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Ancient mitochondrial DNA introgression in the Iberian hare expresses a marginal environmental niche: a consequence of natural selection?

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Conflicts of interest

The authors declare that they have no conflict of interest.

Running title: Niche of *Lepus timidus* mtDNA introgression

Abstract

Aim: The role of mitochondrial DNA (mtDNA) in local adaptation has been thoroughly debated, and the ancient pervasive mtDNA introgression from *Lepus timidus* into *L. granatensis* allows testing this hypothesis. Previous studies have suggested that this mtDNA introgression could have resulted from historical demography, natural selection, or both. Here, we study the environmental niche of the introgressed mtDNA and get insights about the potential selective pressures maintaining the introgressed haplotypes.

Location: Iberian Peninsula

Taxon: *Lepus granatensis*

Methods: We modelled the frequency of mtDNA *L. timidus* lineage within the *L. granatensis* distribution area. The response variable was the presence of *L. timidus* lineage at individual level: animals with *L. timidus* lineage (n=406) vs. the same number of randomly selected points within *L. granatensis* distribution range that were used as background. Finally, the variation in the introgression frequencies were plotted along the environmental gradients (identified by the models), after controlling for latitude.

Results: The model identified environmental gradients (mostly related with high temperature ranges), in addition to latitude, as the more relevant in explaining the pattern of the introgressed animals within the species range. The results showed that i) the niche of the introgressed populations have marginal environmental conditions relative to the complete niche of *L. granatensis*, ii) both latitude and environment, and their overlaid effects, explain the variation in the introgression frequencies, and iii) after controlling for latitude, clines in mtDNA introgression frequency are associated with transitions in the environmental gradients, even when the analyses were restricted to the territory where the introgressed haplotypes occur.

Main conclusions: While the strict demographic explanation for mtDNA introgression in *L. granatensis* implies that geography, resulting from the post-glacial expansion of the species, explains the gradients of introgression, our results show correlations with environmental gradients that are not geographically structured. Our results relate the prevalence of introgressed mtDNA with more extreme climatic conditions in the range of *L. granatensis*, suggesting that environmental selective pressures could have played a role determining the spatial maintenance of mtDNA haplotypes originating from the cold-adapted species. These results are thus relevant to understand the response of the species to the ongoing processes of global change, which will alter the selective pressures by reducing the specific niche of the introgressed mtDNA lineage.

KEYWORDS environmental niche, evolution processes, *Lepus granatensis*, mitochondrial DNA, mountain hare, species distribution

1| INTRODUCTION

The distribution of genetic diversity across the distribution range of species is a product of demographic and selective phenomena, and characterizing and understanding these evolutionary processes is a fundamental goal in evolutionary biology (Arbogast, 2006; Avise, 2000). The genetic variation in space may result not only from intraspecific evolutionary process, but also from secondary introgression between species, and therefore arenas of hybridization offer important opportunities to elucidate the evolutionary consequences of interspecific genetic exchanges (Hewitt, 2001; Keller, Roos, Groeneveld, Fischer, & Zinner, 2010). Mitochondrial DNA (mtDNA) is frequently involved in introgression events (Toews & Brelsford, 2012), and despite being traditionally considered a nearly neutral marker, mtDNA harbours functional elements that regulate mitochondrial functions in interaction with nuclear encoded proteins (Cheviron & Brumfield, 2009; Dowling, Friberg, & Lindell, 2008). Cases of mtDNA replacement have been suggested to directly result from local adaptation (Toews & Brelsford, 2012), or to have important impacts on cytonuclear interactions leading to genetic conflicts (e.g. the mother's curse; Gemmell, Metcalf, & Allendorf, 2004; Smith, Turbill, & Suchentrunk, 2010) and cyto-nuclear coevolution (Beck, Thompson, Sharbrough, Brud, & Llopart, 2015). On the contrary, the abundant cases of mtDNA introgression can be a by-product of the historical demography of the interacting species and sex-biased effects (Currat, Ruedi, Petit, & Excoffier, 2008; Seixas, Boursot, & Melo-Ferreira, 2018). For example, hybridization during range invasions can lead to high introgression frequencies in the invasion front caused by strong drift, which is more pronounced in markers with lower migration rates and smaller effective population sizes (Currat et al., 2008), as mtDNA for female-philopatric species. This effect can be further enhanced by female-biased assortative mating in the hybridization events (Chan & Levin, 2005; Seixas et al., 2018). Models with substantial mtDNA introgression are therefore particularly appealing to understand the causes and consequences of mtDNA exchanges.

In the Iberian Peninsula, ancient hybridization during post-glacial range replacements led to high frequencies of mtDNA introgression from *Lepus timidus*, an arctic-boreal species present in northern Iberia at the last glacial maximum, LGM (e.g. Melo-Ferreira, Alves, Freitas, Ferrand, & Boursot, 2009), into populations of three hare species currently inhabiting the region (*L. castroviejoi*, *L. europaeus* and *L. granatensis*; see Acevedo, Melo-Ferreira, Real, & Alves, 2012), before *L. timidus* disappeared from Southern Europe. Since the description of the introgression (Alves, Ferrand, Suchentrunk, & Harris, 2003), several studies have been conducted to understand the causes and consequences of these ancient hybridization events. The analysis of random autosomal SNP frequency gradients across the range of *L. granatensis* suggested that the hybridization events occurred during a northwards postglacial range expansion of the species from southern Iberia into the range of *L. timidus*, resident in Iberia at the time (with ecological niche modelling suggesting the expansion could have originated closer to central Iberia; Acevedo, Melo-Ferreira, et al., 2012). This demographic model and the effect of drift can thus explain the appreciable amounts of introgression in the invaded territories (Melo-Ferreira et al., 2009). Several patterns of variation of the introgressed haplotypes in the Iberian hare fit this scenario, such as i) the south-north gradient of increasing frequencies of introgressed mtDNA haplotypes of *L. timidus* origin, ii) the northward increase in haplotype diversity among the introgressed haplotypes, iii) sectors of differentiation perpendicular to the introgression limits (e.g. Melo-Ferreira, Alves, Rocha, Ferrand, & Boursot, 2011). Recently, geographically-explicit demographic modelling based on whole-genome data suggested that the observed northwards gradient of introgression at the mtDNA is within the wide frequency variance expected under the "surfing" scenario, considering female philopatry and assortative mating (Seixas et al., 2018). However, this demographic scenario does not exclude that selection could have also played a role determining mtDNA and nuclear DNA introgression and its current geographic patterns, either by promoting local adaptation or resolving genomic conflicts created by demography-driven introgression (Chatfield, Kozak, Fitzpatrick, & Tucker, 2010; Seixas et al., 2018). For example, several nuclear genes show frequencies of introgression that are not compatible with the demographic model patterns of introgression, or that mimic the geographical pattern of mtDNA introgression, some of which have functions in mitochondria (Seixas et al., 2018). Moreover, mtDNA has been shown to have evolved under positive selection in hare species (Melo-Ferreira, Vilela, et al., 2014).

Whether the distribution of mtDNA introgression from *L. timidus* seen today in *L. granatensis* resulted from neutral demography, selective processes, or both, is therefore still a matter of debate (Alves, Melo-Ferreira, Freitas, & Boursot, 2008; Marques et al., 2017; Melo-Ferreira, Boursot, Suchentrunk, Ferrand, & Alves, 2005; Melo-Ferreira et al., 2009; Melo-Ferreira et al., 2011; Melo-Ferreira, Vilela, et al., 2014). While adaptive introgression predicts that the frequency of the introgressed variants depends on the adaptive landscape of the haplotypes in relation with the environment, introgression driven by purely demographic processes strongly relies on the biogeographical and demographic history of the involved species. The detailed study of variations in introgression along environmental gradients is useful to bring light at this respect since gene flow often follows a "stepping-stone model" along environmental gradients in which populations most frequently exchange migrants with neighboring populations that occupy relatively similar habitats (Cheviron & Brumfield, 2009; Räsänen & Hendry, 2008). A study at macroecological scale showed that the current distribution of mtDNA of *L.*

timidus origin in the Iberian Peninsula can be explained by the expected range occupied by the *L. timidus* during LGM (Acevedo et al., 2015), which may suggest some potential for local adaptation. However, this is also consistent with the purely demographic process, with recurrent introgression occurring during the species replacement, and mtDNA resting on the populations of the range previously occupied by the replaced species, given female philopatry (Marques et al., 2017; Melo-Ferreira, Farelo, et al., 2014; Seixas et al., 2018). In this context, here we test the potential of environmental-mediated selection pressures for maintaining *L. timidus* mtDNA introgression in *L. granatensis*. Concretely, we analysed i) whether *L. granatensis* individuals with mtDNA of *L. timidus* lineage are homogenously distributed along the species environmental niche or, in contrast, they occupy a marginal space; ii) the relative role of demography (geography) and environmental gradients (possibly related with adaptive processes) explaining the introgression frequency, and iii) the concordance between introgression frequency and environmental gradients, after controlling for confounding correlations of geographical and environmental gradients. This framework can provide relevant information on the potential adaptive role of the *L. timidus* mtDNA introgression.

2 | MATERIAL AND METHODS

2.1 | Environmental niche of *L. granatensis* introgressed animals

The environmental niche of *L. timidus* introgression within the distribution range of *L. granatensis* was determined using the locations in which the introgressed individuals were detected. For modelling, we used data concerning *L. timidus* introgression from previous studies (see Appendix 1 in Acevedo et al., 2015). A total of 406 individuals with *L. timidus* introgression were modeled against the same number of points randomly located within the current range of *L. granantesis* in the Iberian Peninsula, excluding introduced French populations (Acevedo, Melo-Ferreira, et al., 2012; Bencatel, Sabino-Marques, Álvares, Moura, & Barbosa, 2019.; see Fig. 1). With this model, the most relevant eco-geographical gradients discriminating the locations where the *L. timidus* mtDNA lineage was detected in relation to the overall *L. granatensis* distribution range, were identified.

➔ Figure 1

The niche model was parameterized using an 80% random sample of the data (training dataset) and evaluated against the remaining 20% (validation dataset). Nineteen bioclimatic variables (spatial resolution of approximately 1 km) were obtained from the WorldClim project database (see Table 1; Hijmans, Cameron, Parra, Jones, & Jarvis, 2005). In addition, geographic longitude and latitude were also included in order to account for the purely spatial structure of the data (Legendre, 1993), which would support the a purely demographic-driven process driving mtDNA introgression during species range changes. Prior to modelling, all predictors were standardized and centered in order to facilitate comparability of their relative importance in the model (Gelman, 2008). In addition, predictors were squared in order to explore possible curvilinear relationships with the response variable (Austin, 2007); an initial exploratory analysis of the different predictors showed that all response curves were $y = x + x^2$ type, providing a basis for ignoring polynomial terms higher than quadratic (Engler, Guisan, & Rechsteiner, 2004). The predictors were considered in a multiple logistic regression (Hosmer, Jovanovic, & Lemeshow, 2006), in which mtDNA *L. timidus* lineage vs random points within *L. granatensis* range was used as response variable and the bioclimatic and geographic variables as predictors. A first model was obtained by using forwards-backwards stepwise procedure based on the Akaike`s information criteria (AIC). The quadratic form of the predictors maintained in the first model were tested in a next step for providing better adjust than lineal ones. On the final model, including both lineal and significant quadratic terms, two components of its predictive performance were assessed in the validation dataset, namely discrimination and reliability (Jiménez-Valverde, Acevedo, Barbosa, Lobo, & Real, 2013). The area under the curve of the receiver operating characteristic plot (AUC) was computed using 'ROCR' R package (Sing, Sander, Beerenwinkel, & Lengauer, 2005) to assess the discriminatory power of the model. The reliability of the predicted probabilities was estimated by exploring the calibration plots (Pearce & Ferrier, 2000). These graphs were constructed using the R package 'ggplot2' (Wickham, 2009) by plotting the proportion of occupied evaluation sites against the predicted probability of presence (for the 10 equally wide probability intervals); points located along the 45° line indicate perfect calibration. The Hosmer–Lemeshow goodness-of-fit statistic (H-L; Lemeshow & Hosmer, 1982) was used to statistically assess whether the observed values matched those expected in the calibration plots.

➔ Table 1

Finally, to disentangle the effect of demographic processes and adaptive pressures explaining the introgression pattern, the variation in the introgression frequencies were plotted along the environmental gradient after controlling by the geographical gradients, i.e., after excluding the geographical inertia in the environmental gradient. Using deviance partitioning procedure we specified how much of the variation is explained by each factor (geography and environment), not affected by the collinearity with the other factor, and which proportion is attributable to their overlaid effects (Borcard, Legendre, & Drapeau, 1992; Peres-Neto, Legendre, Dray, & Borcard, 2006; for analytical details see Alzaga et al., 2009). Independently of the geographical gradient, a high concordance between the mtDNA *L. timidus* lineage frequencies and the environmental gradient may indicate that the maintenance of the mtDNA population genetic structure is promoted by environmentally mediated selection pressures (see Cheviron & Brumfield, 2009). If natural selection constrains gene flow, then the transitions in both introgression frequency and environmental variables should occur at the same point along the environmental gradient (coincident cline centers) and the cline widths should be equivalent (concordant cline widths) (Moore & Hendry, 2005).

Finally, considering that mtDNA introgression is possibly restricted to northern Iberia due to the demographic history of the northwards replacement, i.e. it is now present where hybridization occurred in the past (Melo-Ferreira, Farelo, et al., 2014), we conservatively built a new niche model limited to the area that was effectively explored for the species and thus where selective pressures for maintaining introgressed mtDNA haplotypes could have acted, northern Iberia. For this purpose, the new arena for modelling was delimited as the minimum convex polygon (MCP) encompassing all introgressed individuals (see Fig. 1). The analytical approach was then performed as described above, but the model was parameterized using the 406 individuals with introgression against 200 random points located within the previously fitted MCP.

3 | RESULTS

3.1 | Environmental niche of *L. granatensis* introgressed animals

The result of the model built here is interpreted as the main environmental factors modulating the introgression pattern within the *L. granatensis* distribution area in Iberia. The evaluation parameters indicated a good predictive performance (discrimination and reliability) when model predictions were assessed on an independent dataset (20% of data were reserved for model evaluation): AUC=0.86 and H-L=17.12, p=0.03 (see also Figure 2), and the final model explained 43.33% of the deviance.

➔ Figure 2

The statistical model allowed identifying four environmental gradients, in addition to latitude, some of them in their quadratic form, as the more relevant to explain the distribution of mtDNA introgression of *L. timidus* origin (Table 2; see also Fig. 3).

➔ Table 2

➔ Figure 3

Finally, variation partitioning showed that pure effect of latitude explained 16.93% of the deviance, similarly the environmental gradients explained 15.19% and their overlaid effects 11.21%. When the potential effect of latitude is extracted from the environmental gradients (pure effect of environmental gradient), a high concordance is observed between the frequency of introgression and the environmental gradient (Fig. 4A). The same pattern was obtained with the model and subsequent analyses when they were restricted to northern Iberia (Fig. 4B). Results highlighted both geographical (latitude) and environmental (annual mean temperature, isothermality and precipitation of warmest quarter) variables as the main drivers segregating locations with mtDNA introgressed individuals from the background in the potential area of *L. timidus* occupancy at the LGM (Table S1).

➔ Figure 4

4 | DISCUSSION

Understanding the relative contribution of demographic processes and natural selection determining patterns of interspecific genetic exchange is not trivial. In particular, the fact that demographic processes of invasive replacements with hybridization, which can occur recurrently through climate cycles, can lead to appreciable amounts of introgression along the invasion front (Currat et al., 2008; Seixas et al., 2018), advises against simplistic interpretations of intraspecific gradients and high frequencies of introgression as resulting from natural selection. The ancient and currently massive introgression from *L. timidus* mtDNA into the populations of the northern range of *L. granatensis* is one such cases (Acevedo, Melo-Ferreira, et al., 2012; Marques et al., 2017), and a geographic explicit demographic model based on whole-genome variation previously suggested that the empirical gradient of mtDNA introgression in *L. granatensis* is within the wide variance expected from the demographic process of northwards invasion (Seixas et al., 2018). However, this does not preclude that selection could have played a role in the process, either promoting introgression or subsequently shaping its geographic patterns and population prevalence. While a strict demographic model predicts that geography and dispersal are the sole determinants of introgression patterns, here we show that, controlling for geography, gradients of mtDNA introgression still correlate with environmental gradients.

Our results suggest that the *L. granatensis* populations with introgressed mtDNA express a marginal niche in relation to the environmental availability within *L. granatensis* distribution range. Introgressed populations occupy an environmental space characterized by lower temperatures (annual mean temperatures and isothermality) and higher mean diurnal range and mean temperature of wettest quarter than the mean response for *L. granatensis* populations in Iberia. Previous studies showed a strong dependence of *L. timidus* on temperature gradients (Acevedo, Jiménez-Valverde, Melo-Ferreira, Real, & Alves, 2012, Acevedo et al., 2015; Levänen, Kunnasranta, & Pohjoismäki, 2018; Thulin 2003), which is coherent with the environmental characteristics obtained here for the localities with *L. timidus* mtDNA type, where temperatures (annual mean temperature, mean diurnal range, isothermality and mean temperature of wettest quarter) markedly determine their distribution. These works (Acevedo et al., 2012, 2015) suggest that the introgressed haplotypes can now be found where the mountain hare was likely present in the Iberian Peninsula in the past, which is delimited by specific environmental conditions that are marginal to the conditions expressed by the total niche of the host, *L. granatensis*. The restriction of the mtDNA haplotypes to northern Iberia can however simply represent the area of the hybridization events, with female philopatry and reduced intraspecific migration rates impeding the spread of mtDNA introgression to the south (Acevedo et al., 2015; Seixas et al. 2018). Still, the environmental correlation of mtDNA frequency gradients remains along the residuals of the relationship between environmental gradients and latitude, i.e. the environmental effect that is not latitudinally structured (Fig. 4), even when restricting the complete analyses to the northern range of the species. These results show that, at least in part, the prevalence and maintenance of mtDNA introgression in *L. granatensis* is not explained by geography, but is correlated with the environment. This suggests that natural selection could have played a role shaping the spatial pattern of introgressed mtDNA after the ancient hybridization events.

In *L. granatensis*, genome-wide patterns of introgression appear primarily determined by the demographic dynamics of the invasion, coupled with intrinsic genomic incompatibilities that prevent the persistence of introgressed variation on chromosomal regions of low recombination (Seixas et al., 2018). The role of selection maintaining introgressed variation may result from resolving genomic conflicts created by demographic driven introgression, hinted by outlier introgression patterns of male-fertility genes possibly in response to a predominance of foreign mtDNA haplotypes (suggested by Seixas et al., 2018), or driving local adaptation, as suggested by genomic analyses for immune related genes (Seixas et al., 2018). The environmental correlations shown here suggest that mtDNA introgression may have also contributed to local adaptation in the northern, colder, range of the species. Thermal and metabolic adaptation have been the prominent selective factors suggested to drive mtDNA-related adaptation (Boratyński et al., 2011, 2014; Lajbner, Pnini, Camus, Miller, & Dowling, 2018; Bettinazzi, Rodríguez, Milani, Blier, & Breton, 2019). Also, Araújo et al. (2013) suggested that while tolerance to heat is largely conserved across lineages, tolerance to cold varies between and within species. An exchange of the ability to tolerate cold from the cold-adapted *L. timidus* to the temperate *L. granatensis*, allowing it to cope with possibly sub-optimal areas for the species (Rieseberg, 2011), could explain the correlations shown by our work. The results presented here are relevant to understand the response of the species to the ongoing processes of global change (Pauls, Nowak, Bálint & Pfenninger, 2013), which will alter the selective pressures by reducing the specific niche of the introgressed mtDNA lineage. This hypothesis needs to be evaluated, testing functional and fitness differences between mtDNA haplotypes. Given the widespread nature of mtDNA introgression (Toews & Brelsford 2012), our results encourage research in that direction.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are found in Appendices Table S2.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article: **Table S1**. Results of the model developed for explaining *Lepus timidus* mtDNA introgression pattern within the observed range of *L. timidus* during LGM in the Iberian Peninsula. Predictors are listed following the order of entrance (the first at the top) in the stepwise procedure. *B* is the parameter coefficient and its standard error (SE).

Figure S1. Calibration plot on the evaluation dataset (numbers represent the number of individuals considered in each bin). Filled and open symbols indicate bins with ≥ 15 or < 15 localities, respectively.

Table S2. Data available.

BIOSKETCH

Antonio Carpio is a researcher at the Instituto de Investigación en Recursos Cinegéticos. His interests include the integration of macroecology and genomics to extract further insights for conservation biology, with special interest in hunting species.

Authors contributions: AJC, JMF, PA and PCA designed the study; PA analysed the data with the help from AJC; AJC and JMF wrote the paper with contributions from MMH, LF, RC, and PA; PCA, MMH, RC and LF provided data; PA, PAC, RC, MMH and LF commented on later drafts of the manuscript.

Table 1. Explanatory predictors used to model the environmental niche of *Lepus timidus* mtDNA introgression pattern within *L. granatensis* distribution range in the Iberian Peninsula (Hijmans et al., 2005).

Code	**Description**
BIO1	Annual mean temperature
BIO2	Mean diurnal range (mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (* 100)
BIO4	Temperature seasonality (standard deviation *100)
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7	Temperature annual range (BIO5-BIO6)
BIO8	Mean Temperature of wettest quarter
BIO9	Mean Temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter

Table 2. Results of the model developed for explaining *Lepus timidus* mtDNA introgression pattern within *L. granatensis* distribution range in the Iberian Peninsula. Predictors are listed following the order of entrance (the first at the top) in the stepwise procedure. *B* is the parameter coefficient and its standard error (SE).

Predictor	***B* (SE)**	***z*-value**
Latitude	2.819 (0.315)	8.949***
Annual mean temperature	0.675 (0.215)	3.149**
Annual mean temperature^2	-0.659 (0.157)	-4.200***
Isothermality	-0.813 (0.172)	-4.723***
Mean diurnal range	0.781 (0.183)	4.262***
Mean Temperature of wettest quarter	0.243 (0.126)	1.931·
Mean Temperature of wettest quarter^2	0.487 (0.141)	3.459***
Intercept	-0.494 (0.245)	-2.017*

Figure 1. In grey, the distribution of *Lepus granatensis* in the Iberian Peninsula (Palomo Gisbert, & Blanco, 2007; Bentanel et al., 2019). Individuals' introgressed with mtDNA *L. timidus* lineage (red circles) as well the random points (blue circles) within *L. granatensis* range selected for modelling are also shown. Area delimited by minimum polygon convex encompassing all introgressed individuals (green line; see Appendix II)

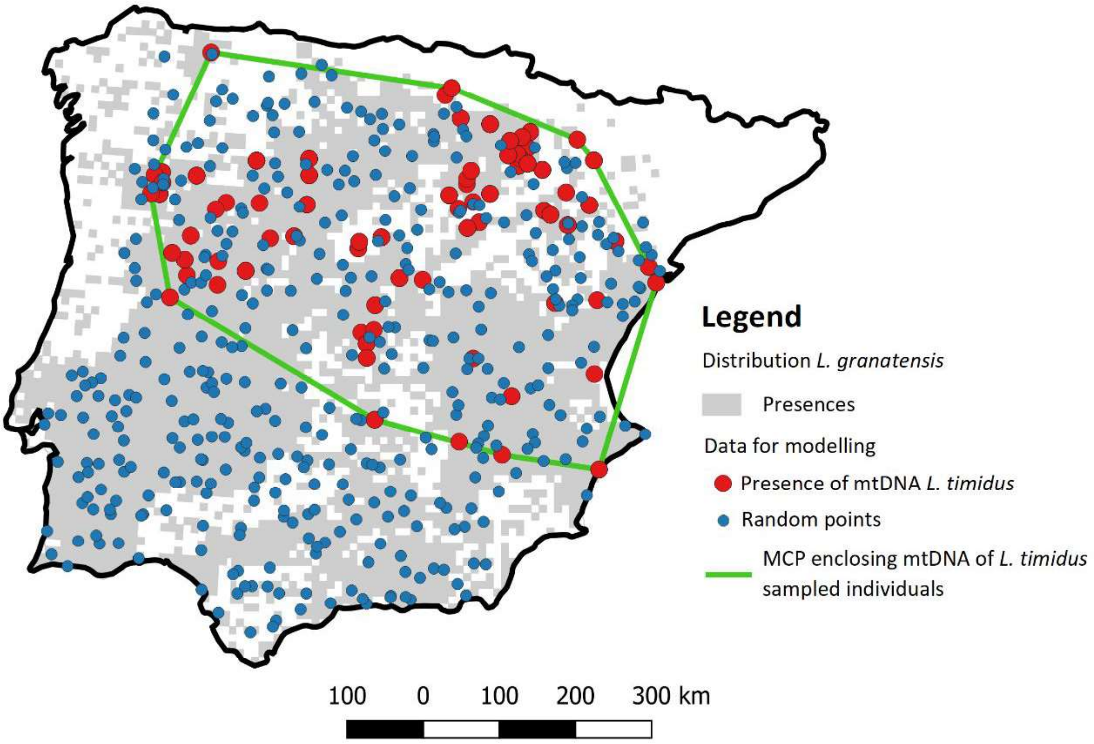


Figure 2. Calibration plot on the evaluation dataset (numbers represent the number of individuals considered in each bin). Filled and open symbols indicate bins with ≥ 15 or < 15 localities, respectively. In bins with < 15 localities, the observed frequency should be considered with caution (Jovani & Tella, 2006).

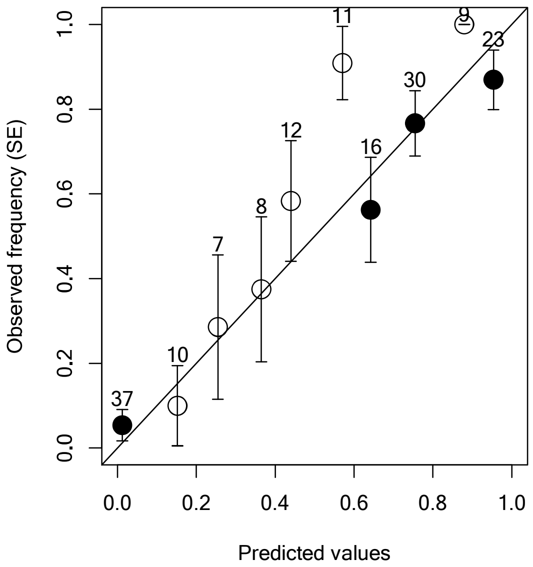


Figure 3. Variation in *Lepus timidus* mtDNA introgression frequency on *L. granatensis* population in relation to the main eco-geographical gradients (variables coded as in Table 1) able to discriminate between introgressed vs non-introgressed *L. granatensis* populations (see Table 2).

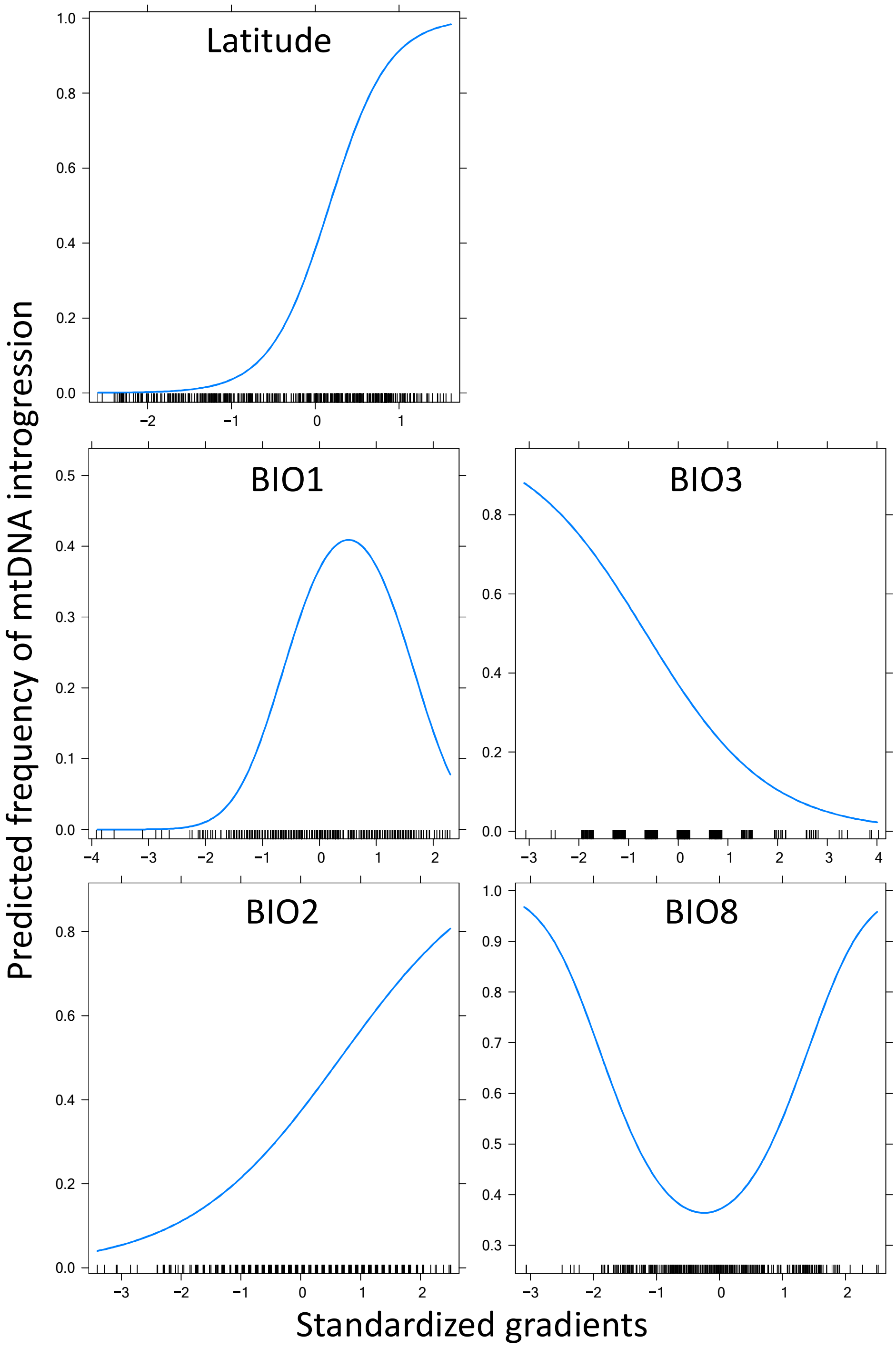
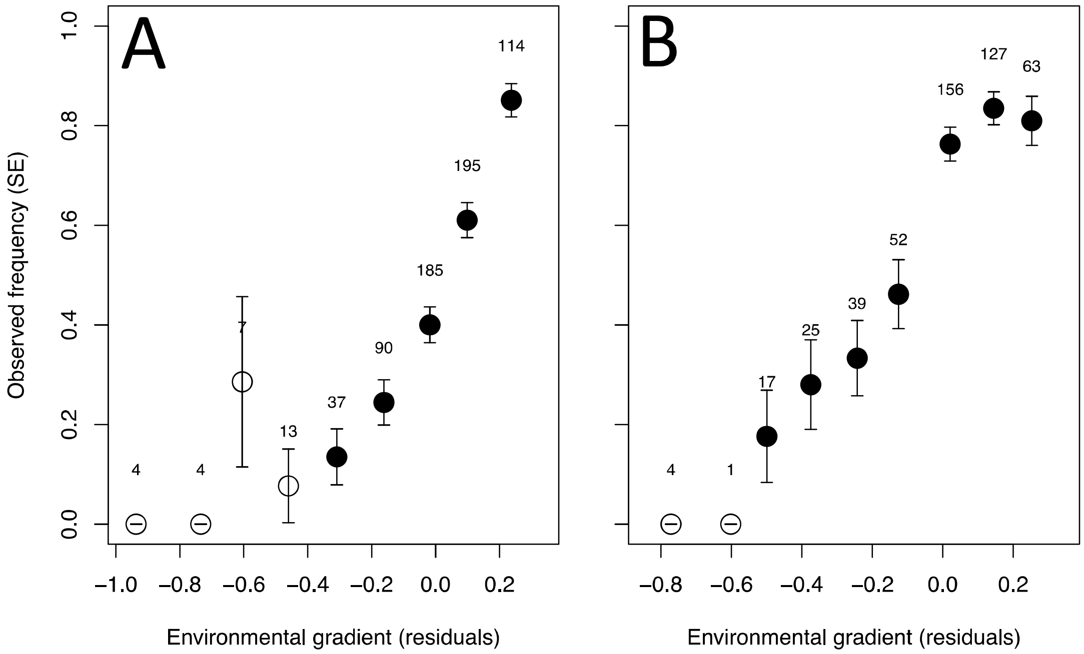


Figure 4. Plots showing variation in the predicted frequencies of *Lepus timidus* mtDNA introgression in *L. granantensis* along the main environmental gradients, after controlling by latitude. A) Results obtained for the model parameterized for overall *L. granatensis* distribution range. B) Results for the model limited to the range in which *L. timidus* mtDNA is now present (see also Appendix II). Filled and open symbols indicate bins with ≥ 15 or < 15 localities, respectively. In bins with < 15 localities, the observed frequency should be considered with caution (Jovani & Tella, 2006).



Supplementary Material

Ancient mitochondrial DNA introgression in the Iberian hare expresses a marginal environmental niche: a consequence of natural selection?

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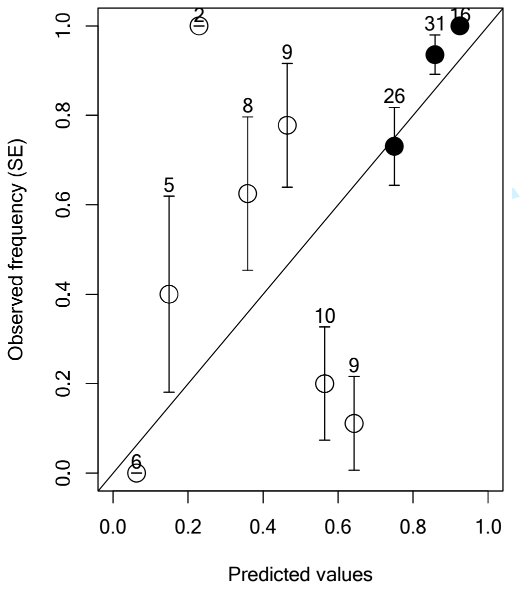
Table S1. Results of the model developed for explaining *Lepus timidus* mtDNA introgression pattern within the observed range of *L. timidus* during LGM in the Iberian Peninsula. Predictors are listed following the order of entrance (the first at the top) in the stepwise procedure. *B* is the parameter coefficient and its standard error (SE).

Predictor	***B* (SE)**	***z*-value**
Latitude	2.330 (0.269)	8.644***
Annual mean temperature	1.213 (0.165)	7.347***
Precipitation of the warmest quarter	-0.501 (0.170)	-2.953**
Isothermality	-0.337 (0.132)	-2.551*
Intercept	0.336 (0.149)	2.257*

Figure S1. Calibration plot on the evaluation dataset (numbers represent the number of individuals considered in each bin). Filled and open symbols indicate bins with ≥ 15 or < 15 localities, respectively.



The model explained 22.6% of the deviance and showed a good predictive performance: AUC=0.81 and H-L=34.9, $p<0.05$ (see also Figure S1).