Where and when local adaptation happens: lessons from the European barn owl (*Tyto alba*)

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Abstract

Climatic variations subject living species to evolutionary stresses and shape their distributions. Since the Last Glacial Maximum (LGM) and the subsequent temperature rise, species (re-) colonised higher latitudes. Among the evolutionary mechanisms involved during this process, local adaptation is thought to be one of the keystones of species persistence. However, its relative role and location in space remain elusive. The Western Palearctic barn owl (*Tyto alba*) recolonised Europe after the LGM (from the Italian and Iberic peninsulas) and offers an excellent opportunity to study local adaptation at the continental scale. Here, we modelled the evolution of the barn owl's ecological niche since LGM and showed that the birds probably followed its shift towards the North during the recolonisation. To identify traces of local adaptation, we scanned the entire genomes of 74 owls from 9 European populations using both Tajima's D and population-specific FST and associated genomic variants to temperature and precipitation through a Redundancy analysis. The combination of these methods yielded a total of 394 genomic windows putatively involved in the local adaptation distributed over all populations. Interestingly, our results identified a large region under selection in both refugial populations, consistent with climate change and ecological convergence in the two peninsulas. This study shed light on the genomic basis of local adaptation of the European barn owl, emphasised the importance of considering the ecological niche as a dynamic entity and paved the way to a better understanding of where and how local adaptation happens in a context of postglacial recolonisation.

Key words

Species Distribution Modelling, Genome scans, Genotype-Environment Association Analysis, postglacial recolonisation, Climate change

Introduction

Earth's climate fluctuates due to many extrinsic factors such as solar activity, changes in orbit or the amount of greenhouse gas in the atmosphere (Munn 2002). The resulting climatic variation affects species' distributions, which in turn shape the diversity of ecosystems around the world (Huntley and Webb 1989; Hewitt 2000; Davis and Shaw 2001). If individuals fail to survive or reproduce in a challenging environment induced by climatic change, local or global extinction of the species might happen (Jackson and Overpeck 2000). When the magnitude of climatic changes is not too wide nor too sudden, species persistence will depend on its ability to migrate and/or cope with and/or adapt to its environment (Lafontaine et al. 2018). Through migration, species will track their niches in space and shift their distribution to survive. However, In order to cope with the new conditions, species can either morphologically change via phenotypic plasticity or through genomic adaptation. With phenotypic plasticity, species will deal with the fluctuations of their environment by changing their phenotypes depending on the biotic and abiotic conditions (Nürnberger 2013) – a process described in plants (Gianoli and González-Teuber 2005; Atkin et al. 2006), insects (Sgrò et al. 2016) or birds (Biquet et al. 2022). However, because plasticity might have an evolutionary cost (Murren et al. 2015) or if the climatic changes exceed the range of possible plastic responses, natural selection will select the more tolerant individuals, and *de facto* induce the adaptation of the entire population.

Since the end of the Last Glacial Maximum (LGM), about 20'000 years ago (Clark et al. 2009), the earth's climate has experienced significant warming. With the rise of temperatures, the continental ice caps that covered part of the Northern and Southern Hemispheres melted, leaving room for many species to (re-)colonise previously unsuitable lands. Extensive

69 literature addressing the postglacial demographic history of European organisms showed that
70 this (re-)colonisation process was conditioned by the distribution of species during the
71 glaciation, combined with the complex landscape of the continent (reviewed in (Hewitt
72 2000)). However, even if the low-resolution genetic data used before the genomic era was
73 sufficient to retrace the past history of the species (such as demographic or migration events),
74 it proved too sparse to describe the complex evolutionary patterns that occurred during the
75 recolonisation, with the interaction of neutral and selective processes. In a theoretical point
76 of view, individuals of a given species might have migrated towards these new lands,
77 potentially inducing a decrease in population size and thus an increase in genetic drift. For
78 these newly founded populations, the environment could either be similar to the one
79 experienced previously or different to some extent. In the latter case, selective pressure could
80 have forced the population to locally adapt to the non-optimal environment. Individuals could
81 also have stayed at the core of their initial distribution (i.e., in the glacial refugia), but local
82 climatic conditions might also have changed since the LGM. Consequently, this change in
83 environmental pressure could have also led to adaptation in individuals who remained in the
84 glacial refugia. Given these different scenarios, the relative role of selective and neutral
85 processes as well as where they take place at the continental level remain elusive and, to our
86 knowledge, rarely addressed.

87 The Western Palearctic barn owl (*Tyto alba*), a nonmigratory raptor, recolonised Northern
88 Europe at the end of the LGM from two main glacial refugia located in the Iberian Peninsula
89 and the Italian / Greek Peninsulas (Cumer, Machado, Dumont, et al. 2022). Nowadays, its
90 range stretches from the Southwest to the Northeast of Europe (Cumer, Machado, Dumont,
91 et al. 2022). Studies investigating the genetic structure across its range showed that
92 populations of this species are weakly genetically differentiated (Antoniazza et al. 2010; Burri

93 et al. 2016; Cumer, Machado, Dumont, et al. 2022). Despite this, individuals from Southern
94 Europe and the Levant show a white phenotype, whereas those from Northern Europe have
95 a more reddish plumage (Antoniazza et al. 2010; Roulin 2013; Antoniazza et al. 2014; Burri et
96 al. 2016). ; Antoniazza et al. 2014 pointed out that this phenotypic variation cannot be
97 explained by purely neutral processes, emphasising that selection for local adaptation was or
98 is still acting on this phenotype. The well-understood history of the barn owl in Europe since
99 the Last Glacial Maximum, combined with the low genetic structure at the continental level,
100 as well as the previous evidence of local adaptation throughout Europe, make this species an
101 interesting model to test and quantify the extent of local adaptation for other traits than color
102 in the context of postglacial recolonisation.

103 Here, we evaluated whether climatic selective pressures could have induced local adaptation
104 in the European barn owl, assessed where in Europe these selective processes might have
105 occurred and how it might have helped the species to cope with its environment. First, we
106 used ecological information to model how the current climatic conditions experienced by the
107 barn owl differ from those during the Last Glacial Maximum. We then quantified the present
108 climatic heterogeneity faced by the barn owl, by evaluating how the current climatic
109 conditions differ spatially at the continental level. Second, we scanned the whole genome of
110 74 owls from 9 different populations to detect traces of selection. Based on the hypothesis
111 that some of the climatic conditions might play a role in local adaptation, we looked for an
112 association between the variants and climatic variables (namely temperature and
113 precipitation) through a Redundancy Analysis. Combining the genome scans and the results
114 from the redundancy analysis allowed us to identify genomic regions and genes potentially
115 selected under the influence of climate in each population. By using climatic information (i.e.
116 temperature and precipitation), we characterise the shift in space of the ecological niche of

117 the barn owl, as well as the shift in climatic conditions through time in the past glacial refugia.
118 Coupled with genomic information, we identified the key biological functions that enable
119 individuals of the same species to cope with a variety of environments. All together, these
120 results give a better understanding of where and how local adaptation happens and shed light
121 on the mechanisms allowing the persistence of species in a changing environment.

122

123 Material and methods

124 The general workflow followed in this study is depicted in Figure 1.

125 Ecological modelling

126 *Species Distribution Modelling*

127 To quantify the extent to which past climatic conditions during LGM faced by the barn owl
128 differ from the current ones (i.e. present), we conducted a Species Distribution Modelling
129 (SDM) to identify suitable areas for the species at both time points. For consistency, SDM was
130 done using the same Maximum Entropy modelling (MaxEnt) (Phillips et al. 2006) procedure as
131 the one described in Cumer et al. (2022). In brief, 19 WorldClim variables were extracted at 5
132 arc min resolution (Hijmans et al. 2005) with the rbioclim R package (Exposito-Alonso 2017).
133 Variables with a correlation equal to or higher than 0.8 were removed. This filtration step led
134 to 7 remaining climatic variables: Mean Diurnal Range (Bio2), Min Temperature of Coldest
135 Month (Bio6), Temperature Annual Range (Bio7), Mean Temperature of Wettest Quarter
136 (Bio8), Precipitation Seasonality (Bio15), Precipitation of Driest Quarter (Bio17), and
137 Precipitation of Coldest Quarter (Bio19). We ran a total of 100 quadratic MaxEnt models (with
138 a regularisation multiplier = 1), each of them using 10 '000 presence points independently

139 sampled from the IUCN distribution map (BirdLife International 2016). For each replicate, we
140 projected the models to the climatic conditions from the Last Glacial Maximum (about 20'000
141 years ago) extracted from the WorldClim database (Hijmans et al. 2005) as well as in the
142 present time. We used the conservative "maximum test sensitivity plus specificity" threshold,
143 commonly used in MaxEnt Modelling (Kebede et al. 2014; Vale et al. 2014), to transform the
144 projected output from the model into binary suitability maps. Finally, we averaged the values
145 among replicates and retained cells as suitable only if they were so in at least 90% of the
146 models, i.e., an average value equal to or larger than 0.9. For the projection in the past, we
147 used the Multivariate Environmental Similarity Surface (MESS) (Elith et al. 2011) approach to
148 identify areas from the past with climatic conditions absent from those in the calibration data.
149 Such geographic regions were considered unsuitable during the LGM.

150 *Quantification of currently suitable climate absent during the LGM*

151 We then determined whether currently suitable areas displayed climatic conditions different
152 from those found during the LGM. To do so, we extracted values of the 7 climatic variables
153 used in the SDM (see *Species Distribution modelling* section for details) at every continental
154 cell of the studied area for both past and present, merged them (for a total of 598'652
155 observations for each climatic variable) and applied a PCA. The first three principal
156 components were retained (Figure S2), explaining up to 91.50 % of the climatic variance. We
157 projected past and present suitable pixels within this PCA space and formed the "3D-climatic
158 space", representing the climatic conditions faced by the barn owl. Next, we aimed to classify
159 the present suitable conditions as "present" or "absent" during the LGM. To do so, a polygon
160 was built within the 3D-climatic space around the suitable conditions from the LGM, using the
161 alphashape3d R package (α = 0.5) (Lafarge and Pateiro-Lopez 2020). We split the current

suitable conditions into two categories: the ones located inside or outside the polygon, respectively “present” or “absent” from the range of LGM conditions. For the second category, we also computed the Euclidean distance between each pixel from the present time outside the polygon and the closest suitable pixel from the past, within the 3D-climatic space. This way, we quantified how much they diverged from the range of conditions experienced by the barn owl during LGM. We rescaled the distance values, by dividing each of them by the maximum to obtain rescaled values ranging between 0 and 1.

Estimation of the shift of climate within suitable areas

For each cell that was suitable during LGM and at present, we quantified the shift in climatic conditions using the 3D-climatic space described above. We computed the Euclidean distance between the past and present positions in the 3D-climatic space for each of them. Distance values were also rescaled from 0 to 1 by dividing each of them by the maximum value.

Individual barn owl sampling

Biological samples

This study took advantage of the datasets of European barn owls previously published by Cumer *et al.* (2022), Machado, Cumer, et al. (2021) and Machado, Topaloudis, et al. (2021). Whole genome sequences were retrieved from the Sequence Read Archive (SRA - Bioprojects PRJNA700797, PRJNA727915 and PRJNA727977, Table S1). To avoid spurious effects caused by strong genetic divergence, we focused on the mainland populations and discarded lineages from Cyprus (Machado, Topaloudis, et al. 2021) and the Canary Islands ((Cumer, Machado, Siverio, et al. 2022).

184 ## *Genetic data preparation*

185 Read mapping, variant discovery and variant filtering were performed according to Cumer *et*
186 *al.* (2022). In brief, raw reads were mapped to the reference barn owl genome (GenBank
187 accession GCA_018691265.1; Machado et al. 2021) with BWA-MEM v.0.7.15 (Li and Durbin
188 2009). Base quality score recalibration (BQSR) was performed in GATK v.4.1.3 using high-
189 confidence calls described in Cumer et al. (2022). Variants were called with GATK's
190 HaplotypeCaller and GenotypeGVCF v.4.1.3 from the recalibrated bam files. Genotype calls
191 were filtered using a hard-filtering approach as suggested for nonmodel organisms, using
192 GATK and VCFtools (Danecek et al. 2011). Details of technical filtration can be retrieved from
193 Cumer *et al.* (2022).

194 We identified pairs of individuals with a relatedness higher than 0.1, to prevent some alleles
195 from being over-represented in the dataset (relatedness statistic based on the method of
196 (Manichaikul et al. 2010), implemented in VCFtools v0.1.14 (Danecek et al. 2011)). We
197 removed one of the two individuals for each identified pair. In the end, this led to a dataset of
198 74 individuals (Table S1).

199 Thereafter, genomic regions whose SNP calling was uncertain were discarded. A further
200 filtering step was the exclusion of regions of the genome where our ability to confidently map
201 reads is limited (i.e. a "mappability" mask). To this end we followed the procedure
202 documented at lh3lh3.users.sourceforge.net/snpable.shtml. Briefly, the reference genome
203 was split into reads of 150 base pairs (bp) with a sliding window of 1bp. These artificial reads
204 were mapped back to the reference using BWA-MEM v.0.7.17. Regions of the sequence
205 where less than 90% of the reads map perfectly and uniquely were discarded by excluding
206 variants using a bed file in VCFtools v0.1.14. Thereafter, only bi-allelic SNPs were kept and

207 filtered for the Minor Allele Frequency (MAF) to be higher than 5% using VCFtools v0.1.14
208 (Danecek et al. 2011), thus removing rare alleles from the data set. Loci with more than 5% of
209 missing data were also removed and 16 scaffolds (out of 60) showing less than 1000 SNPs
210 were discarded from the analysis.

211 At the end of the filtration process, 4'689'284 SNPs from 39 scaffolds were retained,
212 genotyped in 74 European barn owls (10 individuals from the Aegean Islands (AE), 10 from
213 Denmark (DK), 4 from France (FR), 9 from Greece (GR), 9 from Italy (IT), 10 from Israel (IS), 9
214 from Portugal (PT), 5 from Serbia (SB) and 9 from Switzerland (CH)).

215 *Phasing process and evaluation*

216 Phasing and imputation of the individuals was performed in two steps. First, a read-based
217 phasing of each individual was performed using WhatsHap v1.0 (Martin et al. 2016). During
218 this step, haplotypes are reconstructed based on the mapped sequencing reads that cover
219 multiple variants. Second, an estimation-based phasing was done with ShapeIt4 v4.1.3 with
220 default parameters (Delaneau et al. 2019). The latter uses a statistical approach to infer
221 individuals' haplotypes based on the population genotypes (Delaneau et al. 2012) and uses
222 the phase information from WhatsHap.

223 To assess phasing performance, the switch error rate (SER) of the phasing produced by
224 ShapeIt4 was established for each individual (Choi et al. 2018). In brief, we ran a statistical
225 phasing of each individual without considering the read-based phasing from WhatsHap. This
226 phasing was then compared to the "true" local phasing, inferred from the read-based
227 approach (WhatsHap). The estimation of the switch error rate between both phasing sets was
228 done using the switchError code (available at https://github.com/SPG-group/switchError).
229 Among the 74 values, none exceeded 0.7% (Figure S4).

230 # Detection of traces of selection

231 ## *Genome scans in each population*

232 We combined two summary statistics to detect genomic regions putatively under selection:
233 population-specific F_{ST} and Tajima's D, both implemented in the hierfstat R package (Goudet
234 2005; Goudet et al. 2021). The two statistics were computed along the genome in non-
235 overlapping windows of 1 '000 SNPs length for each population independently. To detect
236 outlier windows, (regions with extreme values of positive population-specific F_{ST} and negative
237 Tajima's D), the two statistics were transformed into Z-scores, by subtracting the population
238 mean from each estimate and dividing it by the standard deviation, for each population
239 independently. Z-scores from all populations were then merged and a window was considered
240 an outlier in a given population if its Z-score for Tajima's D was in the lowest 5% and its Z-score
241 for population-specific F_{ST} in the highest 5% of all Z-scores from all populations. This allowed
242 us to focus on regions exhibiting signs of selection (low Tajima's D) with an excess similarity in
243 the population compared to the rest of the genome (high population-specific F_{ST}). See Cumer
244 et al. (2022) for more details about this approach.

245 ## *Landscape genomics*

246 We independently carried out a Genotype-Environment Association (GEA) analysis to assess
247 the relationship between the genotypes of the barn owl and their surrounding environment
248 for all populations simultaneously.

249 Based on the GPS coordinates of the 74 samples (Table S1), we extracted values for the same
250 7 climatic variables as the ones used in the Species Distribution Modelling (namely, Mean
251 Diurnal Range (Bio2), Min Temperature of Coldest Month (Bio6), Temperature Annual Range

252 (Bio7), Mean Temperature of Wettest Quarter (Bio8), Precipitation Seasonality (Bio15),
253 Precipitation of Driest Quarter (Bio17), and Precipitation of Coldest Quarter (Bio19), see
254 *Species Distribution Modelling* section for details). We performed a Principal Component
255 Analysis (PCA) on these climatic data to assess the level of climatic divergence experienced by
256 the sampled barn owls. We retained the first three principal components, explaining 94.97 %
257 of the climatic variance, and determined the differences between individuals within the PCA
258 space.

259 We then associated variants with genomic information using a Redundancy Analysis (RDA).
260 This method relies on a multiple linear regression of the genotypes on a set of climatic
261 predictors. The expected genotypes based on the model (also called fitted values) are
262 extracted and used as input for a PCA. Projection of the principal axes and components in the
263 RDA space (PCA of the predicted genotypes) allows the detection of SNPs that contribute the
264 most to the RDA axis and therefore whose allelic frequency might putatively be driven by the
265 environment (Capblancq and Forester 2021). The imputed genotype matrix was used as the
266 response matrix and we considered the bioclimatic variables extracted for each sampling
267 locality for the multiple linear regression. The RDA was performed using the vegan package
268 (Oksanen et al. 2020) in R.

269 A permutation test was performed to evaluate the significance of the relationship between
270 genotypes and climatic variables (Borcard et al. 2011). In brief, a test statistic (F-statistic) was
271 computed from the regression using the actual data. Afterwards, additional regressions were
272 carried out 999 times on permuted rows of the response data (i.e., the genotype matrix),
273 allowing the establishment of the empirical null distribution of the statistics, to which the
274 observed statistic was compared (Borcard et al. 2011). From this test, we found a significant

275 relationship between the environmental variables and genetic components (Table S3). To
276 select the number of RDA axes to retain, we also performed a permutation test for each axis
277 (n = 100) by following the procedure given by Borcard *et al.* (2011). Based on this method, the
278 first four RDA axes were kept, explaining 69.83 % of the constrained variance (Table S4). To
279 detect loci that strongly contribute to the individual's discrimination in the RDA space (outlier
280 loci), we followed the procedure described in (Capblancq et al. 2018): we computed
281 Mahalanobis distances between each locus and the RDA space's centre using the previously
282 retained four axes. P-values were adjusted for the false discovery rate (FDR) by computing q-
283 values using the qvalue R package (Storey et al. 2022). A value was considered an outlier if its
284 q-value was less than 0.1, following suggestions from Capblancq et al. (2018).

285 ***Concordance between the genome scans and landscape genomics***

286 Because the study aimed at detecting traces of selection linked with local climatic conditions,
287 outlier windows from the population-specific genome scans (see *Genome scans in each*
288 *population* section) which contained at least one SNP associated with the climate in the RDA
289 (see *Landscape genomics* section) were considered as the final list of windows putatively
290 under selection and used for downstream analysis.

291 We then assessed whether the number of climate-associated SNPs located within the outlier
292 windows from the genome scans was higher than expected by chance. We randomly sampled
293 along the genome as many windows as we previously identified as outliers (i.e., from
294 population-specific FST / Tajima's D genome scans, n = 544) and counted the number of
295 climate-associated SNPs in those randomly selected windows. We independently performed
296 this sampling 1'000 times. Based on the annotated barn owl genome (GenBank Assembly
297 Accession: GCA_018691265.1), genes that partially or fully overlapped the final list of outlier

298 windows were considered as potentially involved in local adaptation to climate and we,
299 therefore, extracted a list of genes for each population.

300 Gene Ontology

301 Gene Ontology Enrichment (GOE) was performed using ShinyGo v.076.3 (Ge et al. 2020) to
302 assess in which biological pathways the genes located in the final list of outlier windows could
303 be involved and if some of them might be linked to local adaptation to abiotic conditions.
304 GOEs were run for each set of genes coming from each population separately and one
305 additional GOE was performed using all the outlier genes from all populations. As a baseline
306 set of genes against which the observed enrichment signature was compared - also called
307 background list - we used all the genes annotated in the barn owl genome with coordinates
308 overlapping at least one of the 4'689'284 SNPs.

309 Ecological convergence in glacial refugia

310 Because we found a strong signal of selection shared between the Italian and Portuguese
311 populations (see *Results* section), we investigated in these two populations how the climate
312 has changed since the LGM. We extracted values of the 7 climatic variables used in the
313 ecological modelling for the LGM and today (see *Species Distribution Modelling* section) at the
314 GPS coordinates for the Italian and Portuguese samples. We projected these conditions inside
315 the 3D-climatic space (see *Quantification of currently suitable climate absent during the LGM*
316 section). Then, between each peninsula, we computed the 3D climatic pairwise Euclidean
317 distances between LGM and now for all sampling localities. This way, we assessed the change
318 in the climatic conditions in these two regions since the LGM.

319

Results

The shift of the suitable climatic conditions in Europe

Species Distribution Modelling of the barn owl today and during the LGM (~ 20'000 years ago) allowed us to evaluate how much the climatic conditions experienced by the bird differed between the two time points. During the Last Glacial Maximum, suitable regions were found mostly around the Mediterranean Sea, including the Northern part of Africa, the Iberian Peninsula as well as the Italian and Greek Peninsulas (Figure S1a). Nowadays, the suitable regions expanded to Western, Central and Northern Europe, with almost the entire continent being suitable (Figure S1b).

Based on these suitability maps, we evaluated the differences between the past and current climatic envelopes experienced by the barn owl. To do so, we build a PCA including climatic conditions at both time points with all the continental cells. We retained the three first axes as they explained 91.50 % of the variance. We then reprojected past and present suitable cells in this 3D-climatic space. Past suitable climatic conditions were used to build a past suitable polygon (see the *Quantification of currently suitable climate absent during the LGM* section for details).

31.05 % of the currently suitable areas were found within this past suitable polygon. These cells were thus considered as displaying climatic conditions similar to suitable conditions from the LGM. These areas, in purple surrounded by white in Figure 2a, are mostly located in the Northern part of Africa, the Northern-West part of the Iberian Peninsula, the Western part of France and the British Isles. In Western Europe, today's conditions are highly similar to those present during the LGM, with pixels outside the polygons but coloured in lighter blue, based on their low Euclidean distance to the past suitable polygon (Figure 2a). Finally, contemporary

343 climatic conditions become more and more different from those of the LGM as one moves to
344 central Europe (in light blue to white in Figure 2a). Overall, Western Europe today has similar
345 temperature and precipitation conditions to those in the glacial refugia, 20'000 years ago; The
346 further East we go, the largest the difference with climatic conditions that were present in the
347 Glacial refugia.

348 The areas considered suitable both during the LGM and today were mostly found in the
349 Northern part of Africa, the Iberian Peninsula and the coast of the Italian Peninsula. Within
350 these cells, we quantified the changes in climatic conditions by monitoring the shift of each of
351 them inside the 3D-climatic space (Figure 2b). The regions exhibiting the greatest climate shift
352 were the Iberian Peninsula and areas around the Strait of Gibraltar (in yellow in Figure 2b). In
353 comparison, the change in climatic conditions in the two other Mediterranean Peninsulas
354 (modern day Italy and Greece) was smaller or null (in green in Figure 2b).

355 To complement the general climatic investigation performed at the continental level, we also
356 evaluated how the climatic conditions experienced by the sampled barn owls differed
357 spatially. To do so, we applied a PCA on the climatic values extracted at the 74 GPS coordinates
358 (Figure S3). When reprojected inside the PCA space, individuals were clustered depending on
359 their sampling population. Polygons specific to each population did not overlap, except for
360 Greece and Portugal (Figure S3a) and Greece and Israel (Figure S3b). Overall, sampled barn
361 owls experienced different temperature and precipitation conditions depending on their
362 sampling location.

363 Detection of genomic regions under selection

364 Based on the hypothesis that the climatic condition differences played a role in the local
365 adaptation of the barn owl in the Western Palearctic, we combined a classical genome scan

366 approach with a Gene-Environment Analysis to identify regions of the genome under a
367 potential influence of climate. First, we detected a total of 2'101 outlier windows for Tajima's
368 D (616 privates and 1'485 shared by at least two populations) and 2'101 population-specific
369 F_{ST} outliers windows (987 unique and 1114 shared by at least two populations), corresponding
370 to the respective lowest and highest 5% quantile (Table S2). We combined the signals from
371 these two statistics and retained 544 outlier windows putatively under selection in at least
372 one population (grey squares in Figure 3a). On average, each population contained 76 outlier
373 windows. Portugal and Israel are the two extremes, with respectively 91 and 64 outlier
374 windows detected by our population-specific F_{ST}/Tajima's D genome scans (Table 1). Second,
375 we associated the variants to temperature and precipitation variables through a Redundancy
376 Analysis. According to the regression model, 4.99 % of the genetic variance was explained by
377 climatic variation. This analysis showed a total of 46'547 SNPs significantly associated with
378 climate, spread all over the genome (Figure S6).

379 To focus on selection induced by climate, we overlapped the two signals (i.e., one from
380 genome scans and one from the Redundancy Analysis). We retained genome scan outlier
381 windows in the final list of outliers if they contained at least one SNP associated with the
382 climate according to the RDA. Thus, 394 windows were retained (blue diamond in Figure 3a),
383 encompassing a total of 8'423 SNPs associated with temperature and precipitation. To
384 measure whether the windows identified in the genome scans were enriched in SNPs
385 identified by the Redundancy Analysis compared to the rest of the genome, we randomly
386 sampled 544 windows along the genome and counted the number of climate-associated SNPs
387 in those randomly selected windows. We performed this sampling 1'000 times and the
388 random distribution can be found in Figure S7. The observed number in the final list of

389 windows (8’423 SNPs) was larger than the random counts (mean of 5’395 SNPs) and distant
390 from the distribution.

391 Figure 3b shows the repartition of the 394 outlier windows among the nine populations of
392 European barn owls. A majority of these 394 outlier windows (80 %) were population-specific:
393 316 were private to one single population and 78 were shared between at least two
394 populations (Table 1, Figure 3b). Notably, a large part of the Italian signal was shared with the
395 Portuguese individuals (red square in Figure 3b). Figure 3c pictures the repartition of the
396 outliers along the genome, where each bar represents one of the 39 Super-Scaffolds ordered
397 by physical length. We observed that outlier windows containing climate-associated SNPs
398 (blue segments in Figure 3c) were spread throughout the entire genome. However, we
399 detected a strong signal from Super-Scaffold 22. This signal corresponds to the overlapping
400 windows between the Italian and Portuguese populations (Figure 3b-c; Figure 4). On this
401 portion of the genome, the population-specific F_{ST} increased drastically whereas Tajima’s D
402 dropped in both populations (Figure 4).

403 Gene Ontology

404 We extracted a total of 1’461 genes from the 394 windows putatively under selection by the
405 climate. Among them, 967 were private to one single population (Table S5). Using genes
406 putatively under selection, we ran a Gene-Enrichment analysis. In Switzerland, we observed a
407 significant enrichment in some GO pathways, namely “T cell proliferation”, “adult behaviour”
408 as well as “appendage and limb morphogenesis” (Table S6). For the other populations, the GO
409 enrichment analysis did not yield any significant results. Nonetheless, all the genes were
410 clustered in functional groups, based on their similar functions (Table S7). Among them, we

411 obtained functions related to anatomy, locomotion and immunity. These were common to
412 every population, but with different genes involved in the different populations.

413 Ecological convergence in glacial refugia

414 We then aimed to understand the strong signal of selection shared between individuals from
415 the Italian and Portuguese populations. We extracted the climatic variables during the LGM
416 and today at sampling localities of individuals from both populations. By projecting these
417 climatic conditions into the 3D-climatic space, we confirmed that conditions from the Iberian
418 Peninsula changed more than those from the Italian one (Figure S8). Both Portuguese and
419 Italian conditions shifted slightly along the first axis of the PCA (an axis correlated to bio6 and
420 bio8, two temperature variables), but Portuguese ones shifted more on the second axis of the
421 PCA, getting closer to the Italian conditions. This second axis was mostly negatively correlated
422 with bio19, a precipitation variable (Figure S2).

423 To quantify the evolution of the climatic conditions in the two peninsulas, we computed all
424 the pairwise Euclidean distances between the two sets of sampling localities at both time
425 points. This analysis showed that abiotic conditions at the Italian and Portuguese sampling
426 localities got closer in the climatic space, exhibiting a convergence of the climate in the two
427 peninsulas (Figure S8).

428

429 Discussion

430 Climatic conditions and their past fluctuations are known to have shaped today's distribution
431 and genetic diversity of many species (Huntley and Webb 1989; Hewitt 2000; Davis and Shaw
432 2001). Among several evolutionary mechanisms, genetic local adaptation is expected to play

433 a key role in the persistence of living species (Lafontaine et al. 2018). Here, we used the
434 European barn owl as a study organism to explore where and how local adaptation occurred,
435 in the context of postglacial colonisation. First, we compared the past and current climatic
436 conditions experienced by the European barn owl. We showed that today's climatic conditions
437 found along the West coast of Europe are either similar to or only slightly different from those
438 in the glacial refugia, 20'000 years ago. Nonetheless, we also demonstrated that nowadays,
439 the temperature and precipitation conditions vary spatially, and individuals encounter
440 heterogeneous climates throughout the continent. We scanned the genome of 74 barn owls
441 from the Western Palearctic and identified regions putatively under selection and we
442 independently looked for an association between variation at SNPs and climatic conditions.
443 The combination of these approaches allowed us to detect signals of selection linked to
444 climate scattered along the genome in every population, and identify functional groups
445 putatively involved in the local adaptation of the species. In particular, we found that Super-
446 Scaffold 22 showed important signs of selection linked to climate in two populations, both
447 located in the past glacial refugia (namely the Iberian and Italian Peninsulas). Concordantly,
448 we showed that the climatic conditions within these two regions converged since the LGM.
449 With these results, we highlight the changes in the ecological niche of the barn owl from the
450 Last Glacial Maximum to the present day and how these changes might have influenced the
451 genetic makeup of this non-migratory species. Additionally, our results provide valuable
452 insights into the location and mechanism of local adaptation that might have occurred since
453 the Last Glaciation.

Evolution of the European barn owl ecological niche

During a postglacial recolonisation event, a species might conserve its ecological niche and track it through space via migration, or the niche might evolve, with the species being able to thrive and survive in new environments (Banks et al. 2008). These two scenarios are described in the literature as niche conservation or niche evolution and discriminating between the two often relies on the reconstruction of the past distribution based on fossil data (Hennessy et al. 2001; Banks et al. 2008). In the case of the barn owl, too few records of fossils are described in the literature. Instead, we used Species Distribution Modelling to reconstruct the climatic niche (Wiens et al. 2009) and assumed that the barn owl conserved its niche. This hypothesis has been based on an extensive review from (Peterson 2011), showing that for short-term processes such as distribution shifts, niche conservation is widespread in many phyla such as plants, mammals, birds or invertebrates.

We first modelled the current realised niche through Maximum Entropy modelling (Phillips et al. 2006) before projecting it into the past. Thus, we did not expect to observe conditions suitable in the past but absent from today's distribution. However, this projection allowed us to quantify if some of today's conditions were absent during the LGM. We showed that contemporary climatic conditions along the Atlantic coast up to the UK were present 20'000 years ago (Figure 2a). In other words, barn owls that have migrated to these areas face a similar environment to that experienced in the glacial refugia. In what remains of Western Europe, we showed that conditions were only slightly different to that of the past. Altogether, we suggest that at the end of the LGM and along with the rise of temperature, the favourable conditions of the glacial refugia shifted to the North, which allowed the European barn owl to expand its range, as previously hypothesised by (Machado, Cumer, et al. 2021). However, this

477 only concerns the Western part of Europe and new, different climatic conditions can be found
478 in Central and Eastern Europe.

479 It is difficult to assess whether these new conditions limited the recolonisation of Central and
480 Eastern Europe, nor if the actual conditions constrain the survival of the barn owl because, to
481 our knowledge, no estimates of population sizes are available through the continent.
482 Furthermore, no samples coming from Eastern Europe (white areas in Figure 2a) were
483 available and it is thus difficult to know to which extent these new climatic conditions have
484 led to a local adaptation in these regions. Nonetheless, all populations included in this study
485 face distinct current environments (Figure S3), with temperature and precipitation conditions
486 varying at the continental scale. This current environmental heterogeneity thus enables the
487 search for traces of local adaptation to climatic conditions at the genomic level.

488 Genomic traces of local adaptation

489 Heterogeneous climatic conditions can be the source of major selective pressures for wild
490 species. Because the populations considered in this study face distinct conditions (Figure S3),
491 we scanned the genomes of 74 barn owls for traces of selection linked to the environment. To
492 do so, we combined both genome scans and Genotype-Environment Association analysis. The
493 use of Tajima's D allowed us to detect traces of selection in a set of individuals based on the
494 difference between nucleotide diversity (π) and the number of segregating sites (S). By
495 comparing the value observed in a given region to the expectation under its neutral evolution
496 (i.e. what we observe in the rest of the genome), this approach points to regions with an
497 excess of rare alleles, a sign of recent population expansion or a potential target of recent
498 selection. We complemented this classical approach with the population-specific F_{ST}. This
499 approach allowed us to identify genomic regions with an excess of genetic identity within a

500 population, compared to the genetic identity observed with other populations. Compared
501 with the remainder of the genome, this approach allows for identifying regions of the genome
502 with specific history (i.e., selection event), an approach first implemented by (Cumer,
503 Machado, Siverio, et al. 2022). However, even if the use of genome scans to detect traces of
504 selection is common in the literature, it presents some major caveats. Thus, it has been
505 suggested that background selection and/or regions with low recombination can lead to
506 genomic traces similar to positive selection (Johri et al. 2021; Jensen 2023). Moreover, the
507 agent of selection remains unclear as many selective pressures might have led to such
508 genomic patterns, including predation, competition, immunity or any other biotic or abiotic
509 condition.

510 In addition to these scans, we thus conducted a Redundancy Analysis to identify loci
511 influenced by the abiotic variables (temperature and precipitation), as commonly investigated
512 through this type of approach. Such Genotype-Environment Association Analysis is a powerful
513 tool to detect potential links with the environment (Dauphin et al. 2022), but the observed
514 association might also be due to other factors than selection, such as allele surfing during
515 expansion, drift or the covariation between climatic variable and geography. We, therefore,
516 combined the signals from both methods and retained only outlier windows containing
517 climate-associated SNPs. By using the two approaches together, we targeted regions of the
518 genome being under selection (according to the genome scans) due to the climatic
519 environment (according to the Redundancy Analysis), compensating for the caveats of
520 methods taken independently.

521 In the end, we retained 394 windows containing a total of 8’423 climate-associated SNPS,
522 highlighting areas of the genome potentially involved in local climate adaptation (Figure 3).

523 We showed that the set of windows identified in the first approach was enriched in climate-
524 associated SNPs compared to the remainder of the genome (Figure S7), supporting the role of
525 these regions in the adaptation to the local climate. Through this analysis, we identified 1'167
526 genes putatively under the influence of climate, giving insight into the functional targets of
527 the local adaptation of the barn owl.

528 The functional targets of selection

529 To assess the functional role of the genes within regions putatively under selection, we
530 investigated their ontologies. Despite no significantly enriched pathway within populations,
531 except for Switzerland, genes putatively under selection were clustered based on their known
532 function (Table S7). Surprisingly, this classification did not reveal many pathways directly
533 related to climate but an overall implication of genes involved in immunity (with terms such
534 as "immune system" and "process and immune response"), anatomy (i.e., "anatomical
535 structure morphogenesis", "regulation of the developmental process", "growth") and
536 locomotion. These pathways were involved in the local adaptation of every population - even
537 though the genes identified were different among them (Table S7).

538 Exposure to pathogens and infectious diseases is known to be another major selective
539 pressure in human species as well as in birds (Fumagalli et al. 2011; Shultz and Sackton 2019).
540 Additionally, temperature and rainfall are known to highly influence the composition of the
541 pathogen communities (Clark et al. 2018; Harvey and Voelker 2019). Thus, the heterogeneity
542 of climatic conditions could have influenced the pathogen-associated selective pressures,
543 explaining why we found genes related to immunity under selection by the local climate in the
544 European barn owl (Table S7). To corroborate this effect of climate on immune genes,
545 (O'Connor et al. 2020) demonstrated in birds that the genes forming the major

546 histocompatibility complex class I (MHC-I) show variation in diversity according to humidity.
547 Further work is needed to understand the precise role of these genes, but these results
548 suggest a genomic adaptation due to pathogenic selective pressure.

549 “Anatomical structure morphogenesis” is another recurrent functional group found under
550 selection in each population, encompassing several dozens of genes (Table S7). In line with
551 this result, the GO terms “appendage and limb morphogenesis” was significantly enriched in
552 the Swiss population. These results emphasise the potential link between phenotypic variation
553 and the local adaptation of the barn owl. A previous study identified a significantly positive
554 relationship between body size and the hunted prey size, suggesting that larger owls might be
555 more competitive in hunting larger prey (Romano et al. 2021). (Romano et al. 2020) also
556 highlighted a significant geographical and climatic trend in the diet of this raptor species, with
557 an increase in the proportion of small prey in the diet of colder environments (including
558 Central and Northern Europe). Finally, this variation in body size also co-varies with colour
559 polymorphism (with smaller owls being redder, (Romano et al. 2021), a trait known to be
560 maintained by selection (Antoniazza et al. 2010), and linked to hunting behaviour (Roulin
561 2004; Romano et al. 2021). Taken together, these results suggest an association between the
562 size of the barn owl, their colour, their behaviour and their diet, all possibly linked to the local
563 adaptation of the barn owl to heterogeneous climatic conditions in Europe. Nonetheless, we
564 should take this interplay between biotic and abiotic factors with caution, as this hypothesis
565 mostly relies on Gene Ontology enrichment. This type of analysis, although commonly used
566 and easy to conduct, can also be the source of spurious links between genome scan results
567 and functionally important genes (Pavlidis et al. 2012) and should be the target of further
568 investigation.

Climatic and genomic convergence

Our results highlighted a strong and common signal of selection in the genome in all individuals from both populations located nowadays in glacial refugia (i.e., the Italian and Portuguese populations). We also linked this signal to climatic variables through a Redundancy Analysis, pointing out the role of this region in the adaptation to the local climate (Figure 4).

To explore environmental changes in the glacial refugia since LGM, we investigated the shifts in climatic conditions at the sampling localities of the barn owl from the Italian and Portuguese populations. We did find a smaller change of conditions in the Italian Peninsula compared to the Iberian one (Figure S8), concordantly with what was found in the climate evolution analysis (Figure 2a). However, these results pointed out that, even though Portuguese conditions have changed further, they have become very similar to those currently found in Italy, mostly due to a decreased amount of precipitation between the LGM and today (Figure S8, Figure S2). Taken altogether, these results suggest that the Iberian Peninsula climate changed more and got more similar to that in the Italian Peninsula. Through this climatic convergence, the corresponding selective pressures might have favoured the same alleles in these two distinct populations. Through time, their frequency increased, approaching fixation in each of the two populations and resulting in a genomic convergence on this part of the genome.

The size of the region involved, as well as the pronounced pattern of genetic differentiation (in both F_{ST} and Tajima's D), could also arise due to a chromosomal rearrangement. It has been shown in the literature that chromosomal inversion might facilitate local adaptation despite gene flow (Tigano and Friesen 2016; Wellenreuther and Bernatchez 2018), thus permitting the converging adaptation of the barn owl to the convergent climatic changes in its past refugia.

592 Further investigation in the future will allow us to test this hypothesis as well as understand
593 the molecular mechanisms affected by this genomic region.

594 The genomic resources for local adaptation

595 Based on our results, the recolonisation of Northern territories has been facilitated by
596 favourable conditions shifting North. However, climatic conditions are dynamic everywhere
597 and our results show that conditions in the South have also changed during the last 20'000
598 years (Figure 2b, Fig S8). These changes in climatic conditions across the entire continent
599 drove the environmental heterogeneity that the species faces today. This discrepancy of
600 climatic selective pressures impacted the genome of barn owls from all populations, leaving
601 traces of selection in a wide part of the genome (Figure 3, Figure S5). Given the pattern of
602 selection we have observed, one of the remaining questions concerns the origin of adaptive
603 alleles.

604 One first possibility would imply the occurrences of *de novo* mutations, bringing new
605 favourable alleles in the different populations. Considering the low mutation rate (Smeds et
606 al. 2016), and the need for the mutation to be advantageous and to appear within the right
607 population (Rees et al. 2020), it is not very likely that this mechanism would explain the extent
608 of selection we observed (Figure 3). A more realistic explanation relies on adaptation due to
609 already existing alleles. According to this second scenario, adaptation would have therefore
610 relied on allele sorting of standing variation. This can happen when segregating alleles become
611 advantageous following a change in selective pressures, for example, due to the colonisation
612 of a new habitat or climate changes (Peter and Slatkin 2013: 201). This explanation implied
613 that within glacial refugia, a pool of segregating alleles was maintained in sufficiently large
614 populations (de Filippo et al. 2016). These alleles were then sorted through space by local

615 adaptation, with different environments (biotic and abiotic) acting as a filter on deleterious
616 and/or less advantageous alleles. This whole process has been reported in species as diverse
617 as *Arabidopsis thaliana* and *Drosophila melanogaster* (Barrett and Schluter 2008), birds
618 (Enbody et al. 2022) or in humans, following the 'Out-of-Africa' migration (50'000 - 70'000
619 years ago) (Haber et al. 2019; Rees et al. 2020). The latter example has been corroborated by
620 de Filippo et al. (2016) as they highlighted that a change in selective regime following the 'Out-
621 of-Africa' migration and consequently positive selection of standing variation in some
622 populations was the most likely process explaining the observed genetic diversity.

623 In this study, we did not properly test each of the two scenarios (i.e., an adaptation from *de*
624 *novo* mutation or standing variation). Therefore, further work is required to fully understand
625 how the genome of the European barn owl evolved during the past 20'000
626 years. Investigating the ancestral state of the regions under selection (Keightley and Jackson
627 2018) will be of major interest in the future. Combined with the dating of the mutation
628 appearance (with methods such as the one proposed by (Gandolfo et al. 2014)), as well as the
629 timing of the selection (Albers and McVean 2020), it would allow us to confirm this hypothesis
630 and further understand the mechanisms explaining the local adaptation of the barn owl to its
631 environment.

632 Lessons from the European barn owl

633 Global warming since LGM and the subsequent postglacial expansion has had many
634 consequences for (re-)colonising species. This complex process was dissected by a wide body
635 of literature exploring many aspects of it, from dispersal limitation (Hastings et al. 2004;
636 Marsico and Hellmann 2009; Hargreaves and Eckert 2014), the inability to adapt to potential
637 new conditions (Angert et al. 2008; Holt and Barfield 2011; Polechová and Barton 2015) as

638 well as the genetic load accumulated during an expansion (Excoffier et al. 2009; Hallatschek
639 and Nelson 2010; Peischl et al. 2013; Peischl et al. 2015; Peischl and Excoffier 2015) and it's
640 interaction with local adaptation (Gilbert et al. 2017). In this work, we show that the barn owl
641 recolonised the Northern area by following its niche, but also faced climatic conditions absent
642 during LGM. In both cases, our results show signals of local adaptation in all Northern
643 populations, probably favoured by the absence of strong bottleneck during founding events
644 as well as the relatively high diversity in Northern populations, all combined with a low
645 differentiation of the populations, consistent with ongoing connectivity of these populations
646 (Cumer et al. 2022). This setup of large population sizes, combined with sustained gene flow
647 would have allowed selection for favourable alleles and for adaptation of the populations to
648 their environment during or since the expansion. Altogether, these results emphasise that the
649 evolutionary forces acting on the expanding populations are now well understood and allow
650 us to properly describe the dynamic of the populations in the North.
651 However, our results also show that climatic conditions in the glacial refugia changed while
652 the core niche present during the LGM moved North. At the genomic level, we show that the
653 populations located at the same geographical location as the refugia during the LGM also had
654 to adapt to these climatic changes, with traces of selection in modern populations living in
655 LGM refugia and evidence of a potential genetic convergence in response to an ecological
656 convergence (see *Climatic and genomic convergence* for details). Such adaptation might have
657 been fuelled by the diversity present in these populations, as well as their putative large
658 effective population sizes facilitating adaptation.
659 Our empirical study of the barn owl asserts that Southern populations are also subject to local
660 adaptation. Work on other species in the future will probably show that the barn owl is no
661 exception, with this process probably ongoing in many species. Indeed, to our knowledge, the

consequences of the climatic changes for the populations remaining at the base of the expansion remain mostly unexplored. Considering the past but also ongoing and future climate change, these southern populations are the ones that will face new climatic conditions as the niche is shifting north. Our work paves the way for describing and understanding the genomic bases of local adaptation to change environments but further work is also needed to better understand what mechanisms will allow species to persist and adapt to these challenging conditions.

Conclusion

This work gives insights on the selective processes that have acted on the European barn owl since the Last Glacial Maximum and, more generally, during a postglacial recolonisation event. Through a combined approach of ecological modelling, genome scans and a landscape genomics analysis (RDA), we demonstrate that not only the species adapted to the local climate in territories out of the glacial distribution (Northern part of Europe) but also had to cope through genetic adaptation to an important climatic change in past glacial refugia territories (i.e., Southern part of Europe). This work highlights the importance of considering the evolution of ecological niches through time and space for a better understanding of local adaptation to climate, but also that former glacial refugia are regions where the environment might also undergo substantial changes, driving genetic adaptation.

The effect of postglacial recolonisation on the barn owl genome is probably not limited to Europe. The presence of this species worldwide will allow to test if the pattern we highlighted in this study is also found in other regions. For example, the barn owl is found across North America - where the Northern territories' recolonisation after the Last Glacial Maximum is well documented in birds (Lessa et al. 2003; Weir and Schluter 2004), and facing a continental

685 gradient of environmental conditions. This known recolonisation of Northern territories and
686 the current environmental gradient, coupled with the genetic structure explored at the
687 continental level (Machado et al. 2018), provide a good replicate to corroborate the results
688 found in the present study and expand the conclusions to a sister species.
689 At broader scale, this study of a wild and non-model raptor helps to better understand the
690 mechanisms allowing species to persist through changing environments and where these
691 processes occurred. Such knowledge is of particular interest nowadays, considering the
692 ongoing rapid climate disruption.
693

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700

701 Data Accessibility

702 The raw Illumina reads for the whole-genome sequenced individuals are available in
703 Bioproject PRJNA700797, Bioproject PRJNA727915 and Bioproject PRJNA727977.

704

Authors contribution

HC, TC, and JG designed this study; TC called the variants; AT produced the mappability mask; HC conducted the analyses with the help of TC and suggestions of all authors; HC and TC led the writing of the manuscript with inputs from all authors.

References

Albers PK, McVean G. 2020. Dating genomic variants and shared ancestry in population-scale sequencing data.Barton NH, editor. *PLOS Biol.* 18:e3000586.

Angert AL, Bradshaw Jr HD, Schemske DW. 2008. USING EXPERIMENTAL EVOLUTION TO INVESTIGATE GEOGRAPHIC RANGE LIMITS IN MONKEYFLOWERS. *Evolution* 62:2660–2675.

Antoniazza S, Burri R, Fumagalli L, Goudet J, Roulin A. 2010. Local adaptation maintains Clinal variation in melanin-based coloration of Euopean barn owl (Tyto alba). *Evolution* [Internet]. Available from: http://doi.wiley.com/10.1111/j.1558-5646.2010.00969.x

Antoniazza S, Kanitz R, Neuenschwander S, Burri R, Gaigher A, Roulin A, Goudet J. 2014. Natural selection in a postglacial range expansion: the case of the colour cline in the European barn owl. *Mol. Ecol.* 23:5508–5523.

Atkin OK, Loveys BR, Atkinson LJ, Pons TL. 2006. Phenotypic plasticity and growth temperature: understanding interspecific variability. *J. Exp. Bot.* 57:267–281.

Banks W, Derrico F, Peterson A, Kageyama M, Colombeau G. 2008. Reconstructing ecological niches and geographic distributions of caribou (Rangifer tarandus) and red deer (Cervus elaphus) during the Last Glacial Maximum. *Quat. Sci. Rev.* 27:2568–2575.

Barrett R, Schluter D. 2008. Adaptation from standing genetic variation. *Trends Ecol. Evol.* 23:38–44.

Biquet J, Bonamour S, Villemereuil P, Franceschi C, Teplitsky C. 2022. Phenotypic plasticity drives phenological changes in a Mediterranean blue tit population. *J. Evol. Biol.* 35:347–359.

BirdLife International. 2016. IUCN Red List of Threatened Species: Tyto alba. *IUCN Red List Threat. Species* [Internet]. Available from: https://www.iucnredlist.org/en

Borcard D, Gillet F, Legendre P. 2011. Numerical Ecology with R. New York, NY: Springer New York Available from: http://link.springer.com/10.1007/978-1-4419-7976-6

Burri R, Antoniazza S, Gaigher A, Ducrest A-L, Simon C, The European Barn Owl Network, Fumagalli L, Goudet J, Roulin A. 2016. The genetic basis of color-related local adaptation in a ring-like colonization around the Mediterranean: GENETICS OF CIRCUM-MEDITERRANEAN COLOR ADAPTATION. *Evolution* 70:140–153.

Capblancq T, Forester BR. 2021. Redundancy analysis: A Swiss Army Knife for landscape genomics. *Methods Ecol. Evol.*:2041-210X.13722.

Capblancq T, Luu K, Blum MGB, Bazin E. 2018. Evaluation of redundancy analysis to identify signatures of local adaptation. *Mol. Ecol. Resour.* 18:1223–1233.

744 Choi Y, Chan AP, Kirkness E, Telenti A, Schork NJ. 2018. Comparison of phasing strategies for
745 whole human genomes. *PLOS Genet.* 14:e1007308.

746 Clark NJ, Clegg SM, Sam K, Goulding W, Koane B, Wells K. 2018. Climate, host phylogeny and
747 the connectivity of host communities govern regional parasite assembly.Austin J,
748 editor. *Divers. Distrib.* 24:13–23.

749 Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, Mitrovica JX, Hostetler SW,
750 McCabe AM. 2009. The Last Glacial Maximum. *Science* 325:710–714.

751 Cumer T, Machado AP, Dumont G, Bontzorlos V, Ceccherelli R, Charter M, Dichmann K,
752 Kassinis N, Lourenço R, Manzia F, et al. 2022. Landscape and Climatic Variations Shaped
753 Secondary Contacts amid Barn Owls of the Western Palearctic. *Mol. Biol. Evol.*
754 39:msab343.

755 Cumer T, Machado AP, Siverio F, Cherkaoui SI, Roque I, Lourenço R, Charter M, Roulin A,
756 Goudet J. 2022. Genomic basis of insularity and ecological divergence in barn owls
757 (Tyto alba) of the Canary Islands. *Heredity* 129:281–294.

758 Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G,
759 Marth GT, Sherry ST, et al. 2011. The variant call format and VCFtools. *Bioinforma. Oxf.*
760 *Engl.* 27:2156–2158.

761 Dauphin B, Rellstab C, Wüest RO, Karger DN, Holderegger R, Gugerli F, Manel S. 2022. Re-
762 thinking the environment in landscape genomics. *Trends Ecol.*
763 *Evol.*:S0169534722002774.

764 Davis MB, Shaw RG. 2001. Range Shifts and Adaptive Responses to Quaternary Climate
765 Change. *Science* 292:673–679.

766 Delaneau O, Marchini J, Zagury J-F. 2012. A linear complexity phasing method for thousands
767 of genomes. *Nat. Methods* 9:179–181.

768 Delaneau O, Zagury J-F, Robinson MR, Marchini JL, Dermitzakis ET. 2019. Accurate, scalable
769 and integrative haplotype estimation. *Nat. Commun.* 10:5436.

770 Elith J, Phillips SJ, Hastie T, Dudík M, Chee YE, Yates CJ. 2011. A statistical explanation of
771 MaxEnt for ecologists. *Divers. Distrib.* 17:43–57.

772 Enbody ED, Sendell-Price AT, Sprehn CG, Rubin C-J, Visscher PM, Grant BR, Grant PR,
773 Andersson L. 2022. Large effect loci have a prominent role in Darwin's finch evolution.
774 Evolutionary Biology Available from:
775 http://biorxiv.org/lookup/doi/10.1101/2022.10.29.514326

776 Excoffier L, Foll M, Petit RJ. 2009. Genetic Consequences of Range Expansions. *Annu. Rev. Ecol.*
777 *Evol. Syst.* 40:481–501.

778 Exposito-Alonso M. 2017. rbioclim: Improved getData function from the raster R package to
779 interact with past, present and future climate data from worldclim.org. Available from:
780 github.com/MoisesExpositoAlonso/rbioclim

de Filippo C, Key FM, Ghirotto S, Benazzo A, Meneu JR, Weihmann A, NISC Comparative Sequence Program, Parra G, Green ED, Andrés AM. 2016. Recent Selection Changes in Human Genes under Long-Term Balancing Selection. *Mol. Biol. Evol.* 33:1435–1447.

Fumagalli M, Sironi M, Pozzoli U, Ferrer-Admettla A, Pattini L, Nielsen R. 2011. Signatures of Environmental Genetic Adaptation Pinpoint Pathogens as the Main Selective Pressure through Human Evolution.Akey JM, editor. *PLoS Genet.* 7:e1002355.

Gandolfo LC, Bahlo M, Speed TP. 2014. Dating Rare Mutations from Small Samples with Dense Marker Data. *Genetics* 197:1315–1327.

Ge SX, Jung D, Yao R. 2020. ShinyGO: a graphical gene-set enrichment tool for animals and plants.Valencia A, editor. *Bioinformatics* 36:2628–2629.

Gianoli E, González-Teuber M. 2005. Environmental Heterogeneity and Population Differentiation in Plasticity to Drought in Convolvulus Chilensis (Convolvulaceae). *Evol. Ecol.* 19:603–613.

Gilbert KJ, Sharp NP, Angert AL, Conte GL, Draghi JA, Guillaume F, Hargreaves AL, Matthey-Doret R, Whitlock MC. 2017. Local Adaptation Interacts with Expansion Load during Range Expansion: Maladaptation Reduces Expansion Load. *Am. Nat.* 189:368–380.

Goudet J. 2005. hierfstat, a package for r to compute and test hierarchical F-statistics. *Mol. Ecol. Notes* 5:184–186.

Goudet J, Jombart T, Kamvar ZN, Archer E, Hardy O. 2021. hierfstat: Estimation and Tests of Hierarchical F-Statistics. Available from: https://CRAN.R-project.org/package=hierfstat

Haber M, Jones AL, Connell BA, Asan, Arciero E, Yang H, Thomas MG, Xue Y, Tyler-Smith C. 2019. A Rare Deep-Rooting D0 African Y-Chromosomal Haplogroup and Its Implications for the Expansion of Modern Humans Out of Africa. *Genetics* 212:1421–1428.

Hallatschek O, Nelson DR. 2010. LIFE AT THE FRONT OF AN EXPANDING POPULATION. *Evolution* 64:193–206.

Hargreaves AL, Eckert CG. 2014. Evolution of dispersal and mating systems along geographic gradients: implications for shifting ranges. *Funct. Ecol.* 28:5–21.

Harvey JA, Voelker G. 2019. Host associations and climate influence avian haemosporidian distributions in Benin. *Int. J. Parasitol.* 49:27–36.

Hastings A, Cuddington K, Davies KF, Dugaw CJ, Elmendorf S, Freestone A, Harrison S, Holland M, Lambrinos J, Malvadkar U, et al. 2004. The spatial spread of invasions: new developments in theory and evidence: Spatial spread of invasions. *Ecol. Lett.* 8:91–101.

Hennessy S, Fung P, Scanlon E. 2001. Portable information technologies for supporting graphical mathematics investigations: overview of the PIGMI Project. *J. Comput. Assist. Learn.* 17:219–220.

817 Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405:907–913.

818 Holt RD, Barfield M. 2011. Theoretical Perspectives on the Statics and Dynamics of Species'
819 Borders in Patchy Environments. *Am. Nat.* 178:S6–S25.

820 Huntley B, Webb T. 1989. Migration: Species' Response to Climatic Variations Caused by
821 Changes in the Earth's Orbit. *J. Biogeogr.* 16:5.

822 Jackson ST, Overpeck JT. 2000. Responses of plant populations and communities to
823 environmental changes of the late Quaternary. *Paleobiology* 26:194–220.

824 Jensen JD. 2023. Population genetic concerns related to the interpretation of empirical
825 outliers and the neglect of common evolutionary processes. *Heredity* 130:109–110.

826 Johri P, Riall K, Becher H, Excoffier L, Charlesworth B, Jensen JD. 2021. The Impact of Purifying
827 and Background Selection on the Inference of Population History: Problems and
828 Prospects. *Mol. Biol. Evol.* 38:2986–3003.

829 Kebede F, Moehlman PD, Bekele A, Evangelista PH. 2014. Predicting seasonal habitat
830 suitability for the critically endangered African wild ass in the Danakil, Ethiopia. *Afr. J.*
831 *Ecol.* 52:533–542.

832 Keightley PD, Jackson BC. 2018. Inferring the Probability of the Derived *vs.* the Ancestral Allelic
833 State at a Polymorphic Site. *Genetics* 209:897–906.

834 Lafarge T, Pateiro-Lopez B. 2020. alphashape3d: Implementation of the 3D Alpha-Shape for
835 the Reconstruction of 3D Sets from a Point Cloud. Available from: https://CRAN.R-
836 project.org/package=alphashape3d

837 Lafontaine G, Napier JD, Petit RJ, Hu FS. 2018. Invoking adaptation to decipher the genetic
838 legacy of past climate change. *Ecology* 99:1530–1546.

839 Lessa EP, Cook JA, Patton JL. 2003. Genetic footprints of demographic expansion in North
840 America, but not Amazonia, during the Late Quaternary. *Proc. Natl. Acad. Sci.*
841 100:10331–10334.

842 Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform.
843 *Bioinforma. Oxf. Engl.* 25:1754–1760.

844 Machado AP, Clément L, Uva V, Goudet J, Roulin A. 2018. The Rocky Mountains as a dispersal
845 barrier between barn owl (Tyto alba) populations in North America. *J. Biogeogr.*
846 45:1288–1300.

847 Machado AP, Cumer T, Iseli C, Beaudoing E, Ducrest A-L, Dupasquier M, Guex N, Dichmann K,
848 Lourenço R, Lusby J, et al. 2021. Unexpected post-glacial colonisation route explains
849 the white colour of barn owls (Tyto alba) from the British Isles.
850 *bioRxiv*:2021.04.23.441058.

851 Machado AP, Topaloudis A, Cumer T, Lavanchy E, Bontzorlos V, Ceccherelli R, Charter M,
852 Kassinis N, Lymberakis P, Manzia F, et al. 2021. Genomic consequences of colonisation,

853 migration and genetic drift in barn owl insular populations of the eastern
854 Mediterranean. *Molecular Ecology*, 31:1375– 1388.

855 Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen W-M. 2010. Robust relationship
856 inference in genome-wide association studies. *Bioinformatics* 26:2867–2873.

857 Marsico TD, Hellmann JJ. 2009. Dispersal limitation inferred from an experimental
858 translocation of *Lomatium* (Apiaceae) species outside their geographic ranges. *Oikos*
859 118:1783–1792.

860 Martin M, Patterson M, Garg S, Fischer SO, Pisanti N, Klau GW, Schöenhuth A, Marschall T.
861 2016. WhatsHap: fast and accurate read-based phasing. *bioRxiv*:085050.

862 Munn RE ed. 2002. Encyclopedia of global environmental change. Chichester ; New York:
863 Wiley

864 Murren CJ, Auld JR, Callahan H, Ghalambor CK, Handelsman CA, Heskel MA, Kingsolver JG,
865 Maclean HJ, Masel J, Maughan H, et al. 2015. Constraints on the evolution of
866 phenotypic plasticity: limits and costs of phenotype and plasticity. *Heredity* 115:293–
867 301.

868 Nürnberger B. 2013. Ecological Genetics. In: Encyclopedia of Biodiversity. Elsevier. p. 714–731.
869 Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780123847195000381

870 O'Connor EA, Hasselquist D, Nilsson J-Å, Westerdahl H, Cornwallis CK. 2020. Wetter climates
871 select for higher immune gene diversity in resident, but not migratory, songbirds. *Proc.*
872 *R. Soc. B Biol. Sci.* 287:20192675.

873 Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB,
874 Simpson GL, Solymos P, et al. 2020. vegan: Community Ecology Package. Available
875 from: https://CRAN.R-project.org/package=vegan

876 Pavlidis P, Jensen JD, Stephan W, Stamatakis A. 2012. A Critical Assessment of Storytelling:
877 Gene Ontology Categories and the Importance of Validating Genomic Scans. *Mol. Biol.*
878 *Evol.* 29:3237–3248.

879 Peischl S, Dupanloup I, Kirkpatrick M, Excoffier L. 2013. On the accumulation of deleterious
880 mutations during range expansions. *Mol. Ecol.* 22:5972–5982.

881 Peischl S, Excoffier L. 2015. Expansion load: recessive mutations and the role of standing
882 genetic variation. *Mol. Ecol.* 24:2084–2094.

883 Peischl S, Kirkpatrick M, Excoffier L. 2015. Expansion Load and the Evolutionary Dynamics of a
884 Species Range. *Am. Nat.* 185:E81–E93.

885 Peter BM, Slatkin M. 2013. DETECTING RANGE EXPANSIONS FROM GENETIC DATA: DETECTING
886 RANGE EXPANSIONS FROM GENETIC DATA. *Evolution* 67:3274–3289.

887 Peterson AT. 2011. Ecological niche conservatism: a time-structured review of evidence:
888 Ecological niche conservatism. *J. Biogeogr.* 38:817–827.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecol. Model.* 190:231–259.

Polechová J, Barton NH. 2015. Limits to adaptation along environmental gradients. *Proc. Natl. Acad. Sci.* 112:6401–6406.

Rees JS, Castellano S, Andrés AM. 2020. The Genomics of Human Local Adaptation. *Trends Genet.* 36:415–428.

Romano A, Séchaud R, Roulin A. 2020. Global biogeographical patterns in the diet of a cosmopolitan avian predator. *J. Biogeogr.* 47:1467–1481.

Romano A, Séchaud R, Roulin A. 2021. Geographic variation in body size and plumage colour according to diet composition in a nocturnal raptor. *J. Avian Biol.* 52:jav.02716.

Roulin A. 2004. Covariation between plumage colour polymorphism and diet in the Barn Owl Tyto alba: Plumage colour polymorphism and diet in the Barn Owl. *Ibis* 146:509–517.

Roulin A. 2013. Ring recoveries of dead birds confirm that darker pheomelanic Barn Owls disperse longer distances. *J. Ornithol.* 154:871–874.

Sgrò CM, Terblanche JS, Hoffmann AA. 2016. What Can Plasticity Contribute to Insect Responses to Climate Change? *Annu. Rev. Entomol.* 61:433–451.

Shultz AJ, Sackton TB. 2019. Immune genes are hotspots of shared positive selection across birds and mammals. *eLife* 8:e41815.

Smeds L, Qvarnström A, Ellegren H. 2016. Direct estimate of the rate of germline mutation in a bird. *Genome Res.* 26:1211–1218.

Storey JD, Bass AJ, Dabney A, Robinson D, Warnes G. 2022. qvalue: Q-value estimation for false discovery rate control. Available from: https://bioconductor.org/packages/qvalue/

Tigano A, Friesen VL. 2016. Genomics of local adaptation with gene flow. *Mol. Ecol.* 25:2144–2164.

Vale CG, Tarroso P, Brito JC. 2014. Predicting species distribution at range margins: testing the effects of study area extent, resolution and threshold selection in the Sahara–Sahel transition zone. *Divers. Distrib.* 20:20–33.

Weir JT, Schluter D. 2004. Ice sheets promote speciation in boreal birds. *Proc. R. Soc. Lond. B Biol. Sci.* 271:1881–1887.

Wellenreuther M, Bernatchez L. 2018. Eco-Evolutionary Genomics of Chromosomal Inversions. *Trends Ecol. Evol.* 33:427–440.

Wiens JA, Stralberg D, Jongsomjit D, Howell CA, Snyder MA. 2009. Niches, models, and climate change: Assessing the assumptions and uncertainties. *Proc. Natl. Acad. Sci.* 106:19729–19736.

Tables and Figures

Population	Genome scans outliers		Final outliers	
	Total number	Private	Total number	Private
Aegean	56	32	44	27
Switzerland	75	40	56	32
Denmark	88	58	63	44
France	80	51	53	31
Greece	83	47	61	38
Israel	64	50	47	37
Italy	75	40	49	26
Portugal	91	54	71	45
Serbia	77	53	49	36

Table 1 – Distribution of the different types of outlier windows among the nine populations. **"**Genome-scans outliers" windows describe the number of significant windows from both the population-specific F_{ST} and Tajima's D scans (see *Material and Methods* section for details). "Final outliers" windows were obtained by retaining the initial outlier windows that contained at least one SNP associated with climate based on the RDA. "Total number" refer to the number of window detect in each population. "Private" represent the number of windows exclusive to each of the population.

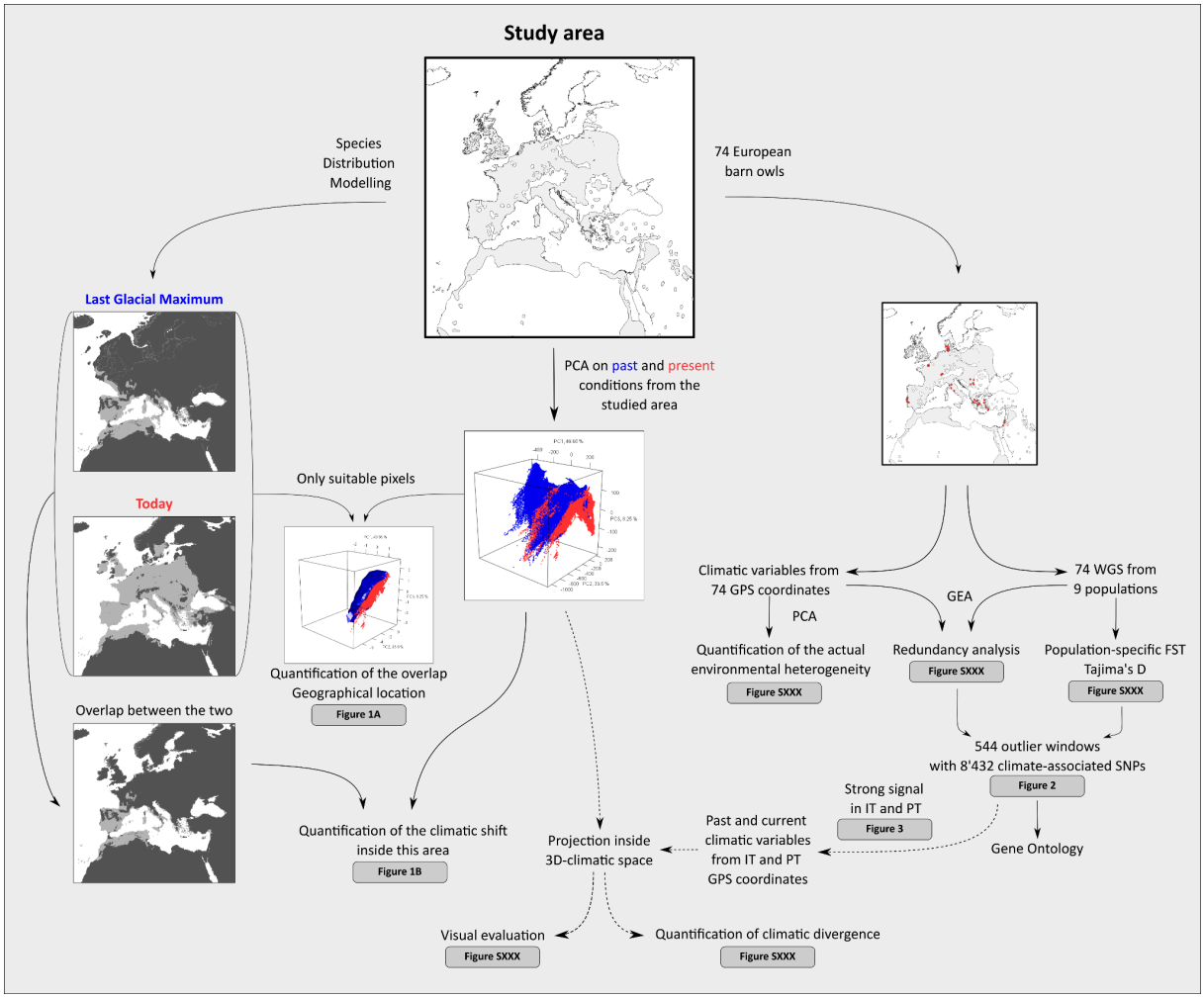


Figure 1 - Schematic representation of the workflow followed in this study. In brief, we modelled the distribution of the European barn owl nowadays and during LGM, 20000 years ago (left part of the scheme), based on the current repartition of the barn owl in the Western Palearctic. This Species Distribution Model was used to explore the evolution of the climatic niche used by the barn owl, as well as its changes in the refugia since the LGM. We combined this climatic analysis with an extensive sampling of 74 barn owls covering the entirety of the study area (right part of the scheme). We used whole genome sequences combined with climatic information, in order to identify genomic evidence of local adaptation in their genome. Based on the strong shared signal of selection in the Italian and Portuguese populations, we further investigated how the climate in the two peninsulas evolved since the last 20'000 years (dashed arrows).

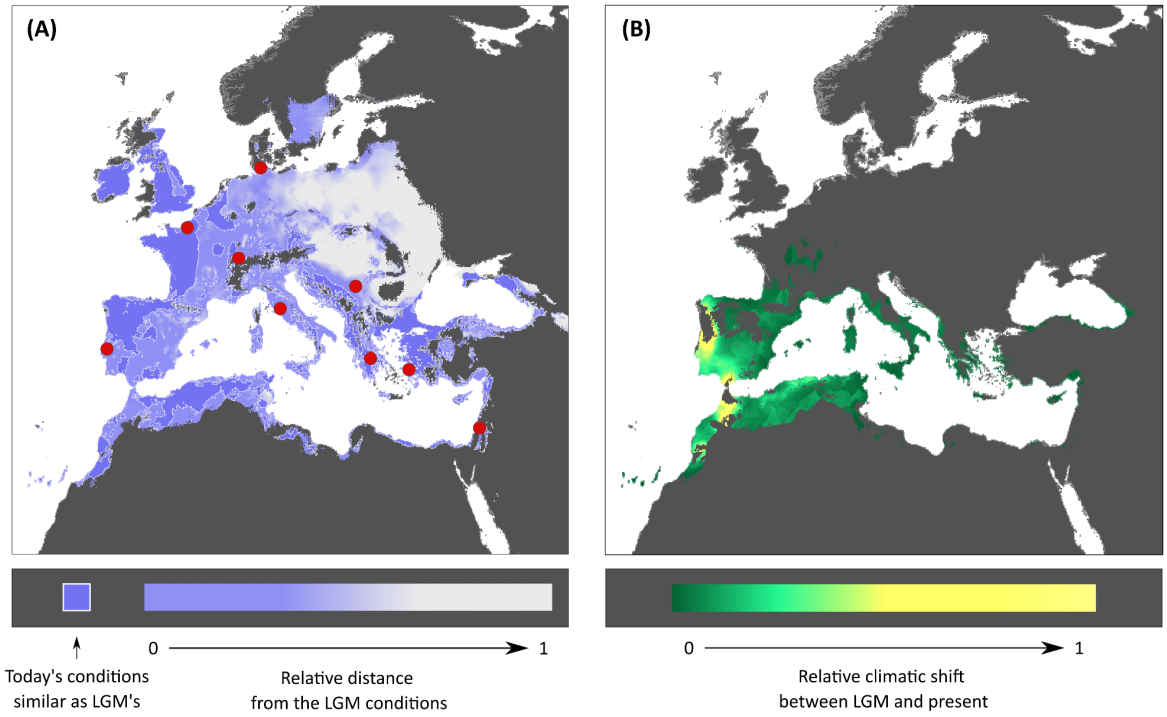
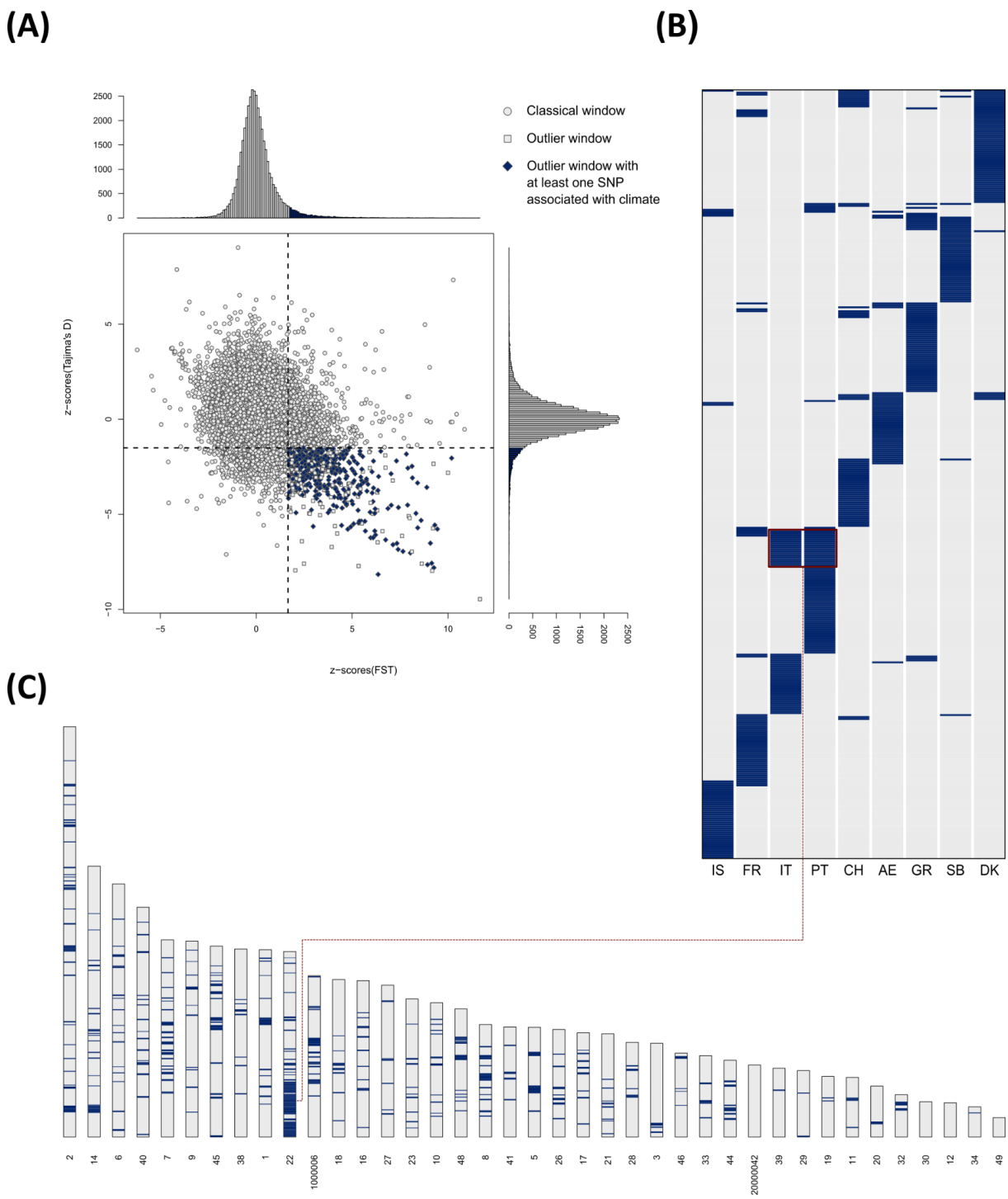


Figure 2 - Suitable climatic conditions for the barn owl and their changes since the LGM. (A) Map showing, for the suitable cells at present, their distance to the suitable conditions from the LGM. Cells in polygons surrounded by a white border have conditions similar to the conditions present in the LGM refugia. Cells outside polygons delimited by a white line were coloured according to their distance to the nearest past suitable pixel in the climatic 3D space. Cells in blue have conditions presenting a low distance (i.e. climatic conditions slightly different) whereas for white cells, it represents highest distances (i.e. climatic conditions highly different, see *Quantification of currently suitable climate absent during the LGM* section for details). Red dots display the average coordinate for the sampling location of each of the nine populations. (B) Map showing the shift of climatic conditions between the LGM and nowadays in the cells suitable at both time points. Dark green to yellow gradient corresponds to the relative shift in conditions between the two time-points, with the higher changes in yellow. Dark grey cells are areas unsuitable now or during LGM.



969

970 **Figure 3 - Genomic traces of selection linked to climate in the European barn owl genome.**
971 (A) Scatterplot containing all the windows from all the nine populations. Vertical and
972 horizontal dashed lines show the top 5% of population-specific F_{ST} and the lowest 5% of
973 Tajima's D respectively. Each association symbol-colour presents a class of window: grey
974 circles represent non outlier windows; grey squares depict outlier windows according to the
975 population-specific F_{ST} and Tajima's D scans; blue diamonds represent outlier windows in both

population-specific F_{ST} and Tajima's D scans and containing at least one SNP associated with climate in the Redundancy Analysis. (B) Repartition of the outlier windows in the different populations. Each column displays one of the 9 studied populations (IS: Israel, FR: France, IT: Italy, CH: Switzerland, AE: Aegean islands, GR: Greece, SB: Serbia, DK: Denmark) whereas each row corresponds to one window containing at least one SNP associated with climate in at least one population. Rows are ordered based on hierarchical clustering. (C) Repartition of the outliers along the genome. Each bar represents one of the 39 Super-Scaffolds, ordered by physical length. Blue segments correspond to an outlier window containing at least one SNP associated with climate in at least one population. The red square highlights the signal of selection in common between the Italian and Portuguese populations. The red dashed line indicates the genomic location of this signal of selection in common between the Italian and Portuguese populations.

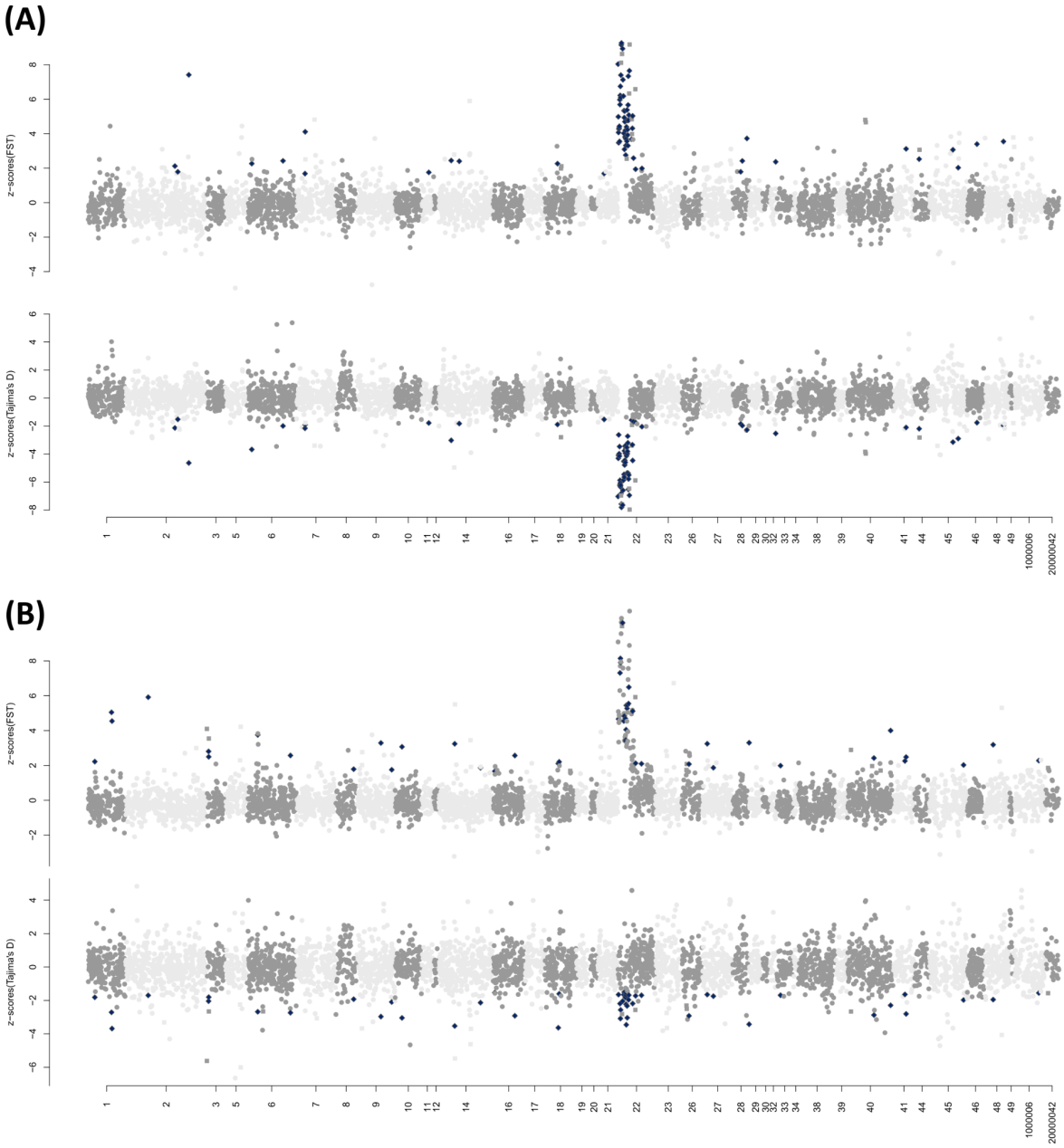


Figure 4 - Population-specific F_{ST}/Tajima's D genome scans for the two populations located in the past glacial refugia area. On each panel: Population-specific F_{ST} is on top whereas Tajima's D is below. Each dot represents a window of 1000 SNPs along the genome. Switching between light and dark grey represents changes of scaffold along the genome. Outlier windows containing at least one SNP associated with climate are highlighted in blue. The names of all the scaffolds are displayed on the y-axis. (A) Genome scans on individuals from Iberian Peninsula (PT). (B) Genome scans on individuals from the Italian Peninsula (IT).