1 Little hope for the polyploid endemic Pyrenean Larkspur (*Delphinium* 2 *montanum*): evidences from population genomics and Ecological Niche 3 Modelling

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20 Abstract

21 Species endemic to restricted geographical ranges represent a particular conservation issue,
22 be it for their heritage interest. In a context of global change, this is particularly the case for
23 plants which belong to high-mountain ecosystems and, because of their ecological

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

40

41 **Keywords** Sky islands, Inbreeding, Tetraploid, Restriction Site Associated DNA Sequencing
 42 (RAD-seq), Species Distribution Modelling, Genotyping By Sequencing (GBS)

43 Introduction

44 Extinction risk associated to climate change is expected not only to increase but to
 45 accelerate for every degree rise in global temperatures (Urban, 2015). In this context, the
 46 number of threatened species by reduced population sizes, habitat degradation and habitat
 47 fragmentation is increasing. Small and fragmented populations are particularly prone to be
 48 affected by drift and inbreeding depression and generally display decreased levels of genetic
 49 diversity (e.g. Bergl et al. 2008; Dixo et al. 2009). The increase of anthropogenic and
 50 environmental pressures, both being intimately bound, represents an unprecedented threat
 51 for vulnerable species. In particular, populations from species of high-mountain ecosystems
 52 might not be able to move upward rapidly enough to keep within their thermal tolerances
 53 and might be doomed on their 'sky islands' (see McCormack et al. 2009, e.g. Kidane et al.
 54 2019, De Gabriel Hernando et al. 2021). Thus, species endemic to specific mountain ranges
 55 represent a major conservation issue, if only for their heritage interest. It is critical to
 56 characterize the distribution of genetic diversity within- and between- their populations to
 57 evaluate the inbreeding risk and study population structure to better understand how
 58 isolated they really are, in order to undertake effective conservation actions (Calevo et al.
 59 2021).

60 The threatened Pyrenean larkspur *Delphinium montanum* DC., 1815 perfectly
 61 illustrates this situation. This plant (Family Ranunculaceae), endemic to the Eastern part of
 62 the Pyrenees (France and Spain), is now only observable at a dozen of localities (two already
 63 got extinct over the 20th century) and its global population size was estimated to range
 64 between 7 800 to 10 300 individuals in total (Aymerich et al. 2020). It occurs between alpine
 65 and subalpine levels, between 1 600 and 2 400 m (López-Pujol et al. 2007). *D. montanum* is
 66 vulnerable as a result of increasing levels of multiple threats. Some of its populations are

located nearby touristic sites (*i.e.* mountain resorts or hiking trails) and are negatively impacted by trampling (Simon et al., 2001). Flowers and fruits predation by herbivores, particularly Pyrenean chamois/isards (*Rupicapra pyrenaica pyrenaica*) was also shown to be responsible for the seed bank's decrease in some populations (Simon et al. 2001, Aymerich 2003). Importantly, differences in population dynamics are observable from one population to another. Some populations are stable, whereas others show decrease in population size (4-Pedraforca) and/or absence of flowering individuals since 2011 (2-Nohèdes). Pioneering work of Simon et al. (2001) and López-Pujol et al. (2007) also suggested relatively low effective size as well as, evidence of inbreeding within-populations and significant population structure. However, these two studies were based on a limited number of genetic markers (*i.e.* 7 and 14 allozyme *loci*, respectively) and populations studied (*i.e.* 2 and 7, respectively).

Implementing a population genomic approach on *D. montanum* could be particularly fruitful to inform conservation strategies by providing a substantial increase in the resolution of the genetic marker dataset and genome complexity reduction protocols such as Reduction site-associated DNA sequencing (or RAD-seq, Baird 2008) allow to get such information on tens to hundreds of individuals. However, the features of the genome of the species make *Delphinium montanum* particularly challenging from a technical and methodological point of view. *D. montanum* is an autotetraploid species (Simon et al. 2001; López-Pujol et al. 2007). Our recent flow cytometry estimates of 10.32 pg/1C for this species (*i.e.* about twice the 1C-value reported for several other diploid *Delphinium* species) corroborates previous knowledge and confirms the large genome size of *D. montanum* (Bertrand et al. unpublished data). A large genome size can be considered has a limitation as itself, be it because in requires higher sequencing efforts and costs to carry out conservation studies. The difficulty

91 to infer genotype and allele frequencies on polyploid species with RAD-seq techniques as
92 well as the restricted set of methods available to analyze such data (see Meirmans and van
93 Tienderen 2013; Dufresne et al. 2014; Meirmans et al. 2018) is also likely to explain the
94 relatively low number of population genomics studies dealing with RAD-seq like techniques
95 on non-model polyploid organisms (Clevenger et al. 2015; van de Peer et al. 2017, Ahmad
96 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 Závěská 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. Závěská 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
106 outputted a higher number of variable sites but signals were found to be overall congruent
107 (Závěská et al. 2019; Brandrud et al. 2020).

108 The recent studies of Závěská 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 can deal with multiple levels of ploidy and is able to use for example population structure, linkage disequilibrium and/or self-fertilization as priors to estimate genotype probabilities. To our best knowledge, this promising and convenient method has been rarely used to implement a population genomic approach on natural populations of polyploid non-model organisms.

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

Materials and Methods

Geographic distribution and population sampling

The Pyrenean Laraspur (*Delphinium montanum*) geographic distribution ranges 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 km, and only one locality is located outside this area: Nohèdes (locality 2), in the

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

We sampled a total of 106 individuals from 9 localities representative of the geographic distribution of *Delphinium montanum* (in the Eastern Pyrenees: 3 sites in France and 6 in Spain) between July and October 2020 (Table 1, Fig. 1, see also Supplementary Appendix S1). At each locality, we collected on individual distant from at least 1 meter from each other, about 1-2 cm² of leaf tissues that were stored in 90% ethanol at 4°C until DNA extraction. *Delphinium montanum* is not legally protected in France but is considered as vulnerable (VU) on the Red List of threatened species of IUCN France (IUCN France, FCBN, AFB, MNHN, 2018). It is legally protected in Catalonia (Decret 172/2008. Catàleg de flora amenaçada de Catalunya) and Spain (Orden AAA/1771/2015. Modificación del catálogo de especies amenazadas). In France, for all sampling done in natural reserves, special authorizations were granted by the 'Direction Départementale des Territoires et de la Mer 66' (DDTM 66). In Spain, we asked for and obtained special permits from the Servei de Fauna i Flora de la Generalitat de Catalunya and Natural Parks (Cadí-Moixeró and Ter-Freser).

Molecular procedures

Genomic DNA extraction and genotyping were subcontracted to LGC Genomics GmbH (Berlin, Germany). Genotyping-By-Sequencing (GBS) was performed following a specific

protocol called nGBS for ‘normalised GBS’. This double digest Restriction site-Associated DNA seq (or RAD-seq) like protocol relies on two restriction enzymes (PstI and ApeKI, in our case) to reduce *D. montanum* genome complexity and includes a normalization step that aims at avoiding repetitive regions. The resulting 106 individually barcoded libraries were sequenced in paired-end mode (2 x 250 bp) on an Illumina NovaSeq 6000 with an expectation of a minimum number of 1.5 million read pairs per sample.

Genomic data processing

We used Stacks v.2.41 (Catchen et al. 2011; 2013) to build loci from Illumina reads, *de novo* (*i.e.* without aligning reads to a reference genome). Stacks consists of a wrapper of several scripts that are usually run sequentially including: *process_radtags* to demultiplex and clean reads, *denovo_map.pl* to build loci within individuals, create a catalog and match all samples against it and *populations* to further filter the SNPs obtained at the population level and compute basic population genetic statistics. We first optimised several key parameters of the pipeline: *-m* (the minimum number of identical raw reads required to form a putative allele), *-M* (the number of mismatches allowed between alleles to form a locus) and *-n* (the number of mismatches allowed between loci during construction of the catalog) on a subset of 12 individuals representative of the whole data set (*i.e.* geographic origin and coverage). As recommended by several authors, we varied *M* and *n* (fixing *M* = *n*) while keeping *m* = 3 (see Paris et al. 2017; Rochette & Catchen 2017). The combination *-m* 3, *-M* 3 and *-n* 3 was found to be the most suitable to maximize the number of SNPs, assembled and polymorphic loci in our case and was used to run Stacks on the whole data set (see Supplementary Appendix S2). As *D. montanum* is a tetraploid species, we also specified the option ‘-X *ustacks:--max_locus_stacks* 5’ (in the *ustacks* script’s) to allow the number of alleles per site

to reach the number of 5 instead of 3 by default. However, as the *populations* script is unable to deal with polyploid genotypes, we followed a different approach to call variants and complete genomic data processing.

We then used polyRAD (Clark et al. 2019), a method implemented in an R-package to call genotypes with uncertainty from sequencing data in polyploids. The Bayesian genotype caller implemented in polyRAD is able to import read depth from the catalog and matches files from Stacks through a specific function called `polyRAD::read_stacks()` to further estimate the probability of each possible genotype for each individual and each locus, taking into account features such as possible levels of ploidy and population structure as priors. In order to get similar filtering parameters than with Stacks and following the polyRAD author's guidelines, we used the following arguments `-min.ind.with.minor. alleles= 5`, `-min.ind.with.reads= 84` and a ploidy of 4. We then plotted the distribution of the ratio of the individual over expected heterozygosity H_{ind}/H_E . The peak of the distribution of H_{ind}/H_E by locus (0.2) was considered to represent well-behaved markers from which inbreeding (F) was estimated at 0.73. From this estimate of F , we then simulated the expected distribution of H_{ind}/H_E if all markers were behaving in a Mendelian fashion. Based on the distribution, we defined minimum and maximum thresholds of 0 and 0.406 comprising 95% of the observations and filtered out markers for which H_{ind}/H_E were outside this interval. We then tested overdispersion (*i.e.* in our case, how much does read depth distribution deviate from what would be expected under binomial distribution) and adjusted its parameter to 6 (from a range of 2 to 14). This information was used to call genotypes while taking into account population genetic structure. The most likely genotypes of the remaining 5 095 loci were then exported in several formats such as genind objects or Structure (.str).

211 **Population genomic analyses**

212 To get an overview of the overall genetic diversity and differentiation among individuals and
 213 populations, we first performed a Principal Component Analysis (PCA) based on a matrix of
 214 106 individuals (as rows) and 5 095 SNPs (as columns) coded as a genind object with the R-
 215 package 'adegenet' (Jombart 2008). We then used Genodive v.3.04 (Meirmans 2020) to
 216 compute expected and observed heterozygosity (H_E and H_O , respectively) as well as the
 217 number of alleles (A) and the number of private alleles was computed with the R-package
 218 'poppr' (Kamvar et al. 2014). The deviation from panmixia was evaluated by computing G_{IS}
 219 and the statistical significance of the obtained values was estimated based on 10 000
 220 permutations, also in Genodive. Overall and pairwise genetic differentiation was also
 221 assessed based on G -statistics (G_{ST} , Nei 1987) as implemented in Genodive in a similar
 222 manner. Pattern of Isolation By Distance (IBD) was assessed by examining the relationship
 223 between linearized G_{ST} s values (*i.e.* $\ln(G_{ST}/(1 - G_{ST}))$) and $\ln$ -geographical distances (after
 224 Rousset 1997) and its statistical significance was tested with a Mantel test between matrices
 225 of pairwise G_{ST} and geographical distance.

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

234

235 **Ecological Niche Modelling**

236 In order to assess the future trends of spatial dynamics of *D. montanum* distribution in a
 237 context of global change, we conducted Ecological Niche Modelling (ENM) analyses based on
 238 known occurrences of the species. Based on 19 bioclimatic layers we downloaded from
 239 CHELSA v.2.1 (Karger et al. 2017), we aimed to i) model current environmental niche and ii)
 240 predict the spatial evolution of the niche in the future. The bioclimatic layers available in
 241 CHELSA are similar to those available in WORLDCLIM (Fick et al. 2017) in a way that they
 242 consist of downscaled Global Climate Models (GCM) output temperature and precipitation
 243 estimates at a maximal resolution of 30 arc seconds (approximately 1 km). In the current
 244 study, we used the most recent version of CHELSA as the methodology and bias correction it
 245 implements have been shown to outperforms WORLDCLIM in mountainous environments
 246 (Karger et al. 2017; Bobrowski et al. 2021). Current climate data correspond to time
 247 averaged variables over the period 1981-2010.

248 Future climates correspond to estimates from phase 3b of the Inter-Sectoral Impact
 249 Model Intercomparison Project (ISI-MIP3b) based on output of phase 6 of the Coupled Model
 250 Intercomparison Project (CMIP6). They consist of the output of five GCMs (GFDL-ESM4,
 251 UKESM1-0-LL, MPI-ESM1-2-HR, IPSL-CM6A-LR and MRI-ESM2-0) and three climate scenario
 252 specifiers (ssp126, ssp370, ssp585). Climate scenarios specifiers reflect different
 253 Representative Concentration Pathways (RCPs) associated with different radiative forcing
 254 values: 2.6, 7.0 and 8.5 W/m² that can be considered as a plausible range of greenhouse gas
 255 concentrations that appear to be optimistic, very likely and pessimistic in term of climate
 256 change, respectively. Annually averaged data were downloaded for three future time
 257 periods: 2011-2040 ("2035"), 2041-2070 ("2055") and 2071-2100 ("2085").

The ENM analyses were conducted thanks to the R-package ENMwizard v.0.3.7 (Heming et al. 2018, see also Gutiérrez et al. 2019; Bagley et al. 2020). We first spatially filtered occurrences to keep only those that were at least 1 km away from each other using the R-package ‘spThin’ (Aiello-Lammens et al. 2015). Due to the relatively small spatial scale of the area under study, this resulted in considering a total of 9 out the 106 occurrences initially available. We then examined raw values for the 19 bioclimatic variables extracted at each occurrence point and noticed that the variable bio19 (“precipitation of coldest quarter”) displayed obviously aberrant (*i.e.* negative) values. This variable bio19 was thus discarded from subsequent analyses. From the 18 remaining variables (bio1 to bio18), we selected the less correlated ones (Person correlation coefficient < 0.75) thanks to the R-package ‘caret’ (Kuhn 2019) and kept five variables: bio5 (“mean daily maximum air temperature of the warmest month”), bio7 (“annual range of air temperature”), bio14 (“precipitation amount of the driest month”) and bio15 (“precipitation seasonality”) for further analyses.

The calibration area for the models was created as a buffer of 1.5° around the minimum convex polygon encompassing all occurrences. We used the maximum entropy method (implemented in MaxEnt ver. 3.4.1, Phillips et al. 2006, 2017) to calibrate model and evaluated model performance thanks to the package ENMeval (Muscarella et al. 2014) as implemented in ENMwizard. We evaluated models using a geographic partition scheme of type “block” and optimized two parameters of MaxEnt: the Regularization Multipliers (RM) and the Feature Classes (FC). RM was varied from 0.5 to 4.5, incremented by 0.5 whereas a suite of 15 FCs (L, for Linear, P, for Product, Q, for Quadratic and H for Hinge) or combination of them were evaluated: L, P, Q, H, LP, LQ, LH, PQ, PH, QH, LPQ, LPH, LQH, PQH, LPQH, resulting in a total of 135 models. Model selection was done by computing the corrected

Akaike Information Criterion (“LowAIC”) and the Area Under the receiver operating characteristic Curve (“AUC”) in the function ENMWizard::calib_md1_b(). The relative importance of the different variables was evaluated with the function ENMWizard::get_cont_permimport(). The best selected model was then spatially projected as current niche. For future predictions, a spatial projection of the consensus of the five CGMs was performed for each time period and each RCPs. Area calculations were carried out after applying a 10-percentile (x10ptp) and a Maximum training sensitivity plus specificity (mtss) threshold rules.

Results

Genetic diversity and differentiation

We obtained a total of 159 million read pairs across the 106 individuals (2 283 178 to 6 266 452 of raw reads per ind., mean 3 761 722, s.d. = 873 055). We assembled a total of 210 756 loci including 22 911 SNPs with Stacks. Average read depth ranged from 27X to 53X based on the combination of parameters we used. After filtering the data with polyRAD, we finally obtained 32 728 loci including 5 095 SNPs.

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

of total alleles A , too, see Table 1). The number of private alleles A_p is consistent with the relative level of geographic isolation of the populations.

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

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

ENMeval analyses identified $RM = 2$ and a combination of Quadratic and Hinge feature (QH) classes as the best-performing parameters for calibrating the final ENM following the “LowAIC” and $M=2$ and a combination of Product and Hinge feature (PH) classes following the “AUC” optimality criteria. The first model had the best corrected Akaike Information Criterion (AICc) score (152.25) and an AICc weight of 0.33 whereas the second one had an AICc score of 174.63 and an AICc weight of $4.53 \cdot 10^{-6}$. These two models had mean omission rates of 0.375 and 0.25 for the 10th percentile and the lowest presence training thresholds, respectively, as well as a mean test AUC of 0.988. Values of these diagnostic metrics are provided for all candidate models in Supplementary Appendix S4. Both models ($RM = 2/PH$ and $RM = 2/QH$) considered bio15 (“precipitation seasonality”) as being the most important

explanatory variable with a contribution of 88.6% and 90.0%, respectively, followed by bio7 (“Temperature annual range (bio5-bio6)”) with 6.2% and 7.1% and bio5 “Maximum temperature of the warmest month” with 5.2 and 3.0%; the contribution of bio14 “Precipitation of the driest month”, being 0% with both models. Current suitable area for *D. montanum* was found to be 2 670 and 2 365 km² following “LowAIC” and “AUC” selection model criteria, respectively (the values were identical whatever the threshold method used). All future consensus spatial projections predict a dramatic decrease of the suitable area (Table 2 and Fig. 4). For RCPs 7.0 and 8.5, the suitable area would have completely disappeared for the time period 2071-2100 (but not for RCP 2.6). We also notice some slightly different outcomes depending on the selection model criteria with RCP 8.5 scenarios giving sometimes higher values than RCP 7.0, and even RCP 2.6 (e.g. time period 2011-2040, “Low AIC” criterion).

Discussion

Conservation genomics on a polyploid species with large genome size

The use of RAD-seq like (nGBS) data with the method implemented in PolyRAD allowed us to successfully address the challenge of conducting a population genomic approach on a plant species that is both polyploid (autotetraploid) and has a large genome size (> 10 Gbp). It confirms the convenience of the workflow we used, specifically calling genotypes in an R-environment, as an alternative to the ones followed in the handful of studies recently published on similar systems (e.g. Závěská et al. 2019; Brandrud et al. 2020; Karbstein et al. 2020; Ahmad et al. 2021). From the > 5 000 SNPs we could rely on, we were able to get an unprecedented genomic picture of the diversity and the differentiation of the species in order to propose appropriate conservation recommendations. The results we obtained at

the genome scale are overall consistent with previous knowledge on population genetics of *D. montanum* (see Simon et al. 2001; López-Pujol 2007).

A set of populations isolated on sky islands

Our results confirm that populations of *D. montanum* can be seen as a set of island-like dwelling systems separated from each other by a matrix of unsuitable habitats and that gene flow has little to no chance to be efficiently maintained within this metapopulation. All populations considered in this study were found to display deficit in heterozygotes ($G_{IS} > 0.315$, $p < 0.05$). These results confirm previous findings of Simon et al. (2001) and López-Pujol et al. (2007) and show that the autotetraploid status of the species does not impede relatively low levels of heterozygosity ($H_o < 0.17$). Polyploidy is expected to be associated with increased genetic variation, a characteristic that provides more diversity to adapt (especially to extreme environments such as mountainous ones) and allows buffering effect against deleterious mutations (see van de Peer et al. 2021 and references therein). From a conservation point of view, polyploidy does not seem to be associated with increased risk of extinction even though plant with big genome size may be more prone to be under threat (Vinogradov 2003; Pandit et al. 2011). As two other closely related congeneric species of *Delphinium montanum*: *D. dubium* and *D. oxysepalum*, that are endemic to the Alps and the Carpathians, respectively, are also tetraploid (López-Pujol et al. 2007), we speculate that polyploidy may have allowed to these species to adapt and survive into these mountainous environments. The open question is whether current levels of genetic diversity may be sufficient or not to allow *D. montanum* populations to overcome inbreeding depression and provide sufficient variation for adaptation in a context of global change. It should be noted that levels of genetic diversity are overall similar among the populations investigated and we

could not associate obvious signs of decline of some of them (e.g. Nohèdes, Pedraforca) to decreased levels in genetic diversity or evidence for deviation from panmixia. This suggests that the evidences of possible decrease in effective size may be too recent to be detectable at the genetic level (see Peery et al. 2012). Unfortunately, the limited number of available methods for polyploids makes effective size estimation and test for bottleneck events cumbersome to perform in such systems.

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

Climate change as the main threat for *Delphinium montanum*

The two previous studies of Simon et al. (2001) and López-Pujol et al. (2007) have listed several threats to explain the decline of *D. montanum*, at least in some particular

401 populations. According to Simon et al. (2001), natural disturbance of habitats due to rock
402 falls and avalanches, trampling because of an overall increase in human visits and animal
403 predation (e.g. caterpillar, Pyrenean chamois/isards and perhaps red and roe deers) as well
404 as competition for insect pollinators with simultaneously blooming species (e.g. *Aconitum*
405 spp.) have thus been considered as more or less serious problems for *D. montanum*. The
406 most recent study of López-Pujol et al. (2007) also emphasized on the consequences of the
407 erosion of genetic diversity, especially the loss of rare alleles associated with weak possibility
408 of gene flow between localities that could be however compensated by relatively high
409 effective size in several populations. Without ruling out all these threats on *D. montanum*
410 populations, our results rather support climate change as the main threat for this species.
411 The future predictions of the area of habitat suitability all agree that the spatial extent of the
412 ecological niche of *D. montanum* is very likely to dramatically decrease, and even disappear
413 by the end of the 21th century, with some differences based on the various scenarios we
414 considered. The current suitable habitat for *D. montanum* has an area that was estimated at
415 about 2500 km², an area that would decrease to about 500 km² (i.e. -75 %), by 2040
416 whatever the scenario envisaged. By 2070, this area would again have decreased to about
417 150 km² based on RCP 2.6 and up to less than 50 km² based on the two other scenarios (RCP
418 7 and RCP 8.5). A Representative Concentration Pathway of 2.6 W/m² has to be considered
419 as a rather optimistic pathway (that would keep global temperature rise below 2°C by 2100).
420 It would require that CO₂ emissions start declining by 2020 and go to zero by 2100 (as well as
421 declines in emission of other greenhouses gases, such as CH₄, SO₂). RCP 7 is usually
422 considered as a baseline rather than a mitigation target. According to RCP 8.5, emissions
423 would continue to rise throughout the 21st century and can be considered as a pessimistic
424 scenario (with an increase in average temperature in the order of 4°C by 2100). The various

output of these scenarios likely explains why the bioclimatic niche of *D. montanum* would have completely disappeared by 2100 for RCP 7 and 8.5 but could have persisted and even started to increase again to about 10% of its current suitable area following the RCP 2.6 scenario.

Environmental changes as the main threat for *D. montanum* populations is also supported from field observations. The population of Nohèdes (2) have not flowered since 2011. The individuals there are very small (*i.e.* a few centimetres high) and remain at a vegetative stage. On this site, we could not notice any evidence of avalanche or landslide that could have altered the habitat. In addition, other plants species present in the vicinity (*e.g.* *Orchis spitzelii*, *Maianthemum bifolium* and *Convallaria majalis*) seem to suffer from flowering default, further supporting that this phenomenon could be non-specific to *D. montanum* and perhaps caused by bioclimatic or at least environmental modifications. There, output from Regional Climate Models (RCMs) predict an increase in mean annual temperature that could reach 3.1 to 4.5°C (+1.5°C already observed since the mean of the period 1961-1990) associated with a decrease in precipitation of up to 25% and perhaps more compared to present-day conditions (Lepinas et al. 2014). According to the same study, precipitations would mostly decrease in summer (-40%) and in spring (-20%) but would remain similar the rest of the year. This in accordance with our ENM predictions suggesting that bio15 “Precipitation seasonality”, the annual coefficient of variation of rainfall may be by far, the most important variable in explaining the bioclimatic niche of *D. montanum*.

Recommendations for conservation

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

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

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

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

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

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

Conclusion

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

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Authors’ Contributions and Conflict of Interest

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. The authors declare no conflict of interest.

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544 **Data Accessibility Statement**

545 Sequencing data have been submitted to the European Nucleotide Archive (ENA;
546 <https://www.ebi.ac.uk/ena/>) under Study with accession n°PRJEB46773 and samples
547 accession n°ERS7180281 (SAMEA9457265) and n°ERS7643565 (SAMEA9965271) to
548 n°ERS7643669 (SAMEA9965375).

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550 **References**

- 551 Ahmad, M., Leroy, T., Krigas, N., Temsch, E.M., Weiss-Scheeweiss, H., Lexer, C., Sehr, E.M.,
552 Paun, O. (2021) Spatial and ecological drivers of population structure in *Alkanna*
553 *tinctoria* (Boraginaceae), a polyploid medicinal herb. *Frontiers in Plant Science*,
554 12:706574.
- 555 Aiello-Lammens, M.E., Boria, R.A., Radosavljevic, A., Vilela, B., Anderson, R.P. (2015) spThin:
556 an R package for spatial thinning of species occurrence records for use in ecological
557 niche models. *Ecography*, 38, 541-545.
- 558 Albrecht, M.A., Edwards, C.E. (2020) Genetic monitoring to assess the success of restoring
559 rare plant populations with mixed gene pools. *Molecular Ecology*, 29, 4037-4039.
- 560 Aymerich, P. (2003) Effects of wild ungulates predation in the conservation of rare plants:
561 two study cases of the Eastern Pyrenees. *Acta Botanica Barcinonensia*, 49, 147-164.
- 562 Aymerich, P., Martínez-Fornés, X.O., Mendez, S., Mangeot, A., Martin, M., Tenas, B. (2019)
563 Seguiment de l'endemisme dels Pireneus orientals *Delphinium montanum* per la
564 xarxa transfronterera FloraCat, Actes del XII Colloqui Internacional de Botànica
565 Pirenaica - Cantàbrica, 147-165.

566 Bagley, J.C., Heming, N.M., Gutiérrez, E.E., Devisetty, U.K., Mock, K.E., Eckert, A.J., Strauss,
567 S.H. (2020) Genotyping-by-sequencing and ecological niche modeling illuminate
568 phylogeography, admixture, and Pleistocene range dynamics in quaking aspen
569 (*Populus tremuloides*). *Ecology and Evolution*, 10, 4609-4629.

570 Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U.,
571 Cresko, W.A., Johnson, E.A. (2008) Rapid SNP discovery and genetic mapping using
572 sequenced RAD markers. *PloS ONE*, 3: e3376.

573 Bergl, R.A., Bradley, B.J., Nsubuga, A., Vigilant, L. (2008) Effects of habitat fragmentation,
574 population size and demographic history on genetic diversity: The Cross River Gorilla
575 in a comparative context. *American Journal of Primatology*, 70, 848-859.

576 Blischak, P.D., Kubatko, L.S., Wolfe, A.D. (2016) Accounting for genotype uncertainty in the
577 estimation of allele frequencies in autopolyploids. *Molecular Ecology Resources*. 16,
578 742-754.

579 Blischak, P.D., Kubatko, L.S., Wolfe, A.D. (2018) SNP genotyping and parameter estimation in
580 polyploids using low-coverage sequencing data. *Bioinformatics*, 34, 407-415.

581 Bobrowski, M., Weidinger, J., Schickhoff, U. (2021) Is new always better? Frontiers in global
582 climate datasets for modelling treeline species in the Himalayas. *Atmosphere* 12: 543.

583 Brandrud, M.K., Paun, O., Lorenzo, M.T., Nordal, I., Brysting, A.K. (2017) RADseq provides
584 evidence for parallel ecotypic divergence in the autotetraploid *Cochlearia officinalis*
585 in Northern Norway. *Scientific Reports*, 7: 5573.

586 Brandrud, M.K., Paun, O., Lorenz, R., Baar, J., Hedrén, M. (2019) Restriction-site associated
587 DNA sequencing of *Nigritella* and *Gymnadenia* (Orchidaceae). *Molecular*
588 *Phylogenetics and Evolution*, 136, 21-28.

589 Brandrud, M.K., Baar, J., Lorenzo, M.T., Athanasiadis, A., Bateman, R.M., Chase, M.W.,
590 Hedrén, M., Paun, O. (2020). Phylogenomic relationships of diploids and the origins of
591 allotetraploids in *Dactylorhiza* (Orchidaceae). *Systematic Biology*, 69, 91-109.

592 Calevo, J., Gargiulo, R., Bersweden, L., Viruel, J., González-Montelongo, C., Rebbas, K.,
593 Boutafia L, Fay, M.F. (2021) Molecular evidence of species- and subspecies-level
594 distinctions in the rare *Orchis patens* s.l. and implications for conservation. *Biodiversity
595 and Conservation*, 30, 1293-1314.

596 Catchen, J., Amores, A., Hohenlohe, P., Cresko, W., Postlethwait, J. (2011) Stacks: building
597 and genotyping loci de novo from short-read sequences. *G3 – Genes, Genomes,
598 Genetics*, 1, 171-182.

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

601 Clark, L.V., Lipka, A.E., Sacks, E.J. (2019) polyRAD: Genotype calling with uncertainty from
602 sequencing data in polyploids and diploids. *G3 – Genes, Genomes, Genetics*, 9, 663-
603 673.

604 Clevenger, J., Chavarro, C., Pearl, S.A., Ozias-Akins, P., Jackson, S.A. (2015) Single Nucleotide
605 Polymorphism identification in polyploids: a review, example, and recommendations.
606 *Molecular Plant*, 8, 831-846.

607 De Gabriel Hernando, M., Fernández-Gil, J., Roa, I., Juan, J., Ortega, F., De la Calzada, F.,
608 Revilla, E. (2021) Warming threatens habitat suitability and breeding occupancy of
609 rear-edge bird specialists. *Ecography*, 44, 1191-1204.

610 Dixo, M., Metzger, J.P., Morgante, J.S., Zamudio, K.R. (2009) Habitat fragmentation reduces
611 genetic diversity and connectivity among toad populations in the Brazilian Atlantic
612 Coastal Forest. *Biological Conservation*, 142, 1560-1569.

613 Dufresne, F., Stift, M., Vergilino, R., Mable, B.K. (2014) Recent progress and challenges in
614 population genetics of polyploid organisms: an overview of current state-of-the-art
615 molecular and statistical tools. *Molecular Ecology*, 23, 40-69.

616 Fick, S.E., Hijmans, R.J. (2017) New 1 km spatial resolution climate surfaces for global land
617 areas. *International Journal of Climatology*, 37, 4302-4315.

618 Fitzpatrick, S.W., Bradburd, G.S., Kremer, C.T., Salerno, P.E., Angeloni, L.M., Funk, W.C.
619 (2020) Genomic and fitness consequences of genetic rescue in wild populations. *Current*
620 *Biology*, 30, 517-522.

621 Frankham, R. (2015) Genetic rescue of small inbred populations: meta-analysis reveals large
622 and consistent benefits of gene flow. *Molecular Ecology*, 24, 2610-2618.

623 Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., François, O. (2014) Fast and efficient
624 estimation of individual ancestry coefficients. *Genetics* 4, 973-983.

625 Frichot, E., François, O. (2015) LEA: An R package for landscape and ecological association
626 studies. *Methods in Ecology and Evolution*, 6, 925-929.

627 Godefroid, S., Piazza, C., Graziano, R., Buord, S., Stevens, A.-D., Aguraiuja, R., Cowell, C.,
628 Weekley, C.W., Vogg, G., Iriondo, J.M., Johnson, I., Dixon, B., Gordon, D., Magnanon,
629 S., Valentin, B., Bjureke, K., Koopman, R., Vicens, M., Venderborght, T. (2011) How
630 successful are plant species reintroductions? *Biological Conservation*, 144, 672-682.

631 Gutiérrez, E.E., Heming, N.M., Penido, G., Dalponte, J.C., Lacerda, A.C.R., Moratelli, R., de
632 Moura Bubadué, J., da Silva, L.H., Wolf, M.M., Marinho-Filho, J. (2019) Climate
633 change and its potential impact on the conservation of the Hoary Fox, *Lycalopex*
634 *vetulus* (Mammalia: Canidae). *Mammalian Biology*, 98, 91-101.

635 Ren, H., Jian, S., Liu, H., Zhang, Q., Lu, F. (2014) Advances in the reintroduction of rare and
636 endangered wild plant species. *Science China Life Sciences*, 57, 603–609.

637 Heming, N.M., Dambros, C., Gutiérrez, E. (2018) ENMwizard: advanced techniques for
638 Ecological Niche Modeling made easy. <https://github.com/HemingNM/ENMwizard>

639 Jombart, T. (2008) adegenet: a R package for the multivariate analysis of genetic markers.
640 *Bioinformatics*, 24, 1403-1405.

641 Kamvar, Z.N., Tabima, J.F., Grünwald, N.J. (2014) Poppr: an R package for genetic analysis of
642 populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2: e281.

643 Karbstein, K., Tomasello, S., Hodač, L., Dunkel, F.G., Daubert, M., Hörandl, E. (2020)
644 Phylogenomics supported by geometric morphometrics reveals delimitation of
645 sexual species within the polyploid apomictic *Ranunculus auricomus* complex
646 (Ranunculaceae). *Taxon*, 69, 1191-1220.

647 Karger, D.N., Conrad, O., Böher, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E.,
648 Linder, H.P., Kessler, M. (2017). Climatologies at high resolution for the earth's land
649 surface areas. *Scientific Data*, 4: 170122.

650 Kidane, Y.O., Steinbauer, M.J., Beierkuhnlein, C. (2019) Dead end for endemic plant species?
651 A biodiversity hotspot under pressure. *Global Ecology and Conservation*, 19: e00670.

652 Kuhn, M. (2019) caret: classification and regression training. [https://CRAN.R-](https://CRAN.R-project.org/package=caret)
653 [project.org/package=caret](https://CRAN.R-project.org/package=caret).

654 Liddell, E., Sunnucks, P., Cook, C.N. (2021) To mix or not to mix gene pools for threatened
655 species management? Few studies use genetic data to examine the risks of both
656 actions, but failing to do so leads disproportionately to recommendations for
657 separate management. *Biological Conservation*, 256: 109072.

658 Lespinas, F., Ludwig, W., Heussner, S. (2014) Hydrological and climatic uncertainties
659 associated with modeling the impact of climate change on water resources of small
660 Mediterranean coastal rivers. *Journal of Hydrology*, 511, 403-422.

661 López-Pujol, J., Orellana, M. R., Bosch, M., Simon, J., Blanché, C. (2007) Low genetic diversity
662 and allozymic evidence for autopolyploidy in the tetraploid Pyrenean endemic
663 larkspur *Delphinium montanum* (Ranunculaceae). *Biological Journal of the Linnean*
664 *Society*, 155, 211-222.

665 Maschinski, J., Albrectht, M.A. (2017) Center for plant conservation's best practice guidelines
666 for the reintroduction of rare plants. *Plant Diversity*, 39, 390-395.

667 McCormack, J.E., Huang, H., Knowles, L.L. (2009) Sky Islands. In: Gillespie, R.G., Clague, D.
668 (eds) *Encyclopedia of Islands*, University of California Press: Berkeley, CA Pp 839-843.

669 Meirmans, P.G., Van Tienderen, P.H. (2013) The effects of inheritance in tetraploids on
670 genetic diversity and population divergence. *Heredity*, 110, 131-137.

671 Meirmans, P.G., Liu, S., van Tienderen, P.H. (2018) The analysis of polyploid genetic data.
672 *Journal of Heredity*, 109, 283-296.

673 Meirmans, P.G. (2020) Genodive version 3.0: Easy-to-use software for the analysis of genetic
674 data of diploids and polyploids. *Molecular Ecology Resources*, 20, 1126-1131.

675 Muscarella, R., Galante, P.J., Soley-Guardia, M., Boria, R.A., Kass, J.M., Uriarte, M., Anderson,
676 R.P. (2014) ENMeval: an R package for conducting spatially independent evaluations
677 and estimating optimal model complexity for Maxent ecological niche models.
678 *Methods in Ecology and Evolution*, 5, 1198-1205.

679 Nei, M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press: New York: 1-514.

680 Pandit, M.K., Pocock, M.J.O., Kunin, W.E. (2011) Ploidy influences rarity and invasiveness in
681 plants. *Journal of Ecology*, 99, 1108-1115.

682 Paris, J.R., Stevens, J.R., Catchen, J. (2017) Lost in parameter space: a road map for stacks.
683 *Methods in Ecology and Evolution*, 8, 1360-1373.

684 Peery, M.Z., Kirby, R., Reid, B.N., Stoelting, R., Doucet-Ber, E., Robinson, S., Vsquez-Carillo,
685 C., Pauli, J.N., Palsbll, P.J. (2012) Reliability of genetic bottleneck tests for detecting
686 recent population declines. *Molecular Ecology*, 21, 3403-3418.

687 Phillips, S.J., Anderson, R.P., Schapire, R.E. (2006) Maximum entropy modelling of species
688 geographic distributions. *Ecological Modelling*, 190, 231-259.

689 Philipps, S.J., Anderson, R.P., Dudk, M., Shapire, R.E., Blair, M.E. (2017) Opening the black
690 box: an open-source release of Maxent. *Ecography*, 40, 887-893.

691 Ralls, K., Ballou, J.D., Dudash, M.R., Eldridge, M.D.B., Fenster, C.B., Lacy, R.C., Sunnucks, P. &
692 Frankham, R. (2018) Call for a paradigm shift in the genetic management of
693 fragmented populations. *Conservation Letters*, 11: e12412.

694 Ren, H., Jian, S.G., Liu, H.W., Zhang, Q.M., Lu, H.F. (2014) Advances in the reintroduction of
695 rare and endangered wild plant species. *Science China Life Sciences*, 57, 603-609.

696 Rochette, N.C., Catchen, J.M. (2017) Deriving genotypes from RAD-seq short read data using
697 Stacks. *Nature Protocols*, 12, 2640-2659.

698 Rousset, F. (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under
699 Isolation by Distance. *Genetics*, 145, 1219-1228.

700 Simon, J., Bosch, M., Molero, J., Blanch, C. (2001) Conservation biology of the Pyrenean
701 larkspur (*Delphinium montanum*): a case of conflict of plant versus animal
702 conservation? *Biological Conservation*, 98, 305-314.

703 UICN France, FCBN, AFB, MNHN (2018) La liste rouge des espces menaces en France –
704 Chapitre Flore vasculaire de France mtropolitaine. Paris, France.

705 Urban, M.C. (2015) Accelerating extinction risk from climate change. *Science*, 348, 571-573.

706 Van de Peer, Y., Mizrachi, E., Marchal, K. (2017) The evolutionary significance of polyploidy.
707 *Nature Review Genetics*, 18, 411-424.

708 Van de Peer, Y., Ahsman, T.-L., Soltis, P.S., Soltis, D.E. (2021) Polyploidy: an evolutionary and
 709 ecological force in stressful times. *Plant Cell*, 33, 11-26.

710 Vinogradov, A.E. (2003) Selfish DNA is maladaptive: evidence from the plant Red List. *Trends*
 711 *in Genetics*, 19, 609-614.

712 Wagner, N.D., He, L., Hörandl, E. (2021) Phylogenomic relationships and evolution of
 713 polyploid *Salix* species revealed by RAD sequencing data. *Frontiers Plant Sciences*, 11,
 714 1077.

715 Whiteley, A.R., Fitzpatrick, S.W., Funk, W.C., Tallmon, D.A. (2015) Genetic rescue to the
 716 rescue. *Trends in Ecology and Evolution*, 30, 42-49.

717 Závcská, E., Maylandt, C., Paun, O., Bertel, C., Božo, F., The STEPPE Consortium,
 718 Schönschwetter, P. (2019) Multiple auto- and allopolyploidisations marked the Pleistocene
 719 history of the widespread Eurasian steppe plant *Astragalus onobrychis* (Fabaceae).
 720 *Molecular Phylogenetics and Evolution*, 139, 106572.

721

722 Tables

723

724 **Table 1** Geographic coordinates (in °) and elevation (in m above sea level) of the sampling
 725 localities, sample size (N), observed (H_O) and expected (H_E) levels of heterozygosity,
 726 deviation from panmixia (G_{IS}), total number of alleles (A) and number of private alleles (A_p)
 727 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	42.277	1.688	2586	12	0.159	0.237	0.346	22 456	524
	Pedregosa									
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	42.283	1,574	2358	12	0.122	0.191	0.376	17 876	4,537
	Cadí									
					106	0.129	0.196	0.361		

728 Geographic coordinates are in WGS84.

729 Population genetic statistics were computed based on 5 095 SNPs. Statistically significant values are indicated

730 in bold ($p < 0.05$).

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

732

733 **Table 2** Current and future *Delphinium montanum* suitable area (in km²) as modelled and

734 predicted from Ecological Niche Modelling under different model selection criteria (LowAIC

735 and AUC) and thresholding methods (10th percentile of training presence, x10ptp and

736 Maximum training sensitivity plus specificity, mtss. Future predictions are reported for

737 several time periods (2011-2040, 2041-2070 and 2071-2100) and various Representative

738 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										

739

740 **Figures**

741

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

744

745 **Figure 2 A)** Principal Component Analysis (PCA) displaying the two first axes (PC1 and PC2)
746 representing 14.85% and 9.21% of the total genetic variance. PCA was computed based on
747 the 106 individuals of *D. montanum* genotyped at 5 095 SNPs whose colours represent
748 sampling localities. **B)** Values of the cross-entropy criterion for a number of clusters ranging
749 from $K = 1$ to 10 (10 sNMF runs each). The optimal number of K was found to be 6. **C)** Barplot
750 of ancestry coefficients obtained from sNMF for 106 individuals for $K = 2$ and $K = 6$, based on
751 5 095 SNPs.

752

753 **Figure 3** Pattern of Isolation By Distance (IBD) across the data set. Linearized G_{ST} values (*i.e.*
754 $\ln(G_{ST}/(1 - G_{ST}))$) are plotted against $\ln$ -geographical distances.

755 **Figure 4** Geographic areas holding suitable climatic conditions for *Delphinium montanum*
756 with projection under present climatic conditions (average for 1981-2010) and consensus
757 projection (from 5 Global Climate Models (CGMs): GFDL-ESM4, UKESM1-0-LL, MPI-ESM1-2-
758 HR, IPSL-CM6A-LR and MRI-ESM2-0), for various plausible climatic scenarios for years 2035
759 (average for 2011-2040), 2055 (average for 2041-2070) and three Representative
760 Concentration Pathways (RCPs): RCP 26, RCP 70 and RCP 8.5. The time period 2071-2100 is
761 not represented since the suitable area would have completely disappeared for RCPs 7.0 and
762 8.5. The Barplot displays current and future area values under various plausible climatic
763 scenarios. These values correspond to “LowAIC” model (“AUC” model being very similar).

Current

Species total suitable area

2011-2040

2041-2070