golondrina, Sierra de Guadarrama: 40°44'N, 4deg00'W; 1300 m a.s.l.) and its habitat is composed by deciduos oak forests (*Quercus pirenaica*), scrub patches where *Cistus laurifolius* predominates and, to a lesser extent, granite outcrops and grasslands. The average annual temperature is 6.2 oC and the average annual rainfall is 1170 mm. The lowland population is located at El Pardo (Madrid: 40deg31'N, 03deg47'W; 650 m a.s.l.), a forest of evergreen holm oaks (*Quercus ilex*) with shrubs of *Cistus ladanifer* . It is separated from the montane population by 32 km in a straight line, and has an average annual temperature of 12.5 oC and an average annual rainfall of 438 mm. *P. algirus* is the most abundant lizard species in both populations, but reaches higher densities at higher altitude (Diaz 1997).

Field sampling and phenotypic analysis

We noosed or captured by hand 10 males and 10 females from each population (N = 40 individuals). Captures were made in the breeding season (April-May) of 2015, and the number of ticks carried by each individual was counted in the field. The lizards were transported to the lab, where all phenotypic traits (snout-vent length, head width and length, hind leg length, number of femoral pores, and body mass) were measured, and photographs of the throat in ventral position were taken to quantify sexual coloration. For that purpose, we processed the photographs with Adobe Photoshop CS6 as explained Llanos-Garrido et al. (2017): we standardized the area of analysis using the 'magnetic loop' tool, we measured the red coloured surface with the 'magic wand' tool (with 30% tolerance) after selecting a random red-colored point, and we then used the 'similar' option of the magic wand, with the same tolerance, to select all areas with a similar coloration. The colored surface was measured as the percentage of colored pixels in the area of analysis. To calculate the red saturation, we use the proportion of the red channel within the RGB channel, that is: R/(R+G+B), where R, G and B are the red, green and blue channels of the graphics card. All these measures were taken blindly with respect to the population of origin. Once all this was done, the lizards were released on their site of capture.

All phenotypic analyses were performed with Statistica software (Statsoft). In order to analyze between populations differences, we used two-way ANOVAs with sex and population as factors and with different morphological measures as the dependent variables. When necessary, we used ANCOVAs to control for the effect of body size. To assess the relationship between tick load and different explanatory variables (e.g. sex, size, or number of femoral pores), analyses were restricted to the Navacerrada population. To summarize variation in male size in this population, we ran a Principal Component Analysis (PCA) that produced a single factor explaining 84.2% of the variation in the data matrix and giving high factor loadings to all size variables (snout-vent length, head length and width, mean leg length, and body mass).

DNA extraction and sequencing

From the tissue samples, we purified DNA for library preparation using the Biotools *Speedtools Tissue DNA Extraction* kit. Next, we used the restriction enzyme *Pst1* to prepare the libraries for genome-wide

genotyping with GBS (*Genotyping by Sequencing*). The sequencing was done in an Illumina HiSeq2500 sequencer, and once the sequences were obtained we characterized single nucleotide polymorphisms (SNPs). For this purpose, we used the UNEAK pipeline, implemented in TASSEL v.3.0 (Bradbury et al. 2007), and specifically designed for samples of species without a reference genome. The sequences were pruned to eliminate sequencing errors using the error rate threshold parameter. The resulting database consisted of 83,648 SNPs with a coverage of 5.68 +- 6.56 (mean +- sd) and missing site rate of 0.49 +- 0.33. We then discarded loci with minor allele frequencies < 0.01, and those that were sequenced in less than 10% of individuals. The final database consisted of 73,291 loci, with a single SNP per locus and a coverage of 6.6 +- 6.75 and a missingness of 0.42 +- 0.31. This dataset was the one used to analyze the neutral variation of our samples.

Characterization of neutral variation and SNPs under selection

Before performing the genetic structure analysis, we use PLINK v1.9 software (Purcell et al. 2007) to prune the SNPs according to their linkage disequilibrium, estimated by correlation coefficients between SNPs. This filtering step was needed because the subsequent structure analysis does not take into account linkage disequilibrium, and this may lead linked SNPs to bias the grouping of individuals. Genetic structure analyses were performed with the Admixture v.1.3 software (Alexander et al. 2009), which allowed us to estimate the ancestries of each individual through maximum likelihood. To determine the number of groups for which the genetic structure model had more predictive power, we used cross validation errors (cv-errors).

We also applied another filtering step before doing the outlier analysis to minimize the false positive rate. We discarded loci whose minor allele frequencies were < 0.05 in any population (thus excluding privative alleles) and loci that could not be sequenced in at least 75% of the individuals in each population. The resulting database consisted of 6,421 SNPs. To identify SNPs putatively under selection, we performed an outlier analysis with Bayescan v.2.1. (Foll and Gaggiotti, 2008), a very conservative method which is not prone to false positives, and is very useful when the number of populations is low (Foll and Gaggiotti 2008). This program uses a logistic regression to split the F_{ST} coefficients into a population-specific effect (β) and a locus-specific effect (α). We selected loci with $\alpha > 0$, suggesting positive selection, and a false discovery rate (corrected by multiple testing) of $q < 0.05$.

Once this was done, we tried to annotate SNPs with the highest values of α. For this purpose, we run a BLASTn analysis against all the NCBI database. In addition, a Chi^2 analysis was performed to determine if any of these loci had allelic frequencies with significant deviations from what was expected under Hardy-Weinberg conditions (H-W). This was done because the environmental differences described above between the two populations could lead not only to divergent selective pressures in some SNPs, but also to respond to a selective pressure present in one of the populations that is absent in the other (in which case, the deviation of allelic frequencies from expected under H-W conditions should occur only in that population).

Environmental analysis

To evaluate environmental variation between El Pardo and Navacerrada, we computed the score of each cell that an individual would have to cross in a theoretical straight-line migration event following valley bottoms on a principal component analysis (PCA) that combined all Bioclim environmental variables (2.0 dataset; cell resolution = 1x1 km; Booth et al. 2014) using R core (R Core Team, 2013). This PCA has been previously used to summarize environmental variation (temperature and humidity) across all the species' range, yielding a single principal axis that opposed dry and warm cells to wet and cold ones (Llanos-Garrido et al., 2021). To assess the environmental gradient between these populations, we also considered the scores at the cells occupied by the populations themselves.

Results

Phenotypic variability

We found significant differences between populations that confirmed the results of previous studies (see Table 2): lizards from the lowland population were smaller, had relatively longer legs, and tended to have more

femoral pores than those from the montane population; in addition, they never presented tick infestation, despite adult ticks being present in their habitat (Table 2). Sexual coloration data were especially scarce from males sampled in this study (few colored males with very weak colorations), and as a consequence differences between populations could not be analyzed. When we only considered lizards from Navacerrada, we found that males had more ticks ($F_{1,18} = 10.85$, $p = 0.004$) and that only the largest males could develop a sexual coloration ($F_{1,8} = 42.17$, $p < 0.001$; Fig. 1). In addition, we detected that, after controlling for the effect of the number of femoral pores (an estimate of each male's investment in chemical signaling) on tick load ($r = 0.733$, $p < 0.001$), uncolored males tended to have more ticks than colored ones (ANCOVA; number of pores: $F_{1,7} = 7.54$, $p = 0.029$; color [as factor]: $F_{1,7} = 3.93$, $p = 0.088$).

Neutral genetic variability and SNPs under selection

The analysis of genetic structure with Admixture yielded a single more plausible model that grouped all samples into a single cluster ($K = 1$, cv-error = 0.382), indicating no apparent genetic structure between both populations. Even when forcing the model towards higher values of K (i.e. less plausible results), we still failed to find any signs of between-populations genetic structure ($K = 2$, cv-error = 0.405; Figure 2).

Regarding outliers analyses, Bayescan did not detect any locus subject to selection (q-values range: 0.883 - 0.910). The largest increase in α, the magnitude of the locus-specific effect of selection, concentrated on four maximum (although low) values, two of which could not be annotated at all (Table 3). Of the other two, one could be annotated with a marginally non-significant E-value of 0.018 (conventional significance value = 0.01), which corresponded to the coding region of a repeated transmembrane ankyrin involved in the cellular response to salicylic acid (inflammatory response) and the regulation of the immune system. The other sequence was annotated with a much higher E-value (0.6) as the coding region of a nuclear photoreceptor protein that regulates gene expression based on the reception of steroids (described mutations point to impairments in the bearer's vision). None of these SNPs presented allelic frequencies that deviated significantly from those expected under H-W conditions after applying the Bonferroni sequential correction (Table 3).

Environmental variability

By using the scree plot criterion, the environmental PCA yielded a single axis (eigenvalue = 0.679) that retained four variables: annual mean temperature (BIO1), max temperature of the warmest month (BIO5), mean temperature of the warmest quarter (BIO10), and annual precipitation (BIO12) (Llanos-Garrido et al. 2021). The warmest/driest cells were located near the lowland population (El Pardo), while the opposite was true for the montane population (Navacerrada). We found significant environmental differentiation between both populations (one-way ANOVA, $F_{1,38} = 4802{,}014$, p-value $<< 0.0001$), as well as relatively high levels of environmental variation along the cells that an individual would have to cross in a theoretical straight-line migration following valley bottoms (mean = -1.563, sd = 0.725). In fact, such variation was much greater than the one found in the geographical cells within any of the two populations, either lowland (mean = -1.069, sd = 0.048; Levene test: $F_{1,28} = 12.048$, $p = 0.002$) or montane (mean = -2.121, sd = 0.043; Levene test: $F_{1,42} = 25.807$, $p < 0.001$).

Discussion

Our results point to a discordance between the absence of genomic divergence and consistent phenotypic differentiation in traits whose adaptive value seems obvious (Iraeta et al. 2006, 2010, 2011, 2013). The fact that, as previously found, in the montane population the load of ectoparasites is related to secondary sexual characteristics of males, and that there are no infestations in the lowland population, shows that the presence of ticks is associated with different selective pressures in both populations (Llanos-Garrido et al. 2017). Apart from their negative effect on the survival of individuals (Salvador et al. 1996), ticks appear to have other significant impacts on fitness, influencing the expression of sexual ornaments that are important for reproduction (Salvador et al. 1996, Llanos-Garrido et al. 2017). In addition, the altitudinal gradient involves many other selective pressures that, while less easy to identify than the one imposed by ticks (but still evident given the environmental differences shown by our PCA scores), generate an undeniable impact

on fitness-related traits such as growth rate, egg size or clutch size (Iraeta et al. 2006, 2010, 2011, 2013). In addition, the adaptive phenotypic differences found in this and earlier studies span the whole life cycle of lizards (e.g. incubation time, hatchling size, juvenile growth rate, and sexual ornamentation and reproductive investment of adults), which should involve a considerable variety of divergent selective pressures underlying their phenotypic differentiation (Roff 2007). Moreover, the fact that these traits maintain their differentiation in common garden conditions (Iraeta et al. 2013) or in reciprocal transplant experiments (Iraeta et al. 2006) reveals a divergent genetic basis between the two populations (De Kort et al. 2014).

However, the background genetic differentiation between these two populations is very low (Díaz et al. 2017, Verdú-Ricoy et al. 2010), even when analytical power is increased with genomic scans. Irrespective of phenotypic differenciation, other populations of *Psammodromus algirus* showed marked genetic isolation despite being much closer (both geographycally and environmentally) than the ones presented here (Díaz et al. 2017, Llanos-Garrido et al. 2019). This is true even at a fragmented metapopulation where suitable habitat patches are as close as hundreds of meters apart, and where subpopulations have diverged less than 100 years ago (Santos et al. 2008, Pérez-Tris et al. 2019). However, this latter scenario occurs at the northern edge of the species' range, where genetic diversity might be lower than at El Pardo or Navacerrada, which occupy the center of the Iberian distribution range (Holt and Keitt 2000, Takahashi et al. 2016). Such decrease in genetic diversity and/or effective population size could have led to a subsequent reduction of migration rate and increased genetic structure in this marginal population (Holt and Keitt 2000, Zalewski et al. 2016, Li et al. 2018; see Deng et al. 2020), whereas the opposite might be true at the center of the species' distribution range. However, as migration rate between El Pardo and Navacerrada is high enough (or divergence time recent enough) to blur any hint of genetic structure, it is nearly impossible to properly estimate gene flow among undefined populations (Pfenninger and Posada, 2002). Thus, elucidating how gene flow affects local adaptation in this system constitutes a distinct challenge, really difficult to circumvent even by increasing our analytical power or by getting longer assemblages.

However, the lack of overall genetic structure should not hinder the genetic differentiation of specific variants on which the basis of adaptive divergence is located. There are other systems in which the background genetic differentiation between phenotypically well-differentiated groups is low, and yet islands of selection whose local divergence outstands above the rest of the genome can still be found (Aguillon et al. 2018). These genome fractions may persist differentiated even in continuous gene flow scenarios between the study groups (Philips et al. 2008, Moody et al. 2015, Poelstra et al. 2014, Shaner et al. 2015). In our case, it seems that the divergence between populations is so recent that it has not translated into a genomic imprint to a scale large enough to be detected with the approach we have used (Aguillon et al al. 2018). However, there is compelling evidence that points to the existence of at least a part of the genome that has diverged, giving rise to the observed phenotypic differences. One possible explanation is that the only divergent regions are those expressed as phenotypes (Safran et al. 2016). These regions would be located in very specific areas of the genome and could be really scarce (Campagna et al. 2015), to the extent that low coverage genotyping approaches could miss them. Another possibility is that the SNPs responsible for the observed adaptive divergence could be located in regions of the genome with low genetic diversity, which would express a peak of divergence at a local genomic scale, but which would escape the outliers detection analysis used in this study (Campbell et al 2018) if the amount of genetic variability is distributed heterogeneously throughout the genome (Feulner et al 2015, Nosil et al. 2009, Poelstra et al. 2014). However, such heterogeneity does not seem to be an issue in our system, because background divergence is very low across all the genome, which suggests that SNPs with almost any degree of divergence could have been detected as outliers.

Finally, there could be no significant divergence at all on a genome-wide scale. It is true that previous studies point to a genetic basis, especially those that included reciprocal transplants or common garden experiments, but it could be the case that the observed differences were the result of a heritable epigenetic response to the environment (Groot et al. 2018, Trerotola et al. 2015). Such epigenetic marks could be inherited only up to a specific number of generations, after which the signal would be lost (Trerotola et al. 2015). To discard this hypothesis, we could confirm previous findings by extending common garden experiments a few generations more (Groot et al 2018), or we could perform a transcriptomic differentiation study (McGirr y Martin 2018).

In summary, we succeeded to demonstrate that genome-wide differentiation was not associated with local adaptation in an altitudinal gradient with marked environment differences between two lizard populations. The phenotypic differentiation pattern of these populations was obviously concordant with a scenario of ecologically-driven divergence, but it did not lead to any detectable genotypic isolation between them. Moreover, and for reasons that remain unveiled, genome-wide differenciation seems much lower than expected by geographic distance. Thus, we uncovered another example of locally (and divergently) adapted populations which remain undifferentiated on a genome-wide scale, a phenomenon that could be more frequent than previously thought due to publication bias against this apparent lack of positive results (Hendry 2009). Highlighting natural systems where divergent adaptation occurs with different degrees of genomic-wide differenciation (including none) is of paramount importance to understand how frequently isolation by adaptation occurs, and to what extent the first incipient stages of ecological speciation are widespread (Feder et al. 2012, Hendry 2009, Shafer and Wolf 2013, Sexton et al. 2014, Krohn et al. 2018).

Data availability statement

Data for this study are available at PANGAEA (Llanos-Garrido, Pérez-Tris, & Díaz, 2019): https://doi.org/10.1594/PANGAEA.908220.

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Table 1. Phenotypic differences found between the populations of El Pardo and Navacerrada in previous studies.

Phenotypic trait	Reference
Shorter incubation times in the montane population.	Iraeta et al. 2006
Larger hatchlings in the lowland population.	Iraeta et al. 2006
Faster growth in lowland juveniles (reciprocal transplant experiment).	Iraeta et al. 2006
Faster growth in lowland juveniles (common garden experiment).	Iraeta et al. 2013
More plastic activity levels in response to food availability in the lowland population.	Iraeta et al. 2008
Larger clutches of smaller eggs in the lowland population.	Iraeta et al. 2008 Iraeta et al. 2013
Longer flight distance for pregnant females in the montane population.	Iraeta et al. 2010
Relatively longer hind limbs in the lowland population.	Iraeta et al. 2011
Larger adult females in the montane population.	Iraeta et al. 2013
Countergradient variation in body size: the genotypes that presumably control the key adaptations of the lowland population (larger eggs and hatchlings, and faster growing juveniles) occur in a low-productivity environment in which lizards grow more slowly and reach a smaller adult size.	Iraeta et al. 2006 Iraeta et al. 2013
More and relatively larger femoral pores in males from the lowland population.	Iraeta et al. 2011
Greater development of the sexual coloration of the head (i.e. larger colored surface) in males from the lowland population.	Iraeta et al. 2011 Llanos-Garrido et al. 2017

Phenotypic trait	**Reference**
Increased saturation of the sexual coloration of the head in males from the montane population.	Llanos-Garrido et al. 2017
Males respond to the activation of the immune system by reducing the extent of the sexual coloration of the head in the lowland population, and its saturation in the montane population.	Llanos-Garrido et al. 2017
Higher rates of infestation by tick nymphs in the montane population (no ticks in the lowland population).	Llanos-Garrido et al. 2017

Table 2. Phenotypic differences between the populations studied in this work: results of one-way ANOVAs with population as factor, and of two-way ANCOVAs (for the last two variables, marked with an asterisk) with population and sex as factors and size (SVL) as the covariate.

Feature	El Pardo (mean ± sd)	Navacerrada (mean ± sd)	Effect of population
Body length	68.5 ± 2.5	71.9 ± 5.1	$F_{1,38} = 6.73$; $p = 0.01$
Head length	10.4 ± 1.2	11.2 ± 1.5	$F_{1,38} = 3.50$; $p = 0.07$
No. of ticks	0.0 ± 0.0	4.4 ± 4.1	$F_{1,38} = 66.77$; $p < 0.001$
Hindlimb length*	22.2 ± 0.4	20.6 ± 0.4	$F_{1,35} = 19.54$; $p < 0.001$
No. of femoral pores*	17.8 ± 0.4	17.1 ± 0.2	$F_{1,35} = 2.56$; $p = 0.12$

Table 3. SNPs with greater divergence between populations detected by Bayescan. The Bayescan result (α and q) and the Chi-square analysis are presented to verify that the allelic frequencies of these SNPs do not differ significantly from what would expected under Hardy Weinberg's equilibrium.

SNP_ID	α (q)	H-W (El Pardo)	H-W (Navacerrada)
14537818	0.067 (0.883)	$\chi^2 = 0.436$; $p = 0.804$	$\chi^2 = 0.900$; $p = 0.638$
17216934	0.059 (0.887)	$\chi^2 = 5{,}445$; $p = 0{,}066$	$\chi^2 = 0.183$: $p = 0.913$
17911336	0.050 (0.891)	$\chi^2 = 7{,}871$; $p = 0{,}020$	$\chi^2 = 1.389$; $p = 0.499$
18623045	0.042 (0.885)	$\chi^2 = 0{,}720$; $p = 0{,}698$	$\chi^2 = 0.106$; $p = 0.949$

Figure legends

Figure 1. From top to bottom and right to left: location of sampling populations, theoretical straight-line dispersal route following valley bottoms between them, altitude profile of this route, and the position of both populations and dispersal route (means and SDs) in an environmental gradient described in detail elsewhere (Llanos-Garrido et al.2021). These environmental changes are based on an environmental PCA that summarizes BIOCLIM variables on temperature and humidity across all the species' range (annual mean temperature, maximum temperature of the warmest month, mean temperature of the warmest quarter, and annual precipitation).

Figure 2. Size differences between males with sexual ornamentation and those without it in Navacerrada (mean ± IC95).

Figure 3. Probability of assignment to groups generated by Admixture for the model with two groups

(K=2). On the left, CV-errors for each of the models analyzed; the most credible model is the one that includes only one group (K=1).

Figure 1.

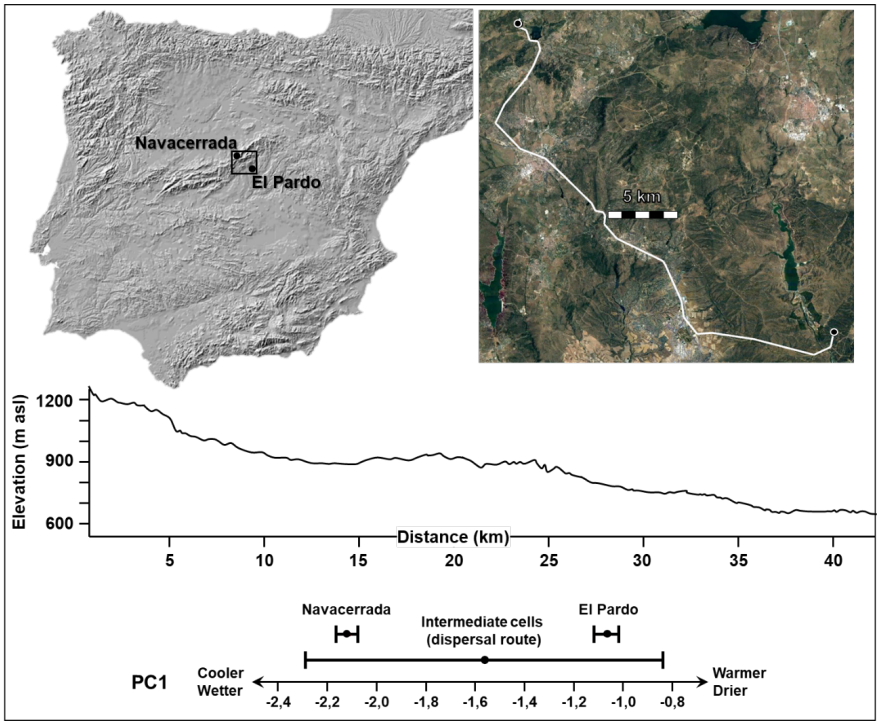


Figure 2.

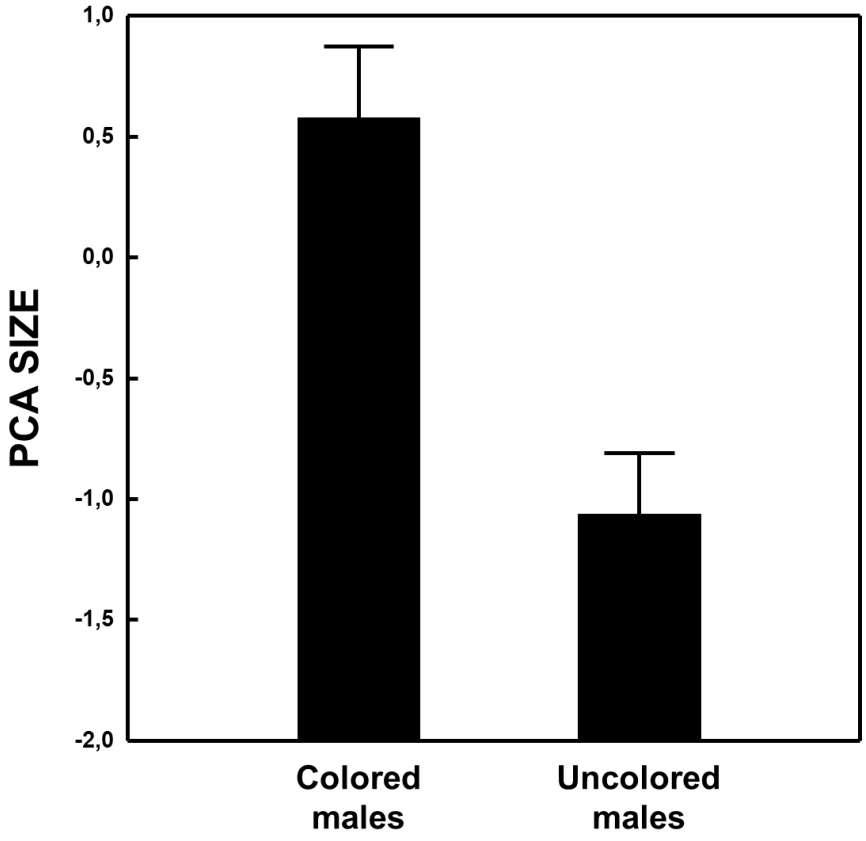
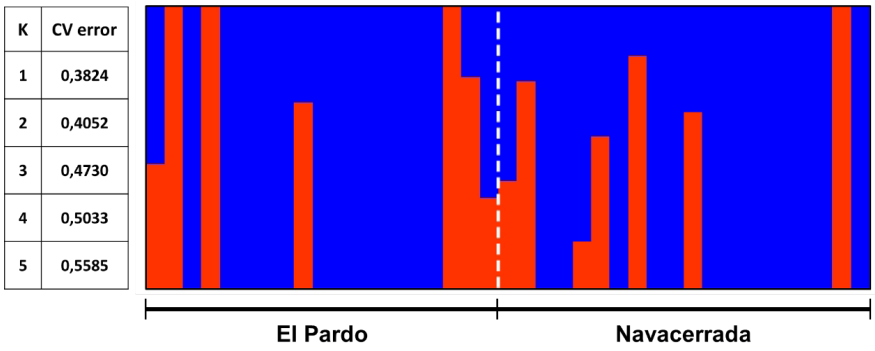


Figure 3.



Bibliography

Aguillon, S. M., L. Campagna, R. G. Harrison, and I. J. Lovette. 2018. A flicker of hope: Genomic data distinguish Northern Flicker taxa despite low levels of divergence. The Auk.

Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research.

Bonhomme, M., C. Chevalet, B. Servin, S. Boitard, J. Abdallah, S. Blott, and M. SanCristobal. 2010. Detecting selection in population trees: The Lewontin and Krakauer test extended. Genetics.

Campagna, L., I. Gronau, L. F. Silveira, A. Siepel, and I. J. Lovette. 2015. Distinguishing noise from signal in patterns of genomic divergence in a highly polymorphic avian radiation. Molecular Ecology.

Campbell, C. R., J. W. Poelstra, and A. D. Yoder. 2018. What is Speciation Genomics? The roles of ecology, gene flow, and genomic architecture in the formation of species. Biological Journal of the Linnean Society.

Chiu, K. W., and P. F. A. Maderson. 1975. The microscopic anatomy of epidermal glands in two species of gekkonine lizards, with some observations on testicular activity. Journal of Morphology.

Cole, C. J. 1966. Femoral glands of the lizard, Crotaphytus collaris. Journal of Morphology.

De Kort, H., K. Vandepitte, H. H. Bruun, D. Closset-Kopp, O. Honnay, and J. Mergeay. 2014. Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species Alnus glutinosa. Molecular Ecology.

Díaz, J. A. 1997. Ecological correlates of the thermal quality of an ectotherm's habitat: A comparison between two temperate lizard populations. Functional Ecology.

Diaz, J. A., A. L. Alonzo-Gomez, and M. J. Delgado. 1994. Seasonal variation of gonadal development, sexual steroids, and lipid reserves in a population of the lizard Psammodromus algirus. Journal of Herpetology.

Díaz, J. A., J. Pérez-Tris, J. L. Tellería, R. Carbonell, and T. Santos. 2005. Reproductive investment of a lacertid lizard in fragmented habitat. Conservation Biology 19:1578–1585.

Díaz, J. A., J. Verdú-Ricoy, P. Iraeta, A. Llanos-Garrido, A. Pérez-Rodríguez, and A. Salvador. 2017. There is more to the picture than meets the eye: adaptation for crypsis blurs phylogeographical structure in a lizard. Journal of Biogeography.

Ellegren, H., L. Smeds, R. Burri, P. I. Olason, N. Backström, T. Kawakami, A. Künstner, et al. 2012. The genomic landscape of species divergence in Ficedula flycatchers. Nature.

Feulner, P. G. D., F. ééJ J. Chain, M. Panchal, Y. Huang, C. Eizaguirre, M. Kalbe, T. L. Lenz, et al. 2015. Genomics of Divergence along a Continuum of Parapatric Population Differentiation. PLoS Genetics.

Foll, M., and O. Gaggiotti. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. Genetics.

Groot, M. P., N. Wagemaker, N. J. Ouborg, K. J. F. Verhoeven, and P. Vergeer. 2018. Epigenetic population differentiation in field- and common garden-grown Scabiosa columbaria plants. Ecology and Evolution.

Harris, R. B., P. Alström, A. Ödeen, and A. D. Leaché. 2018. Discordance between genomic divergence and phenotypic variation in a rapidly evolving avian genus (Motacilla). Molecular Phylogenetics and Evolution.

Iraeta, P., C. Monasterio, A. Salvador, and J. A. Díaz. 2006. Mediterranean hatchling lizards grow faster at higher altitude: A reciprocal transplant experiment. Functional Ecology 20:865–872.

Iraeta, P., C. Monasterio, A. Salvador, and J. A. Díaz. 2011. Sexual dimorphism and interpopulation differences in lizard hind limb length:Locomotor performance or chemical signalling? Biological Journal of the Linnean Society.

Iraeta, P., A. Salvador, and J. A. Díaz. 2013. Life-history traits of two Mediterranean lizard populations: A possible example of countergradient covariation. Oecologia.

Iraeta, P., A. Salvador, C. Monasterio, and J. A. Díaz. 2010. Effects of gravidity on the locomotor performance and escape behaviour of two lizard populations: The importance of habitat structure. Behaviour.

Joo, J. W. J., E. Y. Kang, E. Org, N. Furlotte, B. Parks, F. Hormozdiari, A. J. Lusis, et al. 2016. Efficient and accurate multiple-phenotype regression method for high dimensional data considering population structure. Genetics.

Lawson, L. P., and K. Petren. 2017. The adaptive genomic landscape of beak morphology in Darwin's finches. Molecular Ecology.

Llanos-Garrido, A., J. A. Díaz, A. Pérez-Rodríguez, and E. Arriero. 2017. Variation in male ornaments in two lizard populations with contrasting parasite loads. Journal of Zoology.

Martın, J., and A. Forsman. 1997. Social costs and development of nuptial coloration in male Psammodromus algirus lizards : an experiment. Behavioral Ecology 10:396–400.

McGirr, J. A., and C. H. Martin. 2018. Parallel evolution of gene expression between trophic specialists despite divergent genotypes and morphologies. Evolution Letters.

Moody, K. N., S. N. Hunter, M. J. Childress, R. W. Blob, H. L. Schoenfuss, M. J. Blum, and M. B. Ptacek. 2015. Local adaptation despite high gene flow in the waterfall-climbing Hawaiian goby, Sicyopterus stimpsoni. Molecular Ecology.

Nosil, P., D. J. Funk, and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology.

Palmer, D. H., and M. R. Kronforst. 2015. Divergence and gene flow among Darwin's finches: A genome-wide view of adaptive radiation driven by interspecies allele sharing. BioEssays.

Phillips, B. L., G. P. Brown, J. M. J. Travis, and R. Shine. 2008. Reid's Paradox Revisited: The Evolution of Dispersal Kernels during Range Expansion. The American Naturalist.

Poelstra, J. W., N. Vijay, C. M. Bossu, H. Lantz, B. Ryll, I. Müller, V. Baglione, et al. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. R. Ferreira, D. Bender, J. Maller, et al. 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American Journal of Human Genetics.

Ravinet, M., R. Faria, R. K. Butlin, J. Galindo, N. Bierne, M. Rafajlović, M. A. F. Noor, et al. 2017. Interpreting the genomic landscape of speciation: a road map for finding barriers to gene flow. Journal of Evolutionary Biology.

Roff, D. A. 2007. Contributions of genomics to life-history theory. Nature Reviews Genetics.

Safran, R. J., E. S. C. Scordato, M. R. Wilkins, J. K. Hubbard, B. R. Jenkins, T. Albrecht, S. M. Flaxman, et al. 2016. Genome-wide differentiation in closely related populations: the roles of selection and geographic isolation. Molecular ecology.

Salvador, A., J. P. Veiga, J. Martin, P. Lopez, M. Abelenda, and M. Puerta. 1996. The cost of producing a sexual signal: Testosterone increases the susceptibility of male lizards to ectoparasitic infestation. Behavioral Ecology.

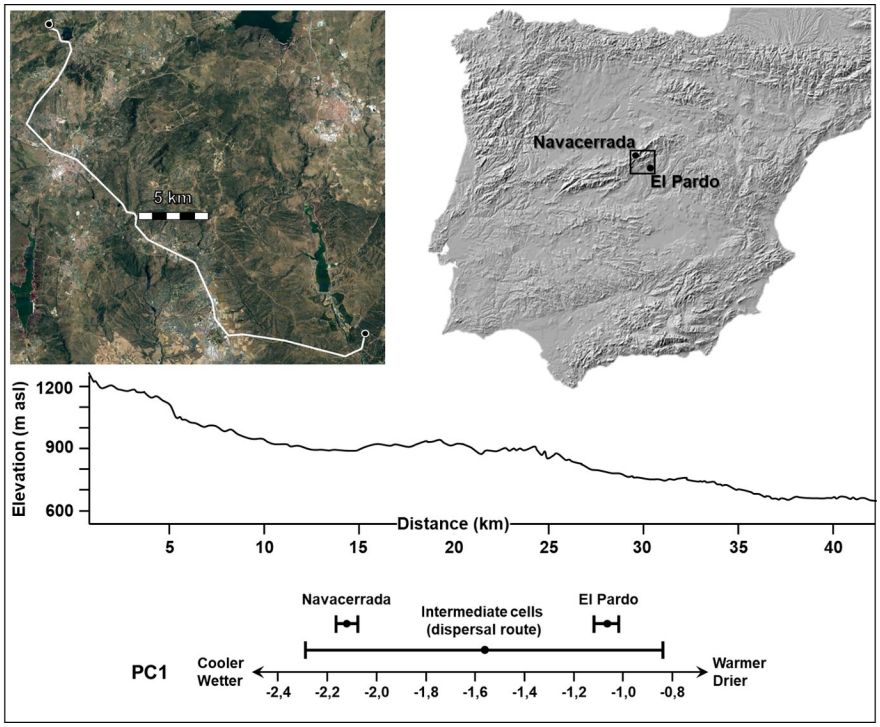
Shaner, P. J. L., T. H. Tsao, R. C. Lin, W. Liang, C. F. Yeh, X. J. Yang, F. M. Lei, et al. 2015. Climate niche differentiation between two passerines despite ongoing gene flow. Journal of Animal Ecology.

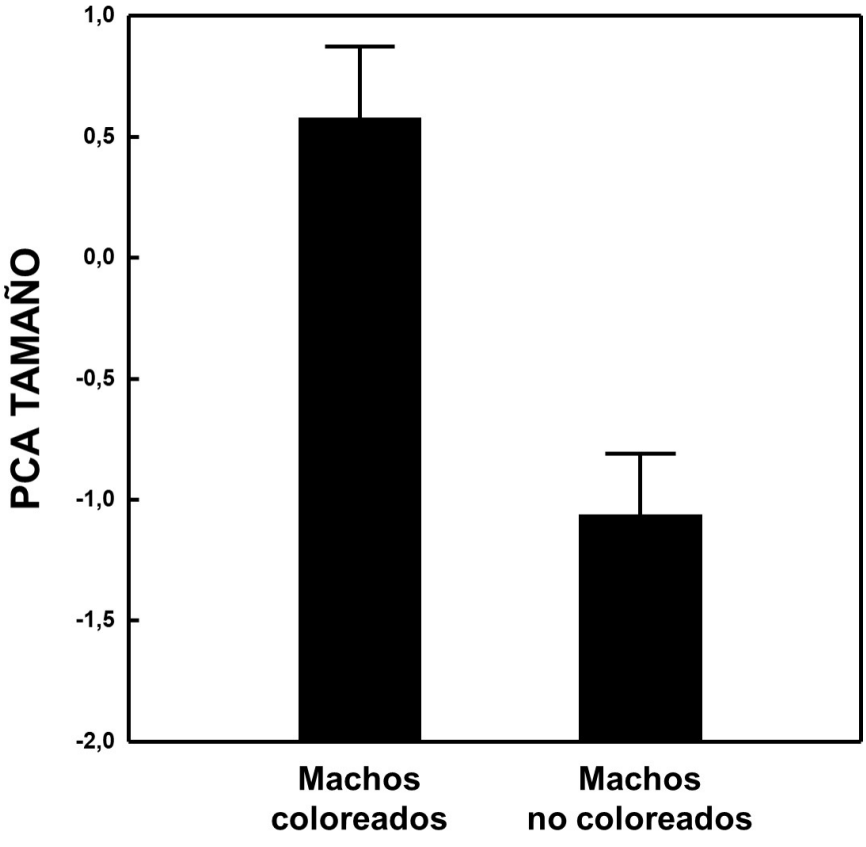
Tigano, A., and V. L. Friesen. 2016. Genomics of local adaptation with gene flow. Molecular Ecology.

Tigano, A., A. J. Shultz, S. V. Edwards, G. J. Robertson, and V. L. Friesen. 2017. Outlier analyses to test for local adaptation to breeding grounds in a migratory arctic seabird. Ecology and Evolution.

Trerotola, M., V. Relli, P. Simeone, and S. Alberti. 2015. Epigenetic inheritance and the missing heritability. Human genomics.

Verdú-Ricoy, J., S. Carranza, A. Salvador, S. D. Busack, and J. A. Díaz. 2010. Phylogeography of Psammodromus algirus (Lacertidae) revisited: Systematic implications. Amphibia Reptilia.





K	CV error
1	0,3824
2	0,4052
3	0,4730
4	0,5033
5	0,5585