MOLECULAR ECOLOGY RESOURCES

**Supplemental Information for:**

# An integrated framework to identify wildlife populations under threat from climate change

Orly Razgour, John B. Taggart, Stephanie Manel, Javier Juste, Carlos Ibáñez, Hugo Rebelo, Antton Alberdi, Gareth Jones, Kirsty Park

## Table of Contents:

MOLECULAR ECOLOGY
RESOURCES

# Appendix 1 – Supplementary Methods

## Ecological niche modelling procedures

We addressed collinearity in predictor variables through removing strongly correlated variables (R > 0.75; calculated in ENMTools, Warren *et al.* 2010), as well as variables that did not contribute to the model. Over-fitting and the effect of adjusting model parameters and regularization were assessed using Akaike Information Criterion (AIC) as implemented in ENMTools. The best fit model based on AIC scores included a regularization value of one, five features (linear, quadratic, product, threshold and hinge) and 1500 iterations. Following the recommendations in Merow *et al.* (2013), we used the raw output during the model comparison stage and the cumulative output to produce our final models and for model evaluation.

## Molecular lab protocols

Genomic DNA was extracted using the Qiagen DNeasy Blood and Tissue extraction kit, and quantified with Qubit® Fluorometer 2.0 and the dsDNA High Sensitivity assay kit (Invitrogen, ThermoFisher Scientific). DNA degradation was assessed through visualisation on1% agarose gels. We excluded samples with low DNA quantity (<2 ng/µl) or quality. To equalise DNA concentration at around 2-3 ng/µl, we diluted samples with high DNA concentrations using the Qiagen elution buffer (EB).

The ddRAD library preparation protocol was based on the methodology originally reported by Peterson *et al*. (2012), with modifications / refinements as described in Manousaki *et al*. (2016). Briefly, each of the 95 DNA samples was simultaneously digested by two high fidelity restriction enzymes (RE): SbfI (CCTGCA|GG recognition site), and SphI (GCATG|C recognition site), both sourced from New England Biolabs (NEB, UK). Digestions were incubated at 37°C for 50 min, using 10 U of each enzyme per microgram DNA in 1× CutSmart Buffer (NEB), in a 6 µL total reaction volume. After cooling the reactions to room temperature, 3 µL of a premade barcode / adapter mix was added to the digested DNA, and incubated at room temperature for 10 min. This adapter mix comprised individual-specific barcoded combinations of P1 (SbfI-compatible) and P2 (SphI-compatible) adapters at 6 nM and 72 nM concentrations respectively, in 1× reaction buffer 2 (NEB). Adapters were compatible with Illumina sequencing chemistry (see Peterson *et al*., 2012). The barcoded adapters were designed such that adapter– genomic DNA ligations did not reconstitute RE sites, while residual RE activity limited concatemerization of genomic fragments. The adapters included an inline five- or seven-base barcode for sample identification. Ligation was performed over 3 hr at 22°C by addition of a further 3 µL of a ligation mix comprising 4 mM rATP (Promega, UK), and 2000 cohesive-end units of T4 ligase (NEB) in 1× CutSmart buffer. The ligated samples were then heat denatured at 65°C for 20 min, cooled, and combined into a single pool. The pooled sample was column-purified (MinElute PCR Purification Kit, Qiagen, UK), and eluted in 100 µL EB buffer (Qiagen, UK). Size selection of fragments, ranging from approximately 400 bp to 700 bp, was performed by agarose gel separation. Following gel purification (MinElute Gel Extraction Kit, Qiagen, UK), the eluted size-

selected template DNA (60 µL in EB buffer) was PCR amplified (13 cycles PCR; 28 separate 12.5-µL reactions, each with 1 µL template DNA) using a high fidelity Taq polymerase (Q5 Hot Start High-Fidelity DNA Polymerase, NEB). The PCR reactions were combined (350 µL total), and column-purified (MinElute PCR Purification Kit). The 55 µL eluate, in EB buffer, was then subjected to a further size-selection cleanup using an equal volume of AMPure magnetic beads (Perkin-Elmer, UK), to maximize removal of small fragments (less than ca. 200 bp). The final library was eluted in 19 µL EB buffer and sequenced, over three consecutive Illumina MiSeq runs (v2 chemistry, 300 cycle kit, 162 bp paired end reads; Illumina, Cambridge, UK).

**Bioinformatics for genomic data analysis**

The MiSeq generated reads were processed using a software pipeline designed specifically for RAD analysis, Stacks (v.1. 17; Catchen *et al*., 2013). First, the 'process_radtags' function was used to demultiplex the individual samples. During this step sequence reads with quality scores below 10, missing either restriction site or with ambiguous barcodes were discarded. Barcodes were removed and all sequences trimmed to be no greater than 148 bases long. For the purposes of this analysis paired-end reads were treated as separate loci, read 2 sequences being appended to read 1 sequence files. These sequences were assigned to RAD loci and genotypes using the 'denovo_map.pl' component of Stacks. The key parameter values employed in identifying RAD loci were; a minimum stack depth of 10 (m=10), a maximum of 2 mismatches allowed in a locus (M=2) in an individual and up to 1 mismatch between loci when building the catalog (n=1). Finally the 'populations' component of Stacks was used to export

filtered data (polymorphic loci containing 1-3 SNPs and present in at least 70% of samples for each population) in PLINK file format (PED and MAP files).

**Population structure analysis methods**

We ran assignment tests in STRUCTURE (Pritchard *et al.* 2000) for population cluster values of K=1-9. We performed five independent runs for each K with 100,000 burn-in iterations followed by 500,000 MCMC steps. We assumed the admixture model and did not include any prior information on populations. The number of distinct clusters was determined using STRUCTURE HARVESTER (Earl & von Holdt 2012) based on the conservative Evanno's method (Evanno *et al.* 2005). We re-ran the analysis for each identified population cluster to look for sub-structuring. Cluster assignment was visualised with DISTRUCT (Rosenberg 2004).

**Outlier scan methods**

Bayescan (Foll & Gaggiotti 2008) was run with 1,000,000 iteration, 50,000 burn-in and 20 pilot runs. Results were visualised in R using the script provided with the Bayescan download package with no modifications, setting false discovery rate (FDR) to 0.05.

LOSITAN (Antao *et al*. 2008) was run with 1,000,000 simulations under the Infinite Alleles mutation model, using the 'Neutral' mean Fst and forcing mean Fst options, and setting FDR to 0.05 and confidence intervals to 0.99.

**Methods for generating resistance cost surfaces for the landscape genetics analysis**

The ENM was transformed into opposite continuous resistance costs, whereby high probability of occurrence (100) received the lowest resistance costs (1), as well as into a categorical layer divided into 10 quantiles. The altitude and slope layers (downloaded from WordlClim, www.worldclim.org) were converted into continuous resistance costs that increased with elevation/slope (range 0-100). The forest cover gradient variable was generated through reclassifying the GlobCover2009 map into five forest cover categories. We also generated a continuous tree cover variable from Hansen *et al*. (2013) percent tree canopy cover map.

**References**

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: a workbench to detect molecular adaptation based on a Fst-outlier method. *BMC Bioinformatics*, **9**, 323.

Catchen J, Hohenlohe P, Bassham S, Amores A, Cresko W (2013) Stacks: an analysis tool set for population genomics. *Molecular Ecology,* **22**, 3124–3140.

Earl DA, von Holdt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resour*ces, **4**, 359–361.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Foll M, Gaggiotti OE (2008) A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, **180**, 977–993.

Hansen MC, Potapov PV, Moore R *et al*. (2013) High resolution global maps of 21st century forest cover change. *Science*, **342**, 850–853.

Manousaki T, Tsakogiannis A, Taggart JB, *et al*. (2016) Exploring a non-model teleost genome through RAD sequencing - Linkage mapping in Common Pandora, *Pagellus erythrinus* and comparative genomic analysis. *G3 Genes Genomes Genetics,* **6**, 509–519.

Merow C, Smith MJ, Silander JA (2013) A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, **36**, 1058–1069.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, **7**(5), e37135.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, 1**55**, 945–959.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.

Warren DL, Glor RE, Turelli M (2010) ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography*, **33**, 607–611.

MOLECULAR ECOLOGY
RESOURCES

## Appendix 2 – LFMM R script

```
## Script for running LFMM through the R package LEA (Frichot & François 2015) based on the tutorial in http://membres-timc.imag.fr/Olivier.Francois/LEA/tutorial.htm

library(LEA)

# Data input from ped file
data = read.table("snp_dataset.ped")
output = ped2lfmm("snp_dataset.ped")

# Input environmental data from text file saved as '.env'
data_env = read.table("rainwarmq.env")

# Running lfmm
project = NULL
project = lfmm("snp_dataset.lfmm", "rainwarmq.env", K = 3, repetitions = 5, iterations = 100000, burnin = 10000, CPU = 4,
               project = "new")

summary(project)

# Get the zscores of each run for K = 3
zs_k3_rain = z.scores(project, K = 3)

# Combine the z-scores using the Stouffer method
zs.stouffer_k3_rain = apply(zs_k3_rain, MARGIN = 1, median)

# Calculate the genomic inflation factor
lambda_k3_rain = median(zs.stouffer_k3_rain^2)/.456

# Calculate adjusted p-values
cp.values_k3_rain = pchisq(zs.stouffer_k3_rain^2/lambda_k3_rain, df = 1, lower = FALSE)
plot(ecdf(cp.values_k3_rain),xlim=c(0,1),ylim=c(0,1))
abline(a=0,b=1)

for (alpha in c(.05,.1,.15,.2)) {
  # expected FDR
  print(paste("expected FDR:", alpha))
  L = length(cp.values_k3_rain)
```

```
# Return a list of candidates with an expected FDR of alpha.
  w = which(sort(cp.values_k3_rain) < alpha * (1:L) / L)
  candidates = order(cp.values_k3_rain)[w]

  # Estimated FDR and True Positives
  estimated.FDR_k3_rain = length(which(candidates <= 350))/length(candidates)
  estimated.TP_k3_rain = length(which(candidates > 350))/50
  print(paste("FDR:", estimated.FDR_k3_rain, "True Positive:", estimated.TP_k3_rain))
}

# Get list of candidate loci using the Benjamini-Hochberg procedure
q = 0.05       # expected false discovery rate
L = length(cp.values_k3_rain)
a <-sort(cp.values_k3_rain)
plot(a)
lines(q*(1:L)/L)
w = which(sort(cp.values_k3_rain) < q * (1:L) / L)
candidates_k3_rain_0.05 = order(cp.values_k3_rain)[w]
print(candidates_k3_rain_0.05)

# Get the p-values
p_k3_rain = p.values(project, K = 3)

# Get the -log10(p-values) for K = 3
mp_k3_rain = mlog10p.values(project, K = 3)

# Write as text files
write.table(zs.stouffer_k3_rain, "zs_k3_rain.txt", sep="\t")
write.table(mp_k3_rain, "log10_k3_rain.txt", sep="\t")
write.table(p_k3_rain, "p_values_k3_rain.txt", sep="\t")
write.table(candidates_k3_rain_0.05, "candidate_loci_k3_rain_0.05.txt", sep="\t")
```

MOLECULAR ECOLOGY
RESOURCES

## Appendix 3 – Supplemental Tables

**Table S1 –** Environment variables included in the ecological niche models and their source.

| Variable | Source | Comments |
|---|---|---|
| Bio7 - Temperature annual range | WorldClim (www.worldclim.org) | |
| Bio10 - Mean temperature of the warmest quarter | WorldClim (www.worldclim.org) | |
| Bio11 - Mean temperature of the coldest quarter | WorldClim (www.worldclim.org) | |
| Bio12 - Annual rainfall | WorldClim (www.worldclim.org) | |
| Bio15 - Rainfall seasonality | WorldClim (www.worldclim.org) | |
| Bio18 - Rainfall during the warmest quarter | WorldClim (www.worldclim.org) | |
| Slope | WorldClim (www.worldclim.org) | |
| Land cover - GlobCover 2009 map | European Space Agency (http://due.esrin.esa.int/page_globcover.php) | reclassified into 10 categories |

MOLECULAR ECOLOGY RESOURCES

**Table S2 –** *Plecotus austriacus* SNPs identified by Bayescan outlier test as potentially under selection and the significance of their correlations (logistic regressions) with summer rainfall (GLMs rain) and temperature (GLMs temp) across the whole study areas (Full) and within Iberia (IB). Significant correlations after Bonferroni correction are highlighted in bold.

| Outlier SNPs | GLMs rain full | GLMs rain IB | GLMs temp full | GLMs temp IB |
|---|---|---|---|---|
| 517 | NS | NS | **P<0.001** | NS |
| 1268 | **P<0.001** | **P<0.001** | NS | NS |
| 1644 | **P<0.01** | P<0.05 | NS | P<0.05 |
| 1911 | NS | NS | **P<0.001** | NS |
| 2196 | zero inflated | zero inflated | zero inflated | zero inflated |
| 3848 | **P<0.01** | NS | **P<0.001** | P<0.05 |
| 3869 | zero inflated | zero inflated | zero inflated | zero inflated |
| 4735 | NS | NS | **P<0.01** | NS |
| 5401 | zero inflated | zero inflated | zero inflated | zero inflated |
| 5874 | **P<0.001** | **P<0.001** | NS | NS |
| 7135 | NS | NS | NS | **P<0.001** |
| 9060 | zero inflated | zero inflated | zero inflated | zero inflated |
| 9306 | zero inflated | zero inflated | zero inflated | zero inflated |
| 9872 | NS | **P<0.01** | NS | NS |
| 10617 | **P<0.001** | **P<0.001** | P<0.01 | NS |
| 12222 | **P<0.01** | NS | **P<0.001** | P<0.05 |
| 14219 | zero inflated | zero inflated | zero inflated | zero inflated |
| 14883 | **P<0.01** | NS | **P<0.001** | P<0.05 |
| 15650 | **P<0.001** | **P<0.01** | **P<0.001** | **P<0.01** |
| 18702 | P<0.01 | NS | P<0.01 | P<0.01 |
| 22104 | P<0.01 | NS | P<0.01 | P<0.01 |
| 22863 | NS | **P<0.01** | NS | NS |
| 23049 | zero inflated | zero inflated | zero inflated | zero inflated |
| 24071 | NS | NS | NS | NS |

MOLECULAR ECOLOGY RESOURCES

**Table S3** – The eight *Plecotus austriacus* SNPs identified as under climate-driven selection by Bayescan, LFMM, logistic regressions (GLMs) and LOSITAN (FDist), with their associated climatic variables and LFMM dataset (Full = whole study area; IB = Iberia only).

| SNP name | Climate variable | LFMM dataset | GLMs | LOSITAN | Comments |
|---|---|---|---|---|---|
| **1268** | Rain | IB | P<0.001 | P<0.01 | |
| **1644** | Rain | Full + IB | P<0.001 | P<0.005 | |
| **3848** | Temperature | IB | P<0.001 | P<0.005 | |
| **5874** | Rain | IB | P<0.001 | P<0.01 | |
| **9872** | Rain | IB | P<0.01 | P<0.005 | |
| **10617** | Temperature + Rain | Full + IB | P<0.001 | NS | Full only for Rain |
| **14883** | Temperature + Rain | IB | P<0.001 | P<0.005 | |
| **15650** | Temperature + Rain | Full + IB | P<0.001 | P<0.005 | Full only for Rain |

MOLECULAR ECOLOGY
RESOURCES

**Tables S4** –Frequencies of potential climate-adaptive alleles in the Iberian *Plecotus austriacus* populations, including overall mean frequencies. Sensitivity is determined based on the overall mean frequency of potential climate-adaptive alleles in the population (high sensitivity <0.5, low sensitivity >0.5) and the number of adaptive alleles present at particularly low frequencies (<0.25), ranging from very high (++), to high (+), medium (0), and low (-).

| SNP | Lisboa | Bizkaia | Girona | Granada | Albacete | Valladolid | Valencia |
|---|---|---|---|---|---|---|---|
| 1268 | 1.00 | 0.50 | 0.33 | 1.00 | 0.89 | 0.60 | 0.50 |
| 1644 | 0.75 | 0.50 | 0.28 | 0.58 | 0.89 | 0.50 | 0.56 |
| 3848 | 0.72 | 0.65 | 0.50 | 0.79 | 1.00 | 0.72 | 0.78 |
| 5874 | 0.94 | 0.50 | 0.33 | 1.00 | 0.89 | 0.60 | 0.50 |
| 9872 | 0.85 | 0.15 | 0.61 | 0.31 | 0.44 | 0.30 | 0.17 |
| 10617 | 0.94 | 0.10 | 0.44 | 0.67 | 0.62 | 0.69 | 0.28 |
| 14883 | 0.31 | 0.30 | 0.20 | 0.69 | 0.56 | 0.50 | 0.06 |
| 15650 | 0.50 | 0.19 | 0.29 | 0.43 | 0.72 | 0.80 | 0.19 |
| **Mean** | 0.75 | 0.36 | 0.37 | 0.68 | 0.75 | 0.59 | 0.38 |
| **Sensitivity** | - | ++ | + | - | - | - | ++ |

MOLECULAR ECOLOGY
RESOURCES

**Table S5** – Genetic differentiation (Fst values) between *Plecotus austriacus* populations based on the neutral dataset (top triangle) and SNPs potentially associated with climate-adaptive genetic variation (bottom triangle). Lis=Lisboa, Bas= Bizkaia, Cat=Girona, Dev=Devon, Dor=Dorset, Gra=Granada, Alb=Albacete, Vld=Valladolid, and Val=Valencia.

| | **Lis** | **Bas** | **Cat** | **Dev** | **Dor** | **Gra** | **Alb** | **Vld** | **Val** |
|---|---|---|---|---|---|---|---|---|---|
| **Lis** | | 0.038 | 0.035 | 0.106 | 0.091 | 0.027 | 0.035 | 0.027 | 0.036 |
| **Bas** | 0.350 | | 0.026 | 0.088 | 0.078 | 0.031 | 0.038 | 0.032 | 0.041 |
| **Cat** | 0.287 | 0.026 | | 0.080 | 0.070 | 0.029 | 0.034 | 0.025 | 0.033 |
| **Dev** | 0.508 | 0.298 | 0.266 | | 0.057 | 0.100 | 0.106 | 0.097 | 0.101 |
| **Dor** | 0.292 | 0.288 | 0.097 | 0.541 | | 0.088 | 0.094 | 0.082 | 0.093 |
| **Gra** | 0.042 | 0.285 | 0.272 | 0.476 | 0.333 | | 0.028 | 0.024 | 0.033 |
| **Alb** | 0.074 | 0.288 | 0.296 | 0.559 | 0.399 | 0.039 | | 0.028 | 0.037 |
| **Vld** | 0.080 | 0.203 | 0.110 | 0.406 | 0.195 | 0.078 | 0.061 | | 0.031 |
| **Val** | 0.319 | 0.001 | 0.022 | 0.334 | 0.234 | 0.302 | 0.277 | 0.195 | |

MOLECULAR ECOLOGY
RESOURCES

**Table S6** – Measure of heterozygosity in neutral SNPs and corresponding relative levels of neutral genetic diversity in *Plecotus austriacus* populations. Relative neutral genetic diversity is ranked from low (Het<0.75) to medium (Het=0.75-0.9) and high (Het>0.9). Neutral sensitivity to future climate change is determined based on the potential contribution of genetic diversity to future adaptive potential (- low sensitivity due to high levels of neutral genetic diversity; 0 medium sensitivity; + high sensitivity due to relatively low levels of genetic diversity).

| Population | Heterozygosity | Neutral Genetic Diversity | Neutral Sensitivity |
|---|---|---|---|
| **Lisboa** | 0.8855 | medium | 0 |
| **Bizkaia** | 0.9638 | high | - |
| **Girona** | 0.9182 | high | - |
| **Devon** | 0.8351 | medium | 0 |
| **Dorset** | 0.6636 | low | + |
| **Granada** | 0.8442 | medium | 0 |
| **Albacete** | 0.9436 | high | - |
| **Valladolid** | 0.9596 | high | - |
| **Valencia** | 0.9711 | high | - |

MOLECULAR ECOLOGY
RESOURCES

**Tables S7** – Results of multiple regressions on distance matrices (MRDM) of genetic distance (Fst) in the *Plecotus austriacus* neutral dataset against geographic distance (geo-dist) and landscape variables for which the sea was assigned a cost of 200 (ENM=ecological niche model, forest=forest cover gradient), and of the residuals of the regression of genetic and geographic distance (Fst~geo-dist) against landscape resistance.

| Variables | $R^2$ | F | P | Comments |
|---|---|---|---|---|
| Fst ~ geo-dist | 0.649 | 63.1 | 0.0001 | |
| Fst ~ ENM continuous | 0.842 | 180.7 | 0.0001 | |
| Fst ~ ENM 10 quantiles | 0.798 | 134.5 | 0.0001 | |
| Fst ~ forest cover gradient | 0.588 | 48.6 | 0.0001 | |
| Fst ~ % tree cover continuous | 0.572 | 45.4 | 0.0001 | |
| Fst ~ altitude | 0.299 | 14.5 | 0.0004 | |
| Fst ~ slope | 0.667 | 68.2 | 0.0001 | |
| Fst ~ ENM + forest | 0.844 | 89.1 | 0.0001 | forest NS |
| Fst ~ ENM + altitude | 0.85 | 93.6 | 0.0001 | altitude NS |
| Fst ~ ENM + slope | 0.842 | 88.3 | 0.0001 | slope NS |
| Fst ~ ENM + forest + altitude | 0.851 | 60.9 | 0.0001 | forest/altitude NS |
| | | | | |
| Residuals(Fst~geo-dist) ~ ENM | 0.197 | 8.3 | 0.005 | |
| Residuals(Fst~geo-dist) ~ forest | NS | | 0.181 | NS |
| Residuals(Fst~geo-dist) ~ altitude | NS | | 0.831 | NS |
| Residuals(Fst~geo-dist) ~ slope | NS | | 0.884 | NS |
| Residuals(Fst~geo_dist) ~ ENM + forest | NS | | 0.614 | NS |
| Residuals(Fst~geo_dist) ~ ENM + altitude | NS | | 0.304 | NS |
| Residuals(Fst~geo-dist) ~ ENM + slope | NS | | 0.184 | NS |

MOLECULAR ECOLOGY
RESOURCES

**Tables S8** – Results of multiple regressions on distance matrices (MRDM) of genetic distance (Fst) in the *Plecotus austriacus* neutral dataset against geographic distance (geo-dist) and landscape variables for which the sea was assigned a cost of 120 (ENM=ecological niche model, forest=forest cover gradient), and of the residuals of the regression of genetic and geographic distance (Fst~geo-dist) against landscape resistance.

| Variables | $R^2$ | F | P | Comments |
|---|---|---|---|---|
| Fst ~ geo_dist | 0.649 | 63.1 | 0.0001 | |
| Fst ~ ENM | 0.715 | 85.4 | 0.0001 | |
| Fst ~ forest | 0.588 | 48.6 | 0.0001 | |
| Fst ~ altitude | NS | | 0.0921 | NS |
| Fst ~ slope | 0.503 | 34.4 | 0.0001 | |
| Fst ~ ENM + forest | 0.757 | 51.4 | 0.0002 | |
| Fst ~ ENM + altitude | 0.754 | 50.5 | 0.0001 | |
| Fst ~ ENM + slope | 0.727 | 43.9 | 0.0001 | slope NS |
| Fst ~ ENM + forest + altitude | 0.776 | 36.9 | 0.0001 | forest/altitude NS |
| | | | | |
| Residuals(Fst~geo_dist) ~ ENM | 0.172 | 7.1 | 0.01 | |
| Residuals(Fst~geo_dist) ~ forest | NS | | 0.355 | NS |
| Residuals(Fst~geo_dist) ~ slope | NS | | 0.632 | NS |
| Residuals(Fst~geo_dist) ~ altitude | NS | | 0.197 | NS |
| Residuals(Fst~geo_dist) ~ ENM + forest | NS | | 0.813 | NS |
| Residuals(Fst~geo_dist) ~ ENM + slope | NS | | 0.567 | NS |
| Residuals(Fst~geo_dist) ~ ENM + altitude | NS | | 0.544 | NS |

MOLECULAR ECOLOGY
RESOURCES

**Table S9** – Combinatorial barcodes used to identify individual bat samples in the pooled ddRAD sequence output. Read 1 sequences begin with P1 barcode; paired Read 2 sequences begin with P2 barcode. Detailed location information is given in Table 1.

| # | Sample ID | Population | P1 barcode (read 1) | P2 barcode (read 2) |
|---|---|---|---|---|
| 1 | Bas_001 | Basque | TCTCTCA | TAGCA |
| 2 | Bas_003 | Basque | GTACACA | TAGCA |
| 3 | Bas_005 | Basque | CTCTTCA | TAGCA |
| 4 | Bas_006 | Basque | CTAGGAC | TAGCA |
| 5 | Bas_008 | Basque | ACGTA | TAGCA |
| 6 | Bas_009 | Basque | AGAGT | TAGCA |
| 7 | Bas_010 | Basque | ATGCT | TAGCA |
| 8 | Bas_011 | Basque | GACTA | TAGCA |
| 9 | Bas_012 | Basque | CAGTCAC | TAGCA |
| 10 | Bas_013 | Basque | GCTAACA | TAGCA |
| 11 | Cat_017 | Catalunya | ACACGAG | TAGCA |
| 12 | Cat_019 | Catalunya | AGGACAC | TAGCA |
| 13 | Cat_021 | Catalunya | TCAGA | AGCTGTC |
| 14 | Cat_023 | Catalunya | GATCG | AGCTGTC |
| 15 | Cat_024 | Catalunya | CATGA | AGCTGTC |
| 16 | Cat_026 | Catalunya | ATCGA | AGCTGTC |
| 17 | Cat_027 | Catalunya | TCGAG | AGCTGTC |
| 18 | Cat_028 | Catalunya | GTCAC | AGCTGTC |
| 19 | Cat_029 | Catalunya | GCATT | AGCTGTC |
| 20 | Cat_031 | Catalunya | CGATA | AGCTGTC |
| 21 | Aqu_P02 | Lisboa | TGCAACA | AGCTGTC |
| 22 | Aqu_P03 | Lisboa | CGTATCA | AGCTGTC |
| 23 | Aqu_P04 | Lisboa | CACAGAC | AGCTGTC |
| 24 | Aqu_P05 | Lisboa | ACTGCAC | AGCTGTC |
| 25 | Aqu_P06 | Lisboa | TCTCTCA | AGTCA |
| 26 | Aqu_P07 | Lisboa | GTACACA | AGTCA |
| 27 | Aqu_P08 | Lisboa | CTCTTCA | AGTCA |
| 28 | Aqu_P09 | Lisboa | CTAGGAC | AGTCA |
| 29 | Aqu_P11 | Lisboa | ACGTA | AGTCA |
| 30 | Aqu_P12 | Lisboa | AGAGT | AGTCA |

MOLECULAR ECOLOGY
RESOURCES

**Table S9 continued**

| # | Sample ID | Population | P1 barcode (read 1) | P2 barcode (read 2) |
|---|---|---|---|---|
| 31 | Pal_051 | Albacete | ATGCT | AGTCA |
| 32 | Pal_052 | Albacete | GACTA | AGTCA |
| 33 | Pal_053 | Albacete | CAGTCAC | AGTCA |
| 34 | Pal_054 | Albacete | GCTAACA | AGTCA |
| 35 | Pal_055 | Albacete | ACACGAG | AGTCA |
| 36 | Pal_056 | Albacete | AGGACAC | AGTCA |
| 37 | Pal_057 | Albacete | TCAGA | TACGTGT |
| 38 | Pal_058 | Albacete | GATCG | TACGTGT |
| 39 | Pal_059 | Albacete | CATGA | TACGTGT |
| 40 | Pal_060 | Albacete | ATCGA | TACGTGT |
| 41 | Gra_071 | Granada | TCGAG | TACGTGT |
| 42 | Gra_072 | Granada | GTCAC | TACGTGT |
| 43 | Gra_073 | Granada | GCATT | TACGTGT |
| 44 | Gra_074 | Granada | CGATA | TACGTGT |
| 45 | Gra_075 | Granada | TGCAACA | TACGTGT |
| 46 | Gra_076 | Granada | CGTATCA | TACGTGT |
| 47 | Gra_077 | Granada | CACAGAC | TACGTGT |
| 48 | Gra_078 | Granada | ACTGCAC | TACGTGT |
| 49 | Gra_079 | Granada | TCTCTCA | GCATA |
| 50 | Val_081 | Valladoid | GTACACA | GCATA |
| 51 | Val_082 | Valladoid | CTCTTCA | GCATA |
| 52 | Val_083 | Valladoid | CTAGGAC | GCATA |
| 53 | Val_085 | Valladoid | ACGTA | GCATA |
| 54 | Val_086 | Valladoid | AGAGT | GCATA |
| 55 | Val_087 | Valladoid | ATGCT | GCATA |
| 56 | Val_088 | Valladoid | GACTA | GCATA |
| 57 | Val_090 | Valladoid | CAGTCAC | GCATA |
| 58 | Val_091 | Valladoid | GCTAACA | GCATA |
| 59 | Val_092 | Valladoid | ACACGAG | GCATA |

MOLECULAR ECOLOGY
RESOURCES

**Table S9 continued**

| # | Sample ID | Population | P1 barcode (read 1) | P2 barcode (read 2) |
|---|---|---|---|---|
| 60 | Val_094 | Valencia | AGGACAC | GCATA |
| 61 | Val_095 | Valencia | TCAGA | GAGATGT |
| 62 | Val_096 | Valencia | GATCG | GAGATGT |
| 63 | Val_097 | Valencia | CATGA | GAGATGT |
| 64 | Val_098 | Valencia | ATCGA | GAGATGT |
| 65 | Val_099 | Valencia | TCGAG | GAGATGT |
| 66 | Val_100 | Valencia | GTCAC | GAGATGT |
| 67 | Val_101 | Valencia | GCATT | GAGATGT |
| 68 | Val_103 | Valencia | CGATA | GAGATGT |
| 69 | Des_104 | Vila Real | TGCAACA | GAGATGT |
| 70 | Des_105 | Vila Real | CGTATCA | GAGATGT |
| 71 | Des_106 | Vila Real | CACAGAC | GAGATGT |
| 72 | Des_107 | Vila Real | ACTGCAC | GAGATGT |
| 73 | Des_108 | Vila Real | TCTCTCA | CGATC |
| 74 | Des_109 | Vila Real | GTACACA | CGATC |
| 75 | Des_110 | Vila Real | CTCTTCA | CGATC |
| 76 | Des_111 | Vila Real | CTAGGAC | CGATC |
| 77 | Dev_U02 | Devon | ACGTA | CGATC |
| 78 | Dev_U03 | Devon | AGAGT | CGATC |
| 79 | Dev_U04 | Devon | ATGCT | CGATC |
| 80 | Dev_U05 | Devon | GACTA | CGATC |
| 81 | Dev_U06 | Devon | CAGTCAC | CGATC |
| 82 | Dev_U07 | Devon | GCTAACA | CGATC |
| 83 | Dev_U08 | Devon | ACACGAG | CGATC |
| 84 | Dev_U09 | Devon | AGGACAC | CGATC |
| 85 | Dev_U10 | Devon | TCAGA | CATCTGT |
| 86 | Dor_U11 | Dorset | GATCG | CATCTGT |
| 87 | Dor_U12 | Dorset | CATGA | CATCTGT |
| 88 | Dor_U13 | Dorset | ATCGA | CATCTGT |
| 89 | Dor_U14 | Dorset | TCGAG | CATCTGT |
| 90 | Dor_U15 | Dorset | GTCAC | CATCTGT |
| 91 | Dor_U16 | Dorset | GCATT | CATCTGT |
| 92 | Dor_U17 | Dorset | CGATA | CATCTGT |
| 93 | Dor_U18 | Dorset | TGCAACA | CATCTGT |
| 94 | Dor_U19 | Dorset | CGTATCA | CATCTGT |
| 95 | Dor_U20 | Dorset | CACAGAC | CATCTGT |

MOLECULAR ECOLOGY
RESOURCES

## Appendix 4 – Supplemental Figures

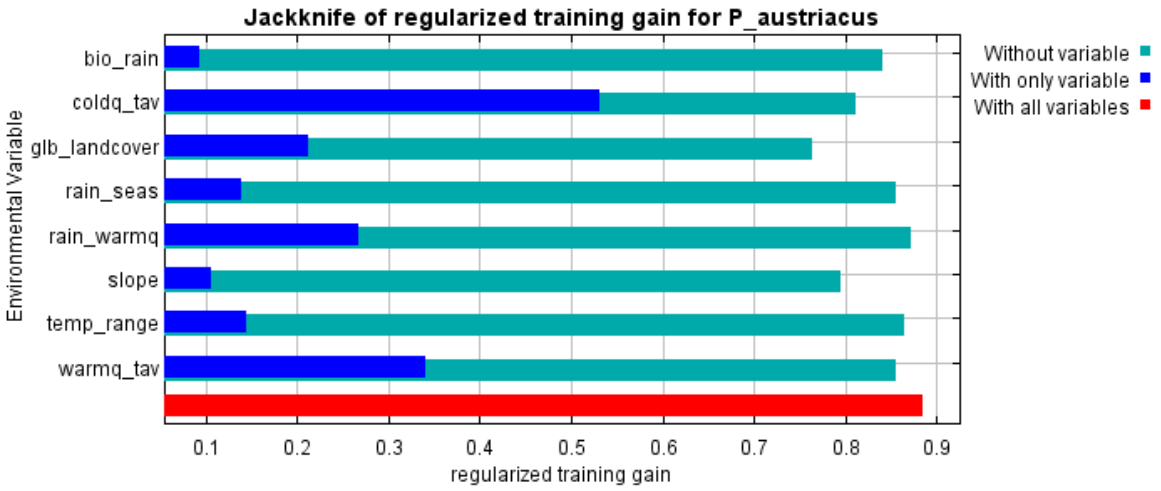


**Figure S1** – Variables included in the ecological niche model and their relative contribution to the model in terms of their effect on increasing model gain when used in isolation (size of dark blue relative to red bar) and on decreasing model gain when omitted from the model (extent of reduction in light blue bars relative to the red bar). bio_rain = annual rainfall, coldq_tav = average temperature of the coldest quarter, glb_landcover = global land cover, rain_seas = rainfall seasonality, rain_warmq = summer rainfall, temp_range = annual temperature range (maximum minus minimum annual temperatures), warmq_tav = average temperature of the warmest quarter.

MOLECULAR ECOLOGY
RESOURCES

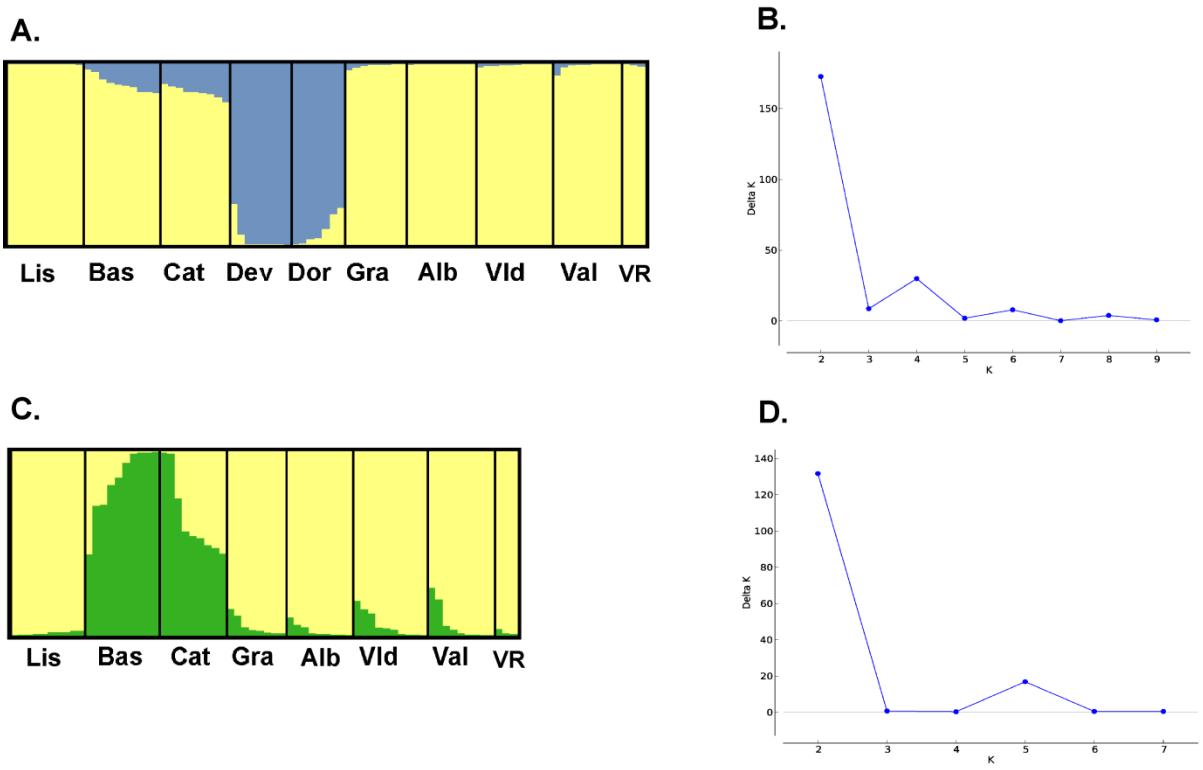


**Figure S2** – Estimates of population structure based on individual-based assignment tests (STRUCTURE) and Evanno's method delta K (B, D) for the full *Plecotus austriacus* dataset (A, B) and within the Iberian cluster (C, D). Lis=Lisboa, Bas= Bizkaia, Cat=Girona, Dev=Devon, Dor=Dorset, Gra=Granada, Alb=Albacete, Vld=Valladolid, and Val=Valencia.

MOLECULAR ECOLOGY
RESOURCES

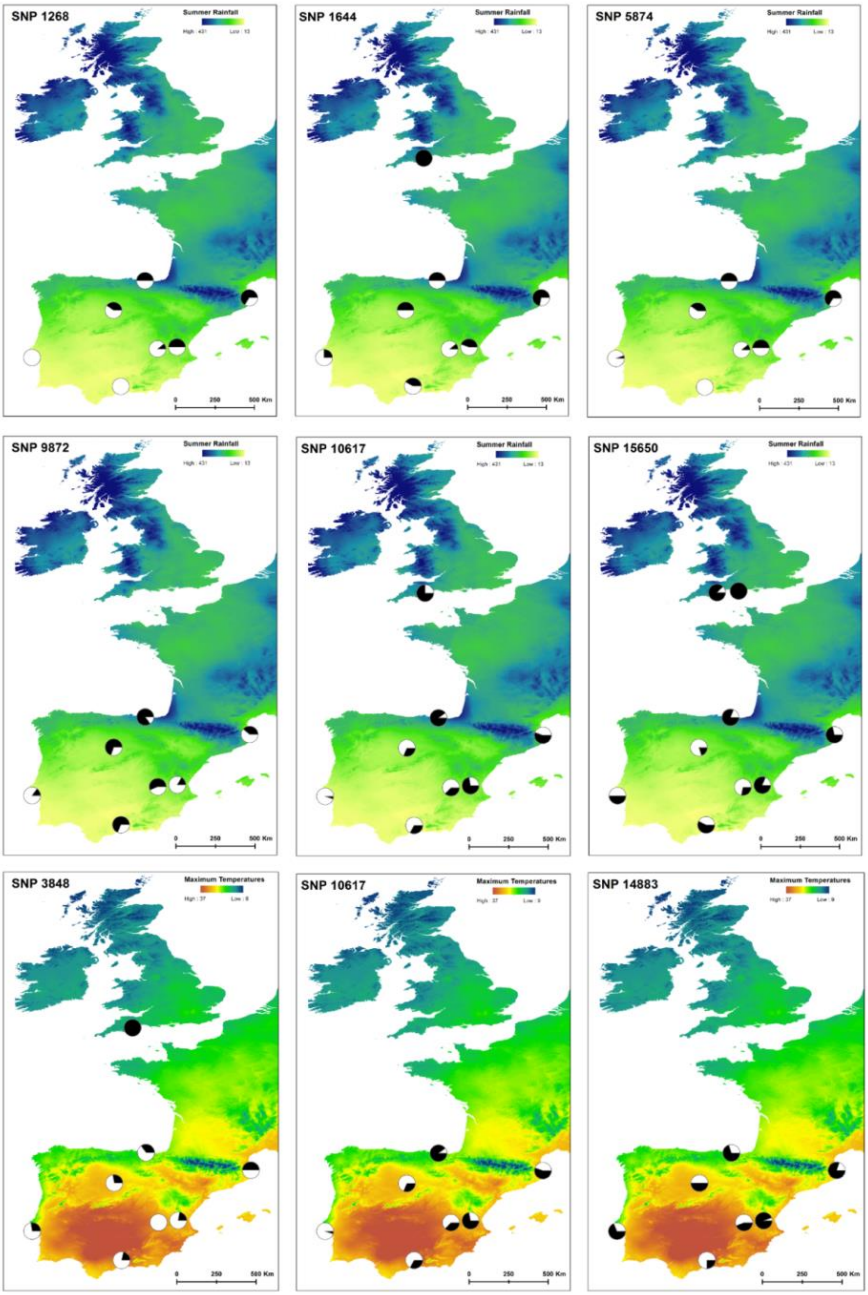


**Figure S3** – Frequency of climate-adaptive SNPs in *Plecotus austriacus* populations, presented over climatic maps (summer rainfall and maximum temperatures). White represents alleles adapted to warmer and dryer conditions. Only Iberian genotypes are shown for SNPs that are only associated with climatic conditions in Iberia.

MOLECULAR ECOLOGY
RESOURCES

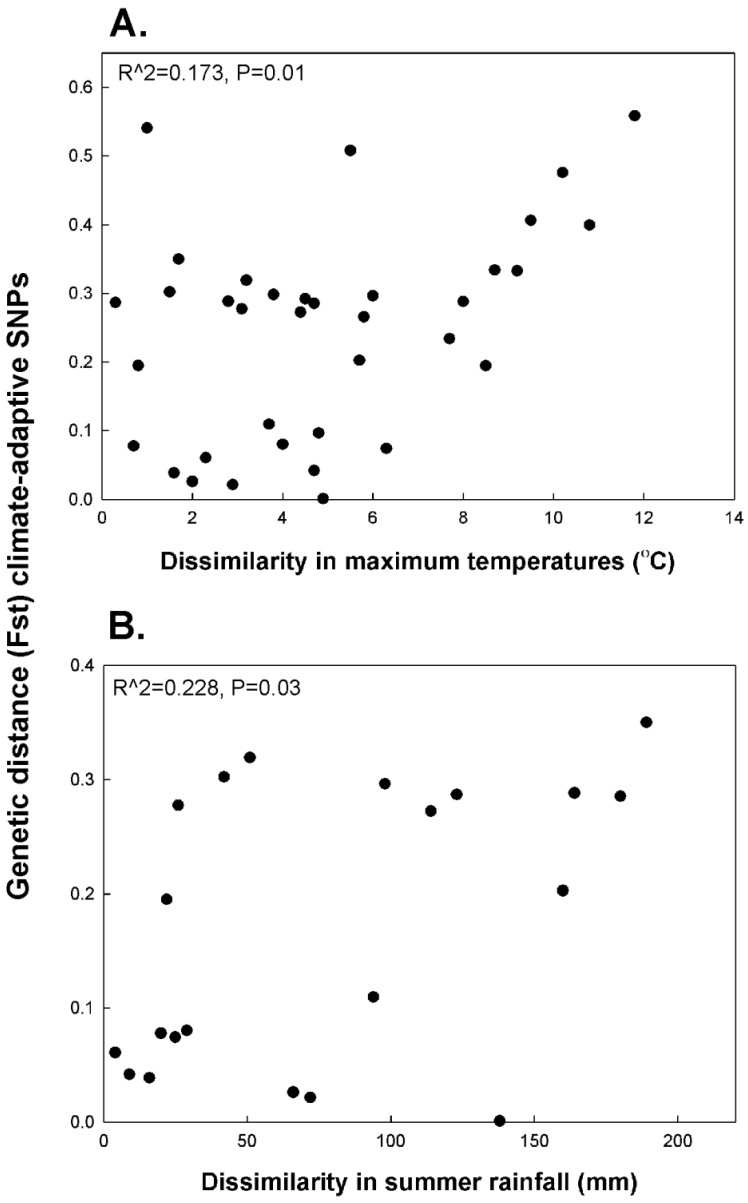


**Figure S4** – Patterns of Isolation by Environment in *Plecotus austriacus* SNPs associated with climate-adaptive genetic variation across the whole study area (A) and within Iberia (B).