Glacial relict Sphagnum fuscum at the Pyrenean rear-edge: genetically depauperate and highly differentiated populations with evidence of a shift to monoicy

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Abstract

Cold-adapted species often persist at their rear-edge in isolated refugia, where they are especially vulnerable to climate change. These populations frequently harbour unique genetic lineages and local adaptations, making them critical for biodiversity conservation. However, fine-scale intraspecific genetic and ecological data are rarely integrated into conservation planning. For the locally threatened peat moss *Sphagnum fuscum* at its southern rear-edge in the Pyrenees, we aim to reveal its regional glacial legacy and reproductive adaptations to extreme fragmentation by studying its distribution, demography, genetic structure, and reproductive biology. Based on these findings, we aim to inform conservation strategies. We genotyped 109 shoots from seven Pyrenean populations using 16 nuclear microsatellite markers. Genetic patterns were analyzed using AMOVA, STRUCTURE, PCoA and UPGMA dendrograms based on Cavalli-Sforza and Edwards' chord distances. We also estimated population sizes and surveyed sporophyte production in the field. Pyrenean populations exhibited extremely low within-population diversity and complete genetic distinctiveness among them. Pyrenean genotypes shared a private allele distinctive from the central populations. Sporophyte production was widespread, although many populations were monoclonal, consistent with regional monoicy. Our results support that *S. fuscum* is a glacial relict in the Pyrenees. Post-glacial contraction explains its current genetic structure. A shift toward monoicy may reflect a local adaptation enhancing persistence under extreme fragmentation. Pyrenean populations likely constitute an evolutionary significant unit, which should be prioritised in conservation planning. Our results provide a basis for designing a genetic rescue by promoting gene flow among these isolated populations.

1. INTRODUCTION

Climate changes and associated shifts in species distribution have been recurrent phenomena throughout the Earth's history. In particular, Quaternary glacial-interglacial cycles (2.58 million years ago to the present) have driven repeated altitudinal and latitudinal shifts in species ranges, shaping the current distribution of biodiversity (Hewitt, 2000; 2004). In Europe, during the Last Glacial Maximum (LGM, ca. 21,000 years ago), temperate flora retreated to glacial refugia in the northern Mediterranean Basin, from where they expanded during the current interglacial (Taberlet et al., 1998; Hewitt, 2000; Eidesen et al., 2013). Conversely, cold-adapted species became widespread across periglacial landscapes that extended during the LGM between southern mountain ranges—such as the Pyrenees, Alps, and Balkans—and the northern ice sheet (Nève and Verlaque, 2009). Many of these cold-adapted taxa now exhibit arctic-alpine distributions, thriving at high latitudes and surviving in isolated populations within mountain refugia at lower latitudes (Varga and Schmitt, 2008). The latter, "rear-edge" populations, are geographically isolated from the species' main distribution and are often surrounded by unsuitable habitat.

Today, the biosphere is undergoing a biodiversity crisis, with extinction rates reaching unprecedented levels (Ceballos et al., 2015). Among the major drivers is anthropogenic climate change, which is already causing shifts in species ranges (Chen et al., 2011; Vitasse et al., 2021; Lenoir and Svenning, 2015) and the extinction of particularly vulnerable taxa or populations (Wiens, 2016). Many studies have attempted to predict how these rear-edge populations will respond to this rapid climate change (Jiménez-Alfaro et al., 2016; Vilà-Cabrera et al. 2019). According to the Centre-Periphery Hypothesis (CPH), rear-edge populations are prone to extinction as they remain confined in small, fragmented and isolated enclaves where there is a great degree of environmental stress (Brown, 1984; Safriel et al., 1994). In addition, they harbor small effective population sizes and a reduced intra-population genetic variation, but a high inter-population genetic diversity (Safriel et al., 1994; Jiménez et al., 2010). These factors put them at risk from demographic stochasticity and reduce their potential to adapt to new conditions. Indeed, the fossil record shows that extinction commonly occurred at rear-edge populations, particularly during periods of rapid climate change (Davis and Shaw, 2001).

However, the performance of rear-edge populations can deviate from the biogeographical CPH predictions because, as reported in several studies, this hypothesis may overlook the effects of ecological interactions and the microclimate heterogeneity, which could buffer macroclimatic trends, specific life-traits, and local adaptations (Hampe & Jump, 2011; Vilà-Cabrera et al., 2019). The result is that many rear-edge populations are supposed to be stable for the long term despite being close to their climatic tolerance limit, becoming climate relicts, remnants of formerly widespread taxa that now persist in stable refugia (Woolbright et al., 2014). These climate relicts may have been exposed to severe selective pressures, developing remarkable local adaptations, such as phenological shifts and increased physiological tolerance (Hampe & Petit, 2005). In addition, bottlenecks and genetic drift have probably driven the evolution of relicts (Woolbright et al., 2014). Both processes, which are not exclusive, have led to patterns of genetic diversity with high levels of variation and uniqueness (Safriel et al., 1994). Therefore, rear-edge populations on southern refugia (e.g., the Pyrenees, Alps, and the Balkans) might harbor more diversity than populations at the center of their area of distribution (Nève & Verlaque, 2009). If this is the case, their uniqueness and antiquity renders them disproportional relevance for the conservation of the genetic diversity, phylogenetic history, and evolutionary potential of species (Hampe & Petit, 2005). Additionally, understanding rear-edge populations is a key factor to improve predictions of the species range shifts driven by ongoing climate change (Thuiller et al., 2008; Hampe and Jump, 2011; Woolbright et al., 2014).

The rear-edge populations are not distributed equally across the geography and landscape. In particular, mires (peat-forming wetlands) are habitats rich in relicts (Hampe and Jump, 2011; Hájková et al., 2015; Jiménez-Alfaro et al., 2016). Their harsh abiotic conditions (anoxia, extreme pH, and nutrient-poor soils) may limit the encroachment of competitors, allowing mire specialists to persist, leading to the creation of biotically limited relicts (Hampe and Jump, 2011). In addition, in a fine-scale, mires act as climatic buffers generating more stable microclimatic conditions (Fernández-Pascual et al., 2015). Despite their resilience, the current dramatic global warming and the reduction of summer precipitation—which will be of particular significance in the Mediterranean Basin (Liu et al., 2022)—may affect the hydrological conditions of mires. A decline in the water table in mires would threaten them, especially those that occur at the rear-edge of the habitat range (Keith et al., 2014), where most of the relicts occur.

Bogs are a specific type of mire characterized by being ombrotrophic, i.e. fed only by precipitation rather than groundwater (minerotrophy) (Lappalainen, 1996; Joosten and Clarke, 2002). In temperate, boreal, and subarctic regions, the vegetation of bogs is dominated by *Sphagnum* mosses, also known as peat mosses. This genus, of cosmopolitan distribution (Michaelis, 2019), has an important role as an ecosystem engineer due to its ability to create an acidic, nutrient-poor, and anoxic habitat that is unsuitable for most other plants, which at the same time improves its fitness outcompeting for light (Breemen, 1995). This habitat's extreme conditions boost its potential as a sink of biotically limited relicts (Slowinski, 2014). Some *Sphagnum* species form clonal colonies known as hummocks—compact, elevated clumps of moss—which are often composed of one or very few genets (Gunnarson et al., 2007; Shaw et al., 2023). Hummock formation is particularly common among some ombrotrophic species, as it helps them remain above the water table in nutrient-poor, acidic environments (Daniels and Eddy, 1990). Bogs cover vast areas of boreal and subarctic zones, but they are scarce and fragmented in southern Europe, primarily confined to high-altitude mountain systems, where they may be reduced to isolated *Sphagnum* hummocks embedded in fen matrices (minerotrophic mires).

Sphagnum fuscum (Schimp.) Klinggr. (subg. *Acutifolia*) is a notable example of a *Sphagnum* species capable of persisting in these marginal environments. It has a wide circumboreal distribution in the Northern Hemisphere but is also found in isolated populations extending into the temperate and sub-meridional zones, restricted to high-elevation mountain ranges in the latter (Kyrkjeeide et al., 2015). *Sphagnum fuscum* is a haploid species that typically forms high and compact hummocks (20000–60000 shoots/m^2), which stand above the water table level (Supplementary Fig. 1). It can reproduce vegetatively, although it frequently produces sporophytes in high densities (Daniels and Eddy, 1990; Sundberg, 2002; Gunnarsson et al., 2007). Although traditionally described as dioicous, *S. fuscum* has also been reported to be polyoicous (also known as polygamous), i.e., populations may be either monoicous or dioicous (Pujos, 1994).

In the Pyrenees, *S. fuscum* reaches one of the southernmost rear-edges, which is highly isolated from the core distribution area. Other isolated localities are known from the French Massif Central (230 km) and a single population in the Cantabrian Mountains in Spain (370 km). There are only 10 known populations in the Pyrenees that inhabit mire systems at margins of alpine lakes, rivulets or springs, mostly above 2000 m. Due to its scarcity in this region, *S. fuscum* has been assessed as Vulnerable in the old French administrative region of Midi-Pyrénées (Sánchez et al., 2015), as Endangered in Catalonia (Sáez et al., 2019), and as Critically Endangered in the Iberian Peninsula and Spain (Cecilia et al., 2007; Garilleti and Albertos, 2012). However, it lacks regional assessment across political borders, which would be biogeographically more coherent. Apart from the currently known occurrences in the Pyrenees, many crucial aspects for the conservation of this species in the region remain largely unexplored—including population size, reproductive capacity, genetic diversity, phylogenetic relationships with populations from other regions, and recent evolution associated with glacial history.

As *S. fuscum* is likely a climate relict in the Pyrenees and is listed as threatened in this region, a deeper understanding of its ecology and evolutionary history is of significant interest for both species and site-specific conservation. This study aims to assess the conservation status of *S. fuscum* in the Pyrenees and explore the relic origin of its extant populations by integrating novel genetic and demographic data. Specifically, our objectives are to: (1) reveal the genetic structure of *S. fuscum* in the Pyrenees; (2) compare the genetic diversity of Pyrenean populations with that of central regions of the range (Canada and Norway); (3) study the occurrence of sexual reproduction in the Pyrenean populations; (4) infer the recent evolutionary history of Pyrenean populations; (5) update the distribution range and estimate the abundance of *S. fuscum* in the Pyrenees; and (6) integrate the previous points to address science-based conservation strategies for the Pyrenean populations of this species. Beyond its species-specific focus, this study aims to emphasize the broader need for detailed ecological and genetic assessments of rear-edge populations, which are often overlooked despite their high conservation value and unique evolutionary trajectories.

2. METHODS

2.1 Sampling sites

Between 2019 and 2021 we sampled seven (out of ten) populations of *S. fuscum* in the Pyrenees (Table 1). This selection covers the maximum geographical and altitudinal amplitude of its regional distribution (Fig. 1). At least one shoot was collected from every hummock occurring at Cauterès (CAU), Coma de Vaca (CDV), Era Planhòla (EPL), Ferreroles (FER), Formiguera (FMP), and Plan deth Horn (PDH). For Estibèra (EST), which is the largest population, a sample of 28 hummocks were randomly selected. A total of 109 shoots were dried and preserved at room temperature.

Table 1

Sphagnum fuscum populations in the Pyrenees *Site*, toponym; *Code*, abbreviation of the site; *First report*, year of the first report for the site; *Author(s)*, author(s) and reference of the first report; *Elevation*, metres above sea level (m); *Number of hummocks*, total number of hummocks at each site, that can be a proxy of the population size ([a] counts on original report; [b] own counts); *Protected Area* (PA), name of the PA; *UICN Management Category*, category of protection of the PA according to the IUCN System (UNEP-WCMC and IUCN, 2022); *Country*, State. *Unknown exact coordinates, it might be the same locality as BGO. Nhèuvielha, Pessons, and Desert del Carlit are considered metapopulations composed of different population nuclei.

Site	Code	Author(s)	Elevation (m)	Number of hummocks	Natural Protected Area	Management Category	Country	Latitude	Longitude
Val de Cauterès	CAU	Hugonnot, in Corriol, 2004	1620	1[a, b]	Parc National des Pyrénées	II	France	42.844012	-0.167969
Nhèuvièlha (Estanh Cap de Long)	ECL	Corriol, 2004	2180	3-7[a]	Parc National des Pyrénées	II	France	42.862814	0.178369
Nhèuvièlha (Varètja)	VAR	Corriol, 2004	2220	11[a]	Aire d'adhésion du Parc National des Pyrénées	V	France	42.869081	0.150478
Nhèuvièlha (Estibèra)	EST	Gauthier, 1990	2280	43[b]	Réserve Naturelle Nationale du Néouvielle	Unknown	France	42.849262	0.163702
Nhèuvièlha (Gorguet)	GOR	Chouard (1929) in Corriol, 2004	2220	NA	Réserve Naturelle Nationale du Néouvielle	Unknown	France	42.817769	0.116675
Plan deth Horn	PDH	Pérez-Haase *et al.*, 2011	1970	20[b]	Site of Community Interest Alt Pallars	Unknown	Spain	42.777337	0.903436
Era Planhòla	EPL	Gauthier, 1991	1840	5[b]	Parc Nacional d'Aigüestortes i Estany de Sant Maurici	V	Spain	42.646006	0.932356
Comapedrosa	CMP	S. Riba, comm. pers.	2250	7[b]	Parc Natural Comunal de les Valls del Comapedrosa	Unknown	Andorra	42.580886	1.442205
Ferreroles	FER	Lazare *et al.*, 2005	2170	15[b]	Non-protected	Non-protected	Andorra	42.601465	1.56029
Pessons (Els Estanys)	EES	Lazare *et al.*, 2005	2410	NA	Ramsar Site and UNESCO's Cultural Landscape of Vall del Madriu-Perafita-Claror	Unknown	Andorra	42.487072	1.654789
Pessons (Els Collells)	ECO	Lazare *et al.*, 2005	2325	10[b]	Non-protected	Non-protected	Andorra	42.517692	1.696025
Formiguera	FMP	Hugonnot, 2012	1455	15[b]	Parc naturel régional des Pyrénées Catalanes	V	France	42.626449	2.117656
Desert del Carlit (Serra de les Llebres*)	LLE	Chevallier *et al.*, 2005	2100–2200?	NA	Parc naturel régional des Pyrénées Catalanes	V	France	42.554042	1.985058
Desert del Carlit (Basses d'en Gombau)	BGO	A. Pérez-Haase, pers. obs.	2140	15[b]	Parc naturel régional des	V	France	42.557563	1.970705

Site	Code	Author(s)	Elevation (m)	Number of hummocks	Natural Protected Area	Management Category	Country	Latitude	Longitude
					Pyrénées Catalanes				
Coma de Vaca	CDV	Pladevall *et al.*, 2023	2180	13[b]	Parc Natural de les Capçaleres del Ter i del Freser	V	Spain	42.398786	2.203188

2.2 Distributional, demographic and reproductive characteristics

To update the distribution range of *S. fuscum* we performed extensive surveys along the Pyrenees to find more populations in suitable sites, complemented by an in-depth review of the existing literature. To delineate the extent of the distribution range, we employed the Extent of Occurrence (EOO) metric as defined by the IUCN (2012). The EOO was estimated using a minimum convex polygon — the smallest polygon in which no internal angle exceeds 180° and that encompasses all known occurrence sites — generated with the online tool GeoCAT (https://geocat.iucnredlist.org/), based on confirmed populations.

We recorded the number of hummocks in each population as a proxy for population size, following the approach of Garilleti and Albertos (2012). For each hummock, we measured its surface area, and its height above the water table. We also assessed sporophyte presence at the population level.

2.3 DNA extraction and genotyping

We used the upper 1.5 – 2.5 cm of the dried shoots (capitulum and first branches) for DNA extraction, which was carried out using the E.Z.N.A. SP Plant DNA Kit (https://omegabiotek.com/) following the manufacturer's instructions. Extractions were performed at the laboratories of the Botanical Institute of Barcelona (IBB).

For genotyping, we used sixteen microsatellites (Supplementary Table 1) that have already been proven to perform successfully for *Sphagnum fuscum* (Shaw et al., 2008; Stenøien et al., 2011; Kyrkjeeide et al., 2015, 2016). Electrophoresis-based genotyping was performed at CD Genomics (https://www.cd-genomics.com/), using GeneMapper™ Software 5 (Applied Biosystems).

2.4 Genetic analysis

We evaluated the levels of genetic diversity of the Pyrenean populations by calculating several metrics at the population level: the proportion of polymorphic loci (*PPL*), number of alleles per locus (*A*), number of multilocus genotypes (hereafter MLGs), number of private alleles per MLG, and haploid diversity per population (*H*). We computed all these parameters using GenAlEx v. 6.5 (Peakall and Smouse, 2012).

To examine the genetic structure of the Pyrenean populations, we applied multiple analytical approaches. First, we conducted a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al. 1992) to partition genetic variation between and within populations. We ran the AMOVA in GenAlEx, with 999 permutations.

Next, we carried out a Bayesian cluster analysi using Structure v. 2.3.4 (Pritchard et al., 2000), implementing an admixture model with correlated allele frequencies. For each *K* value ranging from 1 to 10, we conducted 10 replicate runs, with a burn-in of 100,000 steps followed by 500,000 iterations per run. We identified the most likely number of clusters (K) using the ad hoc statistic ΔK (Evanno et al., 2005).

To further explore genetic relationships, we calculated a pairwise dissimilarity matrix based on Cavalli-Sforza and Edwards' chord distance (D_C) among the Pyrenean MLGs using "hierfstat" R package v. 0.5–11. We visualized this matrix as a heatmap and applied hierarchically clustering with a UPGMA dendrogram, via the R package "pheatmap" v.1.0.12 (Kolde, 2019) in R v. 4.3.1.

At a broader geographical scale, we examined the genetic diversity of *S. fuscum* by performing a Principal Coordinates Analysis (PCoA) based on the D_C dissimilarity matrix. This analysis included additional genotypes from Canada and Norway (available in Kyrkjeeide et al., 2023). We calculated the dissimilarity matrix and the PCoA using the "hierfstat" R package v. 0.5–11. To integrate the data from Kyrkjeeide et al. (2023) with our own dataset, we manually aligned microsatellite loci. We excluded three polymorphic loci (4, 10, and 28) due to missing data in the Norwegian and Canadian samples (Supplementary Table 2). Conversely, we incorporated locus 56, which is monomorphic in the Pyrenean populations but polymorphic in the other regions.

3. RESULTS

3.1 Distributional and demographic characteristics and sexual reproduction

The extensive surveys we performed along the Pyrenees during the period 2019–2021 only allowed us to locate two additional populations: Coma de Vaca (for more details about the discovery; please see Pladevall-Izard et al., 2023), in the Spanish side of the Pyrenees, and Basses d'en Gumbau (BGO), in the French side (Fig. 1; Table 1). The ten populations known up-to-date are located in central and eastern Pyrenees, four in France (two in the Hautes-Pyrénées Department and two in the Pyrénées-Orientales Department), four in Andorra and three in Spain (all in Catalonia), in an altitudinal range between 1455 m and 2410 m (Table 1). The EOO of *S. fuscum* in the Pyrenees was 3,000.363 km^2.

Sphagnum fuscum formed small populations, typically comprising no more than 15 hummocks per site (Table 1). We estimated less than 200 hummocks across the Pyrenees, based on a median of 11 hummocks per population in sites lacking direct censuses. The median surface area of hummocks was 0.75 m² although some reached substantially larger dimensions, up to 12 m² in Ferreroles. The most common growth form was on hummocks elevated 2–60 cm above the water table (Supp. Figure 1). Most hummocks exhibited an ombrotrophic tendency, although some lower hummocks were found near the water table and likely thrived under minerotrophic conditions.

Sporophyte production was frequent in the Pyrenean populations and was observed in five of the seven sites studied (Fig. 2), including Cauterès, where only a single hummock was present. Moreover, sporophyte production within populations varied among hummocks, with some showing no signs of sexual reproduction, whereas others produced sporophytes at varying densities.

3.2 Genetic diversity

Of the 16 microsatellite loci, nine (56.25%) were polymorphic for the Pyrenean populations, yielding 28 alleles (Table 2). Locus 4 was particularly variable, with nine alleles, while the remaining polymorphic loci each harbored two to three alleles each. More than half of the alleles (54%) were private to a single population.

Table 2. Pyrenean *Sphagnum fuscum* genotypes based on nine variable microsatellites *Population,* population codes as in Table 1; *MLG*, code for every different multi-locus genotype; *n*, is the number of gametophytes that belong to each MLG; *Loci*, are the 9 variable microsatellites for *S. fuscum* Pyrenean populations and its numeration corresponds to the originally published nomenclature (Supplementary Table 1). Private alleles are highlighted in green

Population	*MLG*	*n*	Locus 4	Locus 7	Locus 9	Locus 10	Locus 20	Locus 22	Locus 28	Locus 30	Locus 68
CAU	CAU	3	189	188	182	219	295	103	239	131	228
EST	EST1	6	199	188	184	231	295	103	239	127	228
	EST2	21	187	188	184	231	289	103	239	131	228
	EST3	1	201	188	184	231	295	103	239	127	228
PDH	PDH1	10	185	188	186	231	292	103	231	127	228
	PDH2	8	191	192	184	231	292	103	229	131	228
EPL	EPL	11	195	192	184	219	292	97	239	131	228
FER	FER	17	193	188	184	231	292	103	239	131	228
FMP	FMP	17	197	192	184	231	292	103	239	131	228
CDV	CDV	10	197	188	184	231	295	103	239	131	232

We identified 10 MLGs, all private to their respective populations; thus, no MLGs were shared among populations (Table 2). Five populations were monoclonal, each composed of a single MLG. Only two populations (Plan deth Horn and Estibèra) contained more than one MLG (two and three MLGs, respectively). In Estibèra (EST), the multilocus genotype EST3 was a singleton that differed from EST1 by a single tandem repeat at the highly variable locus 4. Formiguera was the only population lacking private alleles. The haploid diversity was obviously null in the monoclonal populations. In Plan deth Horn, the two MLGs occurred in a 10:8 ratio, yielding higher diversity than in Estibèra, where the three MLGs were present but one (EST1) predominated (21:6:1) (Table 3).

Table 3
Diversity Parameters. *Population*, population codes as in Table 1; *H*, haploid diversity per population; *MLG*, code for every different multi-locus genotype; *n*, number of gametophytes that belong to each MLG; *Private alleles*, number of alleles that are unique to each respective MLG. At the bottom, mean ± standard deviation of *H* and the number of private alleles per population.

Population	*H*	MLG	*n*	Private Alleles
CAU	0	CAU	3	2
EST	0.13 ± 0.06	EST1	6	1
		EST2	21	2
		EST3	1	1
PDH	0.27 ± 0.09	PDH1	10	3
		PDH2	8	2
EPL	0	EPL	11	2
FER	0	FER	17	1
FMP	0	FMP	17	0
CDV	0	CDV	10	1
Mean ± sd	0.057 ± 0.02			1.5 ± 0.85

3.3 Genetic structure

The AMOVA showed that most genetic variation (81%) was explained by differences among populations, rather than within them (19%) (Φ_{PT} = 0.808, p = 0.001).

Using STRUCTURE, Bayesian clustering analysis revealed that the most likely number of genetic clusters (K) was nine (Fig. 1), as supported by the Evanno ΔK method (Supplementary Fig. 2). These clusters corresponded well with the nine main MLGs, with EST3 grouping with EST1.

The UPGMA dendrogram based on Cavalli-Sforza and Edwards' chord distances grouped the MLGs into two main clusters: one containing CAU, CDV, FER, EST1, EST2 and EST3 and the other including EPL, FMP and PDH2 (Fig. 3). Notably, PDH1 was sister to both groups and did not cluster with PDH2 despite both MLGs were found in the same population.

The resolution of Pyrenean genotypes decreased when aligned with the dataset from Kyrkjeeide et al. (2023), reducing the number of distinct MLGs from 10 to 8 (i.e., EST1 merged with EST3, and FMP with PDH2). The PCoA's first two axes accounted for 65% of the total variance in the chord distance matrix (Fig. 4). Pyrenean, Norwegian, and Canadian genotypes were clearly separated in the ordination plot. The primary axis (PC1) differentiated the Pyrenean populations from the Norwegian-Canadian group. Locus 56 contributed strongly to this separation, being fixed at 206 bp in the Pyrenees, while alleles in Canadian and Norwegian populations ranged from 209 to 215 bp. Interestingly, a single Norwegian MLG (sample 211) clustered near the Pyrenean group, sharing similar negative PC1 scores. PC2 further separated Norwegian from Canadian samples, with the former exhibiting higher values.

4. DISCUSSION

The findings of this study provide a comprehensive understanding of the genetic structure and reproductive biology of *Sphagnum fuscum* in the Pyrenees. By integrating population genetic structure with reproductive observations, we discuss how glacial dynamics and a shift in the sexual system may have shaped the current distribution and genetic uniqueness of these populations. These insights are particularly relevant for understanding the species persistence at this rear-edge and for informing conservation strategies.

4.1 Genetic diversity and structure

On a large geographic scale, Pyrenean populations are genetically distinct from those in Norway and Canada, showing a fixed private allele, and would therefore be considered as an independent evolutionarily significant unit (ESU). This is another evidence that rear-edge populations of plant species harbour unique genetic lineages, both in the Pyrenees (de Lafontaine et al., 2013; López-Pujol et al., 2013) and

elsewhere (e.g., Gugger et al., 2011, Erichsen et al., 2018; Worth et al., 2021; Balao et al., 2024). However, broader phylogeographic analyses with a more expanded sampling will be needed to assess the global uniqueness of the Pyrenean lineage and to investigate whether similar patterns occur in other rear-edge areas within the entire distribution range of *S. fuscum* Interestingly, the Pyrenean ESU as a whole holds a notable levels of genetic diversity (mostly to differences among populations; see below), as indicated by the wide area occupied by Pyrenean MLGs in the PCoA ordination.

Sphagnum fuscum populations in the Pyrenees exhibit extremely low intrapopulation genetic diversity and strong geographic structuring, with each population harbouring unique MLGs. Only a few studies have assessed the genetic structure of endangered *Sphagnum* species. Terracciano et al. (2012) worked on four populations of *Sphagnum palustre* in the Italian Peninsula (also a rear-edge area for this species) and found that 82% of genetic variation was partitioned among populations according to an AMOVA—remarkably similar to the 81% observed in our Pyrenean populations. In contrast, Scandinavian populations of *Sphagnum angermanicum* showed only 6% variation among populations (Gunnarsson et al., 2005)—what reflects a leading-edge dynamic, which is a radically different biogeographical context. As proposed by Stenøien et al. (2011), S. *angermanicum* populations in Northern Europe may have originated from relatively recent (< 40,000 years) west-to-east colonisation events from North America. Low genetic diversity within and among populations is expected in such leading-edge dynamics due to strong founder effects (Hewitt, 1999, 2000). On the other hand, *S. fuscum* (this work) and *S. palustre* (Terracciano et al.,2012) show higher among-population differentiation in the Mediterranean Basin rear-edge (Italy, the Pyrenees), which is consistent with the persistence of small and isolated populations.

Within-population genetic diversity in the Pyrenean range of *S. fuscum* is critically low—a concerning finding according to the small population paradigm (Ellstrand and Elam, 1993; Frankham, 1996). This outcome was not entirely unexpected, given the high degree of habitat fragmentation and the small size of populations. Similar patterns of reduced diversity have been reported in other peatland mosses with fragmented distributions, such as *Hamatocaulis vernicosus* (Manukjanová et al., 2020) and *Scorpidium revolvens* (Kophimai et al., 2014). Given this genetic landscape, we recommend treating each unique MLG—except for EST1 and EST3, which could be considered as a single unit according to the STRUCTURE analysis—as a separate conservation unit to preserve the full genetic diversity of the Pyrenean ESU.

4.2 Glacial legacy

Our results also provide insights into the evolutionary processes that led to the current situation of *S. fuscum* in the Pyrenees. The clear genetic distinctiveness at a broader scale suggests that *S. fuscum* has persisted in the Pyrenees over long timescales, likely through altitudinal shifts during successive glacial cycles, in prolonged isolation from other lineages.

The most plausible explanation for the current genetic structure of the Pyrenean species range is post-Local Last Glacial Maximum (post-LLGM, from 20 ka to the ca. 11 ka; Calvet et al., 2011) altitudinal upwards migration, consistent with trailing-edge dynamics. We suggest that these populations were established after the retreat of the main glaciers, as the climatic conditions in the Pyrenean lowlands—where peripheral populations may have persisted during the LLGM—became increasingly unsuitable for the species. The current strong genetic differentiation among populations, coupled with low intrapopulation diversity, may be explained by several independent, single-spore colonisation events from these ancient, now-extinct source populations (that would have been located at a lower altitude). A similar pattern of genetic structure has been reported for *Asplenium septentrionale* on isolated erratic boulders, where genetically uniform populations are thought to have originated through single-spore founder events, although this case is not associated with a trailing-edge context (Hepenstrick et al., 2022).

Alternative hypotheses, such as the nunatak hypothesis—i.e., the persistence of populations in unglaciated patches within a glacial landscape (Schneeweiss and Schönswetter 2011)—suggest that the actual populations have remained static, or nearly so, in their present locations during the LLGM, and possibly during earlier glacial maxima. However, this scenario appears less likely, as the current sites were covered by active glaciers during the LLGM (except for Formiguera; Calvet, 2004). Nevertheless, we may be overly confident in dismissing this hypothesis, as long-term persistence in very small suitable patches could equally account for extremely low intrapopulation diversity and highly differentiated populations (Rota et al., 2024).

Another alternative hypothesis is that *S. fuscum* would have recently colonised the studied area from outside the Pyrenees. This hypothesis is less parsimonious, as the patterns of high genetic differentiation among populations found by us would require either (1) multiple independent colonisation events, which seems unlikely or (2) a rapid post-colonisation genetic differentiation, which appears even less likely, A preliminary Mantel test between pairwise D_c and geographical distances did not support a pattern of isolation-by-distance in the Pyrenees, further contradicting the expectation of gradual post-colonisation divergence.

4.3 Sexual system

A particularly striking finding is the apparent monoicy of Pyrenean populations. Although we did not find gametangia, we inferred monoicy based on the presence of sporophytes, even in monoclonal populations. For example, we observed sporophytes in CAU, where only a single hummock occurs. Besides, the absence of sporophytes in Pessons and Desert del Carlit should not be taken as a definitive lack of sexual reproduction or dioicy in these sites, as sporophyte production can fluctuate over years depending on multiple environmental stimulus (Sundberg, 2002). Intrapopulation variation of sporophyte production exists among hummocks, so variation is also expected between populations.

This finding supports Pujos's (1994) earlier assertion that *S. fuscum* is a polyoicous species. However, this author only mentioned this in passing and did not specify the region in which his observation was made. As he studied populations in both Canada and the Pyrenees, it is plausible that the monoicous condition was observed in the latter.

However, the alternative hypothesis of sporophytes arising from hybridization with nearby *Sphagnum* species—particularly within subg. *Acutifolia*—cannot be ruled out. Although it has not been reported for *S. fuscum*, some species of subg. *Acutifolia* exhibit a certain degree of compatibility (Cronberg and Natcheva, 2002; Natcheva and Cronberg, 2007). However, the genotyping results—from gametophytes only—show no evidence of effective introgression [as we performed a preliminary PCoA with other species of subg. *Acutifolia* from Kyrkjeeide et al. (2023) and our samples clustered with *Sphagnum fuscum*]. Moreover, the morphological identification does not raise any doubts in this regard. In addition, the generalized and often high-density sporophyte production strongly suggests the regular sexual reproduction within *S. fuscum.*

From an evolutionary perspective, monoicy could represent an important adaptive trait for *S. fuscum* in the Pyrenees (McDaniel and Perroud, 2012). If self-compatible, monoicy enhances selfing, thereby increasing the chances of long-distance dispersal via spores—structures more specialized compared to gametophyte fragmentation. This reproductive strategy becomes particularly advantageous in conditions where the likelihood of both sexes co-occurring is minimal due to the small population sizes—situations in which dioicy might fail (Patiño, 2018). Within the previously proposed post-glacial evolutionary scenario, it is plausible that this trait was positively selected over dioicy—if dioicy was indeed the ancestral state—during the altitudinal upwards migration to the current refugia or in earlier altitudinal shifts. Although ecologically reasonable, underlying mechanisms for such transition remain unclear. In this case, the classical polyploidy hypothesis can be ruled out, given the presence of a single allele per locus. Unraveling the genomic basis of this shift could offer new insights into sexual system evolution in bryophytes.

4.4 Conservation implications

The extremely small size of most Pyrenean populations makes them highly vulnerable to stochastic events and local extinctions. We recommend implementing intensive demographic monitoring to track trends, identify major threats (e.g., overgrazing; Pérez-Haase, 2019), and enable timely in situ interventions. Monitoring should include hummock counts, surface measurements, assessments of reproductive status, and evaluations of desiccation impacts. During our extensive sampling, we observed that the primary threat to conservation is cattle overgrazing. However, due to unusually long dry spells in recent summers and the likelyhood of their recurrence in the future, we expect desiccation to cause mortality at the tops of the hummocks.

In addition, we advocate for demographic rescue through population reinforcements to reverse the precarious demographic status, particularly at sites showing signs of hummock degradation. Propagating Pyrenean MLGs *ex situ* from minimal material may not only support reinforcement efforts but also safeguard their genetic identity against potential local extinctions. While substantial experience exists in *Sphagnum* propagation—especially in the context of large-scale peatland restoration in the boreal regions—these methods have been developed under ecological conditions quite different from those of the Pyrenean mires (Caporn, 2018; Rochefort and Lode, 2006). Pyrenean mires are smaller and host other endangered species, and are further limited in the availability of suitable microhabitats for the establishment of *S. fuscum.* These constraints make population reinforcement a considerable technical challenge.

From a genetic conservation perspective, we stress the importance of preserving the distinctiveness of the Pyrenean populations. The distinct genetic pool and the evidence of monoicy in the Pyrenees may reflect linked evolutionary processes. We suspect that this shift in the sexual system is related to chromosomal variants that increase the risk of outbreeding depression if divergent genotypes are introduced (Frankham et al., 2011). In the absence of robust ecological, genomic, and fitness data, any attempt to introduce extra-Pyrenean genetic material is considered premature and potentially harmful.

Facilitating gene flow among Pyrenean populations may be a preferable form of genetic rescue, even through assisted colonization of suitable locations. Based on the small population paradigm, this type of genetic rescue has shown success in increasing population size and vigour in various taxa (Frankham, 2015). However, in the case of clonal organisms such as *Sphagnum fuscum*, the goal would not merely be to establish multiple MLGs in close proximity—since hummocks may vegetatively persist over long timespans—but to promote effective crossing between MLGs. If successful, this could yield sexually produced diverse offspring with potentially higher fitness. Nevertheless, we

have specific concerns regarding this scenario, as in Estibèra and Plan deth Horn—sites where more than one MLG coexists and sporophytes have been observed—no evidence of recombinant offspring was found. Several plausible explanations could account for this: physical distance between hummocks of different MLGs, increased likelihood of inbreeding due to monoicy, low frequency of low-frequency MLGs (e.g., in Estibèra), genetic incompatibilities between MLGs, or low probability of successful establishment of new colonies from spores on a local scale (Gunnarson et al., 2007). However, this local limitation does not necessarily contradict the hypothesis that spores have played a key role in long-distance dispersal (see 4.3), as these processes operate at different spatial and temporal scales.

Given the evolutionary uncertainties involved in a genetic rescue, we argue that any attempt should be approached as a controlled experiment. In the absence of robust guarantees regarding the effectiveness and monitorability of the genetic rescue programme, we advocate for a precautionary approach—i.e., favoring population reinforcement using local genotypes.

Fortunately, the vast majority of known *S. fuscum* populations in the Pyrenees are located within protected natural areas. Implementing genetic rescue strategies such as those discussed here, however, would require substantial coordination between conservation authorities across national boundaries (Andorra, Spain, and France). Recent initiatives (e.g., Interreg POCTEFA FLORALAB, FLORALAB+) offer a promising framework for such cooperation. We strongly support such collaborative approaches, which are essential for the effective conservation of biodiversity in a biogeographic region that clearly transcends administrative borders.

STATEMENTS & DECLARATIONS

Competing Interests.

The authors declare no competing interests.

Data accessibility.

All data are available in the tables and supplementary information.

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Author Contribution

JCuscó-B was responsible for data analysis, laboratory work, and manuscript writing. NG-J advised on the design and interpretation of the molecular analyses. JL-P advised on the design of the study’s molecular component, and contributed to the review and editing of the manuscript. EP-I significantly contributed to field sampling, and the review and editing of the manuscript. AP-H (corresponding author) coordinated the project, was responsible for its conceptual design, participated in field sampling, oversaw laboratory work and data analysis, and contributed to manuscript writing.

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Figures

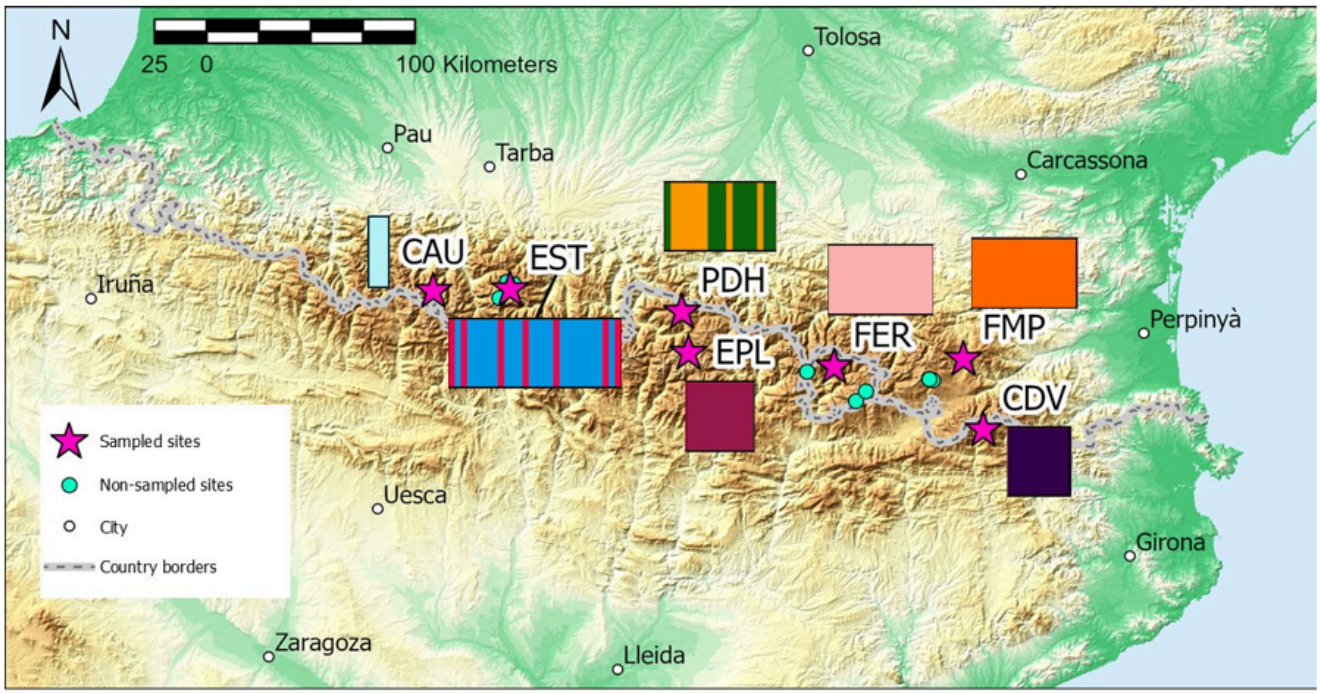

Figure 1

Sphagnum fuscum distribution map in the Pyrenees. Purple stars correspond to the sampling sites (Table 1). The rectangles show the assignment of gametophytes (columns) to the clusters defined by STRUCTURE analysis (best *K*= 9). The turquoise blue dots indicate sites that were not sampled for genotyping (site codes omitted for clarity). From east to west: Desert del Carlit (LLE and BGO), Pessons (EES and ECO), and Comapedrosa (CMP) and two sites from Nhèuvielha (GOR and VAR, close to the sampled EST).

Figure 2

Sphagnum fuscum hummock from Estibèra showing high density of sporophytes Numerous reddish, subspherical capsules (sporophytes) are visible, most of which have already shed their calyptra. The sporophytes are elevated by pseudopodia (gametophyte structures) embedded within pale green perichaetial leaves, which contrast sharply with the typically brown capitula of *S. fuscum*.

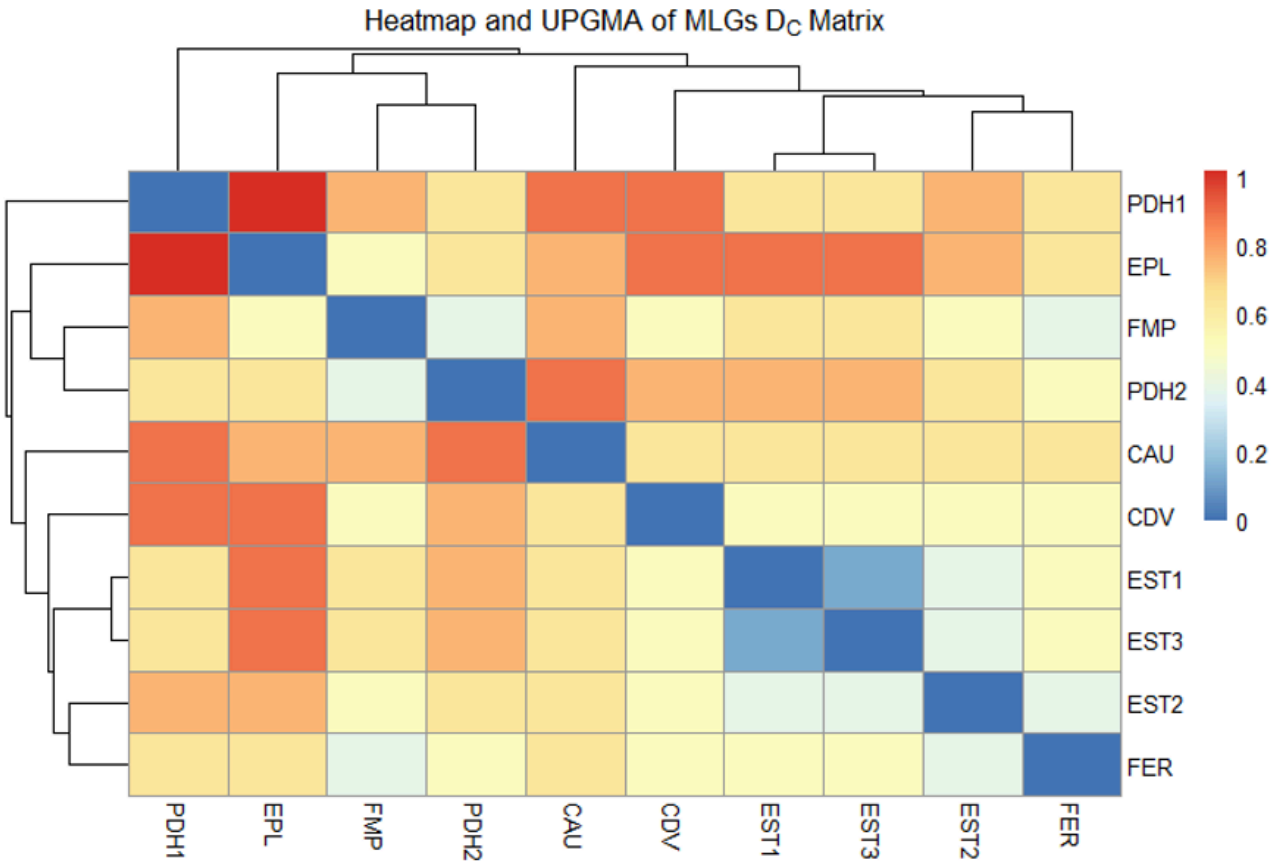

Figure 3

Heatmap and UPGMA based on the pairwise D_C between Pyrenean genotypes. At the top and on the left side, the UPGMA dendrogram based on D_C is represented. The codes of the Pyrenean MLGs are shown at the bottom and right side, as in Table 2. The heatmap represents the D_C between MLGs according to the scale color on the top right side: warmer (red and yellow) colors for distant MLGs and colder (from pale blue to dark blue) colors for similar MLG.

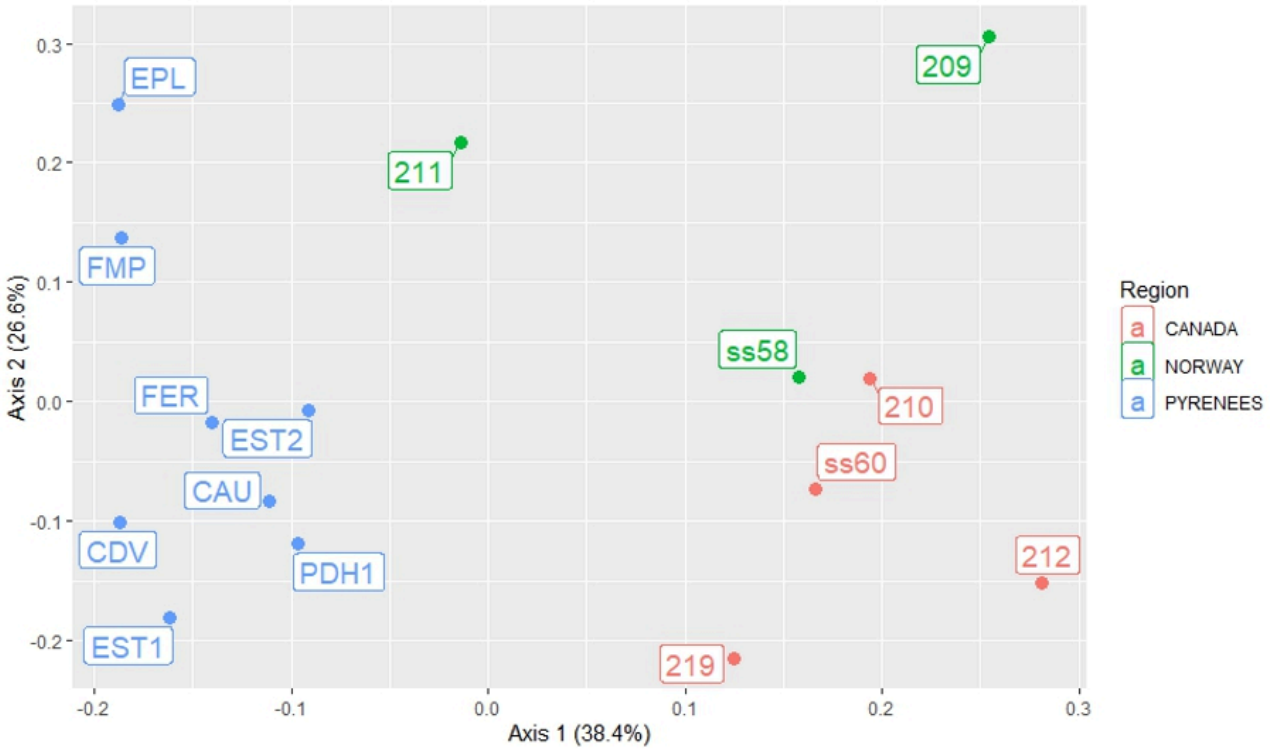

Figure 4

Principal Coordinates Analysis based on pairwise D_C among MLGs. The PCoA shows the coordinates of the Pyrenean, Canadian, and Norwegian populations for the first two axes, which account for 65% of the variance of the dissimilarity matrix.

Supplementary Files

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- Appendix.docx