



**ADAPTIVE VALUE OF MARGINAL POPULATIONS:
INTEGRATING SELECTIVE SIGNALS, NEUTRAL PROCESSES
AND TEMPORAL SCALES**

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5 **2 ADAPTIVE VALUE OF MARGINAL POPULATIONS: INTEGRATING**
6 **3 SELECTIVE SIGNALS, NEUTRAL PROCESSES AND TEMPORAL SCALES**
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1 **1. ABSTRACT**

2 **Background and aims:** The importance of populations inhabiting ecologically marginal
3 areas has been extensively debated and shown to depend, to a large extent, on their
4 adaptive value. In the context of climate change, their ecological originality can give
5 marginal populations the capacity to generate unique gene combinations. In this study,
6 we searched for evidence of genetic diversity of adaptive value in marginal populations,
7 through a genomic analysis that encompasses a multidimensional approach, integrating
8 selective signals, neutral processes and temporal scales.

9 **Methods:** We used a targeted exome sequencing approach to search for genomic
10 signatures of divergent selection between environmentally marginal and core populations
11 of the Mediterranean alpine species *Silene ciliata*. We also analysed their current genetic
12 population structure and demographic history, as well as signatures of selective sweeps
13 at different levels.

14 **Key results:** Populations from core and marginal environments presented similar values
15 of nucleotide diversity, inbreeding, and relatedness. Demographic history played a more
16 relevant role than current environmental conditions in explaining the levels of genetic
17 diversity in marginal populations. We also detected putative signals of diversifying
18 selection among populations inhabiting environmentally marginal and core areas,
19 involving 11 SNPs located in nine genes, as well as five gene pathways.

20 **Conclusions:** Populations currently inhabiting marginal environments can diverge from
21 those occupying core areas due to local adaptation despite low overall genetic
22 differentiation. The presence of polymorphisms or traits adapted to specific environments
23 in marginal populations may be particularly valuable in conservation management and
24 restoration, especially in the context of changing environmental conditions.

25 **Key words:** climate change, demographic inference, environmental gradients, divergent
26 selection, gene pathways, genetic adaptation.

1 2. INTRODUCTION

2 Environmental heterogeneity induces key demographic, genetic and phenotypic
3 differentiation within the natural ranges of species, leading to the identification of *core*
4 and *marginal* areas (Soulé 1973; Pouget *et al.* 2013; Pironon *et al.* 2017). Core areas,
5 characterized by prevalent and, presumably, optimal environmental conditions, host large
6 and, often, genetically diverse populations. In contrast, marginal areas, with atypical and
7 potentially restrictive environmental conditions, present a more complex scenario (Soulé
8 1973; Brown 1984).

9 Historically, marginal populations have been perceived as genetically impoverished and
10 maladapted due to genetic drift and inbreeding, thus harbouring little evolutionary
11 potential (Lynch and Gabriel 1987; Lande 1994; Eckert *et al.* 2008a). However, evidence
12 from some species suggests that these populations may be locally adapted to withstand
13 specific environmental pressures. This makes them particularly valuable in the context of
14 climate change, as their genetic diversity may confer adaptive advantages, enhancing the
15 organisms' chances of survival and reproduction (Barrett and Schluter 2008; Morente-
16 López *et al.* 2021). Despite the contrasting views, most studies have been based on limited
17 genetic information, lacking a genomic perspective (Stillman and Armstrong 2015;
18 Razgour *et al.* 2019). Genomic approaches can help to identify unique adaptations in
19 marginal populations as well as to forecast their responses to future environmental
20 pressures (e.g., Theraroz *et al.* 2024).

21 Genetic adaptation is influenced not only by environmental pressures but also by
22 available genetic diversity, past demographic history and gene flow (Kawecki 2008).
23 High levels of genetic diversity within populations are essential for natural selection and
24 adaptation to take place. Demographic events like bottlenecks can reduce this diversity
25 (Carbognani *et al.* 2019), while population growth can either diminish or augment it,

1 depending on the influx of new genetic variants (Haghighi and Routman 2016). Gene flow
2 can either facilitate adaptation by boosting genetic diversity (Eckert *et al.* 2008b;
3 Morente-López *et al.* 2021; Sacristán-Bajo *et al.* 2025) or hinder it by introducing
4 disadvantageous variants that are removed from the population by natural selection
5 (Lenormand 2002).

6 When genetic adaptation is studied from a genomic perspective, the genomic signature of
7 selection is superimposed on widespread signatures of the population's demographic
8 history. For instance, both bottlenecks and population growth can generate nucleotide
9 variation patterns that mimic selection (Barton and Etheridge 2004; Ramírez-Soriano *et al.*
10 2008). Hence, to identify adaptive genetic variants, it is imperative to employ
11 modelling techniques capable of differentiating between the genetic signatures of
12 selection and those stemming from neutral evolutionary processes (e.g., González-
13 Martínez *et al.* 2017; Mayol *et al.* 2020; Evans *et al.* 2023).

14 Alpine ecosystems exhibit high environmental variability, leading to rapidly shifting
15 selective pressures over short geographical scales (Byars *et al.* 2007; Morente-López *et al.*
16 2022). In this context, *Silene ciliata* Pourret, a well-studied Mediterranean alpine
17 cushion plant, exemplifies key questions in ecology and evolution in marginal plant
18 populations (García-Fernández *et al.* 2012; Lara-Romero *et al.* 2016; Morente-López *et al.*
19 2021, 2022). Field studies have shown lower longevity, lower proportion of flowering
20 plants and fewer flowers, fruits, and seeds per plant in the lower-elevation populations
21 (Giménez-Benavides *et al.* 2007; Giménez-Benavides *et al.* 2011). This results in
22 declining growth rates in low-elevation populations, which face local extinction, while
23 higher-elevation populations in more favourable environments show stable growth rates
24 (Giménez-Benavides *et al.* 2011). In parallel, studies in common-garden conditions, have
25 shown delayed flowering onset, peak and end (Morente-López *et al.*, 2020), lower seed

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3 1 germination (Giménez-Benavides et al. 2007), and greater drought tolerance (García-
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5 2 Fernández et al., 2013) in low-elevation populations. Interestingly, additional research
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7 3 suggests that populations at lower elevations may possess adaptive advantages over their
8
9 4 core counterparts that may be better suited to current and future climatic conditions
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11 5 (Morente-López *et al.* 2020, 2021). Furthermore, in the northern Mediterranean mountain
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13 6 ranges where the species occurs, the last glacial maximum (LGM) have influenced the
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15 7 demographic dynamics and current distributions of many plant species (Felinier 2014;
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17 8 Carbognani *et al.* 2019; Evans *et al.* 2023; Pütz *et al.* 2024), although this aspect has not
18
19 9 yet been studied in *S. ciliata*. This lack of knowledge is significant because demographic
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21 10 history could have influenced not only the current distribution of the species, but also the
22
23 11 observed patterns of genetic adaptation. Understanding these patterns in mountain
24
25 12 populations requires considering both the existence of LGM microrefugia and the
26
27 13 dynamics of rear-edge and leading-edge populations (Hampe & Petit 2005; Opgenoorth
28
29 14 *et al.* 2009; Vila-Cabrera *et al.* 2019). LGM microrefugia represent localized, climatically
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31 15 stable areas where species persisted during glacial periods, often corresponding to
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33 16 present-day rear-edge populations (Rull 2009). Rear edge populations, located at the
34
35 17 oldest and most stable habitat margins, reflect ancestral conditions. In contrast, leading
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37 18 edge populations, found at the most recent and dynamic habitat margins, reflect modern
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39 19 conditions and exhibit rapid adaptations to ongoing environmental changes (Hampe &
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41 20 Petit 2005; Vila-Cabrera *et al.* 2019).

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43 21 In this study, we aimed to assess the adaptive value of marginal populations through a
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45 22 genomic analysis that encompasses both selective and neutral processes, using a
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47 23 multiscale perspective that considers also recent and ancient processes. Our goal was to
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49 24 explore how past climatic fluctuations—particularly the LGM—and present-day
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51 25 environmental gradients have shaped the patterns of genomic variation in core and
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3 1 marginal populations. We sequenced 2,414 candidate genes in six natural populations
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5 2 (three core and three marginal), based on previous research that identified SNPs
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7 3 associated with environmental stress responses (Sacristán-Bajo *et al.* 2019). Using F_{ST} -
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9 4 outlier detection to assess long-term adaptation and selective sweep analyses for more
10
11 5 recent events, we identified potentially adaptive variation across different timescales. Our
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13 6 analysis of adaptive genetic diversity extended to gene pathways to investigate the role
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15 7 of gene networks in adaptation (Olson-Manning *et al.* 2012; Berg and Coop 2014;
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17 8 Exposito-Alonso *et al.* 2018). In addition, we examined spatial genetic structure and
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19 9 population dynamics using demographic inference models. To date, only a few studies
20
21 10 have assessed the adaptive value of marginal populations using combined analyses of
22
23 11 population genetic structure, demographic history and genomic signals of selection (e.g.,
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25 12 Yang *et al.* 2016; Temunović *et al.* 2020; Yuan *et al.* 2023). Moreover, none has applied
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27 13 a multidimensional framework integrating all these perspectives. In our study, we asked
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29 14 the following questions:
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- 35 15 1. Do core and marginal populations differ in levels of genetic diversity, inbreeding
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37 16 or relatedness?
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- 41 17 2. What is the spatial genetic structure of the studied populations? Which candidate
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43 18 genes and biological pathways may underlie adaptive genetic differentiation
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45 19 between core and marginal populations?
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- 48 20 3. Have there been abrupt changes in effective population sizes over time? If so, can
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50 21 they be linked to the LGM, and are there genomic signatures (e.g., of selective
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52 22 sweeps) indicative of adaptation in either rear-edge or leading-edge contexts?
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55 23 We hypothesised that distinct selective pressures operating on core versus marginal
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57 24 populations have driven genetic differentiation between them. We further expected that
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59 25 contemporary environmental stressors have accelerated demographic decline in marginal
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1 populations, reducing their genetic diversity. Finally, we expected that the LGM induced
2 a severe demographic contraction, generating a genetic bottleneck signature still
3 detectable in present-day genetic variation.

4 **3. MATERIALS AND METHODS**

5 **3.1 Study species and populations**

6 *Silene ciliata* Pourr. (Caryophyllaceae) is a dwarf cushion perennial plant that inhabits
7 Mediterranean alpine habitats with marked environmental gradients. It has flowering
8 stems that grow up to 15 cm tall, with each stem bearing 1 to 5 flowers. Its fruit capsules
9 contain up to 100 seeds, which are dispersed by wind in August and September. The seeds
10 are small, averaging 0.59 mg in mass and 1.1 to 1.5 mm in diameter, and lack specialized
11 dispersal structures, making the species primarily barochorous. Pollination is carried out
12 by syrphid flies, bumblebees, and moths. The plant is self-compatible, but self-pollination
13 is limited due to significant protandry (see Lara-Romero et al. 2014 and references
14 therein). The distribution of *S. ciliata* comprises the mountain ranges of the Northern
15 Mediterranean area from Spain to Bulgaria (Kyrkou *et al.* 2015), reaching its
16 southernmost limit in the Sistema Central of the Iberian Peninsula (Spain). All *S. ciliata*
17 populations from the Sistema Central are diploid and have the same phylogenetic origin,
18 as shown by chloroplast DNA analysis (Kyrkou *et al.* 2015). In these areas, the species
19 grows from 1,800 to 2,500 m in dry cryophilic pastures above the tree line. This
20 Mediterranean Alpine ecosystem has a pronounced summer drought combined with high
21 solar radiation, which typically selects for plant xerophytic traits.

22 At the end of the summer of 2013, we collected a minimum of thirty randomly selected
23 plants from six populations located in three sub-mountain ranges of the Sistema Central
24 (Spain): Guadarrama (GDM), Gredos (GRD) and Béjar (BJR) (Figure 1 and Table 1). All
25 sampled plants were separated by at least two meters. The Sistema Central is a southwest-

1 northeast oriented mountain range of approximately 500 km located in the centre of the
2 Iberian Peninsula. GDM, GRD, and BJR are in the western, central and eastern areas of
3 the Sistema Central, respectively. The six studied populations were located at the high
4 and low elevation margins of the sub-mountain ranges (Figure 1 and Table 1). Within
5 each sub-mountain range, the populations were separated by distances ranging from two
6 to four kilometers. High and low elevation margins represent core (optimal) and marginal
7 environmental conditions, respectively, based on the study of the ecological niche and
8 putative selective pressures for the species (Morente-López *et al.* 2022). According to
9 Morente-López *et al.* (2022), core populations inhabit regions where habitat suitability
10 equals or surpasses the highest 33rd percentile of the distribution of ranked suitability
11 values, estimated using the MaxEnt algorithm. Conversely, the marginal populations are
12 found below the lowest 33rd percentile. In marginal populations, vegetation is dominated
13 by dense shrub cover of *Cytisus oromediterraneus* Rivas Mart. et al. and *Juniperus*
14 *communis* L. subsp. *alpina* (Suter) Celak. *S. ciliata* is restricted to small, isolated *Festuca*
15 *curvifolia* Lag. ex Lange pastures, and populations are small, with at most a few hundred
16 individuals. In contrast, core populations are extensive, dominated by *Festuca* fellfields
17 with scarce shrubs, and support many thousands of individuals. Marginal populations are
18 subject to shorter snow cover duration and higher minimum temperatures compared to
19 core populations, reflecting the main climatic gradients relevant to the species (Morente-
20 López *et al.* 2022). Cuttings were obtained from the collected plants, rooted and placed
21 in pots in the Universidad Rey Juan Carlos CULTIVE experimental field ([https://urjc-](https://urjc-cultive.webnode.es)
22 [cultive.webnode.es](https://urjc-cultive.webnode.es)). Resulting plants were grown in common garden conditions before
23 DNA extraction in 2015. Further details on the common garden experiment are provided
24 in Morente-López *et al.* (2020).

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1 3.2 Selection of candidate genes for targeted sequencing

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6 The annotated *Silene latifolia* Poir. and *S. ciliata* transcriptomes (GenBank references:
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8 GCA_900095335 and PRJNA528948, respectively; see Sacristán-Bajo *et al.* 2019) were
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10 used to design an exome capture experiment based on targeted sequencing. We sequenced
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12 a total of 2,414 candidate genes with high-confidence annotations. Candidate genes were
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14 selected considering three factors that address specific aspects related to genetic
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16 differentiation and adaptation in *S. ciliata*. The first set comprised 576 genes carrying
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18 SNPs with unusually high allele frequency differentiation between core and marginal
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20 populations (Sacristán-Bajo *et al.* 2019). Briefly, these SNPs were identified in a previous
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22 study based on 147,118 SNPs distributed along 12,688 sequences obtained from mRNA
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24 libraries of leaf tissue without any prior experimental treatment in common garden
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26 conditions. The second set comprised 489 genes annotated with the Gene Ontology term
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28 “Flower development” (GO: 0009908) or any of the dependent and more specific terms.
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30 These genes are expected to be involved in the development of the flower over time, from
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32 its formation to its mature structure. This set of genes was selected because previous
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34 quantitative genetic studies in *S. ciliata* indicate the existence of important differences,
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36 probably related with contrasted selective pressures, in the flowering phenology of plants
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38 from marginal and core populations (Giménez-Benavides, García-Camacho, *et al.* 2011;
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40 Morente-López *et al.* 2020). Finally, the third set included 1,349 annotated genes
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42 associated with general processes that result in a change in state or activity of a cell or an
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44 organism because of an abiotic stimulus (GO: 0009628 or any of the more specific terms).
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46 This third set was included in the study to detect a broad range of new candidate genes
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48 under divergent selection not previously identified in *S. ciliata*, as previous work on the
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50 species was hampered by low statistical power and may have left much of the potential
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52 adaptive variants unidentified.
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1 3.3 DNA extraction, targeted sequencing and SNP calling

2 We used the DNeasy Plant minikit (QIAGEN, Valencia, USA) to extract and isolate DNA
3 from 94 plants (Table 1), 15-17 for each of the six studied populations. Library
4 preparation covering the targeted sequences followed the manufacturer's protocol for
5 Nugen SeqCap Developer kit. Samples were sequenced as 96-plex on a HiSeq2500 lane
6 with paired-reads of 125 bp. Demultiplexing of reads was carried out with CASAVA
7 1.8.2 (Illumina Inc., San Diego, CA). Reads were masked for adapter contamination with
8 *cutadapt* and low-quality bases were removed using *erne-filter* (ERNE v.1.4.5).
9 Alignment was carried out with BWA MEM v.0.7.10 (<https://github.com/lh3/bwa>). The
10 aligned sequences obtained from the targeted exome sequencing were deposited in
11 GenBank with accession number PRJNA851086. Prior to SNP calling, alignments were
12 filtered for low mapping quality (<10) and reads were realigned around indels using
13 GATK 3.6 (McKenna *et al.* 2010). SNP calling was performed on the entire sample
14 simultaneously using the *UnifiedGenotyper* implemented in the GATK suite, which
15 allowed the initial identification of ca. 185,000 SNPs.

16 Raw SNP data were filtered using VCFtools v0.1.14 (Danecek *et al.* 2011) and *vcffilter*
17 function of VCFLIB (Garrison *et al.* 2022). Only biallelic SNPs with fewer than 20%
18 missing data were kept. Indels were also removed from the dataset. SNPs were then
19 filtered following the hard filtering suggested by GATK's user guide
20 (<https://gatk.broadinstitute.org/>). This stringent filtering reduced the SNP dataset to
21 112,751 SNPs. Further, SNPs from paralogous genes were filtered combining two
22 approaches. First, the SNP dataset was filtered by Hardy-Weinberg disequilibrium and
23 polymorphism excess (Chen *et al.* 2014). To apply this filter, we estimated F_{IS} in sliding
24 windows of 150 bp with a step of 50 bp. Sliding windows with an average F_{IS} lower than
25 -0.5 were filtered (Hohenlohe *et al.* 2013; McKinney *et al.* 2017), which entailed the

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3 1 removal of 27,728 SNPs. Second, for the retained dataset (85,023 SNPs), we applied the
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5 2 *HDplot* method. *HDplot* combines information from the relative proportion of
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7 3 heterozygotes in a population (H) and the deviation of allele-specific reads of each locus
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9 4 from a 1:1 ratio (D), allowing the identification of paralogous loci throughout the range
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11 5 of allele frequencies (McKinney *et al.* 2017). A total of 13,362 SNPs (16%) with H
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13 6 greater than 0.65 and $|D| > 20$ were classified as located in paralogous sequence and
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15 7 subsequently removed, resulting in a final SNP dataset of 71,661 SNPs distributed in
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17 8 2,278 genes.
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22 9 **3.4 Nucleotide diversity, population structure and demographic history**

23 24 25 10 **Nucleotide diversity, inbreeding and relatedness**

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28 11 Nucleotide diversity by population (Tajima's π and Watterson's θ) and standard neutrality
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30 12 tests (Tajima's D , and Fu & Li's D^* and F^*) were computed using mstatspop
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32 13 (<https://github.com/CRAGENOMICA/mstatspop>). Additionally, we estimated the
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34 14 number of polymorphic sites, average inbreeding coefficients (F) and pairwise
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36 15 relatedness (as evaluated by unadjusted A_{jk} statistic) for each population using VCFtools
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38 16 v0.1.14 (Danecek *et al.* 2011).
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42 43 17 **Population genetic structure**

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45 18 We investigated population genetic structure using the hierarchical analysis of molecular
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47 19 variance (AMOVA) implemented in Arlequin v3.5.2 (Excoffier and Lischer 2010). The
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49 20 analysis was conducted at three levels: (1) among mountain ranges; (2) between core and
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51 21 marginal populations within mountain ranges; and (3) within populations. Genetic
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53 22 differentiation was calculated for all three hierarchical levels, leading to estimates for F_{CT}
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55 23 (among mountain ranges), F_{SC} (between core and marginal populations within mountain
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57 24 ranges) and F_{ST} (among populations overall). F_{ST} was also calculated between all pairs of
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1 populations, and the significance levels of pairwise F_{ST} estimates were determined by
2 permutation tests (10,000 permutations). Population genetic structure was also inferred
3 by a Bayesian clustering method using fastStructure, with K groups from $K=1$ to $K=6$ (the
4 number of localities in our sample), and the simple prior. For improved ancestry estimates
5 and to identify subtle structure, the logistic prior was executed at values of K similar to
6 those identified when using the simple prior. Then runs for $K=2$ and $K=3$ (the best K)
7 were repeated ten times, and averaged Q values (i.e., the individual assignment
8 probability to each of the K groups) were used to draw bar plots. As an alternative to
9 AMOVA and fastStructure, we used a Neighbour-net phylogenetic network (NNet)
10 constructed in SplitsTree v 4 (Huson and Bryant 2006), based on uncorrected_p distance
11 with default parameters and 1,000 bootstraps. In order to account for gene flow while
12 inferring the relationships among *S. ciliata* populations, we also used TREEMIX v1.13
13 (Pickrell and Pritchard 2012). This method models patterns of both population splits and
14 admixtures accessing the covariance structure of allele frequencies between populations
15 and performing a Gaussian approximation for genetic drift, improving the fit of the
16 inferred model by enabling migration edges. TREEMIX analyses were implemented with
17 500-SNP bootstrap resampling and considering a different number of migration events,
18 ranging from zero to 10.

19 **Demographic inference**

20 *Demographic inference at population level:* An overall negative Tajima's D indicated an
21 excess of low frequency polymorphisms (see Results), suggesting population growth.
22 Based on these results, we tested multiple demographic models for each population
23 separately using the coalescent approach implemented in Fastsimcoal2 v.2.6.0.2
24 (Excoffier *et al.* 2013). Fastsimcoal2 estimates the composite likelihood of a specific
25 demographic scenario given the observed data as well as population genetic parameters

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3 1 such as divergence time, effective population size and gene flow, using the site frequency
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5 2 spectrum (SFS) as input. Un-folded site frequency spectra (calculated from observed
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7 3 counts of the derived allele) were generated separately for each of the six populations
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10 4 using VCF2SNPs (Liu *et al.* 2018). To maximize statistical power, we used all SNPs,
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12 5 including those in intragenic regions, for demographic inference, assuming most behave
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14 6 neutrally or quasi-neutrally. This assumption is supported by low levels of linkage
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16 7 disequilibrium (mean $r^2 = 0.11$) and a very small proportion of SNPs showing signatures
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18 8 of selection (see Results). We compared three demographic models (Figure 2). The first
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20 9 “Stationary” model represents a null model consisting in a population of constant size and
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22 10 is defined by a single parameter, the current population size (N_{CUR}). The second
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24 11 “Expansion” model is defined by three parameters: N_{CUR} , a time of instantaneous
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26 12 population size change (T_{EXP}) and a population size prior to T_{EXP} . The third “Bottleneck”
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28 13 model is characterized by four parameters: N_{CUR} , a time at which the bottleneck began
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30 14 (T_{BOT}), a population size during the bottleneck event (N_{BOT}), and a population size prior
31
32 15 to T_{BOT} (N_{ANC}). The duration of the bottleneck event was fixed at 100 generations to avoid
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34 16 problems associated with model overparameterization. We performed 100 independent
35
36 17 runs for each population and demographic model consisting of 50 cycles of the Brent
37
38 18 algorithm and 100,000 simulations. The maximum-likelihood runs under each model
39
40 19 were then compared using AIC to select the optimal model for each population. Once the
41
42 20 optimal model was identified, we estimated confidence intervals (CIs) by performing 100
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44 21 parametric bootstraps based on best-model parameters. Mutation rate was estimated from
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46 22 the data assuming a generation time of two years, which reflects the time required for the
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48 23 plant to reach reproductive maturity (Giménez-Benavides *et al.* 2011).

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56 24 *Demographic inference at mountain range level:* Previous studies have suggested
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58 25 historical environmental fluctuations related to glaciations affecting *S. ciliata* populations
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3 1 in central Spain (Morente-López *et al.* 2018). Glacial pulses would have been responsible
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5 2 for shifting *S. ciliata* populations to lower elevations. Postglacial recolonization of higher
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7 3 elevations from lower areas would have originated a front-advancing edge by a subset of
8
9 4 the populations at lower elevation. To better understand the potential effect of
10
11 5 environmental fluctuations during glaciations on *S. ciliata* populations, we used
12
13 6 Fastsimcoal2 to statistically compare the relative fit of multi-population demographic
14
15 7 models (“mountain models”) incorporating three possible demographic scenarios
16
17 8 involving both core and marginal populations (Figure 2b). The first model (“Isolation of
18
19 9 two populations”) was parametrised with the current population size of marginal (N_M)
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21 10 and core populations (N_O), and a time of instantaneous split (T_{DIV}) from a common
22
23 11 ancestral population (N_{ANC}); it represents a simple scenario without effective population
24
25 12 size changes. The second model (“Bottleneck and Isolation of two populations”) also
26
27 13 considered the split of marginal and core populations from a common ancestral
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29 14 population but included a bottleneck event in the ancestral population prior to the split.
30
31 15 This bottleneck event was modelled defining a time when the bottleneck began (T_{BOT}), a
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33 16 population size during the bottleneck event (N_{BOT}) and a population size posterior to T_{BOT}
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35 17 (N_{DIV}). Finally, the third model (“Bottleneck and Divergence of a population”) considered
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37 18 marginal populations as a result of a bottleneck in the ancestral population from which
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39 19 the core populations would have subsequently diverged; this model reflects the suggested
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41 20 glacial dynamics of the species in the Sistema Central mountain range described above.
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43 21 The three models included migration between marginal and core populations after the
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45 22 divergence event (M_{MC} and M_{CM}), as considering gene flow always improved model
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47 23 fitting (data not shown). The modelling set-up and assessment of model performance was
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49 24 the same as described for the models fitted at the population level.
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1 3.5 Detecting distinct signatures of selection in marginal vs core populations

2 Hierarchical F_{ST} -outlier analyses

3 To investigate the contribution of natural selection to genetic adaptation in *S. ciliata*, we
4 combined multiple approaches. First, we searched for signals of divergent selection at the
5 single-locus (SNP) level using hierarchical F_{ST} -outlier analyses implemented in Arlequin
6 and BayeScanHierarchical. For Arlequin, we ran 20,000 simulations under a hierarchical
7 island model with 100 demes per level to establish a null distribution. To control for false
8 positives, we applied a False Discovery Rate (FDR) correction and considered loci with
9 a $1-F_{ST}$ quantile < 0.05 as outliers (Storey et al 2003). BayeScanHierarchical, which
10 extends the standard BayeScan Bayesian likelihood method to hierarchical population
11 structure, was used to identify loci with strong evidence of selection (Foll *et al.* 2014). To
12 ensure adequate statistical power, we set the minimum number of iterations to 10,000, the
13 length of 20 pilot runs to 7,500 iterations, and the burn-in-length to 50,000 iterations. Loci
14 having a Q -value (analogue to a FDR) lower than 5% were considered to be under strong
15 selection.

16 Second, we estimated AFDs (Allele Frequency Differentials) between pairs of core and
17 marginal populations. To reinforce the reliability of our results, we applied a strict
18 criterion, considering a locus as a candidate for selection only if it was identified as an
19 outlier by both hierarchical F_{ST} -outlier analyses and showed consistent AFD patterns
20 across all three pairwise comparisons between core and marginal populations. For the
21 AFD comparisons, the expected number of random significant tests was calculated by
22 multiplying the number of SNPs tested by the probability of AFDs going in the same
23 direction in each comparison by chance (0.5^3), indicating that several thousand SNPs
24 could show consistent AFDs in the same direction by chance. However, combining AFDs

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3 1 with hierarchical F_{ST} -outlier detection at standard FDR of 0.05 allowed us to increase
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5 2 substantially the confidence in our results (FDR of combined test of 0.00625).
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8 **Detection of selective sweeps**

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10 4 To identify recent hard selective sweeps, we employed the SWEEPfinder algorithm
11
12 5 implemented in SweeD v.3.1 (Pavlidis *et al.* 2013). We chose to study hard selective
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14 6 sweeps for their capacity to reveal recent genetic adaptation responding to shifting
15
16 7 environmental pressures. This method has the benefit to derive the null hypothesis from
17
18 8 the background pattern in the data itself, making it more robust to demographic events
19
20 9 such as population growth (Nielsen *et al.* 2005; Pavlidis *et al.* 2013). For each gene,
21
22 10 Composite Likelihood Ratios (CLRs) were calculated at 30 equally-spaced positions
23
24 11 across the target genomic region. To increase statistical power, all CLR scans were run
25
26 12 on unfolded SFS and monomorphic sites were included in the analysis (Pavlidis *et al.*
27
28 13 2013). To further increase power to detect selective sweeps, we only applied the test on
29
30 14 1,758 candidate genes that had 10 SNPs or more, which involved 52,740 SNPs.
31
32 15 Significance thresholds for selective sweeps detected in each population were obtained
33
34 16 by computing the distribution of CLRs in 100 data sets obtained by coalescence
35
36 17 simulations in SFS_CODE (Hernandez 2008). The simulations were based in a
37
38 18 demographic scenario of population expansion after a bottleneck, which was the most-
39
40 19 likely demographic model in all the studied populations based on Fastsimcoal2 (see
41
42 20 Results). To ensure a conservative approach, we only considered selective sweeps with
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44 21 higher likelihood than the maximum obtained in the simulations across all core and
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46 22 marginal populations.
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54 **Selection at the gene pathway level**

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56 24 We used a procedure developed in humans (Daub *et al.* 2013, 2017) and tested in plants
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58 25 (Mayol *et al.* 2020), to detect the joint effect of selection in biological pathways. For this
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3 1 purpose, we used KEGG Automatic Annotation Server to map our 2,278 candidate genes
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5 2 and retained 246 biological pathways that included at least four genes. This threshold was
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7 3 selected to provide sufficient statistical power for subsequent analyses while maintaining
8
9 4 a reasonable number of pathways for biological interpretation. While a larger number of
10
11 5 genes per pathway would provide greater statistical power, it would also significantly
12
13 6 reduce the number of pathways analyzed. We refined this gene-pathway list by removing
14
15 7 non-plant pathways (see a similar approach in Mayol *et al.* 2020), resulting in a final
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17 8 selection of 86 known pathways in plants. Then, for the maximum value of the coefficient
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19 9 of selection *alpha* estimated by SweeD and BayeScanHierarchical, we performed a gene
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21 10 set enrichment approach as in Daub *et al.* (2013). For this purpose, we used the POLYSEL
22
23 11 pipeline (<https://github.com/CMPG/polysel>). This pipeline calculates for each statistic
24
25 12 the SUMSTAT score (Tintle *et al.* 2009), which is the sum of the values for that statistic
26
27 13 of all genes in a given pathway. Then, the pipeline compares SUMSTAT scores, after
28
29 14 different bias corrections, to a null normal distribution of 500,000 random sets created
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31 15 using a sequential random sampling method (Ahrens and Dieter 1985), with the same size
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33 16 as the original pathway. As in Daub *et al.* (2013), we reported the gene pathways that
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35 17 showed evidence of having collectively higher gene selection coefficients (Q -value <
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37 18 0.2), which establishes a probability of finding up to 17 pathways falsely identified as
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39 19 significant by chance alone.

20 **4. RESULTS**

21 **4.1 Nucleotide diversity, population structure and demographic history**

22 **Nucleotide diversity, inbreeding and relatedness**

23 The comparison between core and marginal populations revealed minimal differences in
24 genetic parameters. Nucleotide diversity, inbreeding and relatedness were similar for

1 individuals in both environments (Table 2, all Wilcoxon rank tests: $P \geq 0.1$). Most
2 neutrality tests were negative (only CAM had a positive result; Table 2) indicating an
3 excess of rare polymorphisms, which can be interpreted as a demographic signature of
4 population expansion.

5 **Population genetic structure**

6 Three-level hierarchical analysis of molecular variance (AMOVA, Table S1) showed that
7 most of the genetic variation (91.3%) was found within populations, though a significant
8 portion of the variation (6.7%) occurred among mountain ranges. A small (2%) but
9 significant genetic differentiation was detected between core and marginal populations
10 within mountain ranges. Pairwise F_{ST} values were significant for all population pairs and
11 ranged between 0.0155 and 0.1201 (Table S2), being higher for comparisons between
12 mountain ranges (mean \pm *SD*: 0.086 ± 0.030 , $n=12$) than within mountain ranges (0.022
13 ± 0.015 , $n=3$). The optimal number of genetic groups in the Bayesian clustering analysis
14 performed using fastStructure was $K=2$, with one group corresponding to Guadarrama
15 and the other group corresponding to Bejar and Gredos (i.e., the westernmost) mountain
16 ranges (Figure 3a). The Neighbour-net network (Nnet) exhibited three well defined
17 groups corresponding with the three mountain ranges studied (Figure 3b). Within each
18 mountain range group, individuals from marginal and core populations were not clearly
19 differentiated. The disposition of the three groups within the network indicated that
20 Gredos and Bejar are more closely connected between them than with Guadarrama. Such
21 a partition is also supported by the TREEMIX graph (Figure 3c), which showed evidence
22 of admixture between populations in Bejar (NEG) and Gredos (CAM), and a clear
23 separation of those in Guadarrama (Figure 3c).

24

1 **Demographic inference**

2 *Population level*

3 For all populations, the model incorporating changes in population size due to bottlenecks
4 and subsequent recent expansion (“Bottleneck” model in Figure 2a) was the most
5 supported based on AIC differences ($\Delta AIC > 100$ for all comparisons). In the highest-
6 likelihood parameter set (Figure 4), estimates of ancestral population size (N_{ANC})
7 averaged 44,191 (range 27,425-69,097). The bottleneck started in all populations around
8 4,000–9,500 generations ago, which resulted in a strong reduction of the effective
9 population size (mean $N_{BOT} = 169$, range 54-403). Current population size (N_{CUR}) had a
10 mean of 329 (range 158-525) and represents an increase of between 30% and 75% over
11 N_{BOT} . A recent population expansion was also supported for all populations except CAM
12 by negative neutrality test values (Figure 4; see above).

13 *Mountain range level*

14 Regarding the models fitted for each mountain range, the “Bottleneck and Isolation of
15 two populations” model (Figure 2b) had the lowest AIC scores ($\Delta AIC > 1500$ for all
16 comparisons). The highest-likelihood parameter set (Figure 5) suggests that ancestral
17 populations suffered a bottleneck around 9,000-18,500 generations ago, which reduced
18 drastically their effective population size. After the bottleneck, ancestral populations
19 recovered up to 65% of their previous effective population size. Populations inhabiting
20 current core and marginal areas within each mountain range subsequently diverged
21 around 2,129–13,800 generations ago. After divergence, core and marginal populations
22 were connected by low (i.e., near zero) and asymmetrical gene flow, with higher gene
23 flow from core to marginal populations (mean $M_{CM} = 6.62 \text{ E-}04$ vs. mean $M_{MC} = 7.59 \text{ E-}$
24 05). Estimates of current population sizes were systematically higher for marginal (mean

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3 1 $N_{CUR} = 4,900$, range 2,055-8,195) than for core populations (mean $N_{CUR} = 2,324$, range
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5 2 55-6,821). Estimates of N_{CUR} were also notably lower for westernmost mountain ranges
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7 3 (55 and 96 for Bejar and Gredos, respectively) than for eastern Guadarrama mountain
8
9 4 range (6,821).

5 **4.2 Detecting signatures of selection in marginal vs core populations**

6 **SNP and gene region level**

7 The hierarchical island model in Arlequin identified 750 SNPs as significant F_{ST} outliers
8 ($P < 0.05$ after FDR correction). Out of them, 35 SNPs showed consistent AFDs in all
9 comparisons between pairs of core and marginal populations. Candidate SNPs belonged
10 to 13 different genes. BayeScanHierarchical detected 170 outlier SNPs and 13 of these
11 (belonging to 11 genes) showed consistent AFDs. Eleven SNPs located on nine genes
12 were outliers using both methods (Table 3), and we considered them as potential
13 candidate loci under divergent selection. These potentially adaptive SNPs/genes are
14 involved in regulation of flower development, oxidation-reduction processes, response to
15 light stimulus, UV protection and different stress responses (Table 3). Out of the nine
16 genes, four were among those associated with adaptation to elevation in previous studies
17 (Sacristán-Bajo *et al.* 2019), four were annotated as GO: 0009628 (response to abiotic
18 stimulus) and two as GO: 0009908 (flower development) (Table 3).

19 Overall, composite likelihood ratio tests (CLRTs) found slightly more selective sweeps
20 in core populations (mean = 1,303, range 698-2,478) than in marginal ones (mean =
21 1,186, range 559-2,346). Eighteen selective sweeps located in 10 genes were found in all
22 the populations of the same type (Table 4) and were thus considered as potential candidate
23 gene regions under selection in either marginal or core environments. From them, nine of
24 the 10 genes were found in core populations and only one gene was systematically found
25 in all marginal populations. Only three selective sweeps located in the same gene

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3 1 (PARG1, coding for a poly(ADP-ribose) glycohydrolase) were found in all the studied
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5 2 populations (Table 4). None of the genes with signs of a selective sweep experienced
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7 3 divergent selection according to F_{ST} -outlier analyses (Tables 3 and 4). Five and four of
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9 4 them were involved in different biological processes associated with responses to abiotic
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11 5 stimulus (GO: 0009628) and flower development (GO: 0009908), respectively, and three
12
13 6 were among the candidate genes associated with adaptation to elevation in previous
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15 7 studies (Sacristán-Bajo *et al.* 2019; Table 4).
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20 8 **Gene pathway level**

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23 9 A total of 1,623 out of 2,250 genes mapped on existing KEGG pathway maps for plants
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25 10 (Table S3). Mapped genes were distributed on 86 distinct pathways (Table S3). Five
26
27 11 pathways, involving 47 candidate genes, were enriched for positive values of *alpha*
28
29 12 estimated by BayeScanHierarchical (Q -value < 0.2; Table 4, Table S3) indicating
30
31 13 diversifying selection between core and marginal populations. A low proportion of
32
33 14 candidate gene pathways shared genes, with 42 out of the 47 genes being specific to only
34
35 15 one of the five pathways. None of these genes were among those found significant in
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37 16 analyses at single-gene level (considering both F_{ST} outliers and selective sweeps; Tables
38
39 17 3 and 4, respectively). Enrichment analysis using the maximum value of *alpha* estimated
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41 18 by SweeD did not detect any biological pathways with significant SUMSTAT scores.
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46 19 **5. DISCUSSION**

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49 20 Remarkably, we found low genetic differentiation between core and marginal populations
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51 21 of *S. ciliata*, suggesting that both environments harbour a wealth of genetic variation on
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53 22 which selection could act. The similar levels of genetic diversity observed may reflect the
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55 23 complex demographic history of the species, including range shifts, expansions, and
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57 24 contractions associated with climatic oscillations during and following the LGM. These
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3 1 historical processes likely shaped the current distribution of genetic diversity in *S. ciliata*,
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5 2 enabling both core and marginal populations to retain adaptive potential. This challenges
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7 3 the conventional view that marginal populations are genetically impoverished and
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9 4 stresses their role in future responses to environmental challenges (Morente-López *et al.*
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11
12 5 2021, 2022).

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15 6 Based on current declining growth rates, we hypothesized that *S. ciliata* populations
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17 7 inhabiting marginal areas might be genetically impoverished and lack adaptive value
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19 8 (Lynch and Gabriel 1987; Lande 1994; Eckert *et al.* 2008a). Instead, our study revealed
20
21 9 an unexpected homogeneity in nucleotide diversity, inbreeding, and relatedness across
22
23 10 populations, irrespective of their environmental conditions. Moreover, populations in
24
25 11 marginal environments exhibit larger current population size (N_{CUR}), as estimated by
26
27 12 historical demography models. This similarity in genetic parameters challenges
28
29 13 preconceptions: rather than being genetically deprived, marginal populations possess
30
31 14 genetic diversity comparable to their core counterparts. Genetically-based phenotypic
32
33 15 differentiation between core and marginal populations, as previously documented
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35 16 (García-Fernández *et al.* 2012; Morente-López *et al.* 2020, 2022; Prieto-Benítez *et al.*
36
37 17 2021), together with the presence of genomic adaptive divergence in marginal
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39 18 populations (see Results), emphasize the adaptive value of *S. ciliata* marginal
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41 19 populations. While these marginal populations retain adaptive genetic diversity, climate
42
43 20 change heavily impacts their survival. Environmental conditions at low elevations are
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45 21 constraining the viability of the populations, as evidenced by present-time demographic
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47 22 studies showing declining growth rates and higher local extinction risks at low elevations
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49 23 compared to the stable growth rates in higher elevations (Giménez-Benavides *et al.* 2011;
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51 24 Lara-Romero *et al.* 2014, 2016). Although available adaptive genetic diversity seems not
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53 25 to be enough to adapt to their changing environment, it still may facilitate the adaptation
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1 of higher-elevation populations through adaptive gene flow, as shown in empirical studies
2 (Morente-López *et al.* 2021; Prieto-Benítez *et al.* 2021).

3 Demographic inferences support a bottleneck around the last glacial maximum and
4 subsequent divergence of populations in core and marginal environments. This
5 divergence is plausible even at small spatial scales, given the species' limited dispersal
6 capacity (Lara-Romero *et al.* 2014) and the potential for genetic isolation in
7 heterogeneous landscapes, even despite spatial proximity (Budde *et al.* 2024). While
8 some populations descended to lower elevations, others likely persisted on mountaintops
9 in climatically stable microrefugia (Feliner 2014; Thompson 2020; Pütz *et al.* 2024).
10 These mountaintop populations, consistent with the concept of rear-edge populations
11 (Hampe and Petit 2005; Opgenoorth *et al.* 2009), show lower historical population size
12 (N_{ANC}), marked genetic divergence from marginal populations (as reflected by significant
13 F_{SC}), and a higher number of selective sweeps. These genomic signatures suggest long-
14 term persistence and multiple episodes of positive selection in isolated and stable
15 microrefugia (Colonna *et al.* 2014; Carbognani *et al.* 2019; Deng *et al.* 2021).

16 In contrast, our results suggest that marginal low-elevation populations experienced past
17 expansions during more favourable climatic periods, consistent with the leading-edge
18 dynamics of colonisation into newly suitable environments (Hampe & Petit, 2005).
19 However, progressive warming over the last century has displaced the species' climatic
20 optimum upslope (Giménez-Benavides *et al.* 2007; Morente-López *et al.* 2022), leading
21 to current demographic declines in these now trailing-edge populations (Giménez-
22 Benavides *et al.* 2007; Lara-Romero *et al.* 2014; Morente-López *et al.* 2022). Despite
23 this, their adaptive genetic diversity may still contribute to the evolutionary potential of
24 core populations through adaptive gene flow (Morente-López *et al.* 2021; Prieto-Benítez
25 *et al.* 2022). These findings question typical assumptions about a direct relationship

1 between genetic diversity, range limits and current environmental suitability, highlighting
2 instead the influence of past demographic history in shaping present-day genetic patterns
3 (Duncan *et al.* 2015; Pironon *et al.* 2015; Yang *et al.* 2016).

4 The macroevolutionary forces shaping the distribution and diversity of *S. ciliata* have also
5 influenced microevolutionary processes, such as selection on specific genes and
6 pathways. With respect to individual genes, we detected signals of selection in four genes
7 related with flower development (*APSI*, *NUA*, *CYP71B37*, *atg4*). Previous work at the
8 phenotypic level suggested local adaptation for phenological traits in association with
9 elevation (Giménez-Benavides, García-Camacho, *et al.* 2011; Morente-López *et al.*
10 2020). This is an expected result since in alpine ecosystems flowering phenology is a
11 crucial demographic event (Molau *et al.* 2005; Sedlacek *et al.* 2015), forcing plants to
12 have a fine phenological adjustment to the climate (Scheepens and Sto 2013). Moreover,
13 we also identified candidate genes relevant for abiotic stress response in our study (Table
14 2). These include *PSBO* and *SCPL49*, recognized as drought-stress response genes
15 (Ashburner and The Gene Ontology Consortium 2000; Berardini *et al.* 2015; Shaar-
16 moshe *et al.* 2015), or *GH3*, which controls hypocotyl elongation, a molecular mechanism
17 critical for germination and seedling establishment (Berardini *et al.* 2015).

18 Beyond the level of individual genes, our analysis revealed that adaptation in *S. ciliata*
19 involves complex genetic mechanisms, including selection acting on five metabolic
20 pathways. Four of them were involved in essential metabolic functions that are critical
21 for plant survival, namely thiamine, purine, sulfur and seleno compound metabolisms.
22 Abiotic stress alters plant metabolism, inducing biochemical and physiological responses
23 that create tolerance (reviewed in Ramakrishna and Gill 2019). These metabolic
24 adjustments involve fine regulations of several metabolic pathways including those
25 identified here (Lawlor and Cornic 2002; Yan *et al.* 2014; Heinemann and Hildebrandt

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3 1 2021). The other gene pathway was related to RNA transport from the nucleus to the
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5 2 cytoplasm, which plays pivotal roles in regulation of gene expression. Several studies
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7 3 support the important role that mRNA export mechanisms play in abiotic stress responses
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9 4 of plants (Chinnusamy *et al.* 2008; Ehrnsberger *et al.* 2019; Yeap *et al.* 2019). The
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11 5 detection of this signal at the pathway level suggests that adaptation in species with
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13 6 complex evolutionary histories may involve genetic traits controlled by multiple genes
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15 7 within specific pathways. This challenges the traditional view of adaptation as being
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17 8 driven by single-gene mutations. Remarkably, approaches focused on single loci failed to
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19 9 detect signatures of selection for any of the genes involved in these pathways. Because of
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21 10 their small effect-sizes, genes under “soft” selection are hard to detect by single-locus
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23 11 approaches, although together they can have a large impact on a biological pathway
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25 12 (Olson-Manning *et al.* 2012; Berg and Coop 2014).

26
27 13 The candidate genes and pathways identified in this study offer an initial understanding
28
29 14 of the molecular mechanisms underlying the adaptive responses to marginal and core
30
31 15 environments. However, it is crucial to consider the possibility of false positives. Notably,
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33 16 certain candidate genes revealed multiple significant SNPs, with some of these genes also
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35 17 previously suggested as potentially involved in adaptation in an RNAseq experiment
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37 18 (Sacristan *et al.* 2019). In addition, our strategy was guided by a focus on adaptive
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39 19 molecular signals concurrently identified in the three replicated elevation gradients,
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41 20 aiming at minimizing the occurrence of false positives (Thornton and Jensen 2007).
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43 21 Nevertheless, further confirmation is necessary to ascertain the adaptive nature of
44
45 22 candidate genes and pathways, requiring additional research to establish a clear link
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47 23 between allelic variation and fitness (e.g., Jaramillo-Correa *et al.* 2015; Vangestel *et al.*
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49 24 2018.
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1 **Conclusions**

2 Our findings rejected the hypothesis that marginal populations of *S. ciliata* are
3 genetically impoverished. Instead, they revealed similar levels of genetic diversity in
4 both core and marginal populations, shaped by historical events, with standing genetic
5 variation in marginal populations being essential for adaptation to ongoing climate
6 change. By employing a genomic analysis that encompassed both selective and neutral
7 processes, and adopting a multiscale perspective that considered both recent and ancient
8 dynamics, we provided evidence of past selection in both environments and identified
9 genes potentially associated with flower development and abiotic stress responses.
10 Notably, genetic variation in *S. ciliata* may have partly resulted from selection acting on
11 key metabolic pathways, which highlighted complex mechanisms of adaptation. Further
12 research is needed to validate the adaptive role and fitness effects of these genes, as well
13 as to assess the potential of *S. ciliata* populations to respond to future environmental
14 pressures.

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24

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8. AUTHOR CONTRIBUTIONS

CLR, JML, AGF and JMI designed the experimental study, conducted the fieldwork, and collected the samples. JML with the help of CLR, AGF and JMI performed common garden experiments. CLR, SSB and AGF performed the DNA extractions in the laboratory. CLR and SGM analysed data with the help of SSB and AGF. CLR, SGM and JMI wrote the paper. All authors reviewed the paper and approved the final manuscript.

9. CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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9. LIST OF CAPTIONS

Figure 1. Location of the three main mountain ranges in the Sistema Central of the Iberian Peninsula (Spain) where the study was developed: Bejar (BJR), Gredos (GRD) and Guadarrama (GDM). The three-dimensional coloured map represents an example of the intra-range environmental classification in core (optimal) and marginal areas developed for *S. ciliata* based on ecological niche modelling and putative selective pressures (see Morente-López *et al.* 2022). In each of the three mountain ranges, two populations were selected, one in core (blue) and one in marginal (red) environmental conditions. The approximate locations of the two populations selected in Guadarrama mountain range, Pico de Peñalara (PEN) and Morrena Peñalara (MOR) are depicted in optimal and marginal areas respectively. Black shaded areas in the map represents elevations above 1,500 m. Dashed lines represent political divisions of provinces.

Figure 2. Demographic models tested in Fastsimcoal2. (a) Demographic models tested at population level. Model parameters are bottleneck (T_{BOT}) and expansion (T_{EXP}) times, current (N_{CUR}) and ancestral (N_{ANC}) effective population sizes and population size during the bottleneck event (N_{BOT}). (b) Demographic models tested at mountain range level. Additional model parameters are current population size of marginal (N_M) and core (N_O) populations, time of instantaneous isolation (T_{DIV}) and migration probability from marginal to core (M_{MC}) and core to marginal (M_{CM}) populations (horizontal arrows).

Figure 3. Population genetic structure of *S. ciliata* in the Sistema Central of the Iberian Peninsula (Spain) showing closer relationships between the two western mountain ranges (Gredos and Bejar) and separating the eastern Guadarrama mountain range. Refer to Table 1 for population acronyms. (A) Bayesian cluster analysis (fastStructure). Figure shows the individual bar plot grouped by population and mountain ranges. Bar colours represent individual assignment probability to each of the optimal $K=2$ groups. (B) Neighbour-net (Nnet) phylogenetic network constructed in SplitsTree v4. (C) Population relationships and migration edges inferred by TREEMIX. Arrows are coloured by migration weight and branch lengths are proportional to genetic drift. Notice that the only migration event linked two populations was found in Bejar and Gredos mountain ranges (NEG-CAM).

1 **Figure 4.** Parameters inferred from coalescent simulations with Fastsimcoal2 under the
2 best-supported demographic model for single populations (“Bottleneck” model, see
3 Figure 2a). For each parameter, the point estimate and the lower and upper 95%
4 confidence intervals, generated by parametric bootstrapping, are shown. Model
5 parameters include T_{BOT} (time when the bottleneck began), N_{CUR} and N_{ANC} (current and
6 ancestral population sizes), and N_{BOT} (population size during the bottleneck event).

7 **Figure 5.** Parameters inferred from coalescent simulations with Fastsimcoal2 under the
8 best-supported demographic model fitted at mountain range level (“Bottleneck and
9 Isolation of two populations” model, see Figure 2b). For each parameter, the point
10 estimate and the lower and upper 95% confidence intervals, generated by parametric
11 bootstrapping, are shown. Model parameters include: T_{BOT} and T_{DIV} (bottleneck and
12 instantaneous split times), N_M and N_C (current effective population size of marginal and
13 core populations), N_{ANC} (ancestral population size), N_{BOT} (population size during the
14 bottleneck event), and M_{MC} and M_{CM} (migration from marginal to core and core to
15 marginal populations). The red and blue shades visualise the division of the ancestral
16 population into marginal and core populations.

17 **Figure 6.** Diagram of the gene pathways tested for signals of selection. Five pathways
18 (indicated with red names) were enriched with positive *alpha* values estimated by
19 BayeScanHierarchical, indicating diversifying selection between populations inhabiting
20 core and marginal environments. The size of the nodes (pathways) is proportional to the
21 number of genes involved. The node colour scale represents the SUMSTAT score. A
22 higher SUMSTAT score indicates stronger signals of polygenic selection within the
23 pathway. Edges represent mutual overlap; nodes are connected if one of the sets has at
24 least three of its genes in common with the other gene set. Edge width is proportional to
25 the number of shared genes. Nodes marked with an asterisk indicate gene pathways that
26 are plant-specific. For clarity, the figure represents only the 51 pathways with a
27 SUMSTAT score higher than the median value of the entire dataset (N=86).

28 10. TABLES AND FIGURES

Table 1: *Silene ciliata* populations of the Sistema Central (Spain) used in the targeted resequencing experiment. Geographical and environmental characteristics and sample sizes (*N*) for the experiment are also provided.

Population (ID)	Mountain Range	Environment	<i>N</i>	Latitude	Longitude	Snowpack	MinT (°C)	Elevation (m)
Canchal Negro (NEG)	BJR	Core	15	40°20'20''N	5°41'22''W	0.59	-7.2	2360
Las Cimeras (RUI)	BJR	Marginal	16	40°21'07''N	5°40'59''W	0.27	-6.7	2000
Alto del Morezón (ZON)	GRD	Core	15	40°14'57''N	5°16'08''W	0.62	-7.7	2380
Los Campanarios (CAM)	GRD	Marginal	16	40°15'42''N	5°12'55''W	0.34	-6.0	2000
Pico de Peñalara (PEN)	GDM	Core	17	40°51'02''N	3°57'24''W	0.67	-7.8	2400
Morrena Peñalara (MOR)	GDM	Marginal	15	40°50'11''N	3°57'01''W	0.33	-5.7	1980

ID, identification of each population; Mountain Range, mountain range of origin: Bejar (BJR), Gredos (GRD) and Guadarrama (GDM); Environment, environmental classification; *N*, sample size; Latitude and Longitude, geographical coordinates; Snowpack, snowpack accumulation values in thaw months range from 0 to 1 (values close to 1 indicate deep snowpack, while values close to 0 indicate null snow cover; López-Moreno *et al.* 2007)); MinT, minimum annual temperature; Elevation, refers to the mean elevation (in meters above sea level) estimated for each population (variation in elevation within populations did not exceed 10 meters). Geographical and environmental information extracted from Morente-López *et al.* (2019). Based on Morente-López *et al.* (2022), the main putative selective pressures for *S. ciliata* are snowpack in the thaw months and minimum temperature. These variables were thus crucial to differentiate environmentally marginal and core areas.

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Table 2. Nucleotide diversity, neutrality tests and inbreeding by population.

Pop/Env	L (bp)	Polymorphic sites		Nucleotide diversity		Neutrality tests			Inbreeding	
		N	%	Tajima's π	Watterson's θ	<i>TajD</i>	<i>FuD</i>	<i>FuF</i>	<i>F</i>	Relatedness
NEG/Cor	2,678,634	33,568	47.17	0.00346	0.00338	-0.073	-0.072	-0.077	-0.082	0.056
RUI/Mar	2,684,833	33,747	47.43	0.00337	0.00331	-0.092	-0.134	-0.130	-0.040	0.055
ZON/Cor	2,677,081	34,147	47.99	0.00338	0.00344	-0.231	-0.275	-0.282	-0.044	0.058
CAM/Mar	2,683,788	30,934	43.47	0.00329	0.00303	0.079	0.193	0.173	-0.069	0.053
PEN/Cor	2,679,723	38,336	53.87	0.00346	0.00370	-0.362	-0.471	-0.475	-0.059	0.051
MOR/Mar	2,676,981	34,631	48.67	0.00338	0.00349	-0.263	-0.263	-0.283	-0.041	0.058

TajD, Tajima's *D*; *FuD*, *Fu* & *Li*'s *D**; *FuF*, *Fu* & *Li*'s *F**; *F*, average inbreeding coefficient; Relatedness, average pairwise relatedness statistic (unadjusted A_{jk}), with values around zero indicating unrelated individuals.

Table 3. Candidate genes putatively under divergent selection detected in *Silene ciliata*. “N° SNPs” refers to the number of SNPs detected with Arlequin (ARL) and BayeScanHierarchical approach (BAY). “Category” refers to the group of sequences to which the candidate gene belongs (See section “Selection of genes for sequencing”): A. Response to abiotic stimulus (GO: 0009628); F. Flower development (GO: 0009908); O. Candidate genes related to local adaptation to elevation in previous studies (Sacristán-Bajo *et al.* 2019). “Biological Processes” provides an overview of the main biological processes mediated by the candidate genes.

Gene Id (<i>S. ciliata</i>)	N° SNPs ARL/BAY	Category	Description	Uniprot ID (Best Blast)	Gene ID (Best Blast)	Biological Processes
comp73228	1/1	FO	ATP sulfurylase 1	Q9LIK9	APS1	Hydrogen sulfide biosynthetic process; Response to cytokinin; Response to cadmium ion
m.101530	1/1	A	Oxygen-evolving enhancer protein 1. chloroplastic	P12359	PSBO	Photosystem II assembly and stabilization; response to light intensity, response to water deprivation
m.102611	1/1	FO	Transmembrane protein	Q0WQ99	At4g16850	petal development; positive regulation of cell population proliferation
m.137232	1/1	F	Nuclear-pore anchor	A4GSN8	NUA	Stamen development; Negative regulation of flower development
m.140709	1/1	O	BAG family molecular chaperone regulator 3	Q9LYP4	BAG3	Chaperone-mediated protein folding; positive regulation of cellular process, response to jasmonic acid, response to wounding
m.19977	6/2	FA	Cytochrome P450 71B37	Q9LIP3	CYP71B37	Oxidation-reduction process; response to jasmonic acid, response to wounding, tissue development
m.843	2/2	A	Indole-3-acetic acid-amido synthetase GH3.10	Q9ZNS2	GH3.10	Response to light stimulus
m.87654	1/1	O	Serine carboxypeptidase-like 49	P32826	SCPL49	Proteolysis involved in cellular protein catabolic process
m.8887	1/1	A	Usp domain-containing protein	<u>A5C9Q0</u>	VIT_08s0007g01430	Response to cold; Response to heat; Response to oxidative stress; Chaperone-mediated protein folding

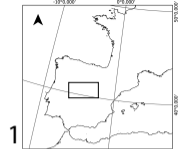
31 **Table 4.** Candidate genes with evidence of selective sweeps for *Silene ciliata* in core or marginal populations. “Category” refers to the group of
 32 sequences to which the candidate gene belongs (See section “Selection of genes for sequencing”): A. Response to abiotic stimulus (GO: 0009628);
 33 F. Flower development (GO: 0009908); O. Candidate genes previously related to local adaptation to elevation in previous studies (Sacristán-Bajo
 34 *et al.* 2019).

Gene Id (<i>S. ciliata</i>)	N° Selective sweeps	Category	Env.	Description	Uniprot ID (Best Blast)	Gene ID (Best Blast)	Biological Processes
m.133396	1	A	Marginal	Proteasome subunit beta type	Q8LD27	PBF1	Defence response to fungus, incompatible interaction; proteasomal protein catabolic; proteolysis
comp65047	3	A	Marginal Core	Poly(ADP-ribose) glycohydrolase	Q9SKB3	PARG1	Cellular response to DNA damage stimulus; Defence response to fungus; Regulation of DNA repair; Response to osmotic stress, oxidative stress and water deprivation; Rhythmic process; Stomatal closure
comp54123	1	O	Core	Carboxylate clamp type tetratricopeptide repeat protein	F4IRM4	PHOX1	Root hair cell development
comp64011	1	A	Core	DNA mismatch repair	F4JP48	MSH4	Homologous chromosome segregation; Mismatch repair
comp76868	1	F	Core	DNA repair protein	Q9SL02	RAD50	Gametophyte development; Chromosome organization involved in meiotic cell cycle; Telomere maintenance; DNA repair
m.117629	4	OF	Core	Protein TPLATE	F4J8D3	TPLATE	Endocytosis; Pollen development

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m.141175	1	O	Core	Uncharacterized protein	A0A0K9RLQ1	SOVF_052510	na
m.28293	1	A	Core	Histone-lysine N-methyltransferase	Q8GZB6	SUVH4	Histone methylation; Maintenance of DNA methylation; Peptidyl-lysine methylation
m.45836	2	O	Core	FAD-binding FR-type domain-containing protein	A0A2P4H4H9	VITISV_024875	Oxidation-reduction process
m.48088	1	A	Core	Sucrose synthase Glycine max	P13708	SS	Nodulation; sucrose metabolic process
m.83703	1	A	Core	ULP_PROTEASE domain-containing protein	A0A0K9S186	SOVF_011010	Proteolysis

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Guadarrama

Guadalajara

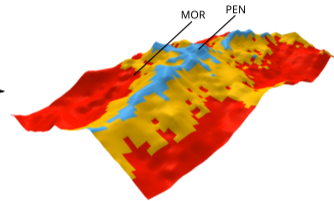
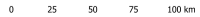
Madrid

Bejar

Gredos

<https://mc.manuscriptcentral.com/aob>

Toledo



Environmental Classification

Optimal

Intermediate

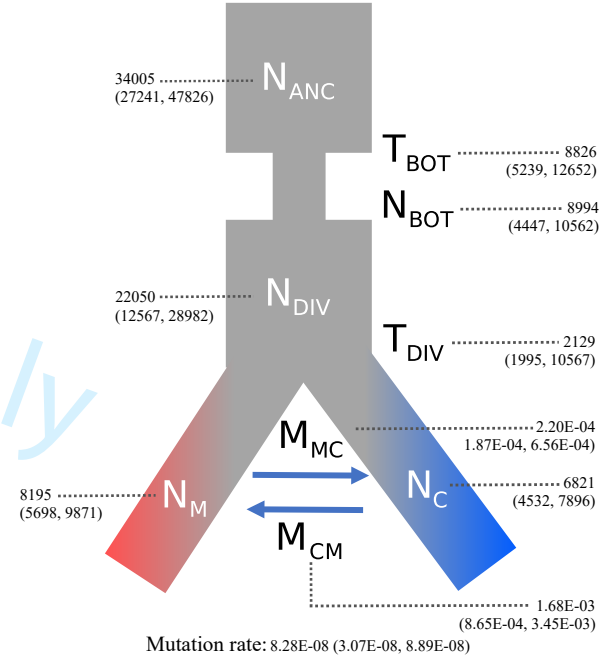
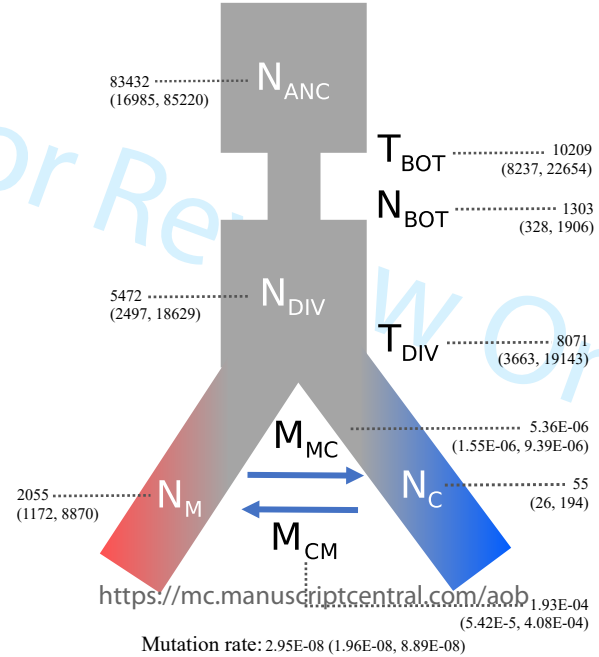
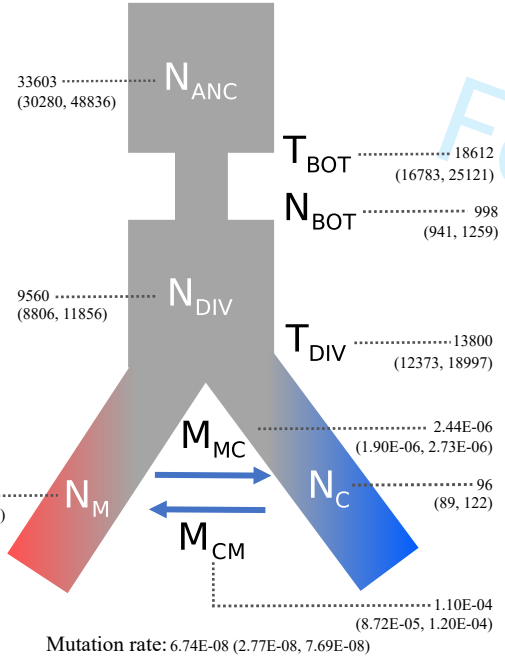
Marginal

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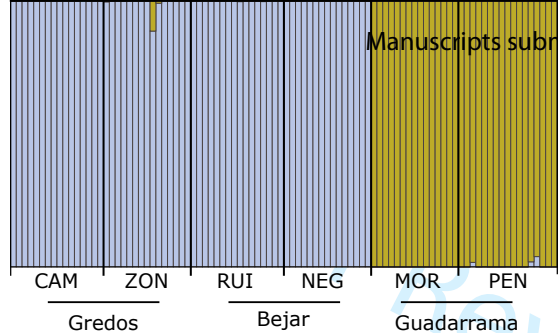
Guadarrama

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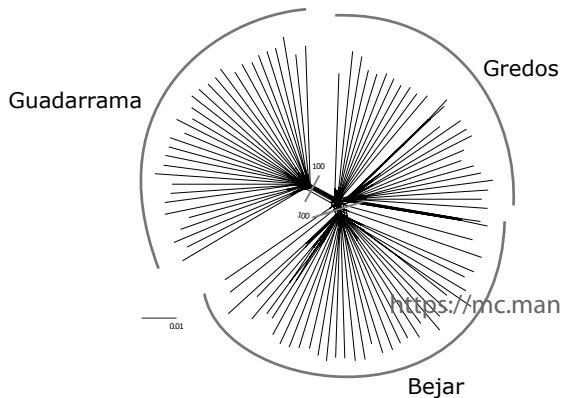


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A)



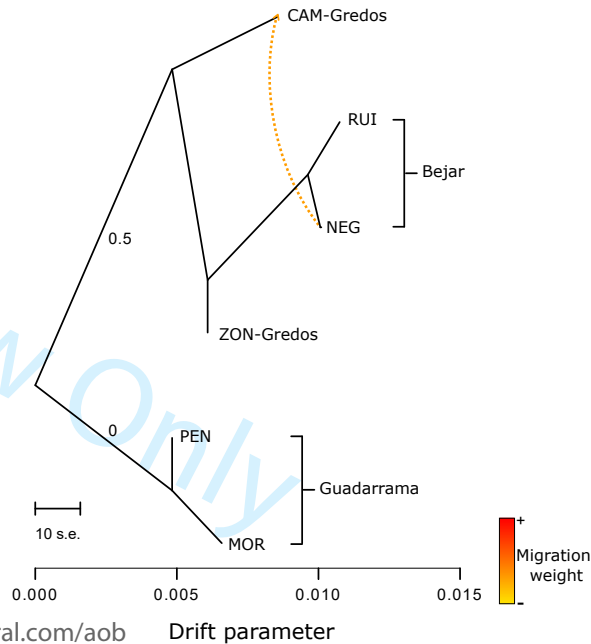
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C)

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Pop/Env	N_{ANC}	N_{CUR}	Mutation Rate	N_{BOT}	T_{BOT}
NEG/Cor	27425 (15519, 95337)	316 (88, 512)	5.66E-08 (3.94E-09, 9.9E-08)	161 (38, 278)	7170 (4010, 9624)
RUI/Mar	29312 (16806, 77050)	393 (53, 613)	4.82E-08 (8.21E-09, 8.96E-08)	114 (20, 472)	7253 (3487-10830)
ZON/Cor	32872 (11390, 83357)	525 (92, 538)	5.24E-08 (2.32E-08, 7.92E-08)	403 (49, 336)	9479 (3684, 9597)
CAM/Mar	33805 (16610, 80001)	184 (119, 478)	6.55E-08 (1.75E-08, 8.5E-08)	138 (36-292)	4223 (3882, 10860)
PEN/Cor	69097 (18843, 94834)	158 (123, 647)	1.02E-07 (1.43E-08, 9.99E-08)	54 (38, 386)	5100 (3237, 9885)
MOR/Mar	62815 (28424, 84521)	400 (98, 577)	3.59E-08 (1.66E-08, 8.58E-08)	146 (29, 310)	9138 (3881, 9813)

Bottleneck

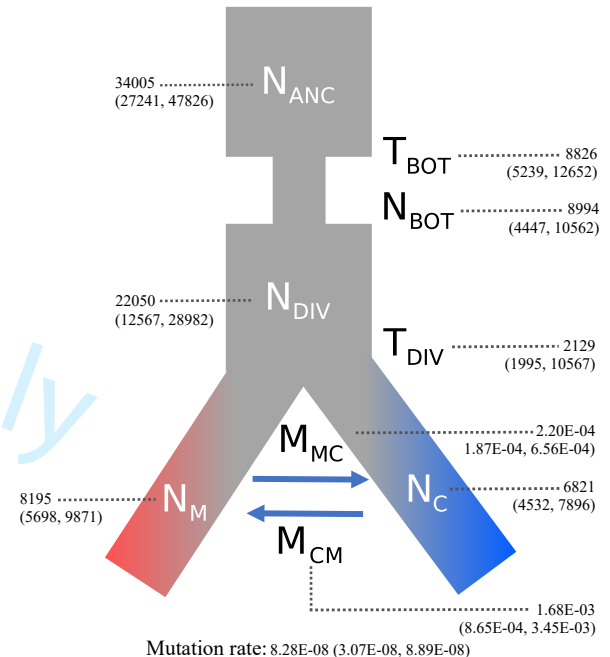
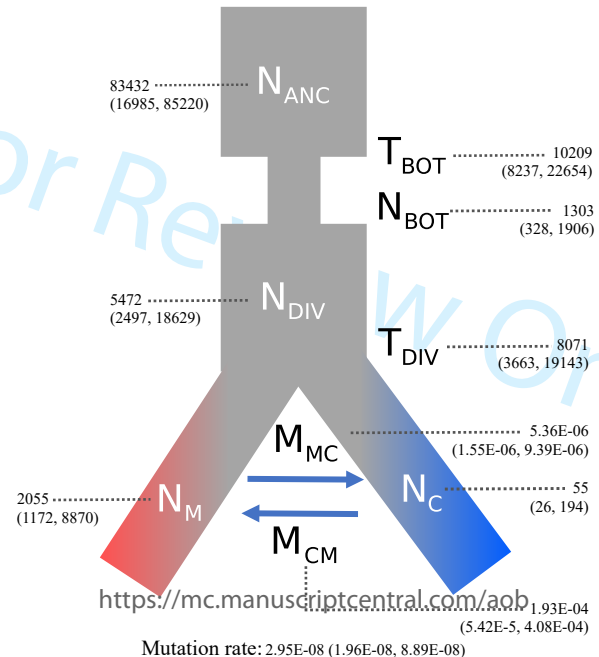
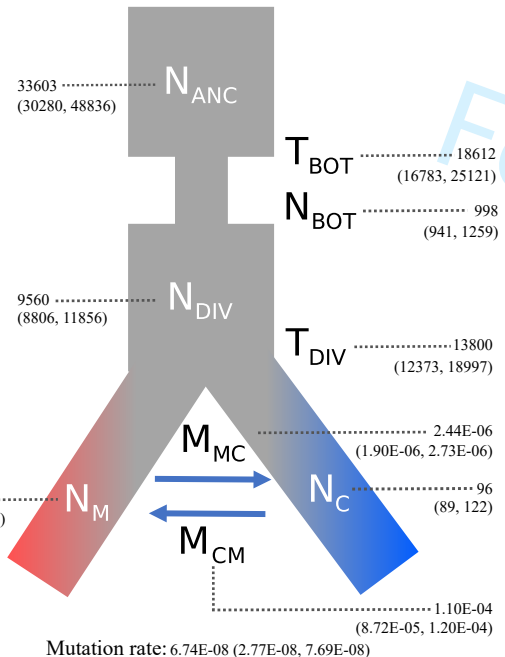
 N_{ANC} N_{BOT} T_{BOT} N_{CUR}

Gredos

Bejar

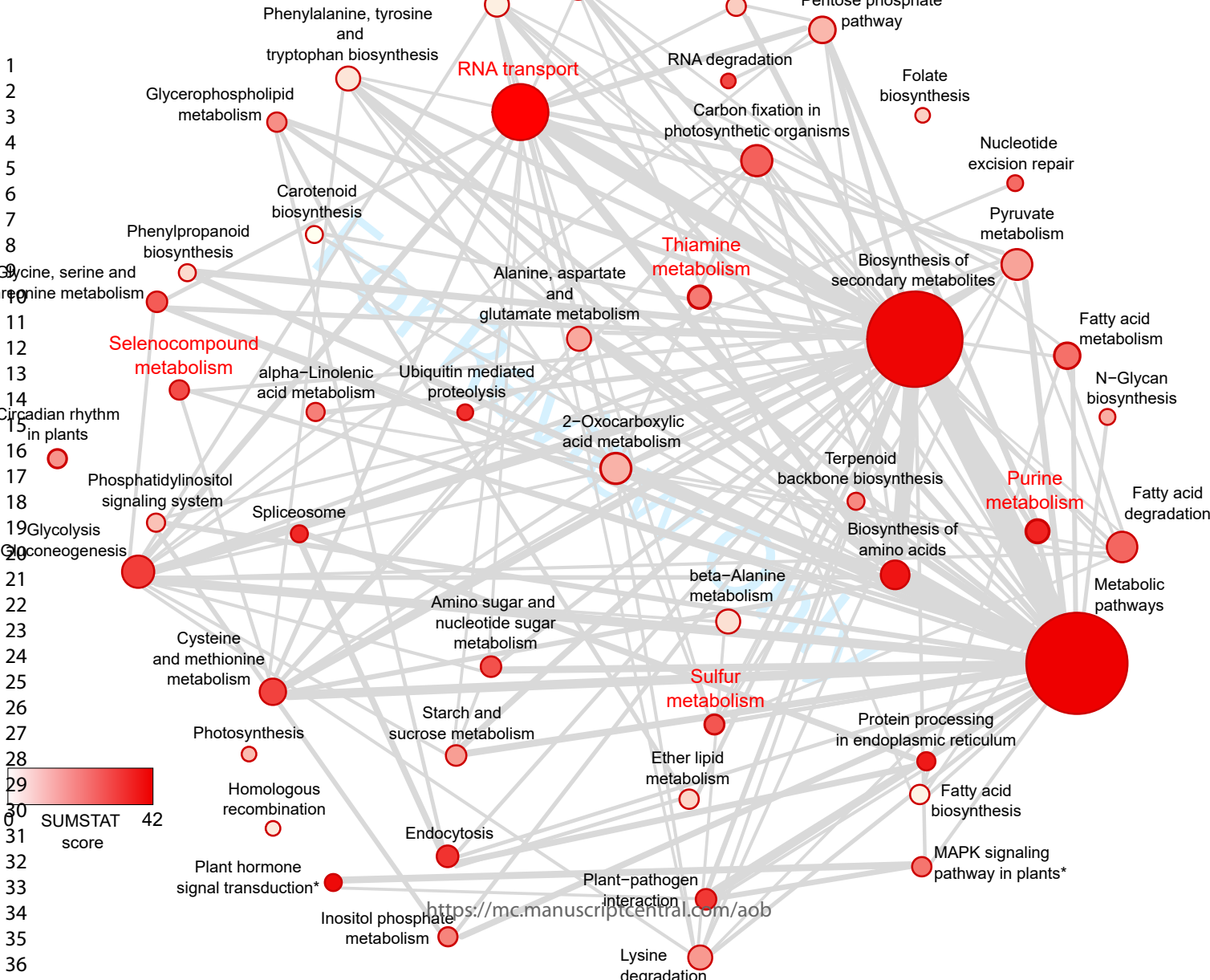
Guadarrama

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1
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3 **1 Original Article**
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5 **2 ADAPTIVE VALUE OF MARGINAL POPULATIONS: INTEGRATING**
6
7 **3 SELECTIVE SIGNALS, NEUTRAL PROCESSES AND TEMPORAL SCALES**
8

9
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33 17 Running title: ADAPTIVE VALUE OF MARGINAL POPULATIONS
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1. ABSTRACT

Background and aims: The importance of populations inhabiting ecologically marginal areas has been extensively debated and shown to depend, to a large extent, on their adaptive value. In the context of climate change, their ecological originality can give marginal populations the capacity to generate unique gene combinations. In this study, we searched for evidence of genetic diversity of adaptive value in marginal populations, through a genomic analysis that encompasses a multidimensional approach, integrating selective signals, neutral processes and temporal scales.

Methods: We used a targeted exome sequencing approach to search for genomic signatures of divergent selection between environmentally marginal and core populations of the Mediterranean alpine species *Silene ciliata*. We also analysed their current genetic population structure and demographic history, as well as signatures of selective sweeps at different levels.

Key results: Populations from core and marginal environments presented similar values of nucleotide diversity, inbreeding, and relatedness. Demographic history played a more relevant role than current environmental conditions in explaining the levels of genetic diversity in marginal populations. We also detected putative signals of diversifying selection among populations inhabiting environmentally marginal and core areas, involving 11 SNPs located in nine genes, as well as five gene pathways.

Conclusions: Populations currently inhabiting marginal environments can diverge from populations those occupying core areas due to local adaptation despite low overall genetic differentiation. The availability presence of polymorphisms or traits adapted to specific environments in marginal populations can may be particularly helpful valuable in conservation management and restoration, especially in the context of changing environments environmental conditions.

1
2
3 1 **Key words:** climate change, demographic inference, environmental gradients, divergent
4 selection, gene pathways, genetic adaptation.
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8 4 **2. INTRODUCTION**

9
10 Environmental heterogeneity induces key demographic, genetic and phenotypic
11 differentiation within the natural ranges of species, leading to the identification of *core*
12 and *marginal* areas (Soulé 1973; ~~Hardie and Hutchings 2010;~~ Pouget *et al.* 2013; Pironon
13 *et al.* 2017). Core areas, characterized by prevalent and, presumably, optimal
14 environmental conditions, host large and, often, genetically diverse populations. In
15 contrast, marginal areas, with atypical and potentially restrictive environmental
16 conditions, present a more complex scenario (Soulé 1973; Brown 1984).
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18

19 Historically, marginal populations have been perceived as genetically impoverished and
20 maladapted due to genetic drift and inbreeding, ~~and~~ thus harbouring little evolutionary
21 potential (Lynch and Gabriel 1987; Lande 1994; Eckert *et al.* 2008a). However, evidence
22 from some species suggests that these populations may be locally adapted to withstand
23 specific environmental pressures. This makes them particularly valuable in the context of
24 climate change, as their genetic diversity may confer adaptive advantages, enhancing the
25 organisms' chances of survival and reproduction (Barrett and Schluter 2008; ~~Hoffmann~~
26 ~~and Sgró 2011;~~ Morente-López *et al.* 2021). Despite the contrasting views, most studies
27 have been based on limited genetic information, lacking a genomic perspective (Stillman
28 and Armstrong 2015; Razgour *et al.* 2019). Genomic approaches can help to identify
29 unique adaptations in marginal populations as well as to forecast their responses to future
30 environmental pressures (e.g., Theraroz *et al.* 2024).
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33 Genetic adaptation is influenced not only by environmental pressures but also by
34 available genetic diversity, past demographic history and gene flow (Kawecki 2008).
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3 1 High levels of genetic diversity within populations are essential for natural selection and
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5 2 adaptation to take place. Demographic events like bottlenecks can reduce this diversity
6
7 3 (Carbognani *et al.* 2019), while population growth can either diminish or augment it,
8
9 4 depending on the influx of new genetic variants (Haghighi and Routman 2016). Gene flow
10
11 5 can either facilitate adaptation by boosting genetic diversity (Eckert *et al.* 2008b; **Hardie**
12
13 6 **and Hutchings 2010**; Morente-López *et al.* 2021; **Sacristán-Bajo et al 2025**) or hinder it
14
15 7 by introducing disadvantageous variants that are removed from the population by natural
16
17 8 selection (Lenormand 2002).

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22 9 When genetic adaptation is studied from a genomic perspective, the genomic signature of
23
24 10 selection is superimposed on widespread signatures of the population's demographic
25
26 11 history. For instance, both bottlenecks and population growth can generate nucleotide
27
28 12 variation patterns that mimic selection (Barton and Etheridge 2004; Ramírez-Soriano *et*
29
30 13 *al.* 2008). Hence, to identify adaptive genetic variants, it is imperative to employ
31
32 14 modelling techniques capable of differentiating between the genetic signatures of
33
34 15 selection and those stemming from neutral evolutionary processes (e.g., González-
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36 16 Martínez *et al.* 2017; Mayol *et al.* 2020; Evans *et al.* 2023).

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41 17 Alpine ecosystems exhibit high environmental variability, leading to rapidly shifting
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43 18 selective pressures over short geographical scales (Byars *et al.* 2007; Morente-López *et*
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45 19 *al.* 2022). In this context, *Silene ciliata* Pourret, a well-studied Mediterranean alpine
46
47 20 cushion plant, exemplifies key questions in ecology and evolution in marginal plant
48
49 21 populations (García-Fernández *et al.* 2012; Lara-Romero *et al.* 2016; Morente-López *et*
50
51 22 *al.* 2021, 2022). Field studies have shown lower longevity, lower proportion of flowering
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53 23 plants and fewer flowers, fruits, and seeds per plant in the lower-elevation populations
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55 24 (Giménez-Benavides *et al.* 2007; Giménez-Benavides *et al.* 2011). This results in
56
57 25 declining growth rates in low-elevation populations faciing, which face local extinction,

1 while higher-elevation populations in more favourable environments ~~showed~~show stable
2 growth rates (Giménez-Benavides, ~~Albert, et al. 2011~~). In parallel, studies
3 in common-garden conditions, have shown delayed flowering onset, peak and end
4 (Morente-López et al., 2020), lower seed germination (Giménez-Benavides et al. 2007),
5 and greater drought tolerance (García-Fernández et al., 2013) in low-elevation
6 populations. Interestingly, ~~phenotypic and quantitative genetic~~additional research
7 suggests that ~~these marginal~~ populations at lower elevations may possess adaptive
8 advantages over their core counterparts, ~~particularly in terms of germination, early~~
9 ~~establishment, and flowering phenology, which~~ that may be better suited to current and
10 future climatic conditions (~~Giménez-Benavides, García-Camacho et al. 2011~~; Morente-
11 López et al. 2020, 2021). Furthermore, in the northern Mediterranean mountain ranges
12 where the species occurs, the last ~~Pleistocene glaciations~~glacial maximum (LGM) have
13 influenced the demographic dynamics and current distributions of many plant species
14 (Feliner 2014; Carbone et al. 2019; Evans et al. 2023; Pütz et al. 2024), although this
15 aspect has not yet been studied in *S. ciliata*. This lack of knowledge is significant because
16 demographic history could have influenced not only the current distribution of the
17 species, but also the observed patterns of genetic adaptation. Understanding these patterns
18 in mountain populations requires considering both the existence of LGM microrefugia
19 and the dynamics of rear-edge and leading-edge populations (Hampe & Petit 2005;
20 Opgenoorth et al. 2009; Vila-Cabrera et al. 2019). LGM microrefugia represent localized,
21 climatically stable areas where species persisted during glacial periods, often
22 corresponding to present-day rear-edge populations (Rull 2009). Rear edge populations,
23 located at the oldest and most stable habitat margins, reflect ancestral conditions. In
24 contrast, leading edge populations, found at the most recent and dynamic habitat margins,

1 reflect modern conditions and exhibit rapid adaptations to ongoing environmental
2 changes (Hampe & Petit 2005; Vila-Cabrera *et al.* 2019).

3 In this study, we ~~soughtaimed~~ to ~~increase the evidence for~~assess the adaptive value of
4 marginal populations through a genomic analysis that encompasses both selective and
5 neutral processes, using a multiscale perspective that considers also recent and ancient
6 processes. Our goal was to explore how past climatic fluctuations—particularly the
7 LGM—and present-day environmental gradients have shaped the patterns of genomic
8 variation in core and marginal populations. We sequenced 2,414 candidate genes in six
9 natural populations (three core and three marginal), based on previous research that
10 identified SNPs associated with environmental stress responses (Sacristán-Bajo *et al.*
11 2019). Using F_{ST} -outlier detection to assess long-term adaptation and selective sweep
12 analyses for more recent events, we identified potentially adaptive variation ~~at~~across
13 different timescales. Our ~~exploration~~analysis of adaptive genetic diversity extended to
14 gene pathways to ~~explore~~investigate the role of gene networks in adaptation (Olson-
15 Manning *et al.* 2012; Berg and Coop 2014; Exposito-Alonso *et al.* 2018). In addition, we
16 examined spatial genetic structure and population dynamics using demographic inference
17 models. To date, only a few studies have assessed the adaptive value of marginal
18 populations using combined analyses of population genetic structure ~~analyses combined~~
19 ~~with,~~ demographic ~~inference model~~history and genomic signals of selection (e.g., Yang
20 *et al.* 2016) ~~or genomic analyses to identify signals of adaptation (e.g.,~~ Temunović *et al.*
21 2020; Yuan *et al.* 2023). Moreover, none has ~~used~~applied a multidimensional ~~approach~~
22 ~~that integrates~~framework integrating all ~~of~~ these perspectives. In our study, we asked the
23 following questions:

- 24 1. Do core and marginal populations ~~have different~~differ in levels of genetic
25 diversity, inbreeding or relatedness?

1 2. What is the spatial genetic structure of the studied populations? Which candidate
 2 genes and biological pathways ~~are potentially involved in explaining~~ may underlie
 3 adaptive genetic differentiation between core and marginal populations?

4 3. Have there been abrupt changes in effective population sizes over time? If so, can
 5 they be ~~dated~~ linked to the ~~last glacial period?~~ And LGM, and are there genomic
 6 signatures (e.g., of selective sweeps) ~~that can be associated to adaptive processes~~
 7 driven by past environmental changes indicative of adaptation in either rear-edge
 8 or leading-edge contexts?

9 We hypothesised that distinct selective pressures operating on core versus marginal
 10 populations have driven genetic differentiation between ~~these groups~~ them. We further
 11 ~~theorized~~ expected that contemporary environmental stressors have accelerated
 12 ~~population~~ demographic decline in marginal populations, ~~leading to reduced~~ reducing their
 13 genetic diversity. ~~Additionally~~ Finally, we expected that the ~~most recent glacial~~
 14 ~~maximum~~ LGM induced a severe demographic contraction, generating a genetic
 15 bottleneck ~~whose~~ signature ~~remains~~ still detectable in ~~the~~ present-day genetic variation ~~of~~
 16 ~~the studied populations.~~

18 3. MATERIALS AND METHODS

19 3.1 Study species and populations

20 *Silene ciliata* Pourr. (Caryophyllaceae) is a dwarf cushion perennial plant that inhabits
 21 Mediterranean alpine habitats with marked environmental gradients. It has flowering
 22 stems that grow up to 15 cm tall, with each stem bearing 1 to 5 flowers. Its fruit capsules
 23 contain up to 100 seeds, which are dispersed by wind in August and September. The seeds
 24 are small, averaging 0.59 mg in mass and 1.1 to 1.5 mm in diameter, and lack specialized

1 dispersal structures, making the species primarily barochorous. Pollination is carried out
2 by syrphid flies, bumblebees, and moths. The plant is self-compatible, but self-pollination
3 is limited due to significant protandry (see Lara-Romero et al. 2014 and references
4 therein). The distribution of *S. ciliata* comprises the mountain ranges of the Northern
5 Mediterranean area from Spain to Bulgaria (Kyrkou *et al.* 2015), reaching its
6 southernmost limit in the Sistema Central of the Iberian Peninsula (Spain). All *S. ciliata*
7 populations from the Sistema Central are diploid and have the same phylogenetic origin,
8 as shown by chloroplast DNA analysis (Kyrkou *et al.* 2015). In these areas, the species
9 grows from 1,800 to 2,500 m in dry cryophilic pastures above the tree line. This
10 Mediterranean Alpine ecosystem has a pronounced summer drought combined with high
11 solar radiation, which typically selects for plant xerophytic traits.

12 At the end of the summer of 2013, we collected a minimum of thirty randomly selected
13 plants from six populations located in three sub-mountain ranges of the Sistema Central
14 (Spain): Guadarrama (GDM), Gredos (GRD) and Béjar (BJR) (Figure 1 and Table 1). All
15 sampled plants were separated by at least two meters. The Sistema Central is a southwest-
16 northeast oriented mountain range of approximately 500 km located in the centre of the
17 Iberian Peninsula. Guadarrama, GredosGDM, GRD, and BéjarBJR are in the western,
18 central and eastern areas of the Sistema Central, respectively. The six studied populations
19 were located at the high and low elevation margins of the sub-mountain ranges (Figure 1
20 and Table 1). Within each sub-mountain range, the populations were separated by
21 distances ranging from two to four kilometers. High and low elevation margins represent
22 core (optimal) and marginal environmental conditions, respectively, based on the study
23 of the ecological niche and putative selective pressures for the species (Morente-López *et*
24 *al.* 2022). According to Morente-López *et al.* (2022), core populations inhabit regions
25 where habitat suitability equals or surpasses the highest 33rd percentile of the distribution

1 of ranked suitability values, estimated using the MaxEnt algorithm. Conversely, the
2 marginal populations are found below the lowest 33rd percentile. In marginal
3 populations, vegetation is dominated by dense shrub cover of *Cytisus oromediterraneus*
4 *Rivas Mart. et al.* and *Juniperus communis* L. subsp. *alpina* (Suter) Celak. *S. ciliata* is
5 restricted to small, isolated *Festuca curvifolia* Lag. ex Lange pastures, and populations
6 are small, with at most a few hundred individuals. In contrast, core populations are
7 extensive, dominated by *Festuca* fellfields with scarce shrubs, and support many
8 thousands of individuals. Marginal populations are subject to shorter snow cover duration
9 and higher minimum temperatures compared to core populations, reflecting the main
10 climatic gradients relevant to the species (Morente-López *et al.* 2022). Cuttings were
11 obtained from the collected plants, rooted and placed in pots in the Universidad Rey Juan
12 Carlos CULTIVE experimental field (<https://urjc-cultive.webnode.es>). Resulting plants
13 were grown in common garden conditions before DNA extraction in 2015. Further details
14 on the common garden experiment are provided in Morente-López *et al.* (2020).

15 **3.2 Selection of candidate genes for targeted sequencing**

16 The annotated *Silene latifolia* Poir. and *S. ciliata* transcriptomes (GenBank references:
17 GCA_900095335 and PRJNA528948, respectively; see Sacristán-Bajo *et al.* 2019) were
18 used to design an exome capture experiment based on targeted sequencing. We sequenced
19 a total of 2,414 candidate genes with high-confidence annotations. Candidate genes were
20 selected considering three factors that address specific aspects related to genetic
21 differentiation and adaptation in *S. ciliata*. The first set comprised 576 genes carrying
22 SNPs with unusually high allele frequency differentiation between core and marginal
23 populations (Sacristán-Bajo *et al.* 2019). Briefly, these SNPs were identified in a previous
24 study based on 147,118 SNPs distributed along 12,688 sequences obtained from mRNA
25 libraries of leaf tissue without any prior experimental treatment in common garden

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3 1 conditions. The second set comprised 489 genes annotated with the Gene Ontology term
4
5 2 “Flower development” (GO: 0009908) or any of the dependent and more specific terms.
6
7 3 These genes are expected to be involved in the development of the flower over time, from
8
9 4 its formation to its mature structure. This set of genes was selected because previous
10
11 5 quantitative genetic studies in *S. ciliata* indicate the existence of important differences,
12
13 6 probably related with contrasted selective pressures, in the flowering phenology of plants
14
15 7 from marginal and core populations (Giménez-Benavides, García-Camacho, *et al.* 2011;
16
17 8 Morente-López *et al.* 2020). Finally, the third set included 1,349 annotated genes
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19 9 associated with general processes that result in a change in state or activity of a cell or an
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21 10 organism because of an abiotic stimulus (GO: 0009628 or any of the more specific terms).
22
23 11 This third set was included in the study to detect a broad range of new candidate genes
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25 12 under divergent selection not previously identified in *S. ciliata*, as previous work on the
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27 13 species was hampered by low statistical power and may have left much of the potential
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29 14 adaptive variants unidentified.
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36 15 **3.3 DNA extraction, targeted sequencing and SNP calling**

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38 16 We used the DNeasy Plant minikit (QIAGEN, Valencia, USA) to extract and isolate DNA
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40 17 from 94 plants (Table 1), 15-17 for each of the six studied populations. Library
41
42 18 preparation covering the targeted sequences followed the manufacturer’s protocol for
43
44 19 Nugen SeqCap Developer kit. Samples were sequenced as 96-plex on a HiSeq2500 lane
45
46 20 with paired-reads of 125 bp. Demultiplexing of reads was carried out with CASAVA
47
48 21 1.8.2 (Illumina Inc., San Diego, CA). Reads were masked for adapter contamination with
49
50 22 *cutadapt* and low-quality bases were removed using *erne-filter* (ERNE v.1.4.5).
51
52 23 Alignment was carried out with BWA MEM v.0.7.10 (<https://github.com/lh3/bwa>). The
53
54 24 aligned sequences obtained from the targeted exome sequencing were deposited in
55
56 25 GenBank with accession number PRJNA851086. Prior to SNP calling, alignments were
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1 filtered for low mapping quality (<10) and reads were realigned around indels using
2 GATK 3.6 (McKenna *et al.* 2010). SNP calling was performed on the entire sample
3 simultaneously using the *UnifiedGenotyper* implemented in the GATK suite, which
4 allowed the initial identification of ca. 185,000 SNPs.

5 Raw SNP data were filtered using VCFtools v0.1.14 (Danecek *et al.* 2011) and *vcffilter*
6 function of VCFLIB (Garrison *et al.* 2022). Only biallelic SNPs with fewer than 20%
7 missing data were kept. Indels were also removed from the dataset. SNPs were then
8 filtered following the hard filtering suggested by GATK's user guide
9 (<https://gatk.broadinstitute.org/>). This stringent filtering reduced the SNP dataset to
10 112,751 SNPs. Further, SNPs from paralogous genes were filtered combining two
11 approaches. First, the SNP dataset was filtered by Hardy-Weinberg disequilibrium and
12 polymorphism excess (Chen *et al.* 2014). To apply this filter, we estimated F_{IS} in sliding
13 windows of 150 bp with a step of 50 bp. Sliding windows with an average F_{IS} lower than
14 -0.5 were filtered (Hohenlohe *et al.* 2013; McKinney *et al.* 2017), which entailed the
15 removal of 27,728 SNPs. Second, for the retained dataset (85,023 SNPs), we applied the
16 *HDplot* method. *HDplot* combines information from the relative proportion of
17 heterozygotes in a population (H) and the deviation of allele-specific reads of each locus
18 from a 1:1 ratio (D), allowing the identification of paralogous loci throughout the range
19 of allele frequencies (McKinney *et al.* 2017). A total of 13,362 SNPs (16%) with H
20 greater than 0.65 and $|D| > 20$ were classified as located in paralogous sequence and
21 subsequently removed, resulting in a final SNP dataset of 71,661 SNPs distributed in
22 2,278 genes.

23 **3.4 Nucleotide diversity, population structure and demographic history**

24 **Nucleotide diversity, inbreeding and relatedness**

1
2
3 1 Nucleotide diversity by population (Tajima's π and Watterson's θ) and standard neutrality
4
5 2 tests (Tajima's D , and Fu & Li's D^* and F^*) were computed using mstatspop
6
7 3 (<https://github.com/CRAGENOMICA/mstatspop>). Additionally, we estimated the
8
9 4 number of polymorphic sites, average inbreeding coefficients (F) and pairwise
10
11 5 relatedness (as evaluated by unadjusted A_{jk} statistic) for each population using VCFtools
12
13 6 v0.1.14 (Danecek *et al.* 2011).
14
15

17 **7 Population genetic structure**

18
19
20 8 We investigated population genetic structure using the hierarchical analysis of molecular
21
22 9 variance (AMOVA) implemented in Arlequin v3.5.2 (Excoffier and Lischer 2010). The
23
24 10 analysis was conducted at three levels: (1) among mountain ranges; (2) between core and
25
26 11 marginal populations within mountain ranges; and (3) within populations. Genetic
27
28 12 differentiation was calculated for all three hierarchical levels, leading to estimates for F_{CT}
29
30 13 (among mountain ranges), F_{SC} (between core and marginal populations within mountain
31
32 14 ranges) and F_{ST} (among populations overall). F_{ST} was also calculated between all pairs of
33
34 15 populations, and the significance levels of pairwise F_{ST} estimates were determined by
35
36 16 permutation tests (10,000 permutations). Population genetic structure was also inferred
37
38 17 by a Bayesian clustering method using fastStructure, with K groups from $K=1$ to $K=6$ (the
39
40 18 number of localities in our sample), and the simple prior. For improved ancestry estimates
41
42 19 and to identify subtle structure, the logistic prior was executed at values of K similar to
43
44 20 those identified when using the simple prior. Then runs for $K=2$ and $K=3$ (the best K)
45
46 21 were repeated ten times, and averaged Q values (i.e., the individual assignment
47
48 22 probability to each of the K groups) were used to draw bar plots. As an alternative to
49
50 23 AMOVA and fastStructure, we used a Neighbour-net phylogenetic network (NNet)
51
52 24 constructed in SplitsTree v 4 (Huson and Bryant 2006), based on uncorrected_p distance
53
54 25 with default parameters and 1,000 bootstraps. In order to account for gene flow while
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1 inferring the relationships among *S. ciliata* populations, we also used TREEMIX v1.13
2 (Pickrell and Pritchard 2012). This method models patterns of both population splits and
3 admixtures accessing the covariance structure of allele frequencies between populations
4 and performing a Gaussian approximation for genetic drift, improving the fit of the
5 inferred model by enabling migration edges. TREEMIX analyses were implemented with
6 500-SNP bootstrap resampling and considering a different number of migration events,
7 ranging from zero to 10.

8 **Demographic inference**

9 *Demographic inference at population level:* An overall negative Tajima's *D* indicated an
10 excess of low frequency polymorphisms (see Results), suggesting population growth.
11 Based on these results, we tested multiple demographic models for each population
12 separately using the coalescent approach implemented in Fastsimcoal2 v.2.6.0.2
13 (Excoffier *et al.* 2013). Fastsimcoal2 estimates the composite likelihood of a specific
14 demographic scenario given the observed data as well as population genetic parameters
15 such as divergence time, effective population size and gene flow, using the site frequency
16 spectrum (SFS) as input. Un-folded site frequency spectra (calculated from observed
17 counts of the derived allele) were generated separately for each of the six populations
18 using VCF2SNPs (Liu *et al.* 2018). To maximize statistical power, we used all SNPs,
19 including those in intragenic regions, for demographic inference, assuming most behave
20 neutrally or quasi-neutrally. This assumption is supported by low levels of linkage
21 disequilibrium (mean $r^2 = 0.11$) and a very small proportion of SNPs showing signatures
22 of selection (see Results). We compared three demographic models (Figure 2). The first
23 "Stationary" model represents a null model consisting in a population of constant size and
24 is defined by a single parameter, the current population size (N_{CUR}). The second
25 "Expansion" model is defined by three parameters: N_{CUR} , a time of instantaneous

1 population size change (T_{EXP}) and a population size prior to T_{EXP} . The third “Bottleneck”
2 model is characterized by four parameters: N_{CUR} , a time ~~of instantaneous population size~~
3 ~~change at which the bottleneck began~~ (T_{BOT}), a population size during the bottleneck event
4 (N_{BOT}), and a population size prior to T_{BOT} (N_{ANC}). The duration of the bottleneck event
5 was fixed at 100 generations to avoid problems associated with model
6 overparameterization. We performed 100 independent runs for each population and
7 demographic model consisting of 50 cycles of the Brent algorithm and 100,000
8 simulations. The maximum-likelihood runs under each model were then compared using
9 AIC to select the optimal model for each population. Once the optimal model was
10 identified, we estimated confidence intervals (CIs) by performing 100 parametric
11 bootstraps based on best-model parameters. Mutation rate was estimated from the data
12 assuming a generation time of two years, which reflects the time required for the plant to
13 reach reproductive maturity (Giménez-Benavides, ~~Albert,~~ *et al.* 2011).

14 *Demographic inference at mountain range level:* Previous studies have suggested
15 historical environmental fluctuations related to glaciations affecting *S. ciliata* populations
16 in central Spain (Morente-López *et al.* 2018). Glacial pulses would have been responsible
17 for shifting *S. ciliata* populations to lower elevations. Postglacial recolonization of higher
18 elevations from lower areas would have originated a front-advancing edge by a subset of
19 the populations at lower elevation. To better understand the potential effect of
20 environmental fluctuations during glaciations on *S. ciliata* populations, we used
21 Fastsimcoal2 to statistically compare the relative fit of multi-population demographic
22 models (“mountain models”) incorporating three possible demographic scenarios
23 involving both core and marginal populations (Figure 2b). The first model (“Isolation of
24 two populations”) was parametrised with the current population size of marginal (N_M)
25 and core populations (N_O), and a time of instantaneous split (T_{DIV}) from a common

1
2
3 1 ancestral population (N_{ANC}); it represents a simple scenario without effective population
4
5 2 size changes. The second model (“Bottleneck and Isolation of two populations”) also
6
7 3 considered the split of marginal and core populations from a common ancestral
8
9 4 population but included a bottleneck event in the ancestral population prior to the split.
10
11 5 This bottleneck event was modelled defining a time ~~of instantaneous population size~~
12
13 6 ~~change when the bottleneck began~~ (T_{BOT}), a population size during the bottleneck event
14
15 7 (N_{BOT}) and a population size posterior to T_{BOT} (N_{DIV}). Finally, the third model
16
17 8 (“Bottleneck and Divergence of a population”) considered marginal populations as a
18
19 9 result of a bottleneck in the ancestral population from which the core populations would
20
21 10 have subsequently diverged; this model reflects the suggested glacial dynamics of the
22
23 11 species in the Sistema Central mountain range described above. The three models
24
25 12 included migration between marginal and core populations after the divergence event
26
27 13 (M_{MC} and M_{CM}), as considering gene flow always improved model fitting (data not
28
29 14 shown). The modelling set-up and assessment of model performance was the same as
30
31 15 described for the models fitted at the population level.
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19 **3.5 Detecting distinct signatures of selection in marginal vs core populations**

20 **Hierarchical F_{ST} -outlier analyses**

21 To investigate the contribution of natural selection to genetic adaptation in *S. ciliata*, we
22 combined multiple approaches. First, we searched for signals of divergent selection at the
23 single-locus (SNP) level using hierarchical F_{ST} -outlier analyses implemented in Arlequin
24 and BayeScanHierarchical. For Arlequin, we ran 20,000 simulations under a hierarchical

1 island model with 100 demes per level to establish a null distribution. To control for false
2 positives, we applied a False Discovery Rate (FDR) correction and considered loci with
3 a $1-F_{ST}$ quantile < 0.05 as outliers (Storey et al 2003). BayeScanHierarchical, which
4 extends the standard BayeScan Bayesian likelihood method to hierarchical population
5 structure, was used to identify loci with strong evidence of selection (Foll *et al.* 2014). To
6 ensure adequate statistical power, we set the minimum number of iterations to 10,000, the
7 length of 20 pilot runs to 7,500 iterations, and the burn-in-length to 50,000 iterations. Loci
8 having a Q -value (analogue to a FDR) lower than 5% were considered to be under strong
9 selection.

10 Second, we estimated AFDs (Allele Frequency Differentials) between pairs of core and
11 marginal populations. To reinforce the reliability of our results, we applied a strict
12 criterion, considering a locus as a candidate for selection only if it was identified as an
13 outlier by both hierarchical F_{ST} -outlier analyses and showed consistent AFD patterns
14 across all three pairwise comparisons between core and marginal populations. For the
15 AFD comparisons, the expected number of random significant tests was calculated by
16 multiplying the number of SNPs tested by the probability of AFDs going in the same
17 direction in each comparison by chance (0.5^3), indicating that several thousand SNPs
18 could show consistent AFDs in the same direction by chance. However, combining AFDs
19 with hierarchical F_{ST} -outlier detection at standard FDR of 0.05 allowed us to increase
20 substantially the confidence in our results (FDR of combined test of 0.00625).

21 **Detection of selective sweeps**

22 To identify recent hard selective sweeps, we employed the SWEEPFINDER algorithm
23 implemented in SweeD v.3.1 (Pavlidis *et al.* 2013). We chose to study hard selective
24 sweeps for their capacity to reveal recent genetic adaptation responding to shifting
25 environmental pressures. This method has the benefit to derive the null hypothesis from

1 the background pattern in the data itself, making it more robust to demographic events
2 such as population growth (Nielsen *et al.* 2005; Pavlidis *et al.* 2013). For each gene,
3 Composite Likelihood Ratios (CLRs) were calculated at 30 equally-spaced positions
4 across the target genomic region. To increase statistical power, all CLR scans were run
5 on unfolded SFS and monomorphic sites were included in the analysis (Pavlidis *et al.*
6 2013). To further increase power to detect selective sweeps, we only applied the test on
7 1,758 candidate genes that had 10 SNPs or more, which involved 52,740 SNPs.
8 Significance thresholds for selective sweeps detected in each population were obtained
9 by computing the distribution of CLRs in 100 data sets obtained by coalescence
10 simulations in SFS_CODE (Hernandez 2008). The simulations were based in a
11 demographic scenario of population expansion after a bottleneck, which was the most-
12 likely demographic model in all the studied populations based on Fastsimcoal2 (see
13 Results). To ensure a conservative approach, we only considered selective sweeps with
14 higher likelihood than the maximum obtained in the simulations across all core and
15 marginal populations.

16 **Selection at the gene pathway level**

17 We used a procedure developed in humans (Daub *et al.* 2013, 2017) and tested in plants
18 (Mayol *et al.* 2020), to detect the joint effect of selection in biological pathways. For this
19 purpose, we used KEGG Automatic Annotation Server to map our 2,278 candidate genes
20 and retained 246 biological pathways that included at least four genes. This threshold was
21 selected to provide sufficient statistical power for subsequent analyses while maintaining
22 a reasonable number of pathways for biological interpretation. While a larger number of
23 genes per pathway would provide greater statistical power, it would also significantly
24 reduce the number of pathways analyzed. We refined this gene-pathway list by removing
25 non-plant pathways (see a similar approach in Mayol *et al.* 2020), resulting in a final

1
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3 1 selection of 86 known pathways in plants. Then, for the maximum value of the coefficient
4
5 2 of selection *alpha* estimated by SweeD and BayeScanHierarchical, we performed a gene
6
7 3 set enrichment approach as in Daub *et al.* (2013). For this purpose, we used the POLYSEL
8
9 4 pipeline (<https://github.com/CMPG/polysel>). This pipeline calculates for each statistic
10
11 5 the SUMSTAT score (Tintle *et al.* 2009), which is the sum of the values for that statistic
12
13 6 of all genes in a given pathway. Then, the pipeline compares SUMSTAT scores, after
14
15 7 different bias corrections, to a null normal distribution of 500,000 random sets created
16
17 8 using a sequential random sampling method (Ahrens and Dieter 1985), with the same size
18
19 9 as the original pathway. As in Daub *et al.* (2013), we reported the gene pathways that
20
21 10 showed evidence of having collectively higher gene selection coefficients (Q -value <
22
23 11 0.2), which establishes a probability of finding up to 17 pathways falsely identified as
24
25 12 significant by chance alone.
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18 **4. RESULTS**

19 **4.1 Nucleotide diversity, population structure and demographic history**

20 **Nucleotide diversity, inbreeding and relatedness**

21 The comparison between core and marginal populations revealed minimal differences in
22 genetic parameters. Nucleotide diversity, inbreeding and relatedness were similar for
23 individuals in both environments (Table 2, all Wilcoxon rank tests: $P \geq 0.1$). Most

1 neutrality tests were negative (only CAM had a positive result; Table 2) indicating an
2 excess of rare polymorphisms, which can be interpreted as a demographic signature of
3 population expansion.

4 **Population genetic structure**

5 Three-level hierarchical analysis of molecular variance (AMOVA, Table S1) showed that
6 most of the genetic variation (91.3%) was found within populations, though a significant
7 portion of the variation (6.7%) occurred among mountain ranges. A small (2%) but
8 significant genetic differentiation was detected between core and marginal populations
9 within mountain ranges. Pairwise F_{ST} values were significant for all population pairs and
10 ranged between 0.0155 and 0.1201 (Table S2), being higher for comparisons between
11 mountain ranges (mean \pm *SD*: 0.086 ± 0.030 , $n=12$) than within mountain ranges (0.022
12 ± 0.015 , $n=3$). The optimal number of genetic groups in the Bayesian clustering analysis
13 performed using fastStructure was $K=2$, with one group corresponding to Guadarrama
14 and the other group corresponding to Bejar and Gredos (i.e., the westernmost) mountain
15 ranges (Figure 3a). The Neighbour-net network (Nnet) exhibited three well defined
16 groups corresponding with the three mountain ranges studied (Figure 3b). Within each
17 mountain range group, individuals from marginal and core populations were not clearly
18 differentiated. The disposition of the three groups within the network indicated that
19 Gredos and Bejar are more closely connected between them than with Guadarrama. Such
20 a partition is also supported by the TREEMIX graph (Figure 4e3c), which showed
21 evidence of admixture between populations in Bejar (NEG) and Gredos (CAM), and a
22 clear separation of those in Guadarrama (Figure 3c).

23 **Demographic inference**

24 *Population level*

1
2
3 1 For all populations, the model incorporating changes in population size due to bottlenecks
4
5 2 and subsequent recent expansion (“Bottleneck” model in Figure 2a) was the most
6
7 3 supported based on AIC differences ($\Delta AIC > 100$ for all comparisons). In the highest-
8
9 4 likelihood parameter set (Figure 4), estimates of ancestral population size (N_{ANC})
10
11 5 averaged 44,191 (range 27,425-69,097). The bottleneck started in all populations around
12
13 6 4,000–9,500 generations ago, which resulted in a strong reduction of the effective
14
15 7 population size (mean $N_{BOT} = 169$, range 54-403). Current population size (N_{CUR}) had a
16
17 8 mean of 329 (range 158-525) and represents an increase of between 30% and 75% over
18
19 9 N_{BOT} . A recent population expansion was also supported for all populations except CAM
20
21
22 10 by negative neutrality test values (Figure 4; see above).

23 24 25 26 27 11 *Mountain range level*

28
29
30 12 Regarding the models fitted for each mountain range, the “Bottleneck and Isolation of
31
32 13 two populations” model (Figure 2b) had the lowest AIC scores ($\Delta AIC > 1500$ for all
33
34 14 comparisons). The highest-likelihood parameter set (Figure 5) suggests that ancestral
35
36 15 populations suffered a bottleneck around 9,000-18,500 generations ago, which reduced
37
38 16 drastically their effective population size. After the bottleneck, ancestral populations
39
40 17 recovered up to 65% of their previous effective population size. Populations inhabiting
41
42 18 current core and marginal areas within each mountain range subsequently diverged
43
44 19 around 2,129–13,800 generations ago. After divergence, core and marginal populations
45
46 20 were connected by low (i.e., near zero) and asymmetrical gene flow, with higher gene
47
48 21 flow from core to marginal populations (mean $M_{CM} = 6.62 \text{ E-}04$ vs. mean $M_{MC} = 7.59 \text{ E-}$
49
50 22 05). Estimates of current population sizes were systematically higher for marginal (mean
51
52 23 $N_{CUR} = 4,900$, range 2,055-8,195) than for core populations (mean $N_{CUR} = 2,324$, range
53
54 24 55-6,821). Estimates of N_{CUR} were also notably lower for westernmost mountain ranges
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1 (55 and 96 for Bejar and Gredos, respectively) than for eastern Guadarrama mountain
2 range (6,821).

3 **4.2 Detecting signatures of selection in marginal vs core populations**

4 **SNP and gene region level**

5 The hierarchical island model in Arlequin identified 750 SNPs as significant F_{ST} outliers
6 ($P < 0.05$ after FDR correction). Out of them, 35 SNPs showed consistent AFDs in all
7 comparisons between pairs of core and marginal populations. Candidate SNPs belonged
8 to 13 different genes. BayeScanHierarchical detected 170 outlier SNPs and 13 of these
9 (belonging to 11 genes) showed consistent AFDs. Eleven SNPs located on nine genes
10 were outliers using both methods (Table 3), and we considered them as potential
11 candidate loci under divergent selection. These potentially adaptive SNPs/genes are
12 involved in regulation of flower development, oxidation-reduction processes, response to
13 light stimulus, UV protection and different stress responses (Table 3). Out of the nine
14 genes, four were among those associated with adaptation to elevation in previous studies
15 (Sacristán-Bajo *et al.* 2019), four were annotated as GO: 0009628 (response to abiotic
16 stimulus) and two as GO: 0009908 (flower development) (Table 3).

17 Overall, composite likelihood ratio tests (CLRTs) found slightly more selective sweeps
18 in core populations (mean = 1,303, range 698-2,478) than in marginal ones (mean =
19 1,186, range 559-2,346). Eighteen selective sweeps located in 10 genes were found in all
20 the populations of the same type (Table 4) and were thus considered as potential candidate
21 gene regions under selection in either marginal or core environments. From them, nine of
22 the 10 genes were found in core populations and only one gene was systematically found
23 in all marginal populations. Only three selective sweeps located in the same gene
24 (PARG1, coding for a poly(ADP-ribose) glycohydrolase) were found in all the studied
25 populations (Table 4). None of the genes with signs of a selective sweep experienced

1
2
3 1 divergent selection according to F_{ST} -outlier analyses (Tables 3 and 4). Five and four of
4
5 2 them were involved in different biological processes associated with responses to abiotic
6
7 3 stimulus (GO: 0009628) and flower development (GO: 0009908), respectively, and three
8
9 4 were among the candidate genes associated with adaptation to elevation in previous
10
11 5 studies (Sacristán-Bajo *et al.* 2019; Table 4).
12
13
14

15 6 **Gene pathway level**

16
17
18 7 A total of 1,623 out of 2,250 genes mapped on existing KEGG pathway maps for plants
19
20 8 (Table S3). Mapped genes were distributed on 86 distinct pathways (Table S3). Five
21
22 9 pathways, involving 47 candidate genes, were enriched for positive values of α
23
24 10 estimated by BayeScanHierarchical (Q -value < 0.2; Table 4, Table S3) indicating
25
26 11 diversifying selection between core and marginal populations. A low proportion of
27
28 12 candidate gene pathways shared genes, with 42 out of the 47 genes being specific to only
29
30 13 one of the five pathways. None of these genes were among those found significant in
31
32 14 analyses at single-gene level (considering both F_{ST} outliers and selective sweeps; Tables
33
34 15 3 and 4, respectively). Enrichment analysis using the maximum value of α estimated
35
36 16 by SweeD did not detect any biological pathways with significant SUMSTAT scores.
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50 19 **5. DISCUSSION**

51 20 Remarkably, we found low genetic differentiation between core and marginal populations
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53 21 of *S. ciliata*, suggesting that both environments harbour a wealth of genetic variation on
54
55 22 which selection could act. The similar levels of genetic diversity observed between core
56
57 23 and marginal populations could, in part, be explained by historical evolutionary
58
59 24 processes, such as past may reflect the complex demographic history of the species.
60

1 ~~including range shifts,~~ expansions, and contractions ~~linked to associated with~~ climatic
2 ~~changes, which have oscillations during and following the LGM. These historical~~
3 ~~processes likely~~ shaped the ~~current distribution of genetic landscape of these~~ diversity in
4 ~~*S. ciliata*, enabling both core and marginal~~ populations: ~~to retain adaptive potential.~~ This
5 ~~finding~~ challenges the conventional view that marginal populations are genetically
6 impoverished. ~~Instead, our results suggest that these populations retain valuable adaptive~~
7 ~~genetic variation that could be essential for responding to~~ and stresses their role in
8 ~~responses to~~ environmental challenges: (Morente-López *et al.* 2021, 2022).

9 Based on current declining growth rates, we hypothesized that *S. ciliata* populations
10 inhabiting marginal areas might be genetically impoverished and lack adaptive value
11 (Lynch and Gabriel 1987; Lande 1994; Eckert *et al.* 2008a). Instead, our study revealed
12 an unexpected homogeneity in nucleotide diversity, inbreeding, and relatedness across
13 populations, irrespective of their environmental conditions. Moreover, populations in
14 marginal environments exhibit larger current population size (N_{CUR}), as estimated by
15 historical demography models. This similarity in genetic parameters challenges
16 preconceptions: rather than being genetically deprived, marginal populations possess
17 genetic diversity comparable to their core counterparts. Genetically-based phenotypic
18 differentiation between core and marginal populations, as previously documented
19 (García-Fernández *et al.* 2012; Morente-López *et al.* 2020, 2022; Prieto-Benítez *et al.*
20 2021), together with the presence of genomic adaptive divergence in marginal
21 populations (see Results), emphasize the adaptive value of *S. ciliata* marginal
22 populations. While these marginal populations retain adaptive genetic diversity, climate
23 change heavily impacts their survival. Environmental conditions at low elevations are
24 constraining the viability of the populations, as evidenced by present-time demographic
25 studies showing declining growth rates and higher local extinction risks at low elevations

1 compared to the stable growth rates in higher elevations (Giménez-Benavides, ~~Albert~~ *et*
2 *al.* 2011; Lara-Romero *et al.* 2014, 2016). Although available adaptive genetic diversity
3 seems not to be enough to adapt to their changing environment, it still may facilitate the
4 adaptation of higher-elevation populations through adaptive gene flow, as shown in
5 empirical studies (Morente-López *et al.* 2021; Prieto-Benítez *et al.* 2021).

6 Demographic inferences support a bottleneck around the last ~~glaciation~~ glacial maximum
7 and subsequent divergence of populations in core and marginal environments. This
8 divergence is plausible even at small spatial scales, given the species' limited dispersal
9 capacity (Lara-Romero *et al.* 2014) and the potential for genetic isolation in
10 heterogeneous landscapes, even despite spatial proximity (Budde *et al.* 2024). While
11 some populations descended to lower elevations, others likely persisted on mountaintops;
12 possibly in protected microhabitats climatically stable microrefugia (Feliner 2014;
13 Thompson 2020; Pütz *et al.* 2024). These mountaintop populations, consistent with the
14 concept of rear-edge populations (Hampe and Petit 2005; Opgenoorth *et al.* 2009),
15 show 2024). ~~This persistence in microrefugia would explain the~~ lower historical
16 population size (N_{ANC}) ~~in current mountaintop core populations (Carbognani *et al.* 2019)~~
17 ~~and their~~, marked genetic divergence from ~~low-elevation~~ marginal populations, ~~(as~~
18 ~~reflected by significant F_{SC} between core and marginal populations. The), and a higher~~
19 number of selective sweeps ~~in core populations further supports this scenario, indicating~~
20 ~~more ancient populations that have undergone.~~ These genomic signatures suggest long-
21 term persistence and multiple episodes of positive selection in isolated and stable
22 microrefugia (Colonna *et al.* 2014; ~~Harris~~ Carbognani *et al.* ~~2020~~ 2019; Deng *et al.* 2021).
23 ~~Our~~ In contrast, our results ~~also~~ suggest that marginal low-elevation populations
24 experienced past expansions, ~~driven by~~ during more favourable environmental
25 conditions climatic periods, consistent with the leading-edge dynamics of colonisation

1 into newly suitable environments (Hampe & Petit, 2005). However, progressive warming
2 over the last century has displaced the species' climatic optimum ~~towards higher~~
3 ~~elevations (Sanz-Elorza *et al.* 2003; Giménez-Benavides *et al.* 2007), explaining current~~
4 ~~demographic patterns~~ upslope (Giménez-Benavides *et al.* 2007; Morente-López *et al.*
5 2022), leading to current demographic declines in these now trailing-edge populations
6 (Giménez-Benavides *et al.* 2007; Lara-Romero *et al.* 2014; Morente-López *et al.* 2022).
7 ~~This scenario represents a 'rear-edge' situation (sensu Hampe and Petit 2005), where~~
8 ~~populations at the low elevation edges played a crucial role in maintaining~~
9 ~~species'~~ Despite this, their adaptive genetic diversity ~~and facilitating adaptation to~~
10 ~~environmental changes. This result questions the~~ may still contribute to the evolutionary
11 potential of core populations through adaptive gene flow (Morente-López *et al.* 2021;
12 Prieto-Benítez *et al.* 2022). These findings question typical assumptions about a direct
13 relationship between genetic ~~variation~~ diversity, range limits and current environmental
14 suitability, ~~suggesting that~~ highlighting instead the influence of past demographic history
15 ~~may still be more important than current environmental conditions~~ in shaping present-day
16 genetic ~~diversity~~ patterns (Duncan *et al.* 2015; Pironon *et al.* 2015; Yang *et al.* 2016).

17 The macroevolutionary forces shaping the distribution and diversity of *S. ciliata* have also
18 influenced microevolutionary processes, such as selection on specific genes and
19 pathways. With respect to individual genes, we detected signals of selection in four genes
20 related with flower development (*APSI*, *NUA*, *CYP71B37*, *atg4*). Previous work at the
21 phenotypic level suggested local adaptation for phenological traits in association with
22 elevation (Giménez-Benavides, García-Camacho, *et al.* 2011; Morente-López *et al.*
23 2020). This is an expected result since in alpine ecosystems flowering phenology is a
24 crucial demographic event (Molau *et al.* 2005; Sedlacek *et al.* 2015), forcing plants to
25 have a fine phenological adjustment to the climate (Scheepens and Sto 2013). Moreover,

1 we also identified candidate genes relevant for abiotic stress response in our study (Table
2
3 2). These include *PSBO* and *SCPL49*, recognized as drought-stress response genes
4
5
6 (Ashburner and The Gene Ontology Consortium 2000; Berardini *et al.* 2015; Shaar-
7
8 moshe *et al.* 2015), or *GH3*, which controls hypocotyl elongation, a molecular mechanism
9
10
11
12 critical for germination and seedling establishment (Berardini *et al.* 2015).
13
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15 Beyond the level of individual genes, our analysis revealed that adaptation in *S. ciliata*
16
17 involves complex genetic mechanisms, including selection acting on five metabolic
18
19 pathways. Four of them were involved in essential metabolic functions that are critical
20
21 for plant survival, namely thiamine, purine, sulfur and seleno compound metabolisms.
22
23
24 Abiotic stress alters plant metabolism, inducing biochemical and physiological responses
25
26 that create tolerance (reviewed in Ramakrishna and Gill 2019). These metabolic
27
28 adjustments involve fine regulations of several metabolic pathways including those
29
30 identified here (Lawlor and Cornic 2002; Yan *et al.* 2014; Heinemann and Hildebrandt
31
32 2021). The other gene pathway was related to RNA transport from the nucleus to the
33
34 cytoplasm, which plays pivotal roles in regulation of gene expression. Several studies
35
36 support the important role that mRNA export mechanisms play in abiotic stress responses
37
38 of plants (Chinnusamy *et al.* 2008; Ehrnsberger *et al.* 2019; Yeap *et al.* 2019). The
39
40 detection of this signal at the pathway level suggests that adaptation in species with
41
42 complex evolutionary histories may involve genetic traits controlled by multiple genes
43
44 within specific pathways. This challenges the traditional view of adaptation as being
45
46 driven by single-gene mutations. Remarkably, approaches focused on single loci failed to
47
48 detect signatures of selection for any of the genes involved in these pathways. Because of
49
50 their small effect-sizes, genes under “soft” selection are hard to detect by single-locus
51
52 approaches, although together they can have a large impact on a biological pathway
53
54 (Olson-Manning *et al.* 2012; Berg and Coop 2014).
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1 The candidate genes and pathways identified in this study offer an initial understanding
2 of the molecular mechanisms underlying the adaptive responses to marginal and core
3 environments. However, it is crucial to consider the possibility of false positives. Notably,
4 certain candidate genes revealed multiple significant SNPs, with some of these genes also
5 previously suggested as potentially involved in adaptation in an RNAseq experiment
6 (Sacristan *et al.* 2019). In addition, our strategy was guided by a focus on adaptive
7 molecular signals concurrently identified in the three replicated elevation gradients,
8 aiming at minimizing the occurrence of false positives (Thornton and Jensen 2007).
9 Nevertheless, further confirmation is necessary to ascertain the adaptive nature of
10 candidate genes and pathways, requiring additional research to establish a clear link
11 between allelic variation and fitness (e.g., Jaramillo-Correa *et al.* 2015; Vangestel *et al.*
12 2018; ~~Nagasaka *et al.* 2022~~).

13 **Conclusions**

14 Our findings ~~rejeete~~rejected the hypothesis that marginal populations of *S. ciliata* are
15 genetically impoverished. ~~Conversely~~Instead, they revealed similar levels of genetic
16 diversity in both core and marginal populations, shaped by historical events, with
17 standing genetic variation in marginal populations being essential for adaptation to ~~on-~~
18 ~~going~~ongoing climate change. By employing a genomic analysis ~~encompassing~~that
19 ~~encompassed~~ both selective and neutral processes, and adopting a multiscale
20 perspective that ~~considers~~considered both recent and ancient ~~processes~~dynamics, we
21 provided evidence of past selection in both environments and identified genes
22 potentially associated with flower development and abiotic stress responses. Notably,
23 genetic variation in *S. ciliata* may ~~have~~partly ~~result~~resulted from selection acting on
24 key metabolic pathways, which ~~underseore~~highlighted complex mechanisms of
25 adaptation. ~~Additional~~Further research is needed to validate ~~these genes'~~the adaptive

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3 1 role and fitness effects of these genes, as well as to assess the potential of *S. ciliata*
4
5 2 populations to respond to future environmental pressures.
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1 9. LIST OF CAPTIONS

2 **Figure 1.** Location of the three main mountain ranges in the Sistema Central of the
 3 Iberian Peninsula (Spain) where the study was developed: Bejar (BJR), Gredos (GRD)
 4 and Guadarrama (GDM). The three-dimensional coloured map represents an example of
 5 the intra-range environmental classification in core (optimal) and marginal areas
 6 developed for *S. ciliata* based on ecological niche modelling and putative selective
 7 pressures (see Morente-López *et al.* 2022). In each of the three mountain ranges, two
 8 populations were selected, one in core (blue) and one in marginal (red) environmental
 9 conditions. The approximate locations of the two populations selected in Guadarrama
 10 mountain range, Pico de Peñalara (PEN) and Morrena Peñalara (MOR) are depicted in
 11 optimal and marginal areas respectively. Black shaded areas in the map represents
 12 elevations above 1,500 m. Dashed lines represent political divisions of provinces.

13 **Figure 2.** Demographic models tested in Fastsimcoal2. (a) Demographic models tested
 14 at population level. Model parameters are bottleneck (T_{BOT}) and expansion (T_{EXP}) times,
 15 current (N_{CUR}) and ancestral (N_{ANC}) effective population sizes and population size
 16 during the bottleneck event (N_{BOT}). (b) Demographic models tested at mountain range
 17 level. Additional model parameters are current population size of marginal (N_M) and
 18 core (N_O) populations, time of instantaneous isolation (T_{DIV}) and migration probability
 19 from marginal to core (M_{MC}) and core to marginal (M_{CM}) populations (horizontal
 20 arrows).

21 **Figure 3.** Population genetic structure of *S. ciliata* in the Sistema Central of the Iberian
 22 Peninsula (Spain) showing closer relationships between the two western mountain
 23 ranges (Gredos and Bejar) and separating the eastern Guadarrama mountain range.
 24 Refer to Table 1 for population acronyms. (A) Bayesian cluster analysis (fastStructure).
 25 Figure shows the individual bar plot grouped by population and mountain ranges. Bar
 26 colours represent individual assignment probability to each of the optimal $K=2$ groups.
 27 (B) Neighbour-net (Nnet) phylogenetic network constructed in SplitsTree v4. (C)
 28 Population relationships and migration edges inferred by TREEMIX. Arrows are
 29 coloured by migration weight and branch lengths are proportional to genetic drift.
 30 Notice that the only migration event linked two populations was found in Bejar and
 31 Gredos mountain ranges (NEG-CAM).

1 **Figure 4.** Parameters inferred from coalescent simulations with Fastsimcoal2 under the
2 best-supported demographic model for single populations (“Bottleneck” model, see
3 Figure 2a). For each parameter, the point estimate and the lower and upper 95%
4 confidence intervals, generated by parametric bootstrapping, are shown. Model
5 parameters include T_{BOT} (time when the bottleneck time began), N_{CUR} and N_{ANC} (current
6 and ancestral population sizes), and N_{BOT} (population size during the bottleneck event).

7 **Figure 5.** Parameters inferred from coalescent simulations with Fastsimcoal2 under the
8 best-supported demographic model fitted at mountain range level (“Bottleneck and
9 Isolation of two populations” model, see Figure 2b). For each parameter, the point
10 estimate and the lower and upper 95% confidence intervals, generated by parametric
11 bootstrapping, are shown. Model parameters include: T_{BOT} and T_{DIV} (bottleneck and
12 instantaneous split times), N_M and N_C (current effective population size of marginal and
13 core populations), N_{ANC} (ancestral population size), N_{BOT} (population size during the
14 bottleneck event), and M_{MC} and M_{CM} (migration from marginal to core and core to
15 marginal populations). The red and blue shades visualise the division of the ancestral
16 population into marginal and core populations.

17 **Figure 6.** Diagram of the gene pathways tested for signals of selection. Five pathways
18 (indicated with red names) were enriched with positive *alpha* values estimated by
19 BayeScanHierarchical, indicating diversifying selection between populations inhabiting
20 core and marginal environments. The size of the nodes (pathways) is proportional to the
21 number of genes involved. The node colour scale represents the SUMSTAT score. A
22 higher SUMSTAT score indicates stronger signals of polygenic selection within the
23 pathway. Edges represent mutual overlap; nodes are connected if one of the sets has at
24 least three of its genes in common with the other gene set. Edge width is proportional to
25 the number of shared genes. Nodes marked with an asterisk indicate gene pathways that
26 are plant-specific. For clarity, the figure represents only the 51 pathways with a
27 SUMSTAT score higher than the median value of the entire dataset (N=86).

28 10. TABLES AND FIGURES

Table 1: *Silene ciliata* populations of the Sistema Central (Spain) used in the targeted resequencing experiment. Geographical and environmental characteristics and sample sizes (N) for the experiment are also provided.

Population (ID)	Mountain Range	Environment	N	Latitude	Longitude	Snowpack	MinT (°C)	Elevation (m)
Canchal Negro (NEG)	BJR	Core	15	40°20'20''N	5°41'22''W	0.59	-7.2	2360
Las Cimeras (RUI)	BJR	Marginal	16	40°21'07''N	5°40'59''W	0.27	-6.7	2000
Alto del Morezón (ZON)	GRD	Core	15	40°14'57''N	5°16'08''W	0.62	-7.7	2380
Los Campanarios (CAM)	GRD	Marginal	16	40°15'42''N	5°12'55''W	0.34	-6.0	2000
Pico de Peñalara (PEN)	GDM	Core	17	40°51'02''N	3°57'24''W	0.67	-7.8	2400
Morrena Peñalara (MOR)	GDM	Marginal	15	40°50'11''N	3°57'01''W	0.33	-5.7	1980

ID, identification of each population; Mountain Range, mountain range of origin: Bejar (BJR), Gredos (GRD) and Guadarrama (GDM); Environment, environmental classification; N , sample size; Latitude and Longitude, geographical coordinates; Snowpack, snowpack accumulation values in thaw months; range from 0 to 1 (values close to 1 indicate deep snowpack, while values close to 0 indicate null snow cover; López-Moreno *et al.* 2007); MinT, minimum annual temperature; Elevation, metres refers to the mean elevation (in meters above sea level.) estimated for each population (variation in elevation within populations did not exceed 10 meters). Geographical and environmental information extracted from Morente-López *et al.* (2019). Based on Morente-López *et al.* (2022), the main putative selective pressures for *S. ciliata* are snowpack in the thaw months and minimum minimum temperature. These variables were thus crucial to differentiate environmentally marginal and core areas.

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Table 2. Nucleotide diversity, neutrality tests and inbreeding by population.

Pop/Env	L (bp)	Polymorphic sites		Nucleotide diversity		Neutrality tests			Inbreeding	
		N	%	Tajima's π	Watterson's θ	<i>TajD</i>	<i>FuD</i>	<i>FuF</i>	<i>F</i>	Relatedness
NEG/Cor	2,678,634	33,568	47.17	0.00346	0.00338	-0.073	-0.072	-0.077	-0.082	0.056
RUI/Mar	2,684,833	33,747	47.43	0.00337	0.00331	-0.092	-0.134	-0.130	-0.040	0.055
ZON/Cor	2,677,081	34,147	47.99	0.00338	0.00344	-0.231	-0.275	-0.282	-0.044	0.058
CAM/Mar	2,683,788	30,934	43.47	0.00329	0.00303	0.079	0.193	0.173	-0.069	0.053
PEN/Cor	2,679,723	38,336	53.87	0.00346	0.00370	-0.362	-0.471	-0.475	-0.059	0.051
MOR/Mar	2,676,981	34,631	48.67	0.00338	0.00349	-0.263	-0.263	-0.283	-0.041	0.058

TajD, Tajima's *D*; *FuD*, *Fu* & *Li*'s *D**; *FuF*, *Fu* & *Li*'s *F**; *F*, average inbreeding coefficient; Relatedness, average pairwise relatedness statistic (unadjusted A_{jk}), with values around zero indicating unrelated individuals.

Table 3. Candidate genes putatively under divergent selection detected in *Silene ciliata*. “N° SNPs” refers to the number of SNPs detected with Arlequin (ARL) and BayeScanHierarchical approach (BAY). “Category” refers to the group of sequences to which the candidate gene belongs (See section “Selection of genes for sequencing”): A. Response to abiotic stimulus (GO: 0009628); F. Flower development (GO: 0009908); O. Candidate genes related to local adaptation to elevation in previous studies (Sacristán-Bajo *et al.* 2019). “Biological Processes” provides an overview of the main biological processes mediated by the candidate genes.

Gene Id (<i>S. ciliata</i>)	N° SNPs ARL/BAY	Category	Description	Uniprot ID (Best Blast)	Gene ID (Best Blast)	Biological Processes
comp73228	1/1	FO	ATP sulfurylase 1	Q9LIK9	APS1	Hydrogen sulfide biosynthetic process; Response to cytokinin; Response to cadmium ion
m.101530	1/1	A	Oxygen-evolving enhancer protein 1. chloroplastic	P12359	PSBO	Photosystem II assembly and stabilization; response to light intensity, response to water deprivation
m.102611	1/1	FO	Transmembrane protein	Q0WQ99	At4g16850	petal development; positive regulation of cell population proliferation
m.137232	1/1	F	Nuclear-pore anchor	A4GSN8	NUA	Stamen development; Negative regulation of flower development
m.140709	1/1	O	BAG family molecular chaperone regulator 3	Q9LYP4	BAG3	Chaperone-mediated protein folding; positive regulation of cellular process, response to jasmonic acid, response to wounding
m.19977	6/2	FA	Cytochrome P450 71B37	Q9LIP3	CYP71B37	Oxidation-reduction process; response to jasmonic acid, response to wounding, tissue development
m.843	2/2	A	Indole-3-acetic acid-amido synthetase GH3.10	Q9ZNS2	GH3.10	Response to light stimulus
m.87654	1/1	O	Serine carboxypeptidase-like 49	P32826	SCPL49	Proteolysis involved in cellular protein catabolic process
m.8887	1/1	A	Usp domain-containing protein	<u>A5C9Q0</u>	VIT_08s0007g01430	Response to cold; Response to heat; Response to oxidative stress; Chaperone-mediated protein folding

31 **Table 4.** Candidate genes with evidence of selective sweeps for *Silene ciliata* in core or marginal populations. “Category” refers to the group of
 32 sequences to which the candidate gene belongs (See section “Selection of genes for sequencing”): A. Response to abiotic stimulus (GO: 0009628);
 33 F. Flower development (GO: 0009908); O. Candidate genes previously related to local adaptation to elevation in previous studies (Sacristán-Bajo
 34 *et al.* 2019).

Gene Id (<i>S. ciliata</i>)	N° Selective sweeps	Category	Env.	Description	Uniprot ID (Best Blast)	Gene ID (Best Blast)	Biological Processes
m.133396	1	A	Marginal	Proteasome subunit beta type	Q8LD27	PBF1	Defence response to fungus, incompatible interaction; proteasomal protein catabolic; proteolysis
comp65047	3	A	Marginal Core	Poly(ADP-ribose) glycohydrolase	Q9SKB3	PARG1	Cellular response to DNA damage stimulus; Defence response to fungus; Regulation of DNA repair; Response to osmotic stress, oxidative stress and water deprivation; Rhythmic process; Stomatal closure
comp54123	1	O	Core	Carboxylate clamp type tetratricopeptide repeat protein	F4IRM4	PHOX1	Root hair cell development
comp64011	1	A	Core	DNA mismatch repair	F4JP48	MSH4	Homologous chromosome segregation; Mismatch repair
comp76868	1	F	Core	DNA repair protein	Q9SL02	RAD50	Gametophyte development; Chromosome organization involved in meiotic cell cycle; Telomere maintenance; DNA repair
m.117629	4	OF	Core	Protein TPLATE	F4J8D3	TPLATE	Endocytosis; Pollen development

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m.141175	1	O	Core	Uncharacterized protein	A0A0K9RLQ1	SOVF_052510	na
m.28293	1	A	Core	Histone-lysine N-methyltransferase	Q8GZB6	SUVH4	Histone methylation; Maintenance of DNA methylation; Peptidyl-lysine methylation
m.45836	2	O	Core	FAD-binding FR-type domain-containing protein	A0A2P4H4H9	VITISV_024875	Oxidation-reduction process
m.48088	1	A	Core	Sucrose synthase Glycine max	P13708	SS	Nodulation; sucrose metabolic process
m.83703	1	A	Core	ULP_PROTEASE domain-containing protein	A0A0K9S186	SOVF_011010	Proteolysis

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