Little hope for the polyploid endemic Pyrenean Larkspur (*Delphinium montanum*): evidences from population genomics and Ecological Niche Modelling

Pascaline Salvado[1], Pere Aymerich Boixader[2], Josep Parera[3], Albert Vila Bonfill[4], Maria Martin[3], Céline Quelennec[3], Jean-Marc Lewin[5], Valérie Hinoux[1,5] & Joris A. M. Bertrand[1]

[1] Laboratoire Génome et Développement des Plantes (LGDP, UMR 5096 UPVD/CNRS), Université de Perpignan Via Domitia, France.

[2] C. Barcelona, 29. 08600 Berga, Spain

[3] Fédération des Réserves Naturelles Catalanes, Prades, France

[4] Parc Natural de Capçaleres del Ter i del Freser, Quelralbs, Spain

[5] Association Charles Flahault, Toulouges, France

Corresponding authors: Joris Bertrand (joris.bertrand@univ-perp.fr) and Valérie Hinoux (valerie.hinoux@univ-perp.fr)

Word count: 6 466

Abstract

Species endemic to restricted geographical ranges represent a particular conservation issue, be it for their heritage interest. In a context of global change, this is particularly the case for plants which belong to high-mountain ecosystems and, because of their ecological requirements, are doomed to survive or disappear on their 'sky islands'. The Pyrenean Larkspur (*Delphinium montanum*, Ranunculaceae) is endemic to the Eastern part of the Pyrenees (France and Spain). It is now only observable at a dozen of localities and some populations show signs of decline, such as a recurrent lack of flowering. Implementing population genomic approach (e.g. RAD-seq like) is particularly useful to understand genomic patterns of diversity and differentiation in order to provide recommendations in term of conservation. However, it remains challenging for species such as *D. montanum* that are autotetraploid with a large genome size (1C-value > 10 pg) as most methods currently available were developed for diploid species. A Bayesian framework able to call genotypes with uncertainty allowed us to assess genetic diversity and population structure in this system. Our results show evidence for inbreeding (mean G_{IS} = 0.361) within all the populations and substantial population structure (mean G_{ST} = 0.403) at the metapopulation level. In addition to a lack of connectivity between populations, spatial projections of Ecological Niche Modelling analyses under different climatic scenarios predict a dramatic decrease of suitable habitat for *D. montanum* in the future. Based on these results, we discuss the relevance and feasibility of different conservation measures.

Introduction

Extinction risk associated to climate change is expected not only to increase but to accelerate for every degree rise in global temperatures (Urban, 2015). In this context, the number of threatened species by reduced population sizes, habitat degradation and habitat fragmentation is increasing. Small and fragmented populations are particularly prone to be affected by drift and inbreeding depression and generally display decreased levels of genetic diversity (e.g. Bergl et al. 2008; Dixo et al. 2009). The increase of anthropogenic and environmental pressures, both being intimately bound, represents an unprecedented threat for vulnerable species. In particular, populations from species of high-mountain ecosystems might not be able to move upward rapidly enough to keep within their thermal tolerances and might be doomed on their 'sky islands' (see McCormack et al. 2009, e.g. Kidane et al. 2019, De Gabriel Hernando et al. 2021). Thus, species endemic to specific mountain ranges represent a major conservation issue, if only for their heritage interest. It is critical to characterize the distribution of genetic diversity within- and between- their populations to evaluate the inbreeding risk and study population structure to better understand how isolated they really are, in order to undertake effective conservation actions (Calevo et al. 2021).

The endangered Pyrenean larkspur *Delphinium montanum* DC., 1815 perfectly illustrates this situation. This plant (Family Ranunculaceae), endemic to the Eastern part of the Pyrenees (France and Spain), is now only observable at a dozen of localities (two already got extinct over the 20th century) and its global population size was estimated to range between 7 800 to 10 300 individuals in total (Aymerich et al. 2020). It occurs between alpine and subalpine levels, between 1 600 and 2 400 m (Lopéz-Pujol et al. 2007), from Serra del Cadí-Pedraforca (Fig. 1, localities 4, 5, 8, and 9) up to the Puigmal massif (localities 1, 3 and 6) via Tosa d'Alp massif (locality 7). There, the distance between the most distant sites is about 60

62 km, and only one locality is located outside this area: Nohèdes (locality 2), in the Madres
63 massif. This latter locality is separated from the Puigmal massif populations by the pit of
64 Cerdagne-Conflent, which could have formed a historical barrier to gene flow. In terms of
65 population size, Cadí-Pedraforca is the most important with up to 70% of the global population
66 size (5 450-6 700 individuals). The sites of the Puigmal massif represent 25 to 30% of the global
67 population (2 200-2 700 individuals). Localities of Madres massif (2) and Tosa d'Alp (7) are
68 numerically low (less than 300, from which none are actually flowering and 35 individuals,
69 respectively).

70 *D. montanum* is endangered as a result of increasing levels of multiple threats and is
71 included in the IUCN (International Union for Conservation of Nature) red list of threatened
72 species, in the Vulnerable (VU) category. Some of its populations are located nearby touristic
73 sites (*i.e.* mountain resorts or hiking trails) and are negatively impacted by trampling (Simon
74 et al., 2001). Flowers and fruits predation by herbivores, particularly Pyrenean chamois/isards
75 (*Rupicapra pyrenaica pyrenaica*) was also shown to be responsible for the seed bank's
76 decrease in some populations (Simon et al. 2001, Aymerich 2003). Importantly, differences in
77 population dynamics are observable from one population to another. Some populations are
78 stable, whereas others show decrease in population size (4-Pedraforca) and/or absence of
79 flowering individuals since 2011 (2-Nohèdes). Two studies previously investigated population
80 genetics of *D. montanum* based on allozymes (Simon et al. 2001; Lopéz-Pujol et al. 2007). They
81 already suggested relatively low effective size as well as, evidence of inbreeding within-
82 populations and significant population structure.

83 The features of the genome of the species also make *Delphinium montanum*
84 particularly challenging from a technical and methodological point of view. *D. montanum* is an
85 autotetraploid species (Simon et al. 2001; Lopéz-Pujol et al. 2007). Our recent flow cytometry

86 estimates of 10.32 pg/1C for this species (*i.e.* about twice the 1C-value reported for several
87 other diploid *Delphinium* species) corroborates previous knowledge and confirms the
88 important genome size of *D. montanum* (Bertrand et al. unpublished data). Implementing a
89 population genomic approach on *D. montanum* thus requires genome complexity reduction
90 protocols such as Reduction site-associated DNA sequencing (or RAD-seq, Baird 2008).
91 However, the difficulty to infer genotype and allele frequencies on polyploid species with such
92 techniques as well as the restricted set of methods available to analyze such data (see
93 Meirmans and van Tienderen 2013; Dufresne et al. 2014; Meirmans et al. 2018) is likely to
94 explain the relatively low number of population genomics studies dealing with RAD-seq like
95 techniques on non-model polyploid organisms (Clevenger et al. 2015; van de Peer et al. 2017,
96 Ahmad 2021).

97 Out the handful of studies that have addressed population genomic questions on
98 polyploid organisms without reference genome, some have considered the genotypes as
99 diploid-like data, an assumption that allows to use classical variant calling tools with default
100 settings (e.g. Brandrud et al. 2017, see also Brandrud et al. 2019; 2020 and Záveská et al.
101 2019). Consensus allelic information may also be coded in the form of ambiguous sites at
102 heterozygous positions (e.g. Wagner et al. 2020). At last, a couple of methods are able to
103 incorporate ploidy-aware calling algorithms and use genotype likelihoods to infer tetraploid
104 genotypes (e.g. Záveská et al. 2019; Brandrud et al. 2020; Karbstein et al. 2020; Ahmad et al.
105 2021). In studies for which both kind of methods were used, the latter one generally outputted
106 a higher number of variable sites but signals were found to be overall congruent (Záveská et
107 al. 2019; Brandrud et al. 2020).

108 The recent studies of Záveská et al. (2019), Brandrud et al. (2020) and Ahmad et al.
109 (2021) used the RAD-seq loci from the catalog generated in the popular pipeline Stacks

110 (Catchen et al. 2011; Catchen et al. 2013) to generate a synthetic reference onto which the
111 raw reads are mapped back before to infer tetraploid genotypes with the approach
112 implemented in EBG (Empirical Bayes Genotyping in Polyploids, Blischak et al. 2018, see also
113 Blischak et al. 2016). As a putative alternative, Clark et al. (2019) proposed a package called
114 PolyRAD to call genotypes with uncertainty from polyploid data in an R environment. PolyRAD
115 can deal with multiple levels of ploidy and is able to use for example population structure,
116 linkage disequilibrium and/or self-fertilization as priors to estimate genotype probabilities. To
117 our best knowledge, this promising and convenient method has been rarely used to
118 implement a population genomic approach on natural populations of polyploid non-model
119 organisms.

120 In this study, we first aimed at reevaluating the conservation status of *Delphinium*
121 *montanum* following such population genomics approach. We used a protocol called
122 normalized Genotyping By Sequencing (nGBS) that provided us with thousands of genetic
123 markers (SNPs) from which we inferred patterns of diversity and differentiation with an
124 unprecedented resolution in order to quantify inbreeding and level of connectivity between
125 populations. We then used an Ecological Niche Modelling (ENM) approach to better
126 understand the bioclimatic features that constraint the current geographic distribution of *D.*
127 *montanum*. Based on several Global Climate Models (CGM), we also predicted the putative
128 evolution of the geographic distribution of the species in the future, at different time periods
129 (2011-2040, 2041-2070 and 2071-2100) and under different, more or less pessimistic but still
130 realistic scenarios of evolution of global warming (or RCPs for Representative Concentration
131 Pathways).

132

133 **Materials and Methods**

134 **Population sampling**

135 We sampled a total of 106 individuals from 9 localities representative of the geographic
136 distribution of *Delphinium montanum* (in the Eastern Pyrenees: 3 sites in France and 6 in
137 Spain) between July and October 2020 (Table 1, Fig. 1, see also Supplementary Appendix S1).
138 At each locality, we collected about 1-2 cm^2 of leaf tissues that were stored in 90% ethanol at
139 4°C until DNA extraction. *Delphinium montanum* is not legally protected in France but is
140 considered as vulnerable (VU) on the Red List of threatened species of IUCN France (UICN
141 France, FCBN, AFB, MNHN, 2018). It is legally protected in Catalonia (Decret 172/2008. Catàleg
142 de flora amenaçada de Catalunya) and Spain (Orden AAA/1771/2015. Modificación del
143 catálogo de especies amenazadas). In France, for all sampling done in natural reserves, special
144 authorizations were granted by the 'Direction Départementale des Territoires et de la Mer 66'
145 (DDTM 66). In Spain, we asked for and obtained special permits from the Servei de Fauna i
146 Flora de la Generalitat de Catalunya and Natural Parks (Cadí-Moixeró and Ter-Freser).

147

148 **Molecular procedures**

149 Genomic DNA extraction and genotyping were subcontracted to LGC Genomics GmbH (Berlin,
150 Germany). Genotyping-By-Sequencing (GBS) was performed following a specific protocol
151 called nGBS for 'normalised GBS'. This double digest Restriction site-Associated DNA seq (or
152 RAD-seq) like protocol relies on two restriction enzymes (PstI and ApeKI, in our case) to reduce
153 *D. montanum* genome complexity and includes a normalization step that aims at avoiding
154 repetitive regions. The resulting 106 individually barcoded libraries were sequenced in paired-
155 end mode (2 x 250 bp) on an Illumina NovaSeq 6000 with an expectation of a minimum
156 number of 1.5 million read pairs per sample.

157

158 **Genomic data processing**

159 We used Stacks v.2.41 (Catchen et al. 2011; 2013) to build loci from Illumina reads, *de novo*
160 (*i.e.* without aligning reads to a reference genome). Stacks consists of a wrapper of several
161 scripts that are usually run sequentially including: *process_radtags* to demultiplex and clean
162 reads, *denovo_map.pl* to build loci within individuals, create a catalog and match all samples
163 against it and *populations* to further filter the SNPs obtained at the population level and
164 compute basic population genetic statistics. We first optimised several key parameters of the
165 pipeline: -*m* (the minimum number of identical raw reads required to form a putative allele),
166 -*M* (the number of mismatches allowed between alleles to form a locus) and -*n* (the number
167 of mismatches allowed between loci during construction of the catalog) on a subset of 12
168 individuals representative of the whole data set (*i.e.* geographic origin and coverage). As
169 recommended by several authors, we varied *M* and *n* (fixing *M* = *n*) while keeping *m* = 3 (see
170 Paris et al. 2017; Rochette & Catchen 2017). The combination -*m* 3, -*M* 3 and -*n* 3 was found
171 to be the most suitable to maximize the number of SNPs, assembled and polymorphic loci in
172 our case and was used to run Stacks on the whole data set (see Supplementary Appendix S2).
173 As *D. montanum* is a tetraploid species, we also specified the option '-*X ustacks:*--
174 *max_locus_stacks* 5' (in the *ustacks* script's) to allow the number of alleles per site to reach
175 the number of 5 instead or 3 by default. However, as the *populations* script is unable to deal
176 with polyploid genotypes, we followed a different approach to call variants and complete
177 genomic data processing.

178 We then used polyRAD (Clark et al. 2019), a method implemented in an R-package to
179 call genotypes with uncertainty from sequencing data in polyploids. The Bayesian genotype
180 caller implemented in polyRAD is able to import read depth from the catalog and matches files
181 from Stacks through a specific function called polyRAD::read_stacks() to further estimate the

182 probability of each possible genotype for each individual and each locus, taking into account
183 features such as possible levels of ploidy and population structure as priors. In order to get
184 similar filtering parameters than with Stacks and following the polyRAD author's guidelines,
185 we used the following arguments *-min.ind.with.minor. alleles= 5*, *-min.ind.with.reads= 84* and
186 a ploidy of 4. We then plotted the distribution of the ratio of the individual over expected
187 heterozygosity H_{ind}/H_E. The peak of the distribution of H_{ind}/H_E by locus (0.2) was considered to
188 represent well-behaved markers from which inbreeding (*F*) was estimated at 0.73. From this
189 estimate of *F*, we then simulated the expected distribution of H_{ind}/H_E, if all markers were
190 behaving in a Mendelian fashion. Based on the distribution, we defined minimum and
191 maximum thresholds of 0 and 0.406 comprising 95% of the observations and filtered out
192 markers for which H_{ind}/H_E were outside this interval. We then tested overdispersion (*i.e.* in our
193 case, how much does read depth distribution deviate from what would be expected under
194 binomial distribution) and adjusted its parameter to 6 (from a range of 2 to 14). This
195 information was used to call genotypes while taking into account population genetic structure.
196 The most likely genotypes of the remaining 5 095 loci were then exported in several formats
197 such as genind objects or Structure (.str).
198
199 **Population genomic analyses**
200 To get an overview of the overall genetic diversity and differentiation among individuals and
201 populations, we first performed a Principal Component Analysis (PCA) based on a matrix of
202 106 individuals (as rows) and 5 095 SNPs (as columns) coded as a genind object with the R-
203 package 'adegenet' (Jombart 2008). We then used Genodive v.3.04 (Meirmans 2020) to
204 compute expected and observed heterozygosity (H_E and H_O, respectively) as well as the
205 number of alleles (*A*) and the number of private alleles was computed with the R-package

206 'poppr' (Kamvar et al. 2014). The deviation from panmixia was evaluated by computing G_{IS}
207 and the statistical significance of the obtained values was estimated based on 10 000
208 permutations, also in Genodive. Overall and pairwise genetic differentiation was also assessed
209 based on *G*-statistics (G_{ST}, Nei 1987) as implemented in Genodive in a similar manner. Pattern
210 of Isolation By Distance (IBD) was assessed by examining the relationship between linearized
211 G_{ST}s values (*i.e.* $\ln(G_{ST}/(1- G_{ST}))$ and ln-geographical distances (after Rousset 1997) and its
212 statistical significance was tested with a Mantel test between matrices of pairwise G_{ST} and
213 geographical distance.

214 To further investigate population structure and characterize putative
215 migration/admixture event, we used sNMF (Frichot et al. 2014) as implemented in the R-
216 package LEA (Frichot & François 2015) to estimate individual ancestry coefficients based on
217 sparse non-negative matrix factorization algorithms. sNMF is particularly suitable for our
218 genotype dataset as it can deal with polyploid data and is fast enough to be applied on
219 hundreds of individuals and thousands of markers with reasonable computation time. The
220 number of genetic clusters was varied from K = 1 to 10, and analyses were run with 10
221 replicates at each value of K.

222

223 **Ecological Niche Modelling**

224 In order to assess the future trends of spatial dynamics of *D. montanum* distribution in a
225 context of global change, we conducted Ecological Niche Modelling (ENM) analyses based on
226 known occurrences of the species. Based on 19 bioclimatic layers we downloaded from
227 CHELSA v.2.1 (Karger et al. 2017), we aimed to i) model current environmental niche and ii)
228 predict the spatial evolution of the niche in the future. The bioclimatic layers available in
229 CHELSA are similar to those available in WORLDCLIM (Fick et al. 2017) in a way that they

230 consist of downscaled Global Climate Models (GCM) output temperature and precipitation
231 estimates at a maximal resolution of 30 arc seconds (approximately 1 km). In the current
232 study, we used the most recent version of CHELSA as the methodology and bias correction it
233 implements have been shown to outperforms WORLDCLIM in mountainous environments
234 (Karger et al. 2017; Bobrowski et al. 2021). Current climate data correspond to time averaged
235 variables over the period 1981-2010.

236 Future climates correspond to estimates from phase 3b of the Inter-Sectoral Impact
237 Model Intercomparison Project (ISIMIP3b) based on output of phase 6 of the Coupled Model
238 Intercomparison Project (CMIP6). They consist of the output of five GCMs (GFDL-ESM4,
239 UKESM1-0-LL, MPI-ESM1-2-HR, IPSL-CM6A-LR and MRI-ESM2-0) and three climate scenario
240 specifiers (ssp126, ssp370, ssp585). Climate scenarios specifiers reflect different
241 Representative Concentration Pathways (RCPs) associated with different radiative forcing
242 values: 2.6, 7.0 and 8.5 W/m^2 that can be considered as a plausible range of greenhouse gas
243 concentrations that appear to be optimistic, very likely and pessimistic in term of climate
244 change, respectively. Annually averaged data were downloaded for three future time periods:
245 2011-2040 ("2035"), 2041-2070 ("2055") and 2071-2100 ("2085").

246 The ENM analyses were conducted thanks to the R-package ENMwizard v.0.3.7
247 (Heming et al. 2018, see also Gutiérrez et al. 2019; Bagley et al. 2020). We first spatially filtered
248 occurrences to keep only those that were at least 1 km away from each other using the R-
249 package 'spThin' (Aiello-Lammens et al. 2015). Due to the relatively small spatial scale of the
250 area under study, this resulted in considering a total of 9 out the 106 occurrences initially
251 available. We then examined raw values for the 19 bioclimatic variables extracted at each
252 occurrence point and noticed that the variable bio19 ("precipitation of coldest quarter")
253 displayed obviously aberrant (*i.e.* negative) values. This variable bio19 was thus discarded

254 from subsequent analyses. From the 18 remaining variables (bio1 to bio18), we selected the
255 less correlated ones (Person correlation coefficient < 0.75) thanks to the R-package 'caret'
256 (Kuhn 2019) and kept five variables: bio5 ("mean daily maximum air temperature of the
257 coldest month"), bio7 ("annual range of air temperature"), bio14 ("precipitation amount of
258 the driest month") and bio15 ("precipitation seasonality") for further analyses.

259 The calibration area for the models was created as a buffer of 1.5° around the
260 minimum convex polygon encompassing all occurrences. We used the maximum entropy
261 method (implemented in MaxEnt ver. 3.4.1, Phillips et al. 2006, 2017) to calibrate model and
262 evaluated model performance thanks to the package ENMeval (Muscarella et al. 2014) as
263 implemented in ENMwizard. We evaluated models using a geographic partition scheme of
264 type "block" and optimized two parameters of MaxEnt: the Regularization Multipliers (RM)
265 and the Feature Classes (FC). RM was varied from 0.5 to 4.5, incremented by 0.5 whereas a
266 suite of 15 FCs (L, for Linear, P, for Product, Q, for Quadratic and H for Hinge) or combination
267 of them were evaluated: L, P, Q, H, LP, LQ, LH, PQ, PH, QH, LPQ, LPH, LQH, PQH, LPQH, resulting
268 in a total of 135 models. Model selection was done by computing the corrected Akaike
269 Information Criterion ("LowAIC") and the Area Under the receiver operating characteristic
270 Curve ("AUC") in the function ENMWizard::calib_mdl_b(). The best selected model was then
271 spatially projected as current niche. For future predictions, a spatial projection of the
272 consensus of the five CGMs was performed for each time period and each RCPs. Area
273 calculations were carried out after applying a 10-percentile (x10ptp) and a Maximum training
274 sensitivity plus specificity (mtss) threshold rules.

275

276 ## Results

277 **Genetic diversity and differentiation**

278 We obtained a total of 159 million read pairs across the 106 individuals. We assembled a total
279 of 210 756 loci including 22 911 SNPs with Stacks. After filtering the data with polyRAD, we
280 finally obtained 32 728 loci including 5 095 SNPs.

281 The two first PCA axes (PC1 and PC2) represented 14.85% and 9.21% of the total
282 genetic variance, respectively (Fig. 2A). This analysis is consistent with limited amount of
283 within-population differentiation and a clear pattern of between-population differentiation.
284 Overall, populations are arranged according to geography, especially along PC1. In term of
285 genetic diversity, all populations display a significant deviation from panmixia: mean G_{IS} =
286 0.361, $p < 0.05$ and population G_{IS} values ranging from 0.315 to 0.426 (all $p < 0.05$). However,
287 G_{IS} values appear as similar from one population to another (this holds true for the number of
288 total alleles *A*, too, see Table 1). The number of private alleles *A*p is consistent with the relative
289 level of geographic isolation of the populations.

290 The degree of genetic differentiation was found to be important overall (mean G_{ST}
291 value = 0.403, $p < 0.05$) and pairwise (with values ranging from 0.156 to 0.527, Supplementary
292 Appendix S3). There is a clear and significant pattern of Isolation By Distance across the
293 dataset ($r = 0.601$, $p = 0.001$, Fig. 3) indicating that gene flow is limited in space at the scale of
294 the *D. montanum* geographic range. The sNMF analysis is also consistent with a strong
295 population structure arranged with geography. Cross-entropy was the lowest for K= 6 (Fig. 2B
296 and 2C). Each cluster corresponds to a sampling site with two exceptions. The individuals from
297 the locality of Orri de Baix (1) were grouped together with the close locality of Cambre d'Aze
298 (6), and the Western localities of the Serra del Cadí (Bastanist (8), Serra Pedregosa (5) and to
299 a lesser extent Pedraforca (4)) formed another group. Only the individuals of Pedraforca (4)
300 showed mixed ancestry coefficients.

301 **Suitable habitats for *D. montanum* and prediction of its spatial dynamics**

302 ENMeval analyses identified RM = 2 and a combination of Quadratic and Hinge feature (QH)
303 classes as the best-performing parameters for calibrating the final ENM following the
304 "LowAIC" and M=2 and a combination of Product and Hinge feature (PH) classes following the
305 "AUC" optimality criteria. The first model had the best corrected Akaike Information Criterion
306 (AICc) score (152.25) and an AICc weight of 0.33 whereas the second one had an AICc score of
307 174.63 and an AICc weight of $4.53.10^{-6}$. These two models had mean omission rates of 0.375
308 and 0.25 for the 10th percentile and the lowest presence training thresholds, respectively, as
309 well as a mean test AUC of 0.988. Values of these diagnostic metrics are provided for all
310 candidate models in Supplementary Appendix S4. Current suitable area for *D. montanum* was
311 found to be 2 670 and 2 365 km^2 following "LowAIC" and "AUC" selection model criteria,
312 respectively (the values were identical whatever the threshold method used). All future
313 consensus spatial projections predict a dramatic decrease of the suitable area (Table 2 and
314 Fig. 4). For RCPs 7.0 and 8.5, the suitable area would have completely disappeared for the time
315 period 2071-2100 (but not for RCP 2.6). We also notice some slightly different outcomes
316 depending on the selection model criteria with RCP 8.5 scenarios giving sometimes higher
317 values than RCP 7.0, and even RCP 2.6 (e.g. time period 2011-2040, "Low AIC" criterion).
318

319 ## Discussion

320 **Conservation genomics on a polyploid species with important genome size**

321 The use of RAD-seq like (nGBS) data with the method implemented in PolyRAD allowed us to
322 successfully address the challenge of conducting a population genomic approach on a plant
323 species that is both polyploid (autotetraploid) and has an important genome size (> 10 Gbp).
324 It confirms the convenience of the workflow we used, specifically calling genotypes in an R-
325 environment, as an alternative to the ones followed in the handful of studies recently

326 published on similar systems (e.g. Záveská et al. 2019; Brandrud et al. 2020; Karbstein et al.
327 2020; Ahmad et al. 2021). From the > 5 000 SNPs we could rely on, we were able to get an
328 unprecedented genomic picture of the diversity and the differentiation of the species in order
329 to propose appropriate conservation recommendations. The results we obtained at the
330 genome scale are overall consistent with previous knowledge on population genetics of *D.*
331 *montanum* (see Simon et al. 2001; López-Pujol 2007).
332
333 **A set of populations isolated on sky islands**
334 Our results confirm that populations of *D. montanum* can be seen as a set of island-like
335 dwelling systems separated from each other by a matrix of unsuitable habitats and that gene
336 flow has little to no chance to be efficiently maintained within this metapopulation. All
337 populations considered in this study were found to display deficit in heterozygotes ($G_{IS} > 0.315$,
338 $p < 0.05$). These results confirm previous findings of Simon et al. (2001) and López-Pujol et al.
339 (2007) and show that the autotetraploid status of the species does not impede relatively low
340 levels of heterozygosity ($H_O < 0.17$). Polyploidy is expected to be associated with increased
341 genetic variation, a characteristic that provides more diversity to adapt (especially to extreme
342 environments such as mountainous ones) and allows buffering effect against deleterious
343 mutations (see van de Peer et al. 2021 and references therein). From a conservation point of
344 view, polyploidy does not seem to be associated with increased risk of extinction even though
345 plant with big genome size may be more prone to be under threat (Vinogradov 2003; Pandit
346 et al. 2011). As two other closely related congeneric species of *Delphinium montanum*: *D.*
347 *dubium* and *D. oxysepalum*, that are endemic to the Alps and the Carpathians, respectively,
348 are also tetraploid (López-Pujol et al. 2007), we speculate that polyploidy may have allowed
349 to these species to adapt and survive into these mountainous environments. The open

350 question is whether current levels of genetic diversity may be sufficient or not to allow *D.*
351 *montanum* populations to overcome inbreeding depression and provide sufficient variation
352 for adaptation in a context of global change. It should be noted that levels of genetic diversity
353 are overall similar among the populations investigated and we could not associate obvious
354 signs of decline of some of them (e.g. Nohèdes, Pedraforca) to decreased levels in genetic
355 diversity or evidence for deviation from panmixia. This suggests that the evidences of possible
356 decrease in effective size may be too recent to be detectable at the genetic level (see Peery
357 et al. 2012). Unfortunately, the limited number of available methods for polyploids makes
358 effective size estimation and test for bottleneck events cumbersome to perform in such
359 systems.

360 The PCA, the clustering analyses, the F_{ST} values as well as the pattern of Isolation By
361 Distance, further confirm limited gene flow between populations and the existence of a
362 population structure that is consistent with geography. The most likely number of clusters
363 found in the sNMF analysis (*K*=6) globally distinguishes populations from the Serra del Cadí
364 (Bastanist (8), Serra Pedregosa (5) and Pedraforca (4)) from those of the Puigmal range (Orri
365 de Baix (1) and Cambre d'Aze (6)) plus the satellite populations of Vallter (3), Nohèdes (2),
366 Tosa d'Alp (7) and Torreta de Cadí (9) (which is the westernmost locality, a bit distant from
367 the others in the Serra del Cadí). Only the locality of Pedraforca (4) shows some ambiguity in
368 population assignment that could indicate a mixed origin. At *K*=2, the intermediate status of
369 Tosa d'Alp (7) (and to a lesser extent of Pedraforca (4)) are also in agreement with its
370 intermediate geographic position and may reflect an ancestral split between Northern and
371 Southern populations. Altogether, these results support that the insect pollinators (mainly
372 bumblebees) and the natural dispersal of seeds produced (barochoric) are not able to
373 maintain gene flow over the geographic distances involved.

374

375 **Climate change as the main threat for *Delphinium montanum***

376 The two previous studies of Simon et al. (2001) and López-Pujol et al. (2007) have listed several
377 threats to explain the decline of *D. montanum*, at least in some particular populations.
378 According to Simon et al. (2001), natural disturbance of habitats due to rock falls and
379 avalanches, trampling because of an overall increase in human visits and animal predation
380 (e.g. caterpillar, Pyrenean chamois/isards and perhaps red and roe deers) as well as
381 competition for insect pollinators with simultaneously blooming species (e.g. *Aconitum* spp.)
382 have thus been considered as more or less serious problems for *D. montanum*. The most
383 recent study of López-Pujol et al. (2007) also emphasized on the consequences of the erosion
384 of genetic diversity, especially the loss of rare alleles associated with weak possibility of gene
385 flow between localities that could be however compensated by relatively high effective size
386 in several populations. Without ruling out all these threats on *D. montanum* populations, our
387 results rather support climate change as the main threat for this species. The future
388 predictions of the area of habitat suitability all agree that the spatial extent of the ecological
389 niche of *D. montanum* is very likely to dramatically decrease, and even disappear by the end
390 of the 21th century, with some differences based on the various scenarios we considered. The
391 current suitable habitat for *D. montanum* has an area that was estimated at about 2500 km^2,
392 an area that would decrease to about 500 km^2 (*i.e.* -75 %), by 2040 whatever the scenario
393 envisaged. By 2070, this area would again have decreased to about 150 km^2 based on RCP 2.6
394 and up to less than 50 km^2 based on the two other scenarios (RCP 7 and RCP 8.5). A
395 Representative Concentration Pathway of 2.6 W/m^2 has to be considered as a rather
396 optimistic pathway (that would keep global temperature rise below 2°C by 2100). It would
397 require that CO_2 emissions start declining by 2020 and go to zero by 2100 (as well as declines

398 in emission of other greenhouses gases, such as CH_4, SO_2). RCP 7 is usually considered as a
399 baseline rather than a mitigation target. According to RCP 8.5, emissions would continue to
400 rise throughout the 21st century and can be considered as a pessimistic scenario (with an
401 increase in average temperature in the order of 4°C by 2100). The various output of these
402 scenarios likely explains why the bioclimatic niche of *D. montanum* would have completely
403 disappeared by 2100 for RCP 7 and 8.5 but could have persisted and even started to increase
404 again to about 10% of its current suitable area following the RCP 2.6 scenario.

405 Environmental changes as the main threat for *D. montanum* populations is also
406 supported from field observations. The population of Nohèdes (2) have not flowered since
407 2011. The individuals there are very small (*i.e.* a few centimetres high) and remain at a
408 vegetative stage. On this site, we could not notice any evidence of avalanche or landslide that
409 could have altered the habitat. In addition, other plants species present in the vicinity (e.g.
410 *Orchis spitzelii, Maianthemum bifolium* and *Convallaria majalis*) seem to suffer from flowering
411 default, further supporting that this phenomenon could be non-specific to *D. montanum* and
412 perhaps caused by bioclimatic or at least environmental modifications. There, output from
413 Regional Climate Models (RCMs) predict an increase in mean annual temperature that could
414 reach 3.1 to 4.5°C (+1.5°C already observed since the mean of the period 1961-1990)
415 associated with a decrease in precipitation of up to 25% and perhaps more compared to
416 present-day conditions (Lespinas et al. 2014). According to the same study, precipitations
417 would mostly decrease in summer (-40%) and in spring (-20%) but would remain similar the
418 rest of the year.

419

420 **Recommendations for conservation**

421 *Ex-situ* conservation would be a way to preserve the genetic diversity of *D. montanum*, but
422 also a bridge for future *in-situ* conservation efforts. A seed collection of different populations
423 of *D. montanum* could be stored in a bank. However, this measure would be of limited interest
424 if the decline's cause of *D. montanum* is actually climate change as keeping dormant seeds will
425 probably not allow this species to adapt to future global changes. Alternatively, conserving
426 genetic diversity in a botanical garden may be a good option to test whether and if so, leave
427 time to plants to adapt to rising temperatures and rainfall modifications. Nevertheless, this
428 approach has potential drawbacks like artificial selection and habitat conversion if *D.*
429 *montanum* adapts to *ex-situ* environment, which may differ from their initial natural
430 environment (Ren et al. 2014). Moreover, if, growing individuals from the all 9 localities
431 studied in an appropriate botanical garden could be a way to set-up *in-situ* conservation,
432 preliminary experiments have showed that *cultivating D. montanum* under experimental
433 conditions in a greenhouse is very difficult because of its environmental requirements
434 (Aymerich, pers. comm.). This confirms that if any, botanical garden would have to be set up
435 at a relatively high elevation (*i.e.* > 1500 m), where environmental conditions are similar to
436 the ecological optimum of the species.

437 We can identify three main methods of *in-situ* conservation that could be envisaged
438 for *D. montanum* (see Godefroid et al. 2011; Ren et al. 2014; Mashinski & Albrecht 2017):
439 reinforcement, translocation and managed relocation (or assisted migration). The first one is
440 reinforcement, which consists in adding *ex-situ* grown individuals in their natural population
441 of origin to increase the total number of individuals, increase the spatial occupancy of the
442 species and thereby reduce extinction risk. On the one hand, the increase in population size
443 provided by reinforcement could contribute to counteract the effect of genetic drift. On the
444 other hand, it would have a limited impact to impede inbreeding as all the individuals would

445 originate from the same genetic pool. The second method consists in translocating individuals
446 (seeds, seedlings or adult plants) from a population to another one with the same
447 ecogeography, to increase genetic diversity (the so-called "genetic rescue") and thus increase
448 the likelihood of population persistence in a changing climate (Mashinski and Albrecht 2017).
449 Our results show that *D. montanum* populations harbor substantial levels of pairwise genetic
450 differentiation which suggest that they have exchanged few or no genetic migrants for many
451 generations and have possibly evolved distinct adaptative differences. As a consequence,
452 gene pool mixing may not be appropriate, since the risk of outbreeding depression could be
453 non-negligible between individuals from the different part of the geographic distribution of *D.*
454 *montanum*. It could be thus careful to first experiment *ex-situ* crossings to check if hybrids are
455 viable, and do not suffer from outbreeding depression. However, the genetic benefits of
456 mixing source populations during genetic rescue have been shown to generally outweigh the
457 risks of outbreeding depression and loss of local adaptation (see Whiteley et al. 2015;
458 Frankham 2015; Ralls et al. 2018, see also Liddell 2021). For example, translocations with
459 mixed source populations of *Arnica montana* showed that genetic variation remained similar
460 or increased across successive environments and some authors documented a 10-fold
461 increase in population size following gene flow between wild Trinidadian guppy populations
462 (Albretch and Edwards 2020; Fitzpatrick et al. 2020).

463 Finally, the third method consists in introducing a new population in a suitable new
464 ecosystem, where *D. montanum* has not been observed yet. This method is called managed
465 relocation or assisted migration (Mashinski and Albrecht 2017). However, it may represent a
466 risk of modifying the ecosystem, by creating competition for space and pollinators with other
467 plants naturally present, or by introducing new pathogens. Thus, these three methods of
468 reinforcement, translocation, and managed relocation can present risks of outbreeding

469 depression and ecosystem destabilization, and their benefits may not outweigh those risks.
470 Moreover, since the main threat for *D. montanum* is climate change, on a longer-scale, all
471 those efforts could be unsuccessful. Keeping individuals in a botanical garden seems to be the
472 least harmful and risky method for natural populations today and it could be a good start for
473 crossing experiments between populations.

474 For the very peculiar situation of Nohèdes's locality (2), which seems to be the most
475 endangered population at the moment, there is still the question of the lack of flowering. To
476 test whether this problem may be associated with the absence of a specific environmental
477 stimulus, we may propose to either i) experimentally modify temperature/water supply, *in*
478 *situ*, ii) translocate some individuals to localities where *D. montanum* seem healthy (e.g. Cadí
479 range) and see if flowering can be restored and/or iii) experimentally modify
480 temperature/water supply in the lab to better understand the conditions required for
481 flowering and figure out the limiting factor in the wild. Alternatively, it may be due to the fact
482 that that this locality is formed by immature individuals and that the local population itself
483 may be recovering from an important mortality event. At the moment, we have no further
484 arguments to support this scenario.

485

486 **Conclusion**

487 Overall, our results confirm that the remaining populations of *D. montanum* show evidence of
488 decreased genetic diversity and evidence of inbreeding at a genomic scale. The degree of
489 genetic structure observable is consistent with a set of strongly isolated populations no longer
490 able to maintain gene flow between their sky islands. In this context, *in-situ* conservation
491 methods are likely to have an effect on the status of the species. Reinforcement would help
492 counteracting decrease in local population sizes, and translocation would help increasing

genetic diversity, buffering inbreeding depression and perhaps, bring the variation required for *D. montanum* to adapt. This being said, *ex-situ* crossings are first recommended to verify the absence of outbreeding depression as local genetic pools were found to differ substantially. As our results support that environmental change may represent the main risk of extinction for *D. montanum* all these measures may be however considered as of limited interest on the long term, if the ecological niche of the species disappears.

Acknowledgements

This study was funded by the interreg-POCTEFA program (FLORALAB Project EFA294/19) and is set within the framework of the "Laboratoires d'Excellences (LABEX)" TULIP (ANR-10-LBX-41) as well as within the framework "École Universitaire de Recherche (EUR)" TULIP-GS (ANR-18-EURE-0019). We also thank Xavier Oliver Martínez-Fornés, Sandra Mendez, Kimberley Goudedranche, Lucie Schaad, Irene Abad, Queralt Tor and all other contributors from the FLORALAB network for the help provided in the field.

Authors Contributions

PS, VH, JAMB analysed the data and wrote the manuscript. PAB, JP, AVB, MM, CC, JML and JAMB contributed to fieldwork. MM, CC and VH coordinated the FLORALAB network and/or project aspects related to this work and JAMB contributed to the conception of the study. All authors have read and approved the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data Archiving

Sequencing data have been submitted to the European Nucleotide Archive (ENA; https://www.ebi.ac.uk.ena) under accession n°PRJEB46773.

References

Ahmad M, Leroy T, Krigas N, Temsch EM, Weiss-Scheeweiss H, Lexer C, et al. (2021) Spatial and ecological drivers of population structure in *Alkanna tinctiria* (Boraginaceae), a polyploid medicinal herb. Front. Plant. Sci. 12: 706574.

Aiello-Lammens ME, Boria RA, Radosavljevic A, Vilela B, Anderson RP (2015) spThin: an R package for spatial thinning of species occurrence records for use in ecological niche models. Ecography 38: 541-545.

Albrecht MA, Edwards CE (2020) Genetic monitoring to assess the success of restoring rare plant populations with mixed gene pools. Mol. Ecol. 29: 4037-4039.

Aymerich P (2003) Effects of wild ungulates predation in the conservation of rare plants: two study cases of the Eastern Pyrenees, Acta Bot. Barc. 49: 147-164.

Aymerich P, Martínez-Fornés, XO, Mendez S, Mangeot A, Martin M, Tenas B (2019) Seguiment de l'endemisme dels Pireneus orientals *Delphinium montanum* per la xarva transfronterera FloraCat, Actes del XII Colloqui Internacional de Botànica Pirenaica - Cantàbrica, 147-165.

Bagley JC, Heming NM, Gutiérrez EE, Devisetty UK, Mock KE, Eckert AJ et al. (2020) Genotyping-by-sequencing and ecological niche modeling illuminate phylogeography, admixture, and Pleistocene range dynamics in quaking aspen (*Populus tremuloides*). Ecol. Evol. 10: 4609-4629.

540 Baird NA, Etter PD, Atwood TS, Currey MC, Shiver AL, Lewis ZA et al. (2008) Rapid SNP
541 discovery and genetic mapping using sequenced RAD markers. PloS ONE 3: e3376.
542 Bergl RA, Bradley BJ, Nsubuga A, Vigilant L. (2008) Effects of habitat fragmentation, population
543 size and demographic history on genetic diversity: The Cross River Gorilla in a
544 comparative context. Am. J. Primatol. 70: 848-859.
545 Blischak PD, Kubatko LS, Wolfe AD (2016) Accounting for genotype uncertainty in the
546 estimation of allele frequencies in autopolyploids. Mol. Ecol. Resour. 16: 742-754.
547 Blischak PD, Kubatko LS, Wolfe AD (2018) SNP genotyping and parameter estimation in
548 polyploids using low-coverage sequencing data. Bioinformatics 34: 407-415.
549 Bobrowski M, Weidinger J, Schickfoff U (2021) Is new always better? Frontiers in global climate
550 datasets for modelling treeline species in the Himalayas. Atmosphere 12: 543.
551 Brandrud MK, Paun O, Lorenzo MT, Nordal I, Brysting AK (2017) RADseq provides evidence for
552 parallel ecotypic divergence in the autotetraploid *Cochlearia officinalis* in Northern
553 Norway. Sci. Rep. 7: 5573.
554 Brandrud MK, Paun O, Lorenz R, Baar J, Hedrén M (2019) Restrition-site associated DNA
555 sequencing of *Nigritella* and *Gymnadenia* (Orchidaceae). Mol. Phylogenetics Evol. 136:
556 21-28.
557 Brandrud MK, Baar J, Lorenzo MT, Athanasiadis A, Bateman RM, Chase MW, Hedrén M et al.
558 (2020). Phylogenomic relationships of diploids and the origins of allotetraploids in
559 *Dactylorhiza* (Orchidaceae). Syst. Biol. 69: 91-109.
560 Calevo J, Gargiulo R, Bersweden L, Viruel J, González-Montelongo C, Rebbas, K et al. (2021)
561 Molecular evidence of species- and subspecies-level distinctions in the rare *Orchis*
562 *patens s.l.* and implications for conservation. Biodivers. Conserv. 30: 1293-1314.

563 Catchen J, Amores A, Hohenlohe P, Cresko W, Postlethwait J (2011) Stacks: building and
564 genotyping loci de novo from short-read sequences. G3 - Genes Genom. Genet. 1: 171-
565 182.
566 Catchen J., Hohenlohe PA, Bassham S, Amores A, Cresko WA (2013) Stacks: an analysis tool
567 set for population genomics. Mol. Ecol. 22: 3124-3140.
568 Clark LV, Lipka AE, Sacks EJ (2019). polyRAD: Genotype calling with uncertainty from
569 sequencing data in polyploids and diploids. G3 - Genes Genom. Genet. 9: 663-673.
570 Clevenger J, Chavarro C, Pearl SA, Ozias-Akins P, Jackson SA (2015) Single Nucleotide
571 Polymorphism identification in polyploids: a review, example, and recommendations.
572 Mol. Plant 8: 831-846.
573 De Gabriel Hernando M, Fernandéz-Gil J, Roa I, Juan J, Ortega F, De la Calzada F, Revilla E
574 (2021) Warming threatens habitat suitability and breeding occupancy of rear-edge bird
575 specialists. Ecography 44:1191-1204.
576 Dixo M, Metzger JP, Morgante JS, Zamudio KR (2009) Habitat fragmentation reduces genetic
577 diversity and connectivity among toad populations in the Brazilian Atlantic Coastal
578 Forest. Biol. Conserv. 142: 1560-1569.
579 Dufresne F, Stift M, Vergilino R, Mable BK (2014) Recent progress and challenges in population
580 genetics of polyploid organisms: an overview of current state-of-the-art molecular and
581 statistical tools. Mol. Ecol. 23: 40-69.
582 Fick SE, Hijmans RJ (2017) New 1 km spatial resolution climate surfaces for global land areas.
583 Int. J. Climatol. 37: 4302-4315.
584 Fitzpatrick, SW, BRadburd GS, Kremer CT, Salerno PE, Angeloni LM, Funk WC (2020) Genomic
585 and fitness consequences of genetic rescue in wild populations. Curr. Biol. 30: 517-522.

586 Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large
587 and consistent benefits of gene flow. Mol. Ecol. 24, 2610-2618.
588 Frichot E, Mathieu F, Trouillon T, Bouchard G, François O (2014) Fast and efficient estimation
589 of individual ancestry coefficients. Genetics 4: 973-983.
590 Frichot E, François O (2015) LEA: An R package for landscape and ecological association
591 studies. Methods Ecol. Evol. 6: 925-929.
592 Godefroid S, Piazza C, Graziano R, Buord S, Stevens A-D, Aguraiuja R, et al. (2011) How
593 successful are plant species reintroductions? Biol. Conserv. 144: 672-682.
594 Gutiérrez EE, Heming NM, Penido G, Dalponte JC, Lacerda ACR, Moratelli R et al. (2019)
595 Climate change and its potential impact on the conservation of the Hoary Fox,
596 *Lycalopex vetulus* (Mammalia: Canidae). Mammalian Biol. 98: 91-101.
597 Ren H, Jian S, Liu H, Zhang Q, Lu F (2014) Advances in the reintroduction of rare and
598 endangered wild plant species. Sci China Life Sci 57, 603–609.
599 Heming NM, Dambros C, Gutiérrez E (2018) ENMwizard: advanced techniques for Ecological
600 Niche Modeling made easy. https://github.com/HemingNM/ENMwizard
601 Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers.
602 Bioinformatics 24: 1403-1405.
603 Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of
604 populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281.
605 Karbstein K, Tomasello S, Hodač L, Dunkel FG, Daubert M, Hörandl E (2020) Phylogenomics
606 supported by geometric morphometrics reveals delimitation of sexual species within
607 the polyploid apomictic *Ranunculus auricomus* complex (Ranunculaceae). Taxon 69:
608 1191-1220.

609 Karger DN, Conrad O, Böher J, Kawohl T, Kreft H, Soria-Auza RW et al. (2017). Climatologies at
610 high resolution for the earth's land surface areas. Scientific Data 4: 170122.
611 Kidane YO, Steinbauer MJ, Beierkuhnlein C (2019) Dead end for endemic plant species? A
612 biodiversity hotspot under pressure. Glob. Ecol. Conserv. 19: e00670.
613 Kuhn M (2019) Caret: classification and regression training. https://CRAN. R-
614 project.org/package=caret.
615 Liddell E, Sunnucks P, Cook CN (2021) To mix or not to mix gene pools for threatened species
616 management? Few studies use genetic data to examine the risks of both actions, but
617 failing to do so leads disproportionately to recommendations for separate
618 management. Biol. Conserv. 256: 109072.
619 Lespinas F, Ludwig W, Heussner S (2014) Hydrological and climatic uncertainties associated
620 with modeling the impact of climate change on water resources of small
621 Mediterranean coastal rivers, Journal of Hydrology 511: 403-422.
622 López-Pujol J, Orellana M, R, Bosch M, Simon J, Blanché C. (2007) Low genetic diversity and
623 allozymic evidence for autopolyploidy in the tetraploid Pyrenean endemic larkspur
624 *Delphinium montanum* (Ranunculaceae). Biol. J. Linn. Soc. 155: 211-222.
625 Maschinski J, Albretcht MA (2017) Center for plant conservation's best practice guidelines for
626 the reintroduction of rare plants. Plant Divers. 39: 390-395.
627 McCormack JE, Huang H, Knowles LL (2009) Sky Islands. In: Gillespie RG, Clague D (eds)
628 *Encyclopedia of Islands*, University of California Press: Berkeley, CA Pp 839-843.
629 Meirmans PG, Van Tienderen PH (2013) The effects of inheritance in tetraploids on genetic
630 diversity and population divergence. Heredity 110: 131-137.
631 Meirmans PG, Liu S, van Tienderen PH (2018) The analysis of polyploid genetic data. J. Hered.
632 109: 283-296.

633 Meirmans PG (2020) Genodive version 3.0: Easy-to-use software for the analysis of genetic
634 data of diploids and polyploids. Mol. Ecol. Res. 20: 1126-1131.
635 Muscarella R, Galante PJ, Soley-Guardia M, Boria RA, Kass JM, Uriarte M. et al. (2014)
636 ENMeval: an R package for conducting spatially independent evaluations and
637 estimating optimal model complexity for Maxent ecological niche models. Methods
638 Ecol. Evol. 5: 1198-1205.
639 Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press: New York: 1-514.
640 Pandit MK, Pocock MJO, Kunin WE (2011) Ploidy influences rarity and invasiveness in plants.
641 J. Ecol. 99: 1108-115.
642 Paris JR, Stevens JR, Catchen J (2017) Lost in parameter space: a road map for stacks. *Methods*
643 Ecol. Evol. 8: 1360-1373.
644 Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Bëer E, Robinson S, et al. (2012) Reliability of
645 genetic bottleneck tests for detecting recent population declines. Mol. Ecol. 21: 3403-
646 3418.
647 Phillips SJ, Anderson, RP, Schapire RE (2006) Maximum entropy modelling of species
648 geographic distributions. Ecological Modelling 190: 231-259.
649 Philipps SJ, Anderson RP, Dudík M, Shapire RE, Blair ME (2017) Opening the black box: an open-
650 source release of Maxent. Ecography 40: 887-893.
651 Ralls K, Ballou JD, Dudash MR, Eldridge MDB, Fenster CB, Lacy RC et al., (2018) Call for a
652 paradigm shift in the genetic management of fragmented populations. Conserv. Lett.
653 11: e12412.
654 Ren H, Jian SG, Liu HW, Zhang QM, Lu HF (2014) Advances in the reintroduction of rare and
655 endangered wild plant species. Sci. China Life Sci.: 57, 603-609.

656 Rochette NC, Catchen JM (2017) Deriving genotypes from RAD-seq short read data using
657 Stacks. Nat. Protoc. 12: 2640-2659.
658 Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under
659 Isolation by Distance. Genetics 145: 1219-1228.
660 Simon J, Bosch M, Molero J, Blanché C (2001) Conservation biology of the Pyrenean larkspur
661 (*Delphinium montanum*): a case of conflict of plant versus animal conservation? Biol.
662 Conserv. 98: 305-314.
663 UICN France, FCBN, AFB, MNHN (2018) La liste rouge des espèces menacées en France –
664 Chapitre Flore vasculaire de France métropolitaine. Paris, France.
665 Urban MC (2015) Accelerating extinction risk from climate change. Science 348: 571-573.
666 Van de Peer Y, Mizrachi E, Marchal K (2017) The evolutionary significance of polyploidy. Nature
667 *Rev. Genet.* 18: 411-424.
668 Van de Peer Y, Ahsman T-L, Soltis PS, Soltis DE (2021). Polyploidy: an evolutionary and
669 ecological force in stressful times. Plant Cell 33: 11-26.
670 Vinogradov AE (2003) Selfish DNA is maladaptive: evidence from the plant Red List. Trends
671 Genet 19: 609-614.
672 Wagner ND, He L, Hörandl E (2021) Phylogenomic relationships and evolution of polyploid
673 *Salix* species revealed by RAD sequencing data. Front. Plant Sci. 11: 1077.
674 Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. *Trends*
675 Ecol. Ecol. 30: 42-49.
676 Záveská E, Maylandt C, Paun O, Bertel C, Božo F, The STEPPE Consortium et al. (2019). Multiple
677 auto- and alloploidisations marked the Pleistocene history of the widespread Eurasian
678 steppe plant *Astragalus onobrychis* (Fabaceae). Mol. Phylogenetics Evol. 139: 106572.
679

680 **Tables**

681

682 **Table 1** Geographic coordinates (in °) and elevation (in m above sea level) of the sampling
683 localities, sample size (*N*), observed (H_O) and expected (H_E) levels of heterozygosity, deviation
684 from panmixia (G_{IS}), total number of alleles (*A*) and number of private alleles (A_p) per locality.

	Locality	Latitude	Longitude	Elevation	*N*	H_O	H_E^*	G_{IS}	*A*	A_p
1	Orri de Baix	42.444	2.119	2115	12	0.117	0.171	**0.343**	17 549	275
2	Nohèdes	42.615	2.263	1758	12	0.101	0.146	**0.336**	19 570	3,704
3	Vallter	42.426	2.264	2141	12	0.095	0.147	**0.378**	16 862	3,483
4	Pedraforca	42.237	1.704	2366	10	0.144	0.244	**0.426**	22 090	1,069
5	Serra Pedregosa	42.277	1.688	2586	12	0.159	0.237	**0.346**	22 456	524
6	Cambre d'Aze	42.451	2.129	2637	12	0.134	0.209	**0.380**	27 075	668
7	Tosa	42.315	1.886	2153	12	0.125	0.177	**0.315**	21 782	3,857
8	Bastanist	42.289	1.688	1979	12	0.165	0.242	**0.332**	26 822	310
9	Torreta de Cadí	42.283	1,574	2358	12	0.122	0.191	**0.376**	17 876	4,537
					106	**0.129**	**0.196**	**0.361**		

685 Geographic coordinates are in WGS84.

686 Population genetic statistics were computed based on 5 095 SNPs. Statistically significant values are indicated
687 in bold ($p < 0.05$).

688 *H_E: unbiased expected heterozygosity measured following Nei (1987) in Genodive.

689

690 **Table 2** Current and future *Delphinium montanum* suitable area (in km^2) as modelled and
691 predicted from Ecological Niche Modelling under different model selection criteria (LowAIC
692 and AUC) and thresholding methods (10th percentile of training presence, x10ptp and
693 Maximum training sensitivity plus specificity, mtss. Future predictions are reported for several
694 time periods (2011-2040, 2041-2070 and 2071-2100) and various Representative
695 Concentration Pathways (RCP 2.6, 7.0 and 8.5).

Model selection criterion	Thresholds methods	Current	2011-2040			2041-2070			2071-2100		
			RCP 2.6	RCP 7.0	RCP 8.5	RCP 2.6	RCP 7.0	RCP 8.5	RCP 2.6	RCP 7.0	RCP 8.5
LowAIC	x10ptp	2670	523	492	526	137	17	24	272	0	0
	mtss										
AUC	x10ptp	2365	459	455	446	161	25	43	287	0	0
	mtss										

696

697 **Figures**

698

699 **Figure 1** Map representing the sampling localities (pink dots) within the extant geographic
700 distribution of *Delphinium montanum*.

701

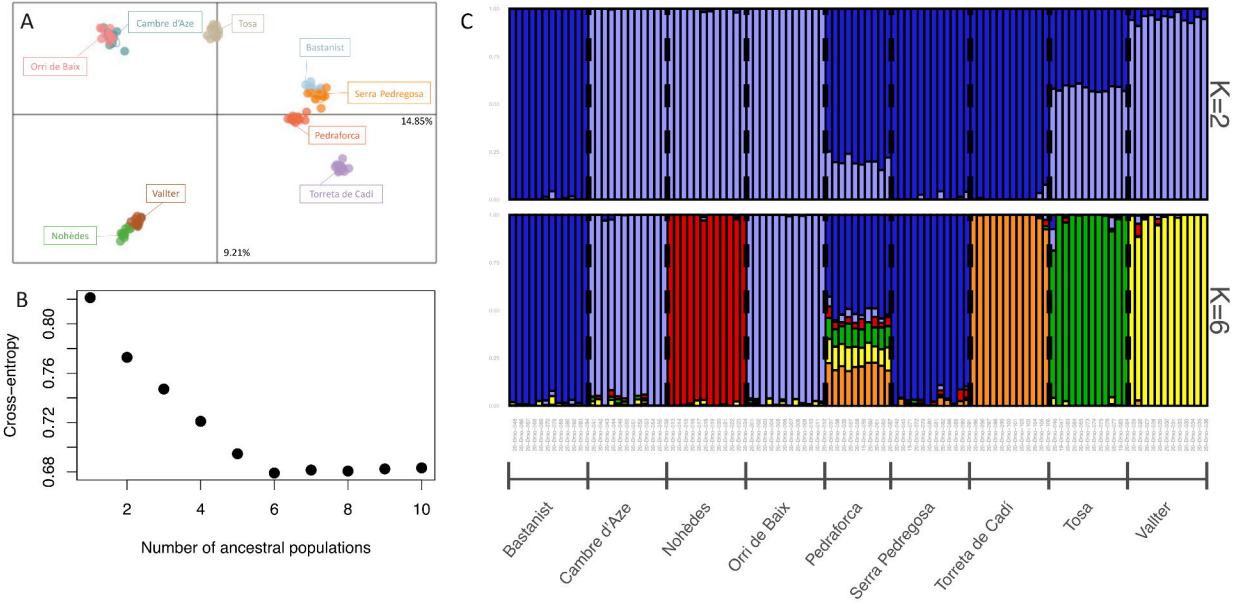
702 **Figure 2 A)** Principal Component Analysis (PCA) displaying the two first axes (PC1 and PC2)
703 representing 14.85% and 9.21% of the total genetic variance. PCA was computed based on the
704 106 individuals of *D. montanum* genotyped at 5 095 SNPs whose colours represent sampling
705 localities. **B)** Values of the cross-entropy criterion for a number of clusters ranging from K = 1
706 to 10 (10 sNMF runs each). The optimal number of K was found to be 6. **C)** Barplot of ancestry
707 coefficients obtained from sNMF for 106 individuals for K = 2 and K = 6, based on 5 095 SNPs.

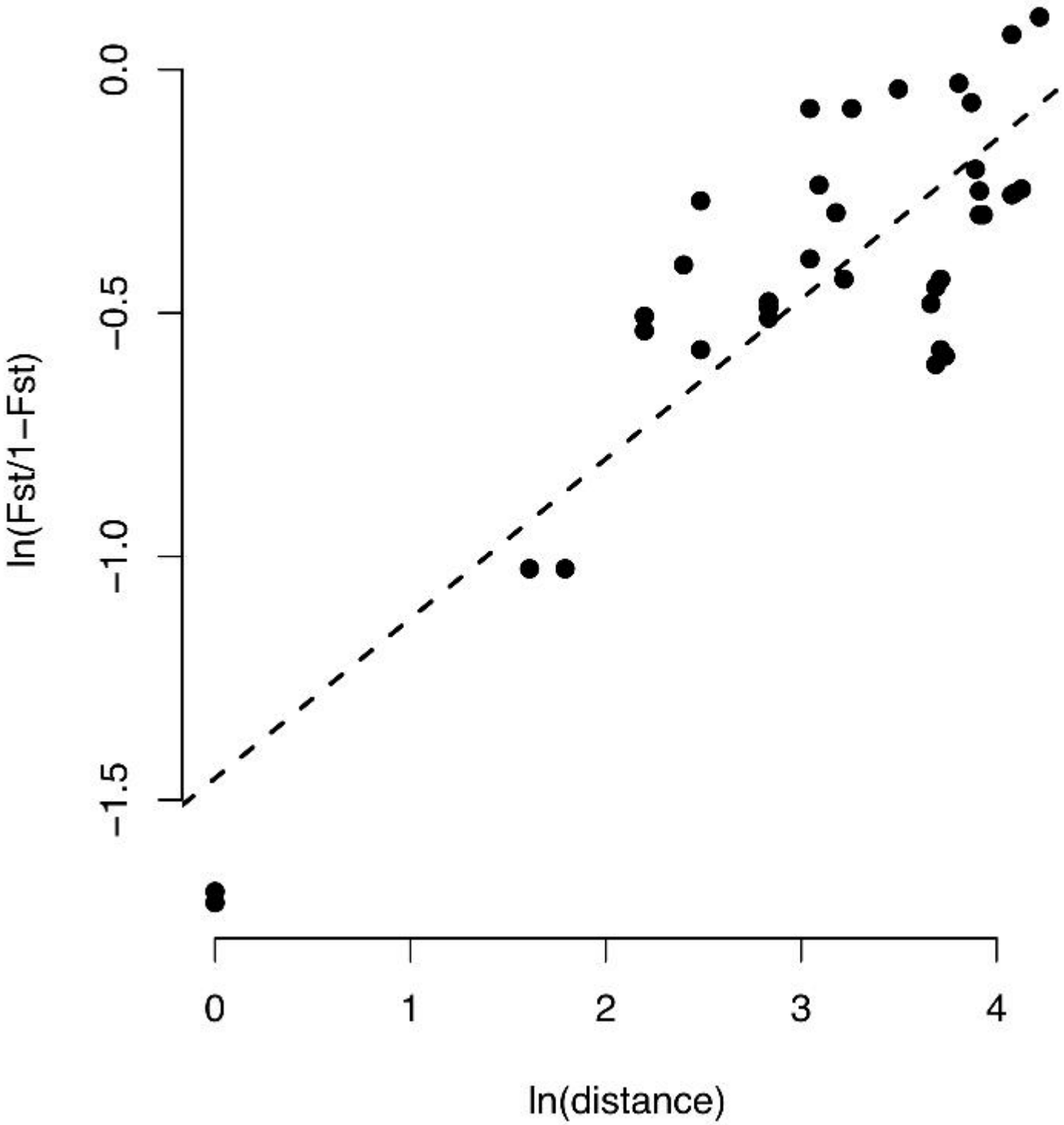
708

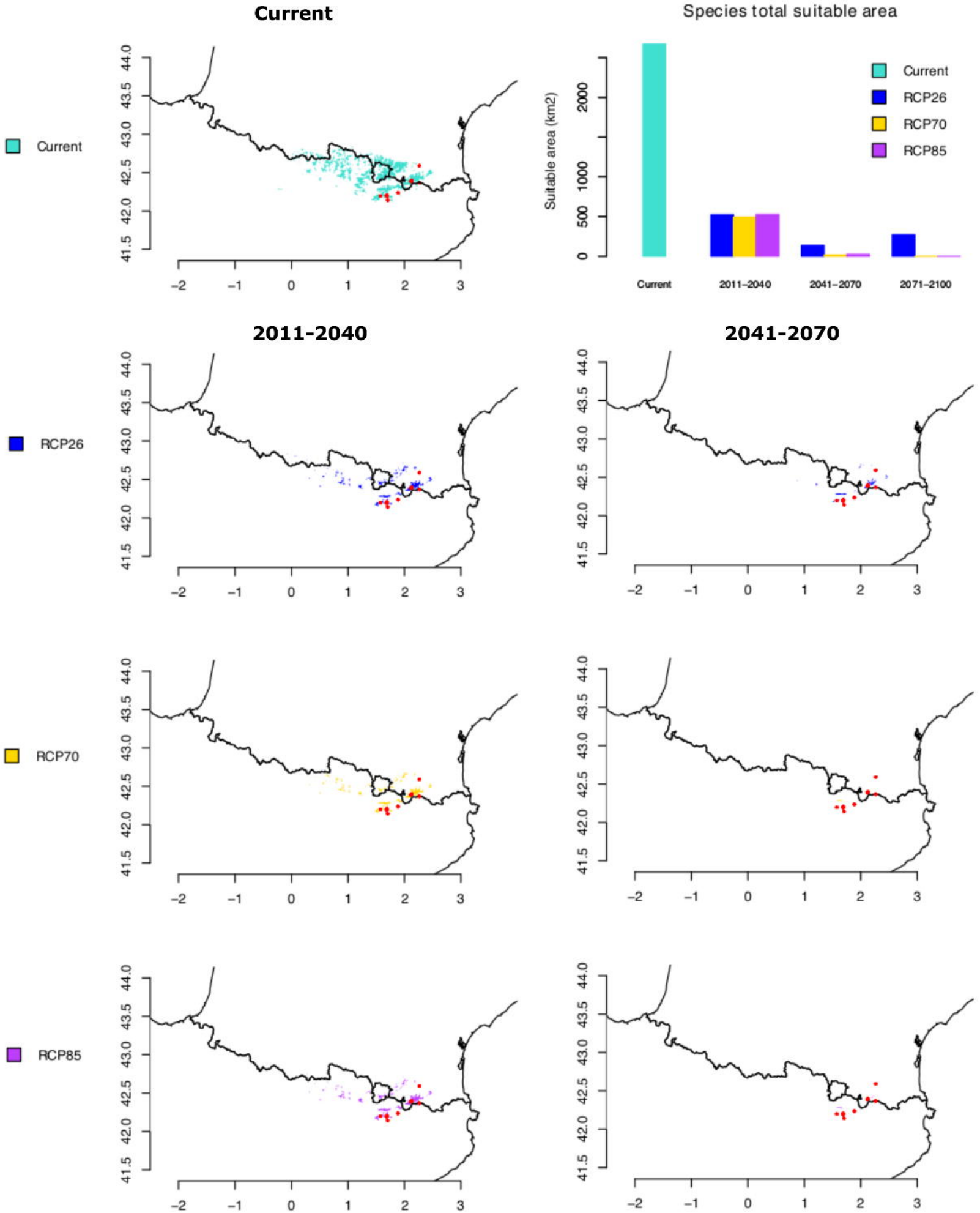
709 **Figure 3** Pattern of Isolation By Distance (IBD) across the data set. Linearized G_{ST}s values (*i.e.*
710 ln(G_{ST}/(1- G_{ST})) are plotted against ln-geographical distances.

Figure 4 Geographic areas holding suitable climatic conditions for *Delphinium montanum* with projection under present climatic conditions (average for 1981-2010) and consensus projection (from 5 Global Climate Models (CGMs): GFDL-ESM4, UKESM1-0-LL, MPI-ESM1-2-HR, IPSL-CM6A-LR and MRI-ESM2-0), for various plausible climatic scenarios for years 2035 (average for 2011-2040), 2055 (average for 2041-2070) and three Representative Concentration Pathways (RCPs): RCP 26, RCP 70 and RCP 8.5. The time period 2071-2100 is not represented since the suitable area would have completely disappeared for RCPs 7.0 and 8.5. The Barplot displays current and future area values under various plausible climatic scenarios. These values correspond to “LowAIC” model (“AUC” model being very similar).

FRANCE
ANDORRA
SPAIN
Mediterranean Sea
2-Nohèdes
1-Orri de Baix
6-Cambre d'Aze
3-Vallter
7-Tosa
9-Torreta de Cadi
8-Bastanist
5-Serra Pedregosa
4-Pedraforca
0
10
20 km


A
Cambre d'Aze
Tosa
Orri de Baix
Bastanist
Serra Pedregosa
14.85%
Pedraforca
Vallter
Torreta de Cadí
Nohèdes
9.21%
B
Cross−entropy
0.68
0.72
0.76
0.80
2
4
6
8
10
Number of ancestral populations
C
K=2
K=6
Bastanist
Cambre d'Aze
Nohèdes
Orri de Baix
Pedraforca
Serra Pedregosa
Torreta de Cadí
Tosa
Vallter


0.0
−0.5
−1.0
−1.5
ln(Fst/1−Fst)
0
1
2
3
4
ln(distance)


Current
Species total suitable area
Current
RCP26
RCP70
RCP85
Suitable area (km2)
0
500
1000
2000
Current
2011–2040
2041–2070
2071–2100
2011-2040
2041-2070
44.0
43.5
43.0
42.5
42.0
41.5
−2
−1
0
1
2
3