Original Article**Range dynamics driven by Quaternary climate oscillations explain the distribution of introgressed mtDNA of *Lepus timidus* origin in hares on the Iberian Peninsula**

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Running title: Quaternary climate oscillations and range of introgressed mtDNA

Word count: 6482 (2 pages for tables and figures)

ABSTRACT

Aim A striking case of mitochondrial DNA (mtDNA) introgression occurs in the Iberian Peninsula: the mtDNA of *Lepus timidus*, a species that inhabited this territory during the Last Glacial Maximum (LGM), is almost fixed in the Iberian range of *Lepus europaeus*, is very frequent in the northern half of Iberia within *Lepus granatensis* and is fixed in *Lepus castroviejo*. Here, we have used a transdisciplinary approach to understand the ecological drivers of the distribution of this introgressed mtDNA in the Iberian hare species.

Methods First, we have modelled the climatic niche of *L. timidus* to predict the favourability for the species during the LGM. Second, we have gathered data regarding *L. timidus* mtDNA introgression for 1137 individuals (139 populations) from the Iberian Peninsula. We have tested whether the climatic favourability for *L. timidus* during the LGM reflects the frequency of the introgressed mtDNA in the carrier hare populations.

Results The climatic favourability for the LGM is positively related to the presence of individuals with *L. timidus* mtDNA (z -value=3.513, $P<0.001$), and discriminates better than by chance between individuals with and without this lineage (AUC=0.712). At the population level, the favourability values relate to the observed frequencies of mtDNA introgression ($r=0.454$, $P<0.001$) and discriminate between populations with and without *L. timidus* mtDNA (AUC=0.835).

Main conclusions The results suggest that the geographic distribution of *L. timidus* mtDNA introgression is linked to *L. timidus* distribution in Iberia during the LGM. Our study helps to understand the role of climate in the reticulated evolutionary history of hares, and is a first step toward understanding the ecological impact of mtDNA introgression on Iberian hares.

Keywords: climatic niche, Iberian hare, *Lepus granatensis*, mountain hare, reticulate evolution, species distribution modelling.

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47 INTRODUCTION

48 Quaternary climatic changes have governed the geographical range dynamics of species
49 worldwide, affecting their spatial distribution and genetic diversity (Hewitt, 2000).
50 Patterns of genetic variation, when interpreted within the context of geography (s.s.
51 phylogeography), can provide important insights into the history of species (Avice,
52 2000). However, research in phylogeography has placed more emphasis on the phylo-
53 component than on geography, despite the inherent information that the latter contains
54 about the evolutionary envelope and ecology of the species (Kidd *et al.*, 2006).

55 The geography of species ranges can be understood by using species distribution
56 models (SDMs), which are descriptive and/or explanatory approaches for the
57 assessment of range drivers and the prediction of species occurrence in space and/or
58 time (see Guisan & Zimmermann, 2000). These approaches have been widely used to
59 address basic questions in ecology (Soberón, 2007) and conservation biogeography
60 (Lomolino, 2004). Moreover, SDMs can provide the information needed to generate
61 and test hypotheses concerning the past structure of genetic diversity (Richards *et al.*,
62 2007; Nogués-Bravo, 2009). When SDMs are analysed in an evolutionary context, they
63 can supply complementary information to that obtained from phylogeography (Diniz-
64 Filho *et al.*, 2013), as both SDMs and phylogeography are focused on the understanding
65 of macroevolutionary patterns, and the microevolutionary processes related to them
66 (Bermingham & Moritz, 1998; Rugg *et al.*, 2006). Phylogeographical evidence can
67 thus be complemented by SDM studies, as the latter provide quantitative hypotheses to
68 test regarding species distribution in past scenarios and a statistical inference of the
69 main determinants of the distribution ranges (Svenning *et al.*, 2011). For instance, the
70 applications of SDMs in evolutionary studies allow researchers to propose and validate
71 hypotheses about the existence and location of glacial refugia (e.g. Carnaval & Moritz,

2008; Acevedo *et al.*, 2012a; de Lima *et al.*, 2014), to explore ecological relationships within and between phylogenetically related taxa (Peterson *et al.*, 1999; Wiens & Graham, 2005; Acevedo *et al.*, 2014) or to reconstruct the conditions that allowed, among other things, historical hybridisation and genetic introgression to occur (Wielstra & Arntzen, 2012).

Hares (*Lepus* spp.) are an important example in the study of hybridisation and genetic introgression (Arnold, 2008). Past and ongoing events of hybridisation and/or mitochondrial DNA (mtDNA) introgression among species of hares are being increasingly reported in literature (e.g. Melo-Ferreira *et al.*, 2012). Most of the cases reported involve the mountain hare, *Lepus timidus*, an arctic/boreal species that is currently widespread in northern Eurasia, from the British Isles to the Russian Far East, with some isolated populations in Scotland, Poland, the Alps and Japan (Smith & Johnston, 2008). The range fluctuations of this species throughout the glacial cycles, attested by fossil records that show the presence of the species during the Upper Pleistocene in areas from where it later disappeared, such as Southern France (Lopez-Martinez, 1980) or the Iberian Peninsula (Altuna, 1970), created multiple contacts with other temperate hare species, thus facilitating hybridisation and introgression, most notably of mtDNA (Alves *et al.*, 2008a). One of the most extreme cases of mtDNA introgression among hares occurs in the Iberian Peninsula (e.g. Melo-Ferreira *et al.*, 2011) where mtDNA of *L. timidus* origin is almost fixed in the Iberian range of *Lepus europaeus*, is very frequent in the northern half of Iberia for *Lepus granatensis* and is fixed in *Lepus castroviejoi* (Alves *et al.*, 2008b; Melo-Ferreira *et al.*, 2012). On the contrary, introgression at nuclear loci seems generally limited (Melo-Ferreira *et al.*, 2009, 2012; but see Melo-Ferreira *et al.*, 2011 for a case of massive X-linked introgression into *L. granatensis*). Molecular data suggest two major time-frames for

these mtDNA introgression events, one during Middle Pleistocene, affecting the common ancestor of *L. castroviejo* and Italian *Lepus corsicanus*, and another at the LGM, affecting all three Iberian species (Melo-Ferreira *et al.*, 2007, 2012). Whether mtDNA introgression from *L. timidus* resulted from neutral demography, adaptive processes or both is a matter of debate (Alves *et al.*, 2008b; Melo-Ferreira *et al.*, 2005, 2009, 2011). While adaptive introgression predicts that the prevalence of the introgressed variants depends on the adaptive landscape of the haplotypes, introgression driven by purely demographic processes strongly relies on the biogeographic and demographic history of the involved species. Theoretical simulations show that situations of competitive replacement with hybridization may lead to massive introgression of genetic variants from the local into the invading species, due to drift in the invasion front and/or recurrent hybridization, and that the pattern should be stronger in markers linked to the least dispersing sex, as mtDNA in species with female philopatry (Currat *et al.*, 2008). This may explain mtDNA introgression from *L. timidus* into *L. granatensis* if the latter competitively replaced the former in northern Iberia by the end of the last glacial period (Melo-Ferreira *et al.*, 2007). A recent study carried out in the contact zone between *L. granatensis* and *L. europaeus* in northern Iberia suggested that recurrent introgression along the invasion front may better explain the patterns of mtDNA differentiation and that the current distribution of mtDNA of *L. timidus* origin in the Iberian hare species could thus be linked to the historical distribution of the species in the region (Melo-Ferreira *et al.*, 2014a).

To date the case of *L. timidus* mtDNA introgression in Iberia has only been addressed by means of molecular approaches. In this work, using SDMs, we test whether the distribution of this mtDNA lineage in other hare species reflects the past distribution of *L. timidus* in Iberia (Melo-Ferreira *et al.*, 2014a). This analysis helps

122 understanding the role of climate-driven range shifts in the reticulated evolutionary
123 history of hares.

124

125 MATERIAL AND METHODS

126 Species data and geographical extent for modelling

127 The current distribution of *L. timidus* in Europe was extracted from The Atlas of
128 European Mammals (Mitchell-Jones *et al.*, 1999), with UTM 50 × 50 km squares as
129 territorial units (Fig. 1). Two procedures were applied in order to select an appropriate
130 geographical background in which to parameterise the species niche model:

131 1) Given that a geographically-biased sampling effort was acknowledged by the
132 authors of the Atlas, we calculated the number of mammal species in each square in
133 order to consider only for modelling those squares in which at least one species was
134 reported. This filter excluded territories from Eastern Europe which coincided with the
135 most incomplete sampling areas identified by the Atlas' authors (A.J. Mitchell-Jones,
136 personal communication), and resulted in the selection of 2557 squares.

137 2) Since the extent of the geographical background (i.e. extent of the study area)
138 has substantial effects on the outputs of species distribution modelling (see Barve *et al.*,
139 2011; Acevedo *et al.*, 2012b), we delimited an adequate territory in order to study *L.*
140 *timidus* distribution within the 2557 squares selected in the previous step. This was done
141 by modelling the species distribution in which the third-degree polynomial of the spatial
142 coordinates were considered as predictors (TSA; Trend Surface Analysis). The
143 geographical background for niche modelling was represented by the localities which,
144 after carrying out the TSA, had a predicted favourability that was higher than the
145 minimum value assigned to a presence (for further details see Acevedo *et al.*, 2012b; see
146 Fig. 1). This procedure selected 1582 squares, of which *L. timidus* was reported in 760.

147 **Determining the species climatic niche**

148 The climatic drivers of *L. timidus* distribution – i.e. its “climatic niche” – were
149 determined on the basis of the locations in which the species currently occurs. The niche
150 model was parameterized using an 80% random sample of the species distribution data
151 (training dataset) and evaluated against the remaining 20% of the data (validation
152 dataset). Nineteen bioclimatic variables (spatial resolution of ~1km) were obtained from
153 the WorldClim project database for the present (see Hijmans *et al.*, 2005): annual mean
154 temperature (BIO1), mean diurnal range (mean of monthly (BIO2=max temp-min
155 temp), isothermality (BIO3=(BIO2/BIO7) * 100), temperature seasonality
156 (BIO4=standard deviation *100); max temperature of warmest month (BIO5), min
157 temperature of coldest month (BIO6), temperature annual range (BIO7=BIO5-BIO6);
158 mean temperature of wettest quarter (BIO8), mean temperature of driest quarter (BIO9),
159 mean temperature of warmest quarter (BIO10), mean temperature of coldest quarter
160 (BIO11), annual precipitation (BIO12), precipitation of wettest month (BIO13),
161 precipitation of driest month (BIO14), precipitation seasonality (BIO15; coefficient of
162 variation), precipitation of wettest quarter (BIO16), precipitation of driest quarter
163 (BIO17), precipitation of warmest quarter (BIO18), and precipitation of coldest quarter
164 (BIO19). Multicollinearity among predictor variables in a model can bias the
165 predictions when the model is transferred outside the range/timeframe in which it was
166 trained, and in which correlation among variables may be different, as in this study. In
167 order to avoid multicollinearity-derived problems, we quantified the variance inflation
168 factor (VIF) in the training dataset in order to exclude those predictors with VIF>10
169 from the analyses prior to modelling (Montgomery & Peck, 1992). VIFs were
170 calculated for each predictor as the inverse of the coefficient of non-determination of
171 the regression of each predictor against all others by using the 'HH' R package

(Heiberger, 2012). The predictors selected after controlling the VIF were considered in a multiple logistic regression (Hosmer & Lemeshow, 1989), in which the *L. timidus* distribution was used as a response variable and the bioclimatic variables selected were used as predictors. The final model was obtained by using a forwards-backwards stepwise procedure based on the Akaike Information Criterion (AIC).

In order to test that our predictions are not biased for the selection of an unique modelling technique, we assessed the consistency of the predictions obtained from logistic regression when they are compared with those derived from seven different techniques widely used in species distribution modelling (namely generalized additive model, boosted regression trees, classification tree analysis, artificial neural network, BIOCLIM and flexible discriminant analysis) and, in addition, an ensemble of their forecasts (see Appendix 1 in the Supporting Information). The results showed that the logistic regression is as robust as obtained by other methods and the predicted pattern for LGM from logistic regression is consistent with the obtained for most explored methods (Fig. S1, Tables S1 and S2). As model's complexity (e.g. Merow *et al.*, 2014) limit the capability of models to be projected, in our study we opted to show results from logistic regression as a simpler, well-known technique able to produce robust inference.

Two components of the model's predictive performance were assessed in the evaluation dataset, namely discrimination and reliability (Jiménez-Valverde *et al.*, 2013). The area under the curve of the receiver operating characteristic plot (AUC) was computed by using the 'ROCR' R package (Sing *et al.*, 2012) to assess the discriminatory power of the model in the evaluation data. The reliability of the predicted probabilities was estimated by exploring the calibration plots (Pearce & Ferrier, 2000). Graphs were constructed using the 'ggplot2' R package (Wickham, 2009) by plotting the

197 proportion of occupied evaluation sites against the predicted probability of presence (for
198 the ten equally sized probability intervals); points located along the 45° line indicate
199 perfect calibration. The Hosmer–Lemeshow goodness-of-fit statistic (H-L; Lemeshow
200 & Hosmer, 1982) was used to statistically assess whether the observed values matched
201 those expected in the calibration plots.

202 The objective of the model calibrated for the present was to identify the climatic
203 favourability for the species. The parameterised model was therefore used to identify
204 the location of the climatically favourable areas for the species during the LGM, when
205 *L. timidus* was present in the Iberian Peninsula. This was done by projecting the model
206 on bioclimatic variables for the LGM (Braconnot *et al.*, 2007), which are available at a
207 spatial resolution of ~5km. Previously, as the models are not able to accurately predict
208 beyond the range of values of the predictors used for training (Campbell, 2004), a
209 multivariate environmental similarity surface (MESS) analysis was developed (Elith *et*
210 *al.*, 2010). The MESS was used to estimate how similar each square in the LGM was to
211 the training dataset (the present), in relation to the predictors retained in the final model.
212 Negative values were obtained for those squares in which at least one predictor had a
213 value that was outside the range of environments represented in the training dataset, and
214 these are therefore climatic conditions that differ from those that the species is currently
215 experiencing, where the uncertainty of the predictions is high. Therefore, the model was
216 not projected to the past in those squares outside the climatic range represented in the
217 training dataset.

218 **Determining *L. timidus* mtDNA introgression in hares from the Iberian Peninsula**

219 We used both data concerning *L. timidus* introgression reported in previous studies
220 carried out by our research group (Alves *et al.*, 2008b; Melo-Ferreira *et al.*, 2011) and
221 new data obtained to improve the representativeness of the populations sampled. A total

of 1137 individuals (139 populations) were analysed, including samples of *L. europaeus* ($n=179$ individuals) and *L. granatensis* ($n=958$ individuals) – see Fig. 1 and Appendix S2.

Total genomic DNA was extracted from frozen liver or ear tissue using the EasySpin Genomic DNA Tissue Kit (Citomed) or the JETQUICK Tissue DNA Purification Kit (Genomed). *Lepus timidus* mtDNA lineage was detected in each of the animals sampled, using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach implemented by Melo-Ferreira *et al.* (2005) or by the amplification and sequencing of a mitochondrial control region fragment, as described by Melo-Ferreira *et al.* (2007).

Relating model predictions for the LGM to *L. timidus* mtDNA in the Iberian Peninsula

Climatic favourability for *L. timidus* occurrence during the LGM was related to the *L. timidus* mtDNA detection in hares from the Iberian Peninsula at both the individual and the population levels. At the individual level we first explored the capability of the predicted probabilities to explain the presence of mtDNA foreign lineage by employing a generalised linear mixed model (GLMM; family "binomial-logit"), in which population was included as a random factor, and which was run with the use of the 'lme4' R package (Bates *et al.*, 2012). We additionally estimated the probability that an animal with *L. timidus* mtDNA lineage chosen at random would be sampled in a square with a higher probability for *L. timidus* occurrence during the LGM than an animal without *L. timidus* mtDNA also chosen at random, i.e. the AUC at the individual level.

The same rationale used at the individual level was also used to assess the predictive performance of the model at the population level. In order to avoid the inclusion of potential bias owing to an insufficient sampling size in the populations

247 sampled, only those populations that contained more than 10 sampled animals were
248 considered in these analyses. The discriminatory capacity of the model predictions to
249 separate populations with and without introgressed lineages, independently of their
250 frequency, was assessed using AUC, and a Pearson correlation was carried out to
251 explore the relationship between the predicted probabilities (probabilities of *L. timidus*
252 occurrence during LGM) and the observed *L. timidus* mtDNA origin frequencies.

254 **RESULTS**

255 From the initial set of predictors, only eight (BIO2, BIO3, BIO5, BIO8, BIO9, BIO15,
256 BIO18 and BIO19) were considered for building up the climatic niche model, as they
257 had all achieved VIFs<10. In the stepwise procedure (Table 1, see also Fig. 2) all the
258 predictors related to temperature were selected earlier than those related to precipitation.
259 The model achieved a good performance in terms of both discrimination (AUC=0.907)
260 and calibration (H-L: $\chi^2=11.28$, $P=0.186$; see Fig. 2) when it was assessed in the
261 validation dataset.

262 Previous to the model's transference to the LGM, the MESS analysis showed
263 that only southern areas of Europe had the LGM climatic conditions included in the
264 gradient represented in the calibration dataset, and the projection of the model to the
265 past was thus restricted to these areas. The results showed that the climatic favourability
266 for *L. timidus* is currently more restricted than it was in the LGM, when climatic
267 conditions in the Mediterranean peninsulas were favourable for it (Fig. 3).

268 The predicted probability for the LGM was significantly related to the presence
269 of individuals with *L. timidus* mtDNA (GLMM: $z\text{-value}=3.513$, $P<0.001$), and there
270 was a better than random discrimination between individuals with and without *L.*
271 *timidus* mtDNA (AUC=0.712). We also obtained consistent results at the population

level: the predicted probabilities related to the observed frequencies of mtDNA introgression (Pearson's $r=0.454$, $n=42$, $P<0.001$) and the discrimination between populations hosting the *L. timidus* mtDNA lineage and populations without this mtDNA were better than by chance (AUC=0.835).

DISCUSSION

Genetic and paleontological data suggest that climate oscillations played an important role in modulating the geographic distribution, evolutionary history and interspecific interactions of *L. timidus* in Western Europe (Altuna, 1970; Lopez-Martinez, 1980; Melo-Ferreira *et al.*, 2007). Ancient hybridisation in areas in which *L. timidus* is currently locally extinct, such as in the Iberian Peninsula, left genetic traces in the native species, which is particularly visible in their mtDNA (Melo-Ferreira *et al.*, 2005; Alves *et al.*, 2008a). A recent genetic study has suggested that the current geographic distribution of mtDNA of *L. timidus* origin in the hare species from the Iberian Peninsula could be a testament to *L. timidus* distribution in the region during the LGM (Melo-Ferreira *et al.*, 2014a). The results reported here confirm this hypothesis from a macroecological perspective.

According to our model (Table 1), *L. timidus* is distributed in cold areas with strong diurnal contrasts in temperature and a high seasonality in the precipitation regime, with very dry summers and very wet winters (e.g. Thulin, 2003; Acevedo *et al.*, 2012c). The four variables first selected in the stepwise procedure, and therefore acting on a larger spatial scale (see Muñoz *et al.*, 2005), were related to temperature. The strong dependence of *L. timidus* on temperature, and in particular on the maximum temperature, reinforces the idea of high sensitivity to ongoing climate change, which had previously been suggested for both this (Acevedo *et al.*, 2012c) and other species

inhabiting the Boreal-Alpine region (Thuiller *et al.*, 2005). Working on a large ecophysiological dataset including terrestrial ectotherms, endotherms and plants, Araújo *et al.* (2013) found that, in contrast to the lower thermal limit of the species climatic niche, the upper thermal limit is highly conserved in nature. As a consequence the species' capacity to adapt to climatic oscillations is principally constrained when the climatic niche of the species is close to its upper limit. The fact that *L. timidus* distribution is strongly limited by maximum temperatures suggests that its realized climatic niche is close to its upper temperature limit. In that case, *L. timidus* would be expected to have been strongly affected by post-glacial climate warming and to be also highly affected by ongoing climate change. This prediction is in agreement with the results obtained in previous works (Acevedo *et al.*, 2012c). This study thus provides new support for the potential role of climate in the decreasing demographic trend of this species in some European countries (e.g. Thulin, 2003), and we strongly suggest that climate must be considered among the potential factors that limit the viability of populations of *L. timidus* in Western Europe (Smith & Johnston, 2008).

Our model of *L. timidus* climatic favourability at the LGM produced a plausible scenario for the past distribution of this species in Iberia, with relevant insights into the biogeographical settings that drove mtDNA introgression into other Iberian hare species, in particular *L. granatensis*, despite the local extinction of *L. timidus* in the region. In keeping with theoretical predictions of Currat *et al.* (2008), the northwards gradient of increasing frequencies of mtDNA introgression of *L. timidus* origin into *L. granatensis* and the signs of expansion of the introgressed lineage, conform to a model of competitive replacement with hybridization of the resident *L. timidus* by an invading *L. granatensis* after the LGM, if the first was present in northern Iberia and the latter expanded from the south, spreading the traces of introgression into the newly colonized

territory (Melo-Ferreira *et al.*, 2005, 2007). Analyses of genetic diversity among the native *L. granatensis* mtDNA haplotypes further refined this model, suggesting that the species may have expanded from a refugium in central Iberia, and that the post-glacial expansion of the species may have been centrifugal (Melo-Ferreira *et al.*, 2011). This hypothesis agrees with SDMs, which suggest that central Iberia (area around Cáceres) was strongly suitable for *L. granatensis* at the LGM (Acevedo *et al.*, 2012a).

Interestingly, the genetic isolation by distance of native *L. granatensis* haplotypes is maximized if this area is considered the origin of a post-glacial expansion. In addition, Acevedo *et al.* (2012a) suggested that some other areas, in the north and the south, were also suitable for the species at the LGM. The present work suggests that *L. timidus* may have been widespread in northern Iberia at the LGM, which could have then impeded the presence of *L. granatensis* in the region. These areas could have nevertheless acted as points of attraction, defining the post-glacial colonization routes of an expanding *L. granatensis*. Similar range replacements may also explain massive introgression into *L. europaeus*, which likely have arrived to Iberia after the LGM (Stamatis *et al.*, 2009).

Another interesting pattern has been inferred from molecular data and deserves consideration here: the introgressed haplotypes of *L. timidus* origin show strong east-west structure in northern Iberia (Melo-Ferreira *et al.*, 2007). Considering this model of northwards range replacement with hybridization of the resident *L. timidus* by an invading *L. granatensis*, two phenomena may have contributed to this structured pattern of introgression: i) the fixation of different introgressed alleles by drift in the invasion front and amplification of these variants in the wave of colonisation; and/or ii) recurrent hybridisation with a structured *L. timidus* population along the invasion front. A recent molecular study suggested that recurrent hybridisation was probably involved, and that

the areas in which the introgressed haplotypes are currently found might thus represent the ancestral distribution of *L. timidus* in Iberia during the LGM (Melo-Ferreira *et al.*, 2014a). The results presented here agree with this hypothesis, by showing that the climatic favourability for the presence of *L. timidus* in northern Iberia during the LGM is correlated with the frequency of introgression (Fig. 3).

The biogeographical scenarios put forward to explain mtDNA introgression do not exclude the possibility that natural selection may have favoured mtDNA introgression or explain the persistence of the introgressed haplotypes in northern Iberia. Indeed, the comparison of the demographic signals of the introgressed and native mtDNA lineages in northern Iberia suggest that the former may have outcompeted the latter (Melo-Ferreira *et al.*, 2011). Also, a recent study has shown evidence of positive selection in the evolution of mtDNA in hares, particularly in the arctic lineage, of which *L. timidus* is part (Melo-Ferreira *et al.*, 2014b). Our results suggest that the current distribution of mtDNA of *L. timidus* origin in the Iberian Peninsula could mimic a particular ecological niche closely related to that expressed by *L. timidus* in Western Europe, which may indicate some potential for local adaptation. Even though our results do not demonstrate an adaptive advantage of *L. timidus* mtDNA in hares from Iberia (or of any other genes that may have been affected by ancient hybridisation events), they provide hypotheses to be tested in future field studies (Richards *et al.*, 2007). Future studies based on sampling populations across the introgression gradient are needed to characterise fitness-related parameters in both introgressed and non-introgressed individuals, and to provide conclusive evidence regarding the ecological role of *L. timidus* mtDNA in hares from Iberia. Also, given the close interaction of mitochondrial and nuclear encoded peptides in key aspects of energy metabolism, the inspection of

putative co-evolution and co-introgression of nuclear genes would shed light onto the adaptive nature of historical introgressive hybridization in this system.

373

374 **ACKNOWLEDGMENTS**

375 Sally Newton kindly reviewed the manuscript for grammar. We thank the Societas
376 Europaea Mammalogica and Tony Mitchell-Jones for providing the distribution data
377 used to prepare the Atlas of European mammals. P.A. is currently supported by the
378 Spanish Ministerio de Economía y Competitividad (MINECO) and Universidad de
379 Castilla-La Mancha (UCLM) through a ‘Ramón y Cajal’ contract (RYC-2012-11970).
380 J.M.-F. and R.C. hold FCT Investigator and postdoctoral grants, respectively, from the
381 Fundação para a Ciência e a Tecnologia (FCT), funded by Programa Operacional
382 Potencial Humano (POPH) – Quadro de Referencia Estrategico Nacional (QREN) from
383 the European Social Fund and by the Portuguese Ministerio da Educacao e Ciencia
384 (IF/00033/2014 and SFRH/BPD/64365/2009, respectively). This work is funded by
385 FEDER funds through the Operational Programme for Competitiveness Factors -
386 COMPETE and by National Funds through FCT - Foundation for Science and
387 Technology under the EXPL/AAG-MAA/1082/2013 and FCOMP-01-0124-FEDER-
388 041082. Partial found was also obtained from FCT-ANR/BIA-EVF/0250/2012 and by
389 the Spanish Ministry of Agriculture, Food and Environment, Spanish National Park’s
390 Network (project 1098/2014).

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SUPPORTING INFORMATION

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

Appendix S1 Assessing the reliability of the past model predictions: comparative

analyses of seven – plus an ensemble of forecasts – techniques .

Appendix S2 Introgression frequencies at the population level used in this manuscript.

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BIOSKETCH

Pelayo Acevedo 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. In addition, he studies the factors affecting the distribution and abundance of pathogens, and their hosts and vectors, through fragmented habitats.

Author contributions: P.A., J.M.-F. and P.C.A. conceived the ideas; J.M.-F., L.F. and R.C. compiled introgression data and performed the genetic analyses; P.A. calibrated the niche models and carried out the statistical analyses; P.A., J.M.-F., L.F., B.B., R.R., R.C. and P.C.A. participated in the discussion of the results and wrote the manuscript.

Editor: Miles Silman

Table 1. Results of the model developed for the current distribution of *Lepus timidus*. Predictors are listed following the order of entrance (the first at the top) in the stepwise procedure. *B* parameter coefficient and its standard error (*SE*), *z-value* test statistics. All coefficients are significant at $P<0.01$.

Predictor	<i>B</i> (<i>SE</i>)	<i>z-value</i>
Max temperature of warmest month	-0.067 (0.005)	-13.121
Isothermality	-0.207 (0.024)	-8.766
Mean diurnal range	0.097 (0.012)	8.171
Mean temperature of driest quarter	0.001 (1E-4)	5.274
Precipitation seasonality	0.038 (0.013)	2.931
Precipitation of warmest quarter	-0.008 (0.002)	-4.314
Precipitation of coldest quarter	0.007 (0.002)	4.232
Intercept	12.162 (1.136)	10.704

FIGURE LEGENDS

Figure 1. (a) Current distribution of *Lepus timidus* in Western Europe; presence data were referred to UTM 50 × 50 km grid cells and were taken from Mitchell-Jones *et al.* (1999). The arena for modelling that was delimited according to trend surface analysis (TSA) using a third-degree polynomial of the spatial coordinates applied to the occurrence localities of *L. timidus* (see Acevedo *et al.*, 2012b. (b) Distribution of the studied populations of *L. granantensis* and *L. europaeus* is shown.

Figure 2. (a) Probability of *Lepus timidus* occurrence in Western Europe for the present according to the climate niche of the species presented in Table 1. (b) Calibration plots showing the relationship between the predicted probability of occurrence for the model and the observed proportion of evaluation localities currently occupied by *L. timidus*. The number of evaluation localities in each interval of probability is also shown.

Figure 3. (a) Probability of *Lepus timidus* occurrence in Western Europe during the last glacial maximum (LGM; ~21 ky before the present) according to the climate niche of the species presented in Table 1. Territories with climatic conditions in the LGM beyond those considered when modelling the climate niche of *L. timidus* were delimited by means of a multivariate environmental similarity surface (MESS; see Elith *et al.*, 2010) and were not used to represent the probability of species occurrence. (b) Frequencies of the *L. timidus* mtDNA lineages (section in black) in populations with a sample size of ≥ 10 ; only the presence (grey boxes) or absence (white boxes) of *L. timidus* mtDNA lineage is shown for the remaining sample populations.

Fig.1

Fig. 2

Fig. 3

Supplementary material – Journal of Biogeography

Range dynamics driven by Quaternary climate oscillations explain the distribution of introgressed mtDNA of *Lepus timidus* origin in hares on the Iberian Peninsula -

- P. Acevedo, J. Melo-Ferreira, L. Farelo, B. Beltran-Beck, R. Real, R. Campos, P. C. Alves

Appendix S1. Assessing the reliability of the past model predictions: comparative analyses of seven – plus an ensemble of forecasts – techniques.

When modelling species distribution discrepancies among predictions obtained from different techniques can be produced. An operational solution to account for this variability is to simultaneously apply more than one technique and/or to fit ensembles of forecasts and analyze the resulting range of uncertainties with consensus methodologies (e.g. Araújo and New, 2007). We here showed the consistency of the predictions obtained from logistic regression when they are compared with those derived from seven different techniques widely used in species distribution modelling (namely Generalized Additive Model [GAM], Boosted Regression Trees [GBM], Classification Tree Analysis [CTA], Artificial Neural Network [ANN], BIOCLIM and Flexible Discriminant Analysis [FDA]) and, in addition, an ensemble of their forecasts. Analyses were performed with biomod2 using default specifications for each technique (for further details see e.g. Thuiller *et al.*, 2009, 2014).

We have followed the same analytical design described in the main text. Briefly, previous to modelling we have delimited the geographical background using a Trend Surface Analyses (Acevedo *et al.*, 2012). The same background-delimited arena was used for parameterizing models from all techniques. Data were randomly split in calibration (80%) and validation (20%). Results suggested that all single techniques,

except BIOCLIM, performed adequately according to discrimination measures, concretely Cohen's Kappa, true kill statistic [TSS] and AUC (see Table S1). Only techniques with TSS>0.6 were considered for ensemble and the predictive performance of ensemble was similar to that achieved by other techniques.

Table S1. Predictive performance measures of each technique when they were assessed on the independent validation dataset.

Technique/Parameter	Kappa	TSS	AUC
Logistic regression	0.855	0.856	0.975
GBM	0.867	0.868	0.984
GAM	0.927	0.928	0.994
CTA	0.809	0.811	0.915
ANN	0.836	0.837	0.956
BIOCLIM	0.400	0.398	0.699
FDA	0.858	0.859	0.974
Ensemble of forecasts	0.885	0.886	0.987

Fig. S1. Climatic suitability for *Lepus timidus* occurrence in Western Europe during the Last Glacial Maximum (LGM). Territories with climatic conditions in the LGM beyond those considered when modelling the climate niche of *L. timidus* were delimited (grey-areas) by MESS (Elith *et al.*, 2010) and excluded of the analyses.

Cartographically, there is also a strong consistency when models were hindcasted to the Last Maximum Glacial (LGM; see Fig. S1). After the exclusion of territories with climatic conditions in the LGM beyond those considered when modelling the climate niche of *Lepus timidus* – defined by means of a multivariate environmental similarity surface (MESS; see Elith *et al.*, 2010) – predictions of species favourability from the different techniques were strongly correlated (Table S2).

Table S2. Pearson's correlation coefficients among predictions for LGM from different techniques on the area do not excluded after MESS analyses ($n=949$; $P<0.001$ in all cases). BIOCLIM did not achieve the threshold of $TSS>0.6$ and therefore it was not included in the ensemble and was excluded of these analyses.

Technique	GBM	GAM	CTA	ANN	FDA	Ensemble of forecasts
Logistic regression (main text)	0.832	0.679	0.763	0.755	0.607	0.863
GBM	-	0.701	0.964	0.807	0.605	0.909
GAM		-	0.622	0.694	0.774	0.886
CTA			-	0.750	0.497	0.845
ANN				-	0.600	0.863
FDA					-	0.828

Overall, the results showed that the logistic regression is as robust as obtained by other methods and the predicted pattern for LGM from logistic regression is consistent with the obtained for most explored methods. As model's dimensionality (Peterson, 2011) and complexity (e.g. Merow *et al.*, 2014) limit the capability of models to be projected, in our study we opted to show results from logistic regression as a simpler, well-known technique able to produce robust inference.

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Appendix S2. Raw data for the number of individuals of *Lepus granatensis* (LGR) and *Lepus europaeus* (LER) with mtDNA of *Lepus granatensis* lineage (GRA), *Lepus timidus* lineage (TIM) and *L. europaeus* lineage (EUR). The number of individuals that were published in previous studies (PUB data) and those which are new for this study (NEW data) are also shown.

Species	Code	XCOORD	YCOORD	Size	mtDNA lineage			Source	
					GRA	TIM	EUR	PUB data	NEW data
LGR	ALA1	-2.698387	42.910000	15	11	4	0	15	0
LGR	ALA1	-2.554819	42.550332	44	27	17	0	44	0
LGR	ALB	-1.858542	38.994350	3	3	0	0	3	0
LGR	ALB	-2.156911	39.207123	4	4	0	0	4	0
LGR	ALB	-1.797291	39.237885	6	6	0	0	6	0
LGR	ALB	-2.603424	38.700745	12	11	1	0	12	0
LGR	ALC	-0.133537	41.051037	24	5	19	0	24	0
LGR	ALI	-0.490686	38.345997	20	18	2	0	20	0
LGR	ALI	-8.164983	37.876057	25	25	0	0	25	0
LGR	AMD	-6.840460	40.619846	3	1	2	0	3	0
LGR	AMD	-6.886843	40.803907	15	10	5	0	8	7
LGR	ARR	-7.986761	38.723221	21	21	0	0	21	0
LGR	BEN	-5.814288	42.010277	25	4	21	0	25	0
LGR	BRG	-6.756738	41.806114	11	2	9	0	11	0
LGR	CAC	-6.370961	39.471329	20	20	0	0	20	0
LGR	CBR	-7.172305	39.934232	24	24	0	0	24	0
LGR	CHV	-7.473165	41.741779	1	1	0	0	0	1
LGR	CHV	-7.306936	41.828781	3	0	3	0	0	3
LGR	CHV	-7.423473	41.790836	2	0	2	0	0	2
LGR	CRD	-4.485322	37.405640	6	6	0	0	6	0
LGR	CRE	-3.642048	38.910183	2	2	0	0	2	0
LGR	CRE	-3.374207	38.995762	12	12	0	0	12	0
LGR	CRE	-3.562719	38.521481	8	8	0	0	8	0
LGR	CRE	-3.910044	38.980040	11	11	0	0	3	8
LGR	CRN	-8.133835	43.000793	4	4	0	0	4	0
LGR	CRN	-8.408066	43.076374	2	2	0	0	2	0
LGR	CUE	-2.381535	39.690079	20	19	1	0	20	0
LGR	FCR	-6.942384	40.971539	1	1	0	0	1	0
LGR	FCR	-7.087496	40.877239	3	0	3	0	3	0
LGR	GER	2.765149	42.118183	1	1	0	0	0	1
LGR	GRN	-3.598557	37.177338	25	25	0	0	25	0
LGR	GRN	-2.929444	37.428333	1	1	0	0	0	1
LGR	GRN	-3.061281	37.182758	3	3	0	0	0	3
LGR	GRN	-2.780160	37.795662	1	1	0	0	0	1
LGR	GRN	-2.851806	37.790000	1	1	0	0	0	1
LGR	GRN	-2.927338	37.607674	1	1	0	0	0	1
LGR	GRN	-2.908233	37.529099	1	1	0	0	0	1
LGR	HLV	-6.944722	37.261421	2	2	0	0	0	2
LGR	HUE	-0.690008	42.275848	2	0	2	0	2	0
LGR	HUE	-0.433141	42.018589	5	0	5	0	5	0
LGR	JAE	-3.371246	38.011425	1	1	0	0	0	1
LGR	VBP	-8.889770	37.102550	8	8	0	0	8	0
LGR	LRD	0.538652	41.500664	10	0	0	10	0	10

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LGR	LUG	-7.556758	43.009739	1	1	0	0	0	1
LGR	MAD	-3.351601	40.232246	3	3	0	0	3	0
LGR	MAD	-3.523562	40.650913	22	8	14	0	22	0
LGR	MER	-7.660666	37.641514	12	12	0	0	12	0
LGR	MNT	-7.732006	41.850042	8	8	0	0	0	8
LGR	MOS	-0.449361	40.362770	1	0	1	0	0	1
LGR	MUR	-1.243637	38.033028	7	7	0	0	7	0
LGR	NAV1	-1.640235	41.974884	10	1	9	0	0	10
LGR	NAV1	-1.482148	42.187229	3	0	3	0	0	3
LGR	NAV1	-1.442261	42.378651	7	0	7	0	0	7
LGR	NAV1	-1.573341	42.319778	3	1	2	0	0	3
LGR	NAV1	-2.082455	42.476921	25	1	24	0	25	0
LGR	NAV1	-1.258404	41.922241	10	0	8	2	0	10
LGR	NAV1	-1.612827	42.064831	1	0	1	0	0	1
LGR	NAV1	-1.653248	42.119507	6	0	6	0	0	6
LGR	NAV1	-1.798200	42.101800	17	11	6	0	0	17
LGR	NAV1	-1.765864	42.277707	28	5	23	0	0	28
LGR	NAV1	-1.490363	41.999619	22	1	20	1	0	22
LGR	PAN	-8.901123	38.793315	29	29	0	0	29	0
LGR	PTM	-8.515796	37.169695	13	13	0	0	13	0
LGR	POL	-6.611051	43.271709	1	0	1	0	0	1
LGR	SAL	-5.184983	41.124939	8	6	2	0	8	0
LGR	SAL	-5.260701	41.047848	2	2	0	0	2	0
LGR	SAL	-6.359504	40.801659	1	1	0	0	1	0
LGR	SAL	-5.897394	41.206612	1	1	0	0	1	0
LGR	SAL	-6.359352	40.522388	1	0	1	0	1	0
LGR	SAN	-8.685994	39.236668	16	16	0	0	16	0
LGR	SEN	-6.271231	41.499542	1	0	1	0	1	0
LGR	SEN	-6.503643	41.307781	1	1	0	0	1	0
LGR	SEN	-6.808799	41.090080	3	1	2	0	3	0
LGR	SEN	-6.431105	41.415914	16	15	1	0	16	0
LGR	SES	-7.625556	40.298332	3	3	0	0	3	0
LGR	SES	-7.266131	40.538349	1	1	0	0	1	0
LGR	SES	-7.089524	40.351662	6	4	2	0	6	0
LGR	SES	-7.392667	40.514511	2	2	0	0	2	0
LGR	SEV	-5.764025	37.840450	1	1	0	0	1	0
LGR	SEV	-5.344389	37.711113	7	7	0	0	7	0
LGR	SOR	-2.463772	41.764431	1	0	1	0	1	0
LGR	SOR	-2.272888	41.308670	2	0	2	0	2	0
LGR	SOR	-2.365938	41.552589	1	0	1	0	1	0
LGR	SOR	-2.599558	41.474736	1	0	1	0	1	0
LGR	SOR	-2.458889	41.830002	1	0	1	0	1	0
LGR	SOR	-2.743580	41.634573	8	4	4	0	8	0
LGR	SOR	-2.457360	41.242658	4	0	4	0	4	0
LGR	SOR	-2.095995	41.648623	6	2	4	0	6	0
LGR	SOR	-2.392854	41.923808	2	0	2	0	2	0
LGR	TAR	0.480001	40.544415	3	0	3	0	0	3
LGR	TAR	0.373882	40.734818	10	1	9	0	0	10
LGR	TCA	-4.976654	42.049622	13	6	7	0	13	0
LGR	TCA	-4.780810	41.915787	1	1	0	0	1	0
LGR	TCA	-4.969508	41.855160	8	2	6	0	8	0

LGR	TER	-1.103675	40.341839	1	0	1	0	1	0
LGR	TOL	-4.027323	39.862831	26	19	7	0	26	0
LGR	TOR	-4.995759	41.504719	22	17	5	0	22	0
LGR	VAL	-0.376288	39.469910	5	4	1	0	5	0
LGR	VLP	-7.329707	41.569266	49	18	31	0	0	49
LGR	VLP	-7.431022	41.439006	1	1	0	0	0	1
LGR	VLP	-7.417325	41.675739	1	1	0	0	0	1
LGR	VLP	-7.467154	41.571795	22	21	1	0	0	22
LGR	VLP	-7.291761	41.706713	8	7	1	0	0	8
LGR	ZAM	-5.745313	41.507324	1	0	1	0	1	0
LGR	ZAM	-5.557500	41.351877	14	14	0	0	14	0
LGR	ZAR	-0.889581	41.648792	1	0	1	0	1	0
LGR	ZAR	-1.244587	41.438297	7	2	5	0	7	0
LGR	ZAR	-0.875759	41.257969	10	0	10	0	10	0
LGR	ZAR	-1.192911	42.249786	1	1	0	0	1	0
LGR	ZAR	-1.616740	41.395985	1	1	0	0	1	0
LGR	ZAR	-1.144898	41.385502	5	0	5	0	5	0
LGR	ZAR	-0.526964	41.488827	14	0	11	3	11	3
LER	ALA2	-2.860516	42.727879	1	0	1	0	1	0
LER	ALA2	-2.352369	42.669483	2	0	0	2	2	0
LER	ALA2	-2.992245	42.846432	1	0	1	0	1	0
LER	ALA2	-2.918374	42.712128	1	0	1	0	1	0
LER	ALA2	-3.098279	42.848080	1	0	1	0	1	0
LER	ALA2	-2.581248	42.882163	61	17	44	0	61	0
LER	ALA2	-2.583586	42.625389	11	11	0	0	11	0
LER	BAR	1.975309	42.244564	4	0	0	4	0	4
LER	BAR	1.818061	42.203552	4	0	0	4	0	4
LER	BAR	1.872023	42.080879	8	0	0	8	0	8
LER	CAN	-3.983120	43.183715	15	0	10	5	15	0
LGR	CAN	-3.921429	43.394523	1	0	1	0	0	1
LER	GER	2.103915	42.316177	1	0	0	1	0	1
LER	GER	2.874128	41.889561	5	0	0	5	0	5
LER	GER	3.056821	42.197102	4	1	0	3	0	4
LER	GER	2.365142	42.313667	4	0	0	4	0	4
LER	HUE	0.035862	42.230381	1	0	0	1	0	1
LER	JAC	-0.547055	42.571716	3	0	3	0	3	0
LER	JAC	-0.983557	42.586388	3	0	3	0	3	0
LER	JAC	-0.677305	42.627350	1	0	1	0	1	0
LER	JAC	-0.889167	42.641388	5	0	4	1	5	0
LER	LRD	0.899247	41.575160	8	0	0	8	0	8
LER	NAV2	-1.370966	42.786083	3	0	3	0	3	0
LER	NAV2	-1.645104	42.733093	1	0	1	0	1	0
LER	NAV2	-2.170814	42.895634	1	0	1	0	1	0
LER	NAV2	-1.002983	42.720951	1	0	0	1	1	0
LER	VLC	-3.572031	42.938454	28	0	28	0	28	0