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1 *Running title: Genetic changes along distribution of a lizard*

2 **Genetic and demographic trends from rear to leading edge are explained by**
3 **climate and forest cover in a cold adapted ectotherm**

4 Andréaz Dupoué ^{1,2*}, Audrey Trochet ¹, Murielle Richard ¹, Mahaut Sorlin ¹, Michaël Guillon
5 ^{3,4}, Jules Teulière Quillet ¹, Clément Vallé ¹, Cyrielle Rault ¹, Maud Berroneau ³, Matthieu
6 Berroneau ³, Olivier Lourdais ⁴, Pauline Blaimont ^{5,6}, Romain Bertrand ¹, Gilles Pottier ⁷,
7 Olivier Calvez ¹, Olivier Guillaume ¹, Hugo Le Chevalier ¹, Jérémie Souchet ¹, Jean François
8 Le Galliard ^{3,8}, Jean Clobert ^{1#}, Fabien Aubret ^{1,9#}

9 ¹ Station d'Ecologie Théorique et Expérimentale de Moulis, CNRS UMR 5321, 09200, Saint Giron,
10 France | ² iEES Paris, Sorbonne Université, CNRS, UMR 7618, Tours 44-45, 4 place Jussieu, 75005,
11 Paris, France | ³ Cistude Nature, Chemin du Moulinat, 33185, Le Haillan, France | ⁴ Centre d'Etudes
12 Biologiques de Chizé - ULR CNRS UMR 7372, 79360 Beauvoir sur Niort, France | ⁵ Department of
13 Ecology & Evolutionary Biology, University of California, Santa Cruz, 1156 High Street, Santa Cruz,
14 CA 95064, USA | ⁶ Department of Biology, Rider University, 2083 Lawrenceville Road,
15 Lawrenceville, NJ 08648, USA | ⁷ Nature en Occitanie, Maison de l'Environnement de Midi-Pyrénées,
16 14 rue de Tivoli, 31000 Toulouse, France | ⁸ Centre de recherche en écologie expérimentale et
17 prédictive (CEREPEP-Ecotron IleDeFrance), Ecole normale supérieure, CNRS UMS 3194, 78 rue du
18 château, 77140 Saint-Pierre-lès-Nemours, France | ⁹ Behavioural Ecology Lab., School of Molecular
19 and Life Sciences, Curtin University, Brand Drive, WA 6102, Bentley, Australia

20 * Authors correspondence: andreaz.dupoue@gmail.com

21 # Co-senior authors

22

23

24 **Abstract**

25 **Aim:** Determining whether altitudinal shifts in species distributions leave molecular
26 footprints on wild populations along their range margins from rear to leading edge.

27 **Location:** South-west France.

28 **Methods:** We compared the demographic and genetic variation in 42 wild populations of the
29 Western oviparous subclade B2 of a cold adapted lizard (*Zootoca vivipara louislantzi*). These
30 populations can be divided into four ecological units across altitudinal clines in South-west
31 France (rear edge: <100 m, admixture zone: 100-500 m, continuous range: 500-1300 m and
32 leading edge: >1300 m above sea level).

33 **Results:** Within the rear edge were found the highest levels of inbreeding, genetic
34 differentiation and evidence of interrupted gene flow compared to central or colonising areas.
35 Within the leading edge, altitudinal range expansion occurred over the last centuries and
36 populations showed relatively low genetic diversity. These demographic and genetic trends
37 were better explained by inhospitable (warm and dry) climate conditions and forest cover.

38 **Main conclusions:** This empirical evidence illustrates that molecular footprints of climate
39 conditions and habitat quality on wild population trends can be perceived after recent events,
40 which should be of particular importance to accurately understand and anticipate human-
41 induced global change on wild species and ecosystems.

42 **Keywords.** Abundance, climate, colonization, ectotherm, forest cover, gene flow, inbreeding,
43 population decline, structuration

44

45 **Introduction**

46 Biodiversity is facing a global loss of habitats resulting in altered species and biotic
47 interactions worldwide (Ceballos et al., 2017; Plotnick et al., 2016; Wiens, 2016). Causes are
48 multiple, often synergetic, and have various footprints from genes to ecosystems (Pecl et al.,
49 2017). As a consequence, many species' distributions are shifting (Lenoir et al., 2020;
50 VanDerWal et al., 2013) and these range shifts have direct impacts on processes and patterns
51 of population genetics (Carvalho et al., 2019; Hampe & Petit, 2005; Templeton et al., 2001).
52 In particular, once populations at the rear edge become isolated, the loss of connectivity and
53 immigration progressively leads to an increase in inbreeding through genetic drift, one of the
54 main precursor of decline in small populations (Frankham, 2010; Hampe & Petit, 2005). In
55 contrast, at the leading edge, newly favourable habitats should be colonized by few
56 individuals resulting in founder events, which implies lower genetic diversity associated with
57 small groups of emigrants and recurrent extinction events (Hampe et al., 2013; Hampe &
58 Petit, 2005; Nadeau & Urban, 2019; Vilà-Cabrera et al., 2019; Waters et al., 2013). Studies
59 have challenged these general assumptions since genetic diversity does not necessarily
60 correlate with biogeography (Eckert et al., 2008; Pironon et al., 2017) nor does it explain
61 population decline (Tobler et al., 2013). This prompts for empirically testing patterns of
62 genetic variation and phylogenetic history along colonization gradients to better understand
63 how population genetic diversity or structure correlate with range shifts of wild populations.

64 Terrestrial ectotherms are well suited organisms for this purpose since their ecology
65 and demography tightly depend on climate conditions and landscape structure (Le Galliard et
66 al., 2012). These organisms are characterized by a strong thermal dependence on their
67 performance, which may explain their spatial distribution (Buckley et al., 2012). Under
68 temperate climates, ectotherm distributions at a continental scale have been strongly
69 influenced by past Quaternary climatic oscillations resulting in successive range expansions

70 and range contractions in glacial refugia (Hewitt, 2000). These historical changes must be
71 considered to address current patterns of genetic variation at a regional scale, especially to
72 characterise the recent impacts of climate change. Indeed, terrestrial ectotherms in their
73 trailing range edge (low latitudes and low altitudes) are now increasingly challenged by
74 recurrent temperatures nearing their upper thermal safety margin (Sunday et al., 2014).
75 Despite a relatively high potential of phenotypic plasticity, once temperatures rise above
76 maximal critical temperatures, individuals at the rear edge of the distribution are exposed to
77 greater risks of overheating and dehydration, which can impair the activity time and incur
78 physiological costs (Dupoué et al., 2018, 2020; Le Galliard et al., 2012; Rozen-Rechels et al.,
79 2019; Sinervo et al., 2010). These extreme climate conditions may constrain lifetime
80 reproductive success and/or longevity, eventually leading to a loss of reproductive individuals
81 and population extirpation (Bestion et al., 2015; Dupoué, Rutschmann, Le Galliard, Clobert,
82 et al., 2017; Lourdais et al., 2017).

83 The present comparative study assessed relative abundance and genetic profiles in
84 natural populations of the Western oviparous subclade B2 of the common lizard (*Zootoca*
85 *vivipara louislantzi*) along an altitudinal cline. This boreal Squamate is a cold adapted
86 specialist combining freezing tolerance to supercooling physiological adaptations (Voituron et
87 al., 2002), as well as reproductive bimodality with distinct oviparous and viviparous
88 populations across its distribution range (Surget-Groba et al., 2006). The viviparous form has
89 colonized the Eurosiberian region from Western Europe to Hokkaido island in Japan being
90 restricted by the polar circle (Surget-Groba et al., 2006). In the Southwest margin of its range,
91 the oviparous form (*Z. v. louislantzi*) experiences a spatially restricted distribution that has
92 been structured across altitude most likely due to temperatures and anthropogenic habitat
93 fragmentation. Populations were affiliated to four ecological units defined *a priori* and
94 following the classification established by Hampe & Petit (2005). This allows for the

95 examination of recolonization dynamics and how multiple demographic trajectories shape
96 genetic variation since the Last-Glacial-Maximum (LGM). In the rear edge within 100 m
97 above sea level (ASL), man-made draining of natural habitats (peatbogs and marshes)
98 occurred in the mid-19th century, which has isolated populations into a few suitable patches of
99 humid areas [boggy islets (Berroneau, 2014)]. Surrounding environments became
100 inhospitable because of human landscape modification and recent climate warming, thus
101 hindering any emigration from these populations. Similar anthropogenic-driven isolations
102 have occurred in the foothills of the French Pyrenees Mountains between 100 and 500 m
103 ASL, where populations are enclaved within forest-isolated peat bogs. This area is located
104 between populations from the rear edges and those of the continuous range, now forming the
105 Admixture zone [*sensu* (Hampe & Petit, 2005)], a lineage mixing area between previously
106 allopatric sub-clades (Rius & Darling, 2014). On the other hand, in its continuous range from
107 500 to 1,300 m ASL, populations occupy various habitat types mostly composed by river
108 ecosystems and forest borders. Finally, populations are now expanding above 1,300 m ASL
109 and colonizing highland meadows, which represent the leading edge.

110 We used microsatellite polymorphisms to assess how 42 natural populations across
111 this cline differed in genetic structure, diversity and connectivity. We anticipated that
112 Pyrenean ascension occurred a few times after the LGM, similar to the latitudinal expansion
113 of the viviparous form of this species (Horreo et al., 2018). Additionally, we examined how
114 human-induced environmental threats may additively affect lizard populations by testing the
115 specific influence of recent climate conditions and habitat characteristics on demographic and
116 genetic markers. Because rear edge populations are exposed to increasingly hostile (warm and
117 dry) thermal conditions, they should also experience high levels of differentiation and
118 inbreeding reflecting range contraction (Hampe & Petit, 2005). Conversely, in the wave front
119 of the expanding margin, we predicted higher segregation and lower genetic diversity if

120 colonisation occurs through founder events (Hampe et al., 2013; Nadeau & Urban, 2019;
121 Waters et al., 2013). Finally, we expected demographic and genetic variation to be negatively
122 impacted by constraining climate conditions and low habitat quality. We tested this prediction
123 by checking the influence of two climate variables (i.e., air temperature and precipitation flux)
124 and two habitats characteristics (i.e., forest cover and wetland potential) that are known to
125 drive ecophysiological adjustments and local adaptations in this species (Dupoué,
126 Rutschmann, Le Galliard, Clobert, et al., 2017; Dupoué, Rutschmann, Le Galliard, Miles, et
127 al., 2017; Lorenzon et al., 1999; Rozen-Rechels et al., in press; Rutschmann et al., 2016,
128 2020).

129

130 **Material and methods**

131 *Sampled populations among four ecological units*

132 We caught 627 individuals from 42 populations between April-August 2017 and in May
133 2018. Populations were selected and considered different based on natural barriers (rivers,
134 forests, altitude) and a minimal 2 km plane geographic distance (~300 m of altitude
135 differential). Following capture, lizards were swabbed in the buccal cavity (Beebee, 2008),
136 and samples were immediately stored in TE buffer and frozen at -20°C the same day to
137 optimize DNA conservation until extraction. In each population, we estimated the relative
138 abundance as the number of lizards captured divided by the time spent on the population and
139 the number of people (range: 2 – 4 persons). As a heliothermic thermoregulator, common
140 lizards rely on solar radiation to regulate body temperature, and capture probability may
141 depend on weather conditions. We limited this bias by sampling all populations under
142 relatively similar weather conditions (< 50% cloud cover) during the activity peak time (10:30
143 - 17:30) as long as basking opportunities remained available. In addition, cloud cover (0-50

144 %) had no statistical influence on abundance estimate ($p = 0.626$) and a similar index
145 provided a reliable proxy of absolute density in another study (Dupoué, Rutschmann, Le
146 Galliard, Clobert et al., 2017). Populations were divided into the four ecological units based
147 on their altitude thresholds as described above (100, 500, and 1,300 m) and the associated
148 landscapes (Table S1). This included the boggy islets in the rear edge ($n = 55$ lizards among 4
149 populations), the forest enclaves of the admixture zone (137 lizards among 9 populations), the
150 mixed habitats of the continuous range (242 lizards among 16 populations), and the highland
151 meadows in the leading edge (198 lizards among 13 populations).

152 *Climatic conditions and habitat characteristics in sampled populations*

153 For each population, we extracted two climate variables (temperature and precipitations) of
154 recent climate conditions from CHELSA database, where climatology is averaged between
155 1981 and 2005 (Karger et al., 2017, 2020) and is available at a high resolution of 30 Arcsec
156 ($\sim 1 \text{ km}^2$). For each population, we considered the annual near-surface (2 m) air temperature
157 (Fig. S1A) and annual cumulative precipitation flux corrected by orographic effects (Fig.
158 S1B). We also considered two descriptors of habitat quality relevant for this species: forest
159 cover and wetland potential. We estimated forest cover at a precision of 30 m from the
160 aboveground live woody biomass density for the year 2000 (Fig. S1C), derived from many
161 studies (<http://www.globalforestwatch.org/>). Wetland potential (the probability for the habitat
162 to be a humid zone ranging from 0 to 3; 0: dry environments; 1: good probability of wetland
163 area; 2: strong probability of wetland area; 3: very strong probability of wetland area) was
164 obtained at a precision of 50 m (Fig. S1D), calculated from pedological and hydrogeological
165 information (Berthier et al., 2014). This index provides a continuous measure of the hydric
166 environment in each population, which fitted well with our direct observation in the field
167 (Dupoué, *pers. obs.*).

168 *Genetic data*

169 Genomic DNA was extracted from swabs using the DNeasy 96 Blood and Tissue Purification
170 Kit (Qiagen™, Hilden, Germany) according to the manufacturer's instructions. We
171 amplified sixteen microsatellite markers (Table S2) in 5µl reaction volumes with 1µl of PCR
172 master Kit (MPBiomedicals) and 5-10ng of template DNA. PCR products were run on an ABI
173 3730 DNA Analyser (Applied Biosystems) with Genescan-600Liz size standard. Genotyping
174 was performed with Genemapper 5.0 (Applied Biosystems). We performed an initial
175 examination on the dataset to retain the loci with limited allele dropout (< 18% of missing
176 data). Ambiguous genotypes were amplified and sized a second time. Out of 16 initial loci, 5
177 loci were rejected from further analyses due to an excess of missing data (Table S2). Null
178 alleles and linkage disequilibrium among remaining loci was tested using GENEPOP 4.7.0
179 (Rousset, 2008) following 10,000 iterations. The 11 loci did not present significant evidence
180 for null alleles (Table S2), nor linkage disequilibrium (Table S3) and were used in the
181 following analyses. We used COLONY software (Jones & Wang, 2010) to identify and
182 remove relatives in each population (Table S4) given that they may alter subsequent analyses
183 (Rodríguez-Ramilo & Wang, 2012). The final dataset included n = 599 individuals.

184 *Statistical analyses*

185 Genetic metrics

186 We used R software (R Development Core Team 2015) and the *DiveRsity* R-package (Keenan
187 et al., 2013) to estimate genetic diversity in each population including expected
188 heterozygosity (H_e), observed heterozygosity (H_o), deviation test to Hardy-Weinberg
189 equilibrium (HWE), and allelic richness (A_r). We used the same package to estimate
190 heterozygote deficit within a population (F_{IS}) and genetic differentiation between populations
191 (F_{ST}) after 1,000 bootstraps. In all these analyses, we excluded one population (Lagunas,
192 Table S1) given that the sample size (n = 6 lizards) was too small to obtain reliable estimates
193 of population genetic diversity. We used the *poppr* R-package (Kamvar et al., 2015) to

194 analyse the molecular variance (AMOVA) and check how variation in genetic diversity
195 differed i) among ecological units, ii) among or within populations, and iii) within
196 individuals. We used the program BOTTLENECK (Piry et al., 1999) to check if any
197 populations experienced a recent bottleneck. Shortly after a bottleneck event, heterozygosity
198 increases more rapidly than allelic diversity (Piry et al., 1999). The program tests the
199 heterozygosity excess and compares H_o and H_e (Wilcoxon test) after 1,000 iterations. We
200 considered a two phase model including a 95% of stepwise mutation model and a variance of
201 12 as recommended (Piry et al., 1999).

202 Genetic structure

203 We used the Bayesian clustering analysis of the program STRUCTURE 2.3.3 (Pritchard et al.,
204 2000) to determine whether populations could be regrouped without *a priori* information. We
205 determined how many clusters (K) best fit with our data, based on ΔK method (Earl &
206 VonHoldt, 2012). To do so, we ran 10 independent simulations for each K ranging from 1 to
207 42, with 500,000 iterations and a burn-in period of 100,000 steps. We adjusted the burn-in
208 period to 200,000 steps in another simulation with K ranging from 1 to 10, to check that the
209 initial burn-in period was sufficiently long to ensure model convergence. We compared two
210 outputs (alpha and likelihood) from the program, illustrating relatively few admixtures
211 between population (alpha < 1) and good model convergence (Fig. S2). We estimated
212 structuration of each population as the highest probability (maximal Q-value) for an
213 individual to be affiliated to a cluster (Fig. 1).

214 We complemented this analysis by using another method for the examination of
215 population structure. We considered a method based on Discriminant Analysis of Principal
216 Component (DAPC) proposed by Jombart et al., (2010) and implemented in the *adegenet* R
217 package (Jombart, 2008). This multivariate method generates models for a range of K clusters
218 and calculate Bayesian Information Criterion (BIC) after 10,000 iterations (Jombart et al.,

219 2010). Optimal K is chosen following a stepping stones approach, for the minimum K when
220 BIC decreases by a negligible amount (Fig. S3).

221 Genetic connectivity

222 We examined the degree of isolation-by-distance (IBD) using Mantel tests implemented in the
223 *adegenet* R package. We compared the relationships between pairwise genetic distances and
224 pairwise Euclidian geographic distances either globally (i.e., analysis on the entire dataset) or
225 locally (i.e., analyses within each ecological unit). We considered chord genetic distances
226 (D_C) between populations defined by (Cavalli-Sforza & Edwards, 1967), since these are more
227 likely to retrieve correct relations among populations (Takezaki & Nei, 1996). P-values were
228 generated by comparing observed and predicted distributions after 1,000 iterations.

229 Effect of environmental conditions on demography and genetic diversity

230 We used AICc based model selection and averaging from the *MuMIn* R-package (Barton,
231 2019) to examine the additive impacts of climate conditions and habitat characteristics on
232 lizard abundance (calculated from field data) and genetic diversity [calculated with *DiveRsity*
233 R package (Keenan et al., 2013)]. We ran four independent sets of 15 linear mixed model [R
234 package *nlme* (Pinheiro et al., 2016)] comparisons, using either demographic (lizard
235 abundance) or genetic markers (A_r , F_{IS} and F_{ST}) as response variables, and environmental
236 covariates alone or in addition (see models detailed in Table 1). Level of correlation between
237 environmental variables was below 0.42, thus limiting the risk of multicollinearity issue
238 (Dormann et al., 2013). In all models, ecological unit was set as a random term to account for
239 spatial interdependence of populations. For each demographic or genetic parameter, we
240 inferred the best models from the AICc score [$\Delta AICc < 2$ (Burnham & Anderson, 2002)], and
241 calculated the conditional model average to test for effect size and significance of fixed co-

242 variates. We verified that residuals of all selected models (Table 1) respected normality
243 conditions (Shapiro test: all $p > 0.104$).

244 Pyrenean colonisation scenarios

245 We used Approximate Bayesian Computation (ABC) to test different Pyrenean colonisation
246 scenarios using DIYABC 2.1.0 (Cornuet et al., 2010). First, we determined if variation in
247 population genetics within and between ecological units coincided to an ancient or a recent
248 Pyrenean colonisation. We followed the same methodology as described in (Ursenbacher et
249 al., 2015) and generated $3 \cdot 10^6$ simulations to compare the posterior probabilities (logistic
250 regression) of three scenarios (Fig. 2) including: a simultaneous split among the four
251 ecological units (scenario 1), an altitudinal progressive colonisation (scenario 2) and a
252 simultaneous split in the Pyrenees regions differing from the rear edge (scenario 3). We
253 restricted the number of tested scenarios by ordering ecological units based on their altitudinal
254 class (Fig. 2). However, we did not test for scenarios with higher proximity between the rear
255 and the leading edge compared to others units since it has no biological meaning. Once the
256 most likely scenario was identified, we further ran $4 \cdot 10^6$ iterations to estimate the parameters
257 (effective population size: N , and divergence time: t). We defined $N1 < N2 < N3 > N4$ and $t3$
258 $> t2 > t1$ as conditional priors (Fig. 2) and we considered the mean number of alleles, the
259 mean gene diversity, the mean size variance, and, between two samples, the F_{ST} , the mean
260 index of classification, the shared allele distance, and the $(d\mu)^2$ distance as summary statistics.
261 We checked the performance of each ABC analysis (scenario comparison and parameters
262 estimation) by simulating 500 pseudo-observed datasets, comparing their posterior
263 distribution, and compiling relative error (Cornuet et al., 2010). We used a generation time of
264 3 years based on the equation $T = \alpha + [s/1(1-s)]$ (Lande et al., 2003) where α is the age at
265 maturity and s is the annual survival rate. Survival and age at maturity are likely to vary along
266 altitude (Heulin et al., 1997), so we considered adult survival rate of 0.51 (low and

267 intermediate altitudes) or 0.32 (high altitudes) and a recruitment in reproduction at one year
268 old (low and intermediate altitudes) or 2 years old (high altitudes).

269 **Results**

270 Genetic diversity

271 Analyses of molecular variance showed that genetic diversity varied mostly within individuals
272 (86.8%), but a substantial and significant part of variation was also explained within
273 populations (4.1%) and between populations (7.4%) followed by a minor variation between
274 ecological units (1.7%, see Table S5). Within population diversity and population
275 differentiation varied among ecological units (A_r : $F_{3,37} = 3.7$, $p = 0.020$; F_{IS} : $F_{3,37} = 4.6$, $p =$
276 0.008 ; F_{ST} : $F_{3,37} = 4.2$, $p = 0.012$). A_r was the highest in the admixture zone and F_{IS} were
277 highest in the rear edge and decreased along altitudinal clines (Fig. 3a & 3b). F_{ST} was higher
278 in both the rear and leading edge but the lowest in the admixture zone (Fig. 3c). We did not
279 find any evidence for a recent bottleneck ($p > 0.216$), except in one population (BTM, $p =$
280 0.002).

281 Genetic structure

282 Both the Bayesian spatial modelling of genetic population structure and the ordination method
283 gathered populations into $K = 6$ clusters (Table S6 & Fig. S2). Clustering was relatively
284 heterogeneous between and within ecological units, and rather dependent on population
285 locality (Fig. 1). Yet, structuration (i.e., the probability for an individual to be affiliated to a
286 cluster) differed significantly between ecological units ($F_{3,38} = 3.5$, $p = 0.024$) since there was
287 a lower structuration in the admixture zone compared to the 3 other ecological units (pairwise
288 comparisons, all $p < 0.032$).

289 Genetic isolation

290 Isolation by distance (IBD) was highly significant globally ($p = 0.001$, Fig. 4a). Locally
291 however, IBD differed among ecological units and according to isolation level. IBD was non-
292 significant at both the rear edge ($p = 0.276$, Fig. 4b) and the admixture zone ($p = 0.320$, Fig.
293 4c), where all pairwise geographic distances were below 60 km. Instead, significant IBD was
294 found in both the continuous range ($p = 0.006$, Fig. 4d) and the leading edge ($p = 0.003$, Fig.
295 4e) where many pairwise geographic distances were above 60 km.

296 Effect of past and present environmental conditions on demography and genetic diversity

297 Spatial variation in lizard abundance was correlated solely to air temperature (Table 1, Fig.
298 5a), with the most scattered populations associated to warm environments. Ar was the lowest
299 under warm and dry climates, and in open habitats (Table 1, Fig. 5b). Variation in both F_{IS}
300 and F_{ST} were better explained by additive constraints in temperature and forest cover (Table
301 1, Fig. 5c & 5d). That is, inbreeding and differentiation is higher in warm and open habitats
302 (Table 1).

303 Pyrenean colonisation scenarios

304 The most likely scenario was the colonization scenario 2 with a posterior probability of $0.86 \pm$
305 0.04 (Fig. 2). We compared logistic distribution in 500 simulated datasets to estimate a global
306 posterior predictive error of 0.17, a type I error (probability of rejecting scenario 2 when it is
307 the correct scenario) of 0.33 and a type II error (probability of choosing scenario 2 when it is
308 an incorrect scenario) of 0.22. Effective population size considerably varied between
309 ecological units ranging from 1,500 in the leading edge to 9,000 reproductive individuals in
310 the continuous range (Table 2). The split between the rear edge and the other populations
311 occurred 750 generations ago, thereby placing the start of the Pyrenean ascension to around
312 2,000 years ago (Table 2). The divergence in the other ecological units occurred very recently

313 in the last millennium, with evidence of high elevation colonization between 100 and 700
314 years before present (Table 2).

315

316 **Discussion**

317 Genetic diversity was relatively high amongst lizards or across populations in the
318 geographic area and reasonably follows the theoretical predictions of the rear-leading edges
319 model of population genetic diversity and structure (Hampe et al., 2013; Hampe & Petit,
320 2005). That is, populations at the rear edge experience strong levels of inbreeding (loss of
321 heterozygosity). Associated with this, pairwise genetic distances were not correlated to
322 geographic distances neither within the rear edge nor in the admixture zone, probably due to
323 recently interrupted gene flow. From the continuous range to the leading edge, populations are
324 mostly isolated by distance, which suggests continuous distance-limited gene flow over time.
325 However, lower allelic richness and high differentiation in the leading edge populations imply
326 that they likely originated via small groups of immigrants through founder events (Hampe et
327 al., 2013; Nadeau & Urban, 2019; Waters et al., 2013). Lower inbreeding levels in these
328 colonized areas might for instance reflect a purge of inbred individuals during recruitment
329 (Hampe et al., 2013), since this species can optimise mate choice for that purpose (Richard et
330 al., 2009).

331 Our results provide new insights on genetic diversity and structuring of the subclade
332 B2 of *Zootoca vivipara*. Concomitantly with the geographic distribution of six genetic units
333 highlighted here, previous studies have shown that the strong genetic structuration of the
334 clade B is strongly shaped by mountain barriers and phylogeography (Horreo, Breedveld, et
335 al., 2019; Horreo, Peláez, et al., 2019; Milá et al., 2013). Genetic introgression between
336 southern Pyrenees (in Spain) and northern Pyrenees (in France) subclades is also common

337 among high altitude populations following secondary contacts associated with range shifts in
338 warmer climates (Horreo, Breedveld, et al., 2019; Milá et al., 2013). This pattern of genetic
339 introgression in the high elevation mountain range may explain why a population such as
340 ARA sampled at high altitude and near a contact zone (Table S1) may have its own genetic
341 structure. In our study, we further found that the highest allelic richness and lowest
342 differentiation or structuration was found in the admixture zone, as this area may represent a
343 mixing zone between genetic lineages of the rear edge populations and those from the
344 continuous range (Hampe & Petit, 2005). Yet, mixing between populations arises despite
345 obvious isolation and interrupted gene flow. Therefore, we hypothesize this specific pattern in
346 the admixture zone to reflect ancient (structuration) versus recent (isolation) events caused by
347 climate conditions and habitat fragmentation (Päckert et al., 2019). Surprisingly however, we
348 found little evidence for bottlenecks (only one population likely due to a local isolation by a
349 road), contrary to Horreo, Peláez, et al., (2019) who documented recent bottlenecks and
350 significant heterozygote deficiencies in most clade B populations. This difference can be
351 caused by methodological bias [n = 11 loci in the present study, n = 28 loci in (Horreo,
352 Peláez, et al., 2019)] and a resulting lack of statistical power to detect recent bottlenecks.
353 Further investigations including more microsatellites will help to better track recent genetic
354 changes in the studied area.

355 A complementary study of the clade B populations further suggested that recent global
356 warming may explain low migration rates and recent bottlenecks (Horreo, Peláez, et al.,
357 2019). In support of a scenario of range contraction and expansion together with genetic
358 structuring shaped by global changes, we showed here that climate conditions and forest
359 cover explained additively variation in lizard demography and population genetics.
360 Specifically, warmer temperatures in lowland populations were associated with lower lizard
361 abundance and higher levels of inbreeding and genetic differentiation. Therefore, in addition

362 to relatively low effective population size, populations at the hot margin of the distribution
363 may be at greater risk of extinction (Cornetti et al., 2015; Templeton et al., 2001). Indeed,
364 comparative studies in contemporary populations of the viviparous form of this species have
365 shown that heat and drought represent important causal determinants of physiological stress,
366 reduction of behavioural activity and eventually population collapse (Bestion et al., 2015;
367 Dupoué et al., 2018, 2020; Dupoué, Rutschmann, Le Galliard, Clobert, et al., 2017; Massot et
368 al., 2008). Furthermore, forest cover systematically exhibited an additive influence on within-
369 population diversity, inbreeding, and genetic differentiation. That is, contemporary
370 populations located in the most open habitats exhibit higher inbreeding under warm climates
371 (low altitude) and experience poorer allelic richness and higher differentiation under cold
372 climates (high altitude). Although this species typically occupies open habitats (meadows,
373 peat-bogs, heathlands) to perform daily activities, mixed habitats with proximity to dense
374 forests might represent more intact habitat landscapes. In addition, lizards in populations next
375 to or mixed with forest patches are better capable of adjusting their breeding phenology
376 (Rutschmann et al., 2016) and their thermoregulation behaviour (Rozen-Rechels et al., in
377 press). Mixed forest habitats might therefore allow lizards to buffer negative consequences of
378 extreme weather events. Our population genetics study thus suggests that this cold-adapted
379 ectotherm may maintain small populations, especially at low altitudes, depending on
380 proximity to forest cover, possibly serving as ‘thermal refuges’ during resting periods. Hence,
381 the preservation of these habitats might represent a key conservation action to protect this
382 lizard and the associated cortege of species (Jofré et al., 2016). There is now a critical need
383 for developing genetically informed ecological niche models to better predict future species
384 distributions under cumulative constraints of climate change and landscape structuration
385 (Carvalho et al., 2019; Ikeda et al., 2017; Razgour et al., 2019).

386 Our results also revealed that the altitudinal colonisation of the Pyrenees started over
387 the past two millennia (< 2 kya), much more recently than we expected (~10 kya following
388 the LGM). Splits were particularly recent between the foothill and upward populations (~400
389 years), and between highland populations (~100 years), thus confirming a rapid and recent
390 range expansion. Errors in scenario selection and parameters estimations were relatively low
391 and in the range of acceptable error to be confident in observed results (Cornuet et al., 2010).
392 The most likely and parsimonious scenario for such recent ascension relates to the combined
393 effects of habitat restructuration and recent climate change. Following the LGM, the present
394 continuous range and leading edges were likely impassable for such a heliothermic lizard
395 given that above 1,000 m, the Pyrenees were at the time entirely covered by dense pine forest
396 (Davasse et al., 1996). As unravelled by palynology, this area was mostly deforested during
397 the Middle Ages to develop pastoralism (Galop et al., 2003; Zanon et al., 2018), which
398 probably opened new habitats and a new climbing path for this species. Additionally, climate
399 conditions in the Pyrenees likely became suitable only after the Little Ice Age when the
400 mountain glaciers retreated starting since approximately 1850. Our findings are consistent
401 with similar insights related to the recent genetic differentiation (~200 years) in the same
402 geographic area of the Pyrenean Desman (*Galemys pyrenaicus*), an endemic semiaquatic
403 mammal affiliated to rivers and streams (Gillet et al., 2017).

404 Retracing population history of wild species is important if we are going to correctly
405 interpret current distributions and foresee future impacts of global warming on demographic
406 trends. In short, this study provides a rare example supporting general theoretical predictions
407 stemming from the rear-leading edge conceptual framework (Hampe et al., 2013; Hampe &
408 Petit, 2005; Nadeau & Urban, 2019; Razgour et al., 2013; Vilà-Cabrera et al., 2019; Waters et
409 al., 2013). Rear edge populations showed the alarming lowest genetic diversity and highest
410 inbreeding, interrupted gene flows and lower population size, which put them at greater risk

411 of extinction. Ground-dwelling ectotherms usually display relatively low dispersal rates
412 (Stevens et al., 2014), yet our study challenged this generality and suggested fast colonisation
413 capabilities in the common lizard likely facilitated by past habitat connectivity. Together, our
414 results therefore illustrate the importance of integrating genetic diversity, differentiation and
415 colonisation capabilities to accurately characterise and anticipate the potential footprints of
416 global change on wild species and ecosystems.

417 **References**

- 418 Barton, K. (2019). *MuMIn: Multi-Model Inference*. R Package Version 1.43.6. [https://cran.r-](https://cran.r-project.org/package=MuMIn)
419 [project.org/package=MuMIn](https://cran.r-project.org/package=MuMIn)
- 420 Beebee, T. J. C. (2008). Buccal swabbing as a source of DNA from squamate reptiles.
421 *Conservation Genetics*, 9(4), 1087–1088. <https://doi.org/10.1007/s10592-007-9464-2>
- 422 Berroneau, M. (2014). Lézard vivipare *Zootoca vivipara* (Lichtenstein, 1823). In C. Nature
423 (Ed.), *Atlas des amphibiens et reptiles d'Aquitaine* (pp. 106–109). Association Cistude
424 Nature.
- 425 Berthier, L., Bardy, M., Chenu, J.-P., Guzmova, L., Laroche, B., Lehmann, S., Lemercier, B.,
426 Martin, M., Mérot, P., Squividant, H., Thiry, E., & Walter, C. (2014). *Enveloppes des*
427 *milieux potentiellement humides de la France métropolitaine - notice*
428 *d'accompagnement. Programme de modélisation des milieux potentiellement humides de*
429 *France*. Ministère d'Ecologie, du Développement Durable et de l'Energie.
- 430 Bestion, E., Teyssier, A., Richard, M., Clobert, J., & Cote, J. (2015). Live fast, die young:
431 Experimental evidence of population extinction risk due to climate change. *PLoS*
432 *Biology*, 13(10), e1002281. <https://doi.org/10.1371/journal.pbio.1002281>
- 433 Buckley, L. B., Hurlbert, A. H., & Jetz, W. (2012). Broad-scale ecological implications of

434 ectothermy and endothermy in changing environments. *Global Ecology and*
435 *Biogeography*, 21(9), 873–885. <https://doi.org/10.1111/j.1466-8238.2011.00737.x>

436 Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a*
437 *practical information-theoretic approach, 2nd edn.* Springer.

438 Carvalho, S. B., Torres, J., Tarroso, P., & Velo-Antón, G. (2019). Genes on the edge: A
439 framework to detect genetic diversity imperiled by climate change. *Global Change*
440 *Biology*, 25(12), 4034–4047. <https://doi.org/10.1111/gcb.14740>

441 Cavalli-Sforza, L. L., & Edwards, A. W. F. (1967). Phylogenetic analysis models and
442 estimation procedures. *American Journal of Human Genetics*, 19(012), 233–257.

443 Ceballos, G., Ehrlich, P. R., & Dirzo, R. (2017). Biological annihilation via the ongoing sixth
444 mass extinction signaled by vertebrate population losses and declines. *Proceedings of the*
445 *National Academy of Sciences of the United States of America*, 114(30), E6089–E6096.
446 <https://doi.org/10.1073/pnas.1704949114>

447 Cornetti, L., Ficetola, G. F., Hoban, S., & Vernesi, C. (2015). Genetic and ecological data
448 reveal species boundaries between viviparous and oviparous lizard lineages. *Heredity*,
449 115(6), 517–526. <https://doi.org/10.1038/hdy.2015.54>

450 Cornuet, J. M., Ravigné, V., & Estoup, A. (2010). Inference on population history and model
451 checking using DNA sequence and microsatellite data with the software DIYABC
452 (v1.0). *BMC Bioinformatics*, 11, 401. <https://doi.org/10.1186/1471-2105-11-401>

453 Davasse, B., Galop, D., & Rendu, C. (1996). Paysages du Néolithique à nos jours dans les
454 Pyrénées de l'est d'après l'écologie historique et l'archéologie pastorale. *XVIIe*
455 *Rencontres Internationales d'archéologie et d'histoire d'Antibes*, 577–599.

456 Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G.,

457 Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E.,
458 Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013).
459 Collinearity: A review of methods to deal with it and a simulation study evaluating their
460 performance. *Ecography*, *36*(1), 027–046. [https://doi.org/10.1111/j.1600-](https://doi.org/10.1111/j.1600-0587.2012.07348.x)
461 [0587.2012.07348.x](https://doi.org/10.1111/j.1600-0587.2012.07348.x)

462 Dupoué, A., Blaimont, P., Rozen-Rechels, D., Richard, M., Meylan, S., Clobert, J., Miles, D.
463 B., Martin, R., Decencière, B., Agostini, S., & Le Galliard, J.-F. (2020). Water
464 availability and temperature induce changes in oxidative status during pregnancy in a
465 viviparous lizard. *Functional Ecology*, *34*(2), 475–485. [https://doi.org/10.1111/1365-](https://doi.org/10.1111/1365-2435.13481)
466 [2435.13481](https://doi.org/10.1111/1365-2435.13481)

467 Dupoué, A., Rutschmann, A., Le Galliard, J.-F., Clobert, J., Angelier, F., Marciau, C., Ruault,
468 S., Miles, D. B., & Meylan, S. (2017). Shorter telomeres precede population extinction in
469 wild lizards. *Scientific Reports*, *7*, 16976. <https://doi.org/10.1038/s41598-017-17323-z>

470 Dupoué, A., Rutschmann, A., Le Galliard, J.-F., Clobert, J., Blaimont, P., Sinervo, B., Miles,
471 D. B., Haussy, C., & Meylan, S. (2018). Reduction of baseline corticosterone secretion
472 correlates with climate warming and drying across wild lizard populations. *Journal of*
473 *Animal Ecology*, *87*(5), 1331–1341. <https://doi.org/10.1111/1365-2656.12843>

474 Dupoué, A., Rutschmann, A., Le Galliard, J.-F., Miles, D. B., Clobert, J., DeNardo, D. F.,
475 Bruschi, G. A., & Meylan, S. (2017). Water availability and environmental temperature
476 correlate with geographic variation in water balance in common lizards. *Oecologia*, *185*,
477 561–571. <https://doi.org/10.1007/s00442-017-3973-6>

478 Earl, D. A., & VonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and
479 program for visualizing STRUCTURE output and implementing the Evanno method.
480 *Conservation Genetics Resources*, *4*(2), 359–361. <https://doi.org/10.1007/s12686-011->

481 9548-7

482 Eckert, C. G., Samis, K. E., & Loughheed, S. C. (2008). Genetic variation across species'
483 geographical ranges: The central-marginal hypothesis and beyond. *Molecular Ecology*,
484 *17*(5), 1170–1188. <https://doi.org/10.1111/j.1365-294X.2007.03659.x>

485 Frankham, R. (2010). Inbreeding in the wild really does matter. *Heredity*, *104*(2), 124–124.
486 <https://doi.org/10.1038/hdy.2009.155>

487 Galop, D., Mazier, F., López Sáez, J. A., & Vanni re, B. (2003). Palynologie et histoire des
488 activit s humaines en milieu montagnard. Bilan provisoire des recherches et nouvelles
489 orientations m thodologiques sur le versant nord des Pyr n es. *Arch ologie Du Midi*
490 *M di val*, *21*(1), 159–170. <https://doi.org/10.3406/amime.2003.1407>

491 Gillet, F., Cabria Garrido, M. T., Blanc, F., Fournier-Chambrillon, C., N moz, M., Sourp, E.,
492 Vial-Novella, C., Zardoya, R., Aulagnier, S., & Michaux, J. R. (2017). Evidence of fine-
493 scale genetic structure for the endangered Pyrenean desman (*Galemys pyrenaicus*) in the
494 French Pyrenees. *Journal of Mammalogy*, *98*(2), 523–532.
495 <https://doi.org/10.1093/jmammal/gyx002>

496 Hampe, A., Pemonge, M. H., & Petit, R. J. (2013). Efficient mitigation of founder effects
497 during the establishment of a leading-edge oak population. *Proceedings of the Royal*
498 *Society B: Biological Sciences*, *280*(1764), 20131070.
499 <https://doi.org/10.1098/rspb.2013.1070>

500 Hampe, A., & Petit, R. J. (2005). Conserving biodiversity under climate change: the rear edge
501 matters. *Ecology Letters*, *8*(5), 461–467. [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2005.00739.x)
502 [0248.2005.00739.x](https://doi.org/10.1111/j.1461-0248.2005.00739.x)

503 Heulin, B., Osenegg-Leconte, K., & Michel, D. (1997). Demography of a bimodal

504 reproductive species of lizard (*Lacerta vivipara*): survival and density characteristics of
505 oviparous populations. *Herpetologica*, 53(4), 432–444.

506 Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405(6789), 907–
507 913. <https://doi.org/10.1038/35016000>

508 Horreo, J. L., Breedveld, M. C., Lindtke, D., Heulin, B., Surget-Groba, Y., & Fitze, P. S.
509 (2019). Genetic introgression among differentiated clades is lower among clades
510 exhibiting different parity modes. *Heredity*, 123(2), 264–272.
511 <https://doi.org/10.1038/s41437-019-0201-7>

512 Horreo, J. L., Peláez, M. L., Breedveld, M. C., Suárez, T., Urieta, M., & Fitze, P. S. (2019).
513 Population structure of the oviparous South-West European common lizard. *European*
514 *Journal of Wildlife Research*, 65(1), 11. <https://doi.org/10.1007/s10344-018-1242-6>

515 Horreo, J. L., Pelaez, M. L., Suárez, T., Breedveld, M. C., Heulin, B., Surget-Groba, Y.,
516 Oksanen, T. A., & Fitze, P. S. (2018). Phylogeography, evolutionary history and effects
517 of glaciations in a species (*Zootoca vivipara*) inhabiting multiple biogeographic regions.
518 *Journal of Biogeography*, 45(7), 1616–1627. <https://doi.org/10.1111/jbi.13349>

519 Ikeda, D. H., Max, T. L., Allan, G. J., Lau, M. K., Shuster, S. M., & Whitham, T. G. (2017).
520 Genetically informed ecological niche models improve climate change predictions.
521 *Global Change Biology*, 23(1), 164–176. <https://doi.org/10.1111/gcb.13470>

522 Jofré, G. M., Warn, M. R., & Reading, C. J. (2016). The role of managed coniferous forest in
523 the conservation of reptiles. *Forest Ecology and Management*, 362, 69–78.
524 <https://doi.org/10.1016/j.foreco.2015.11.044>

525 Jombart, T. (2008). adegenet: A R package for the multivariate analysis of genetic markers.
526 *Bioinformatics*, 24(11), 1403–1405. <https://doi.org/10.1093/bioinformatics/btn129>

527 Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal
528 components: a new method for the analysis of genetically structured populations. *BMC*
529 *Genetics*, *11*, 94. <https://doi.org/10.1186/1471-2156-11-94>

530 Jones, O. R., & Wang, J. (2010). COLONY: A program for parentage and sibship inference
531 from multilocus genotype data. *Molecular Ecology Resources*, *10*(3), 551–555.
532 <https://doi.org/10.1111/j.1755-0998.2009.02787.x>

533 Kamvar, Z. N., Brooks, J. C., & Grünwald, N. J. (2015). Novel R tools for analysis of
534 genome-wide population genetic data with emphasis on clonality. *Frontiers in Genetics*,
535 *6*(JUN), 208. <https://doi.org/10.3389/fgene.2015.00208>

536 Karger, D. N., Conrad, O., Böhrer, J., Kawohl, T., Kreft, H., Soria-Auza, R. W.,
537 Zimmermann, N. E., Linder, H. P., & Kessler, M. (2017). Climatologies at high
538 resolution for the earth's land surface areas. *Scientific Data*, *4*, 170122.
539 <https://doi.org/10.1038/sdata.2017.122>

540 Karger, D. N., Dabaghchian, B., Lange, S., Thuiller, W., Zimmermann, N. E., & Graham, C.
541 H. (2020). High resolution climate data for Europe. In *EnviDat* (Version 1.0).
542 <https://doi.org/doi:10.16904/envidat.150>

543 Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). DiveRcity:
544 An R package for the estimation and exploration of population genetics parameters and
545 their associated errors. *Methods in Ecology and Evolution*, *4*(8), 782–788.
546 <https://doi.org/10.1111/2041-210X.12067>

547 Lande, R., Engen, S., & Saether, B. E. (2003). *Stochastic population dynamics in ecology and*
548 *conservation*. Oxford University Press.

549 Le Galliard, J.-F., Massot, M., Baron, J. P., & Clobert, J. (2012). Ecological effects of climate

550 change on European reptiles. In J. Brodie, E. Post, & D. Doak (Eds.), *Wildlife*
551 *conservation in a changing climate* (pp. 179–203). University of Chicago Press.

552 Lenoir, J., Bertrand, R., Comte, L., Bourgeaud, L., Hattab, T., Murienne, J., & Grenouillet, G.
553 (2020). Species better track the shifting isotherms in the oceans than on lands. *Nature*
554 *Ecology and Evolution*, 4, 1044–1059. <https://doi.org/10.1038/s41559-020-1198-2>

555 Lorenzon, P., Clobert, J., Oppliger, A., & John-Alder, H. (1999). Effect of water constraint on
556 growth rate , activity and body temperature of yearling common lizard (*Lacerta*
557 *vivipara*). *Oecologia*, 118(4), 423–430. <https://doi.org/10.1007/s004420050744>

558 Lourdais, O., Dupoué, A., Guillon, M., Guiller, G., Michaud, B., & DeNardo, D. F. (2017).
559 Hydric “costs” of reproduction: pregnancy increases evaporative water loss in the snake
560 *Vipera aspis*. *Physiological and Biochemical Zoology*, 90(6), 663–672.
561 <https://doi.org/10.1086/694848>

562 Massot, M., Clobert, J., & Ferrière, R. (2008). Climate warming, dispersal inhibition and
563 extinction risk. *Global Change Biology*, 14(3), 461–469. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2486.2007.01514.x)
564 [2486.2007.01514.x](https://doi.org/10.1111/j.1365-2486.2007.01514.x)

565 Milá, B., Surget-Groba, Y., Heulin, B., Gosá, A., & Fitze, P. S. (2013). Multilocus
566 phylogeography of the common lizard *Zootoca vivipara* at the Ibero-Pyrenean suture
567 zone reveals lowland barriers and high-elevation introgression. *BMC Evolutionary*
568 *Biology*, 13(1), 192. <https://doi.org/10.1186/1471-2148-13-192>

569 Nadeau, C. P., & Urban, M. C. (2019). Eco-evolution on the edge during climate change.
570 *Ecography*, 42(7), 1280–1297. <https://doi.org/10.1111/ecog.04404>

571 Päckert, M., Ait Belkacem, A., Wolfgramm, H., Gast, O., Canal, D., Giacalone, G., Lo Valvo,
572 M., Vamberger, M., Wink, M., Martens, J., & Stuckas, H. (2019). Genetic admixture

573 despite ecological segregation in a North African sparrow hybrid zone (Aves,
574 Passeriformes, *Passer domesticus* × *Passer hispaniolensis*). *Ecology and Evolution*,
575 9(22), 12710–12726. <https://doi.org/10.1002/ece3.5744>

576 Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I.-C., Clark, T.
577 D., Colwell, R. K., Danielsen, F., Evengård, B., Falconi, L., Ferrier, S., Frusher, S.,
578 Garcia, R. A., Griffis, R. B., Hobday, A. J., Janion-Scheepers, C., Jarzyna, M. A.,
579 Jennings, S., ... Williams, S. E. (2017). Biodiversity redistribution under climate change:
580 Impacts on ecosystems and human well-being. *Science*, 355(6332), eaai9214.
581 <https://doi.org/10.1126/science.aai9214>

582 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R, C. T. (2016). *nlme: Linear and*
583 *Nonlinear Mixed Effects Models* (R package version 3.1-128). [http://cran.r-](http://cran.r-project.org/package=nlme)
584 [project.org/package=nlme](http://cran.r-project.org/package=nlme)

585 Pironon, S., Papuga, G., Villellas, J., Angert, A. L., García, M. B., & Thompson, J. D. (2017).
586 Geographic variation in genetic and demographic performance: new insights from an old
587 biogeographical paradigm. *Biological Reviews*, 92(4), 1877–1909.
588 <https://doi.org/10.1111/brv.12313>

589 Piry, S., Luikart, G., & Cornuet, J.-M. (1999). BOTTLENECK: A computer program for
590 detecting recent reductions in the effective population size using allele frequency data.
591 *Journal of Heredity*, 90(4), 502–503. <https://doi.org/10.1093/jhered/90.4.502>

592 Plotnick, R. E., Smith, F. A., & Lyons, S. K. (2016). The fossil record of the sixth extinction.
593 *Ecology Letters*, 19(5), 546–553. <https://doi.org/10.1111/ele.12589>

594 Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using
595 multilocus genotype data. *Genetics*, 155(2), 945–959.

596 Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J., Ibáñez, C., Puechmaille, S. J.,
597 Novella-Fernandez, R., Alberdi, A., & Manel, S. (2019). Considering adaptive genetic
598 variation in climate change vulnerability assessment reduces species range loss
599 projections. *Proceedings of the National Academy of Sciences of the United States of*
600 *America*, *116*(21), 10418–10423. <https://doi.org/10.1073/pnas.1820663116>

601 Razgour, O., Juste, J., Ibáñez, C., Kiefer, A., Rebelo, H., Puechmaille, S. J., Arlettaz, R.,
602 Burke, T., Dawson, D. A., Beaumont, M., & Jones, G. (2013). The shaping of genetic
603 variation in edge-of-range populations under past and future climate change. *Ecology*
604 *Letters*, *16*(10), 1258–1266. <https://doi.org/10.1111/ele.12158>

605 Richard, M., Losdat, S., Lecomte, J., De Fraipont, M., & Clobert, J. (2009). Optimal level of
606 inbreeding in the common lizard. *Proceedings of the Royal Society B: Biological*
607 *Sciences*, *276*(1668), 2779–2786. <https://doi.org/10.1098/rspb.2009.0319>

608 Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the
609 success of colonising populations? *Trends in Ecology & Evolution*, *29*(4), 233–242.
610 <https://doi.org/10.1016/j.tree.2014.02.003>

611 Rodríguez-Ramilo, S. T., & Wang, J. (2012). The effect of close relatives on unsupervised
612 Bayesian clustering algorithms in population genetic structure analysis. *Molecular*
613 *Ecology Resources*, *12*(5), 873–884. <https://doi.org/10.1111/j.1755-0998.2012.03156.x>

614 Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the GENEPOP
615 software for Windows and Linux. *Molecular Ecology Resources*, *8*(1), 103–106.
616 <https://doi.org/10.1111/j.1471-8286.2007.01931.x>

617 Rozen-Rechels, D., Dupoué, A., Lourdais, O., Chamaillé-Jammes, S., Meylan, S., Clobert, J.,
618 & Le Galliard, J.-F. (2019). When water interacts with temperature: Ecological and
619 evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. *Ecology*

620 *and Evolution*, 9(17), 10029–10043. <https://doi.org/10.1002/ece3.5440>

621 Rozen-Rechels, D., Rutschmann, A., Dupoué, A., Blaimont, P., Chauveau, V., Miles, D. B.,
622 Guillon, M., Richard, M., Badiane, A., Meylan, S., Clobert, J., & Le Galliard, J. (n.d.).
623 Interaction of hydric and thermal conditions drive geographic variation in
624 thermoregulation in a widespread lizard. *Ecological Monographs*.

625 Rutschmann, A., Miles, D. B., Le Galliard, J.-F., Richard, M., Moulherat, S., Sinervo, B., &
626 Clobert, J. (2016). Climate and habitat interact to shape the thermal reaction norms of
627 breeding phenology across lizard populations. *Journal of Animal Ecology*, 85(2), 457–
628 466. <https://doi.org/10.1111/1365-2656.12473>

629 Rutschmann, A., Rozen-Rechels, D., Dupoué, A., Blaimont, P., de Villemereuil, P., Miles, D.
630 B., Richard, M., & Clobert, J. (2020). Climate dependent heating efficiency in the
631 common lizard. *Ecology and Evolution*, 10(15), 8007–8017.
632 <https://doi.org/10.1002/ece3.6241>

633 Sinervo, B., Mendez-de-la-Cruz, F., Miles, D. B., Heulin, B., Bastiaans, E., Villagrán-Santa
634 Cruz, M., Lara-Resendiz, R., Martínez-Mendez, N., Calderón-Espinosa, M. L., Meza-
635 Lázaro, R. N., Gadsden, H., Avila, L. J., Morando, M., De la Riva, I. J., Victoriano
636 Sepulveda, P., Rocha, C. F. D., Ibarguengoytía, N. R., Aguilar Puntriano, C., Massot,
637 M., ... Sites, J. W. (2010). Erosion of lizard diversity by climate change and altered
638 thermal niches. *Science*, 328, 894–899. <https://doi.org/10.1126/science.1184695>

639 Stevens, V. M., Whitmee, S., Le Galliard, J.-F., Clobert, J., Böhning-Gaese, K., Bonte, D.,
640 Brändle, M., Matthias Dehling, D., Hof, C., Trochet, A., & Baguette, M. (2014). A
641 comparative analysis of dispersal syndromes in terrestrial and semi-terrestrial animals.
642 *Ecology Letters*, 17(8), 1039–1052. <https://doi.org/10.1111/ele.12303>

643 Sunday, J. M., Bates, A. E., Kearney, M. R., Colwell, R. K., Dulvy, N. K., Longino, J. T., &

644 Huey, R. B. (2014). Thermal-safety margins and the necessity of thermoregulatory
645 behavior across latitude and elevation. *Proceedings of the National Academy of Sciences*
646 *of the United States of America*, *111*(15), 5610–5615.
647 <https://doi.org/10.1073/pnas.1316145111>

648 Surget-Groba, Y., Heulin, B., Guillaume, C.-P., Puky, M., Semenov, D., Orlova, V.,
649 Kupriyanova, L., Ghira, I., & Smajda, B. (2006). Multiple origins of viviparity, or
650 reversal from viviparity to oviparity? The European common lizard (*Zootoca vivipara*,
651 Lacertidae) and the evolution of parity. *Biological Journal of the Linnean Society*,
652 *87*(1996), 1–11. <https://doi.org/10.1111/j.1095-8312.2006.00552.x>

653 Takezaki, N., & Nei, M. (1996). Genetic distances and reconstruction of phylogenetic trees
654 from microsatellite DNA. *Genetics*, *144*(October), 389–399.

655 Templeton, A. R., Robertson, R. J., Brisson, J., & Strasburg, J. (2001). Disrupting
656 evolutionary processes: The effect of habitat fragmentation on collared lizards in the
657 Missouri Ozarks. *Proceedings of the National Academy of Sciences of the United States*
658 *of America*, *98*(10), 5426–5432. <https://doi.org/10.1073/pnas.091093098>

659 Tobler, U., Garner, T. W. J., & Schmidt, B. R. (2013). Genetic attributes of midwife toad
660 (*Alytes obstetricans*) populations do not correlate with degree of species decline. *Ecology*
661 *and Evolution*, *3*(9), 2806–2819. <https://doi.org/10.1002/ece3.677>

662 Ursenbacher, S., Guillon, M., Cubizolle, H., Dupoué, A., Blouin-Demers, G., & Lourdais, O.
663 (2015). Postglacial recolonization in a cold climate specialist in western Europe: patterns
664 of genetic diversity in the adder (*Vipera berus*) support the central–marginal hypothesis.
665 *Molecular Ecology*, *24*(14), 3639–3651. <https://doi.org/10.1111/mec.13259>

666 VanDerWal, J., Murphy, H. T., Kutt, A. S., Perkins, G. C., Bateman, B. L., Perry, J. J., &
667 Reside, A. E. (2013). Focus on poleward shifts in species’ distribution underestimates

668 the fingerprint of climate change. *Nature Climate Change*, 3(3), 239–243.
669 <https://doi.org/10.1038/nclimate1688>

670 Vilà-Cabrera, A., Premoli, A. C., & Jump, A. S. (2019). Refining predictions of population
671 decline at species' rear edges. *Global Change Biology*, 25(5), 1549–1560.
672 <https://doi.org/10.1111/gcb.14597>

673 Voituron, Y., Mouquet, N., De Mazancourt, C., & Clobert, J. (2002). To freeze or not to
674 freeze ? An evolutionary perspective on the cold hardiness strategies of overwintering
675 ectotherms. *The American Naturalist*, 160(2), 255–270. <https://doi.org/10.1086/341021>

676 Waters, J. M., Fraser, C. I., & Hewitt, G. M. (2013). Founder takes all: Density-dependent
677 processes structure biodiversity. *Trends in Ecology & Evolution*, 28(2), 78–85.
678 <https://doi.org/10.1016/j.tree.2012.08.024>

679 Wiens, J. J. (2016). Climate-related local extinctions are already widespread among plant and
680 animal species. *PLoS Biology*, 14(12), e2001104.
681 <https://doi.org/10.1371/journal.pbio.2001104>

682 Zanon, M., Davis, B. A. S., Marquer, L., Brewer, S., & Kaplan, J. O. (2018). European forest
683 cover during the past 12,000 years: A palynological reconstruction based on modern
684 analogs and remote sensing. *Frontiers in Plant Science*, 9(March), 253.
685 <https://doi.org/10.3389/fpls.2018.00253>

686

687

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703 **Ethics**

704 The collection of samples was approved by the "Conseil Scientifique Régional du Patrimoine
705 Naturel (CSRPN)" of the region Occitanie. Procedures complied with current French
706 regulations permit (#2017-s-02) relating to an authorization of capture, marking, transport,
707 detention, use and release of protected lizard species.

708 **Author contributions**

709 AD and AT conceived the study together with JC and FA. AD, MR, MS, JTQ, CV, CR,
710 MauB, MatB, OL and PB captured lizards. MR and MS performed molecular assays. AD, AT

711 and MG performed the data analyses. RB and MG participated in environmental data
712 extraction. MauB, MatB and GP initially identified lizard populations sampled in this study.
713 AT, RB, OC, OG, HLC, JS, JFLG, JC, and FA were involved in funding and planning this
714 work. AD led the sampling design and draft the manuscript. All authors commented the
715 article and gave final approval for publication.

716 **Biosketch**

717 Andréaz Dupoué is interested in depicting which are the proximate mechanisms driving
718 population extirpation. His main researches at the interface between molecular ecology and
719 ecological physiology emphasize the importance of water based trade-offs in the evolution of
720 wild squamate reptiles.

721 The research team lead multi-specific studies on Pyrenean and European ectotherms.

722

723 **Data accessibility**

724 The data supporting this article are available at the Zenodo Repository at

725 <http://doi.org/10.5281/zenodo.4266021>

726 **Table 1. Model selection comparing the impacts of climate (temperature and precipitations) and habitat quality (forest cover and**
727 **wetland potential) on lizard demography (abundance) and genetic profile [allelic richness (A_r), inbreeding (F_{IS}) and differentiation**
728 **(F_{ST})]. For each population parameter, the table reports the $\Delta AICc$ based linear mixed model comparison and full averages of fixed covariates.**
729 Ecological unit was set as a random term in all models to account for spatial interdependence.

Abundance	model	df	logLik	AICc	$\Delta AICc$	w_i	term	estimate	SE	z value	Pr(> z)
	temperature	4	-73.9	156.87	0	0.45	(Intercept)	-0.005	0.254	0.02	0.986
	forest + temperature	5	-73.73	159.12	2.25	0.15	temperature	-0.476	0.096	4.80	0.000 ***
	temperature + wetland	5	-73.79	159.25	2.38	0.14	forest	-0.002	0.004	0.54	0.593
	precipitation + temperature	5	-73.89	159.44	2.57	0.12	wetland	0.134	0.290	0.45	0.655
	forest + temperature + wetland	6	-73.65	161.71	4.84	0.04	precipitations	0.056	0.309	0.18	0.860
	forest + precipitation + temperature	6	-73.72	161.83	4.96	0.04					
	precipitation + temperature + wetland	6	-73.75	161.9	5.02	0.04					
	forest + precipitation + temperature + wetland	7	-73.62	164.53	7.66	0.01					
	(Null)	3	-79.21	165.06	8.19	0.01					
	precipitation	4	-78.75	166.59	9.72	0					
	wetland	4	-78.96	167	10.13	0					
	forest	4	-79.03	167.14	10.27	0					
	precipitation + wetland	5	-78.35	168.36	11.49	0					
	forest + precipitation	5	-78.71	169.09	12.22	0					
	forest + wetland	5	-78.74	169.16	12.28	0					

	model	df	logLik	AICc	Δ AICc	w_i	term	estimate	SE	z value	Pr(> z)
	forest + precipitation + wetland	6	-78.32	171.03	14.16	0					
Ar	forest + precipitation + temperature + wetland	7	-12.03	41.45	0	0.38	(Intercept)	-0.009	0.057	0.16	0.875
	forest + precipitation + temperature	6	-13.54	41.55	0.1	0.36	temperature	0.117	0.026	4.27	0.000 ***
	precipitation + temperature + wetland	6	-14.65	43.76	2.31	0.12	precipitations	0.237	0.077	2.97	0.003 **
	precipitation + temperature	5	-16.11	43.93	2.48	0.11	forest	0.002	0.001	2.13	0.033 *
	forest + temperature + wetland	6	-16.5	47.48	6.03	0.02	wetland	-0.122	0.073	1.61	0.108
	temperature + wetland	5	-18.63	48.97	7.52	0.01					
	forest + temperature	5	-19.2	50.11	8.66	0					
	temperature	4	-21.24	51.6	10.15	0					
	forest + precipitation	5	-20.84	53.39	11.94	0					
	forest + precipitation + wetland	6	-19.77	54.01	12.56	0					
	wetland	4	-22.59	54.29	12.83	0					
	forest + wetland	5	-21.39	54.49	13.04	0					
	precipitation	4	-22.85	54.82	13.37	0					
	forest	4	-22.93	54.97	13.52	0					
	(Null)	3	-24.23	55.12	13.67	0					
	precipitation + wetland	5	-21.79	55.29	13.84	0					
F_{1S}	forest + temperature	5	44.26	-76.8	0	0.44	(Intercept)	0.008	0.014	0.52	0.606

forest + temperature + wetland	6	44.86	-75.24	1.55	0.2	
forest + precipitation + temperature	6	44.76	-75.05	1.75	0.18	
forest + precipitation + temperature + wetland	7	45.14	-72.89	3.91	0.06	
forest + precipitation	5	41.59	-71.47	5.32	0.03	
forest	4	39.92	-70.72	6.08	0.02	
forest + wetland	5	41.22	-70.72	6.08	0.02	
forest + precipitation + wetland	6	42.28	-70.09	6.71	0.02	
temperature	4	39.04	-68.98	7.82	0.01	
temperature + wetland	5	39.48	-67.25	9.55	0	
precipitation + temperature	5	39.32	-66.93	9.87	0	
(Null)	3	36.42	-66.2	10.6	0	
precipitation	4	37.31	-65.51	11.29	0	
wetland	4	37.26	-65.41	11.39	0	
precipitation + temperature + wetland	6	39.7	-64.94	11.86	0	
precipitation + wetland	5	38.03	-64.34	12.46	0	
<i>F</i> _{ST}	model	df	logLik	AICc	ΔAICc	<i>w</i> _{<i>i</i>}
	forest + precipitation + temperature	6	98.41	-182.34	0	0.38
	forest + precipitation + temperature + wetland	7	98.89	-180.38	1.97	0.14
	forest + temperature	5	95.95	-180.19	2.16	0.13
	forest + temperature + wetland	6	97.01	-179.56	2.79	0.09

temperature	0.017	0.006	2.67	0.008	**
forest	-0.001	0.000	3.19	0.001	**
wetland	0.018	0.018	1.00	0.317	
precipitations	-0.019	0.019	0.94	0.346	
term	estimate	SE	z value	Pr(> z)	
(Intercept)	-0.0003	0.0043	0.07	0.947	
temperature	-0.0046	0.0018	2.53	0.012	*
precipitations	-0.0097	0.0051	1.83	0.068	
forest	-0.0002	0.0001	2.54	0.011	*

forest	4	93.95	-178.79	3.55	0.06	wetland	0.0051	0.0049	0.99	0.321
forest + precipitation	5	94.67	-177.63	4.71	0.04					
precipitation + temperature	5	94.65	-177.58	4.76	0.04					
forest + wetland	5	94.31	-176.91	5.44	0.03					
(Null)	3	91.43	-176.22	6.13	0.02					
temperature	4	92.62	-176.13	6.22	0.02					
precipitation + temperature + wetland	6	95.13	-175.79	6.55	0.01					
temperature + wetland	5	93.55	-175.39	6.96	0.01					
forest + precipitation + wetland	6	94.79	-175.11	7.23	0.01					
wetland	4	91.83	-174.55	7.79	0.01					
precipitation	4	91.69	-174.27	8.07	0.01					
precipitation + wetland	5	91.94	-172.16	10.18	0					

730

731 df: degree of freedom, logLik: log likelihood, Δ AICc: difference in corrected Akaike Information Criterion relative to model with lowest AICs.
732 Highlighted models (grey area) are based on Δ AICc < 2. Highlighted variables (bolded characters) are based on $\alpha < 0.05$.

733

734 **Table 2. Posterior estimations of demographic parameters (means and 95% CI)**
735 **following $4 \cdot 10^6$ simulations and splits designed under scenario 2 (see Fig. 2).** Table
736 reports the effective population size (N_e) of each ecological unit, and the generation time
737 before sampling associated with each out three divergence times (split of the rear edge: t1,
738 split of the admixture zone: t2, and separation of the leading edge from continuous range: t3;
739 see Fig. 2). For each parameter, we estimated estimation accuracy by generating 500 datasets
740 and compile the Relative Median Absolute Error (RMAE) based on their posterior
741 distributions. The corresponding number of years were assumed considering a generation time
742 of three years. See text for details.

743

Ecological units	N_e	95% CI	RMAE			
Rear edge (< 100 m)	6,100	2,170 – 10,030	0.169			
Admixture zone (100-500 m)	6,390	2,580 – 10,200	0.146			
Continuous range (500-1300 m)	8,730	2,710 – 14,750	0.104			
Leading Edge (> 1300 m)	1,600	1,014 – 2,186	0.171			
	Generations	95%CI	RMAE	Generation time	Years	95%CI
t1	37	21 – 53	0.262	2.5	92.5	52.5 – 132.5
t2	191	108 – 274	0.211	2.0	382	216 – 548
t3	748	536 – 960	0.120	2.0	1,496	1,072 – 1,920

744

745 **Figure captions**

746 **Fig. 1. Genetic structuration of 42 natural populations of the common lizard (*Z. v.***
747 ***louislantzi*) gathered into 4 ecological units associated with the recent shift in**
748 **distribution of the Western clade.** Individuals ($n = 599$) were clustered without *a priori* into
749 $K = 6$ clusters (one colour per K) following a Bayesian approach (top panel). We used the
750 maximal proportion of individuals affiliated to a cluster within a population (as illustrated by
751 the 42 pie charts) to represent an index of population structuration. Although structuration
752 depended on ecological units (mean \pm SE on the right panel), the different clusters depended
753 on geographic localisation rather than ecological units (bottom panels).

754

755 **Fig. 2. Three demographic scenarios of Pyrenean ascension in the common lizards.** For
756 each ecological unit, the total population size (N) was kept constant. Posterior estimates of
757 10^6 simulations for each scenario were compared using logistic regression and scenario 2 was
758 retained with the highest posterior probability (mean \pm 95% CI: 0.74 ± 0.06), compared to
759 scenario 1 (0.10 ± 0.04) and scenario 3 (0.16 ± 0.17). The selected scenario associates three
760 divergence times between ecological units across Pyrenean ascension (split of the rear edge:
761 t_1 , split of the admixture zone: t_2 , and separation of the leading edge from continuous range:
762 t_3).

763

764 **Fig. 3. Comparison of population genetics (means \pm SE) among ecological units and**
765 **associated predictions (grey polygons).** (a) Within-populations genetic diversity decreases
766 across altitude much like (b) inbreeding, while (c) genetic differentiation follow a non-linear
767 trend following our predictions.

768

769

770 **Fig. 4. Population connectivity tested by isolation by distance (IBD) both globally and**
771 **locally.** Significant relationships between genetic distance and geographic distance are
772 symbolized with line types (solid: significant, dashed: non-significant) together with a 2-
773 dimensional kernel density estimation to illustrate population continuity. (a) Globally, genetic
774 distances are associated with geographic distance and continuous, (b,c) IBD was non-
775 significant in isolated populations, and (d,e) IBD was significant but discontinuous in
776 connected populations.

777

778 **Fig. 5. Variation of demographic and genetic markers with climate conditions and**
779 **habitat characteristics.** The line and surfaces are drawn from predictions of the best linear
780 mixed models selected by the best conditional average (Table 1). The color gradient
781 highlights the values from low (blue) to high range (orange). a) Lizard abundance was
782 negatively correlated solely to air temperature, while b) within-population diversity, c)
783 inbreeding or d) population differentiation were influenced by additive effects of air
784 temperature and forest cover.



Rear edge
<100m

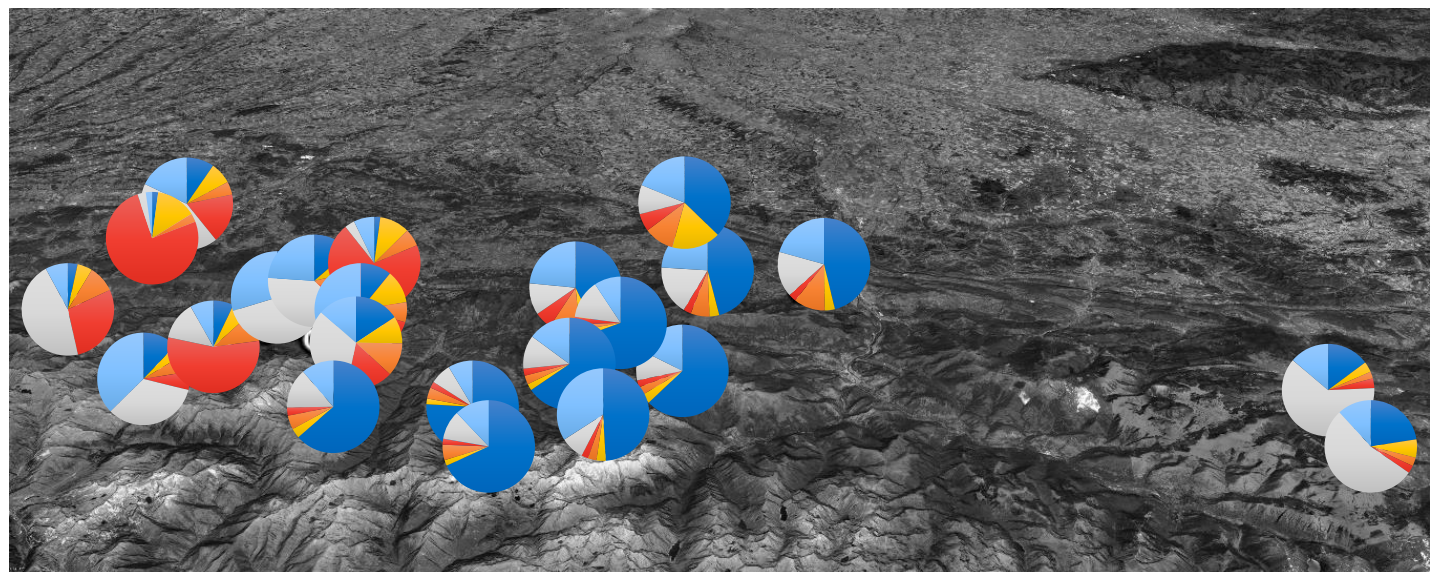
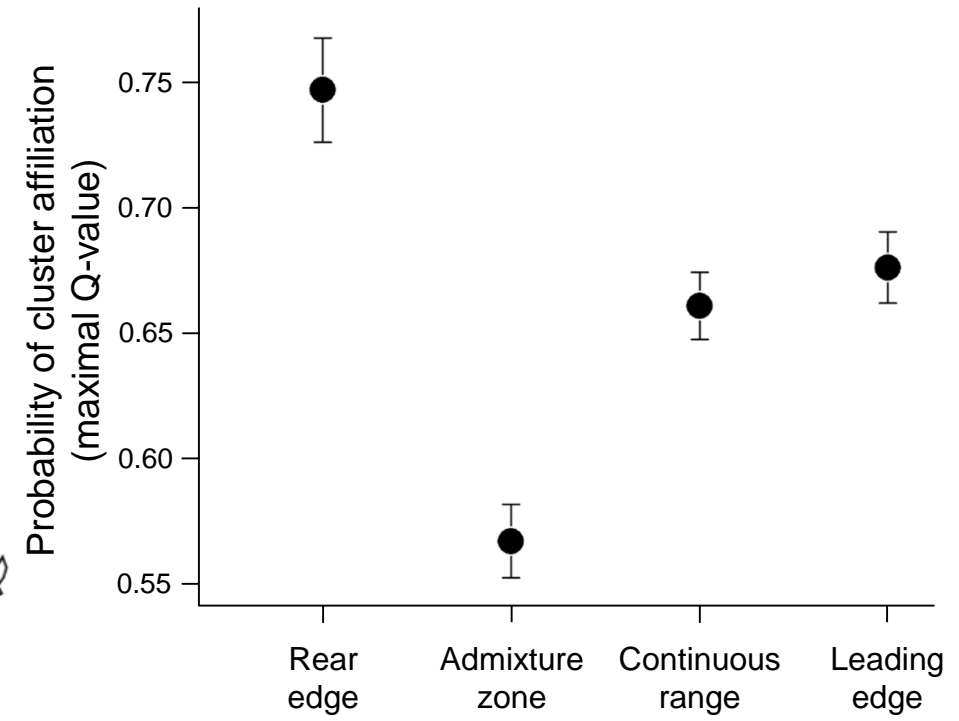
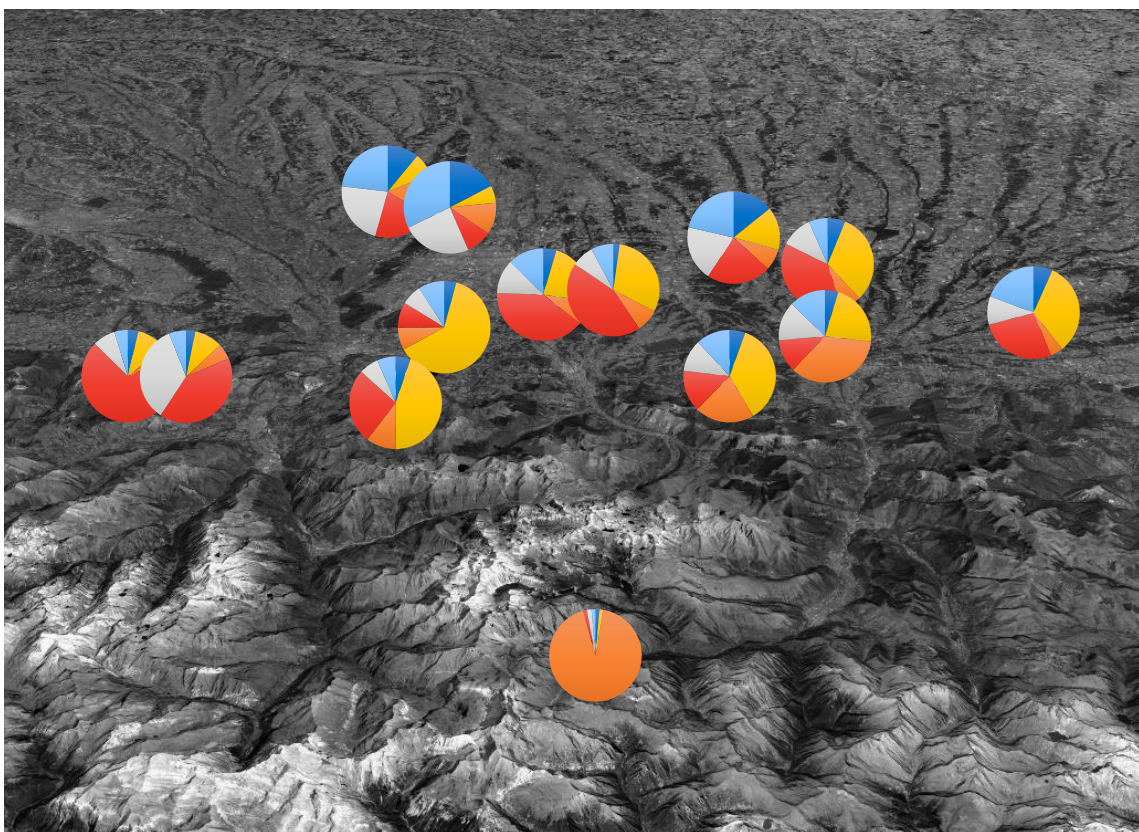
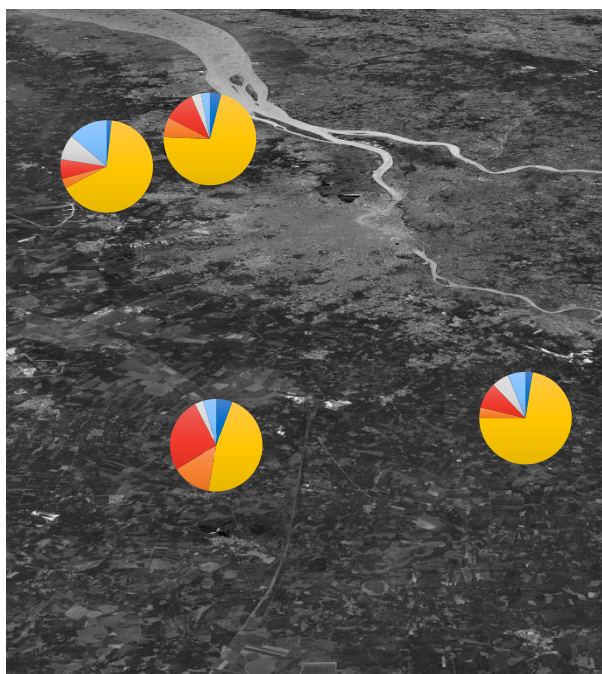
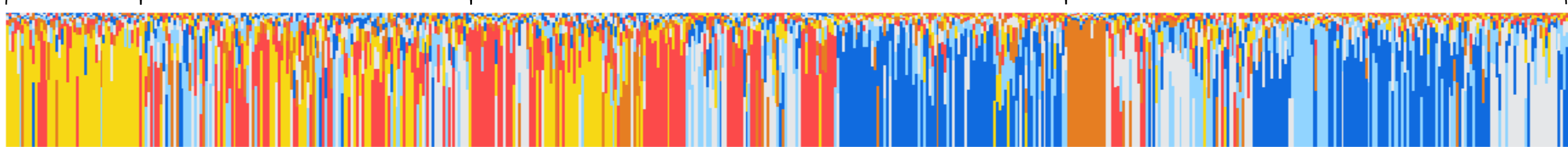
Admixture zone
100 - 500 m

Continuous range
500 - 1300 m

Leading edge
>1300 m

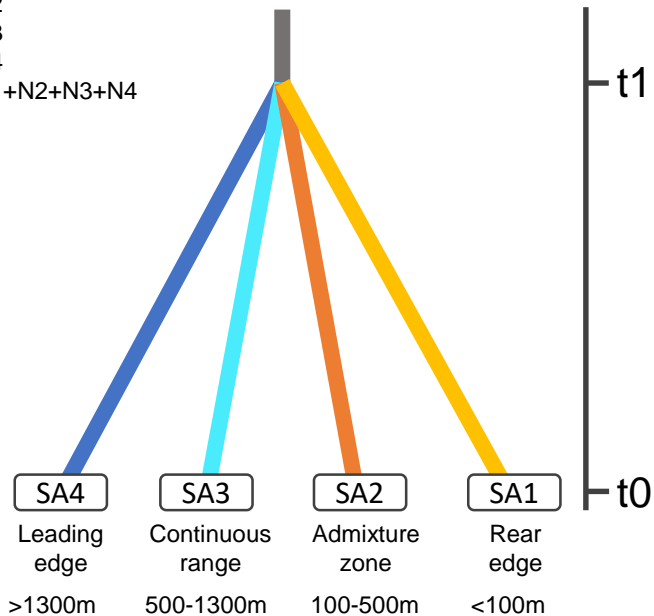
Q-value

1
0.8
0.6
0.4
0.2
0



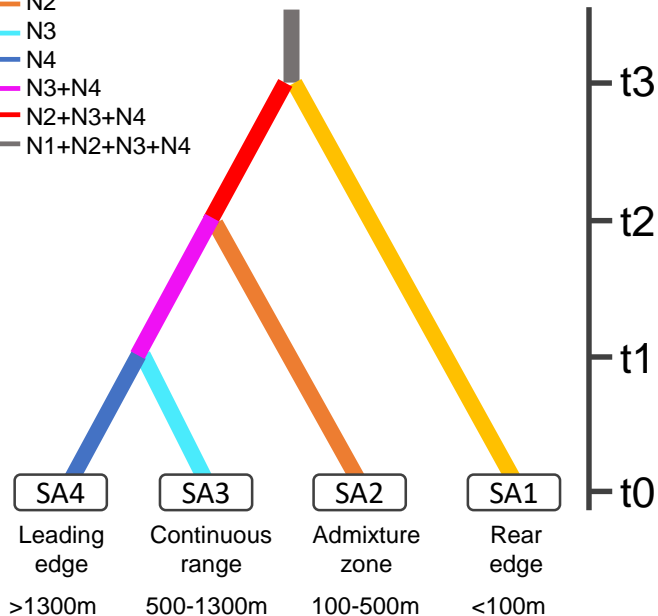
Scenario 1

— N1
 — N2
 — N3
 — N4
 — N1+N2+N3+N4



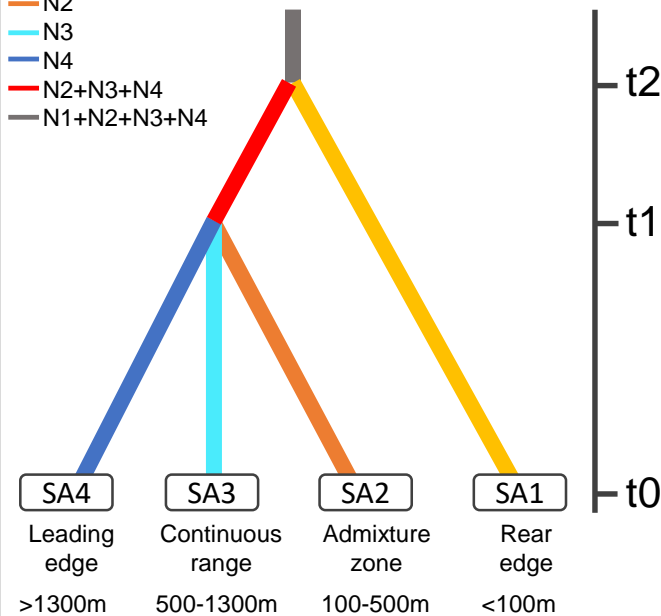
Scenario 2

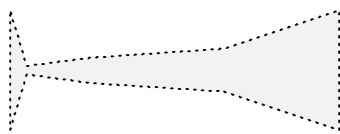
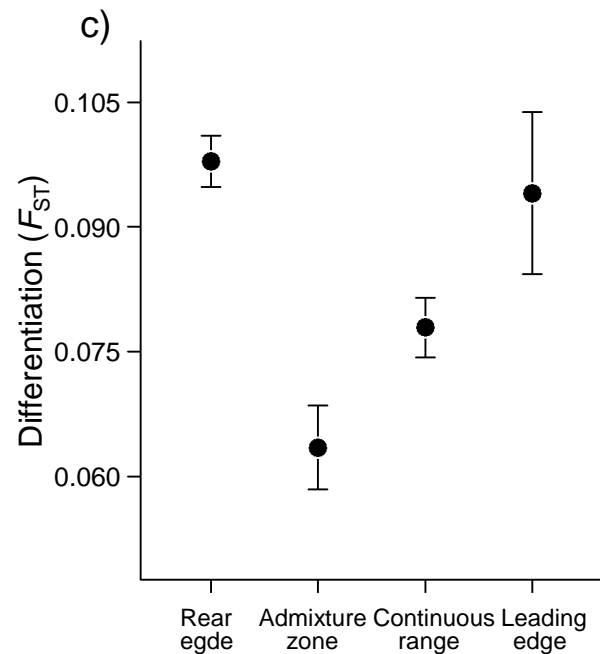
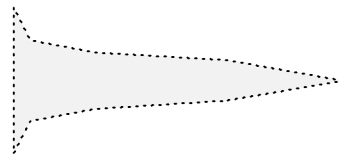
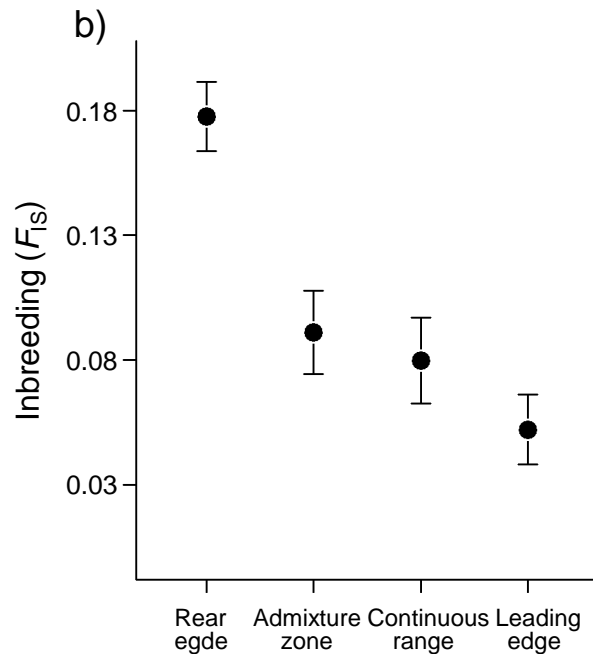
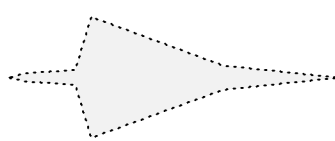
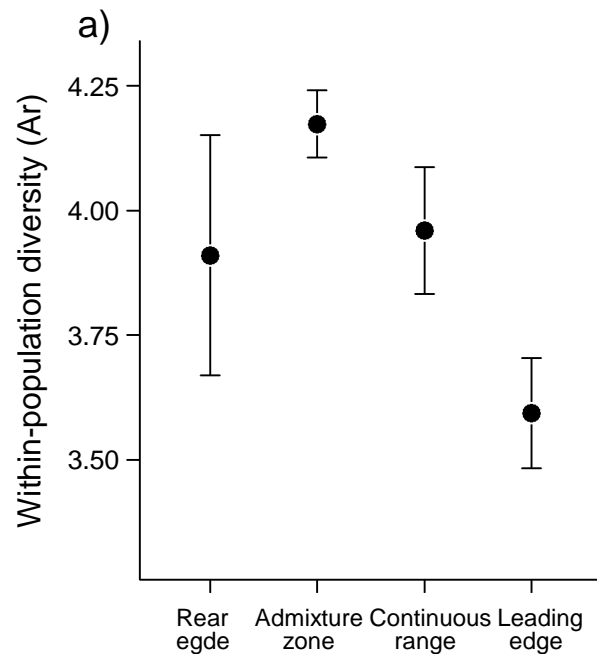
— N1
 — N2
 — N3
 — N4
 — N3+N4
 — N2+N3+N4
 — N1+N2+N3+N4



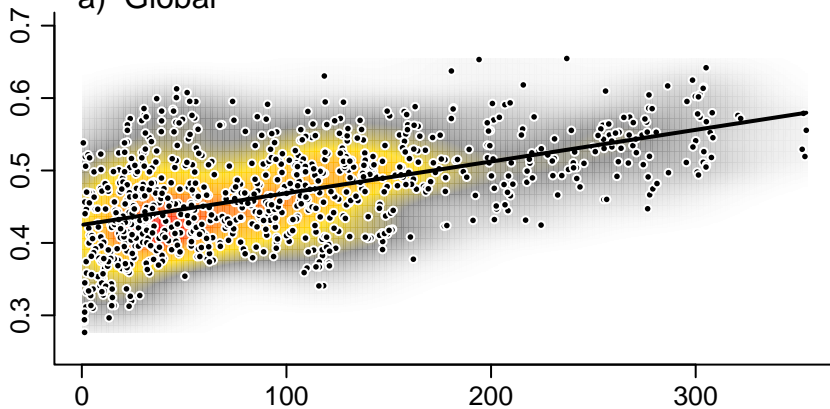
Scenario 3

— N1
 — N2
 — N3
 — N4
 — N2+N3+N4
 — N1+N2+N3+N4

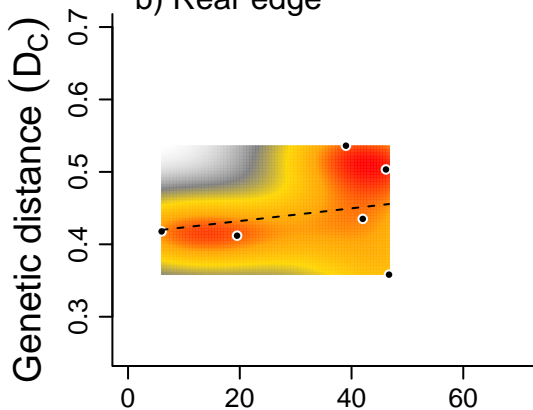




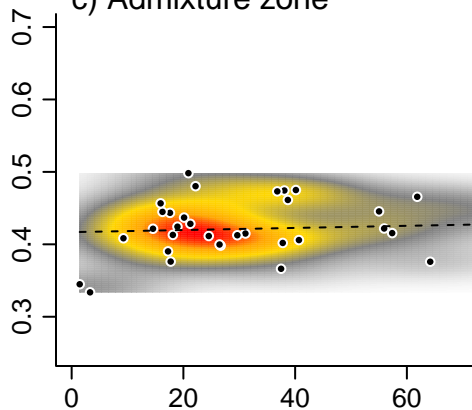
a) Global



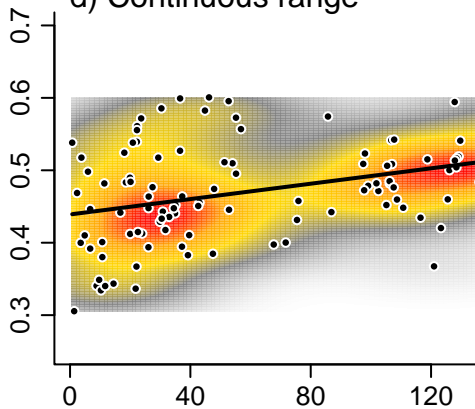
b) Rear edge



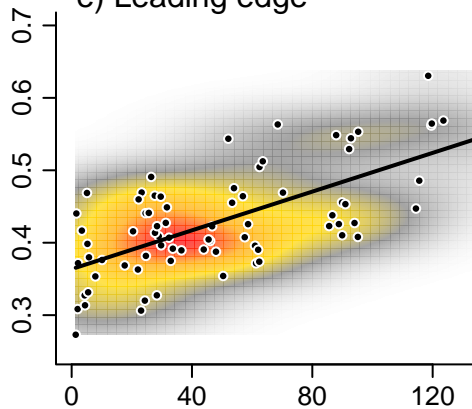
c) Admixture zone



d) Continuous range



e) Leading edge



Geographic distance (km)

