



# Living on the edge—Genomic and ecological delineation of cryptic lineages in the high-elevation specialist *Erebia nivalis*

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## Abstract

1. Cold-adapted species at high elevations may be especially impacted by global warming since they may be limited in their capacity to adapt to changing conditions or may be prevented from shifting their distributions upwards if no suitable habitats are available.
2. The latter may be true for *Erebia nivalis*, a high-elevation specialist that mainly occurs in the Austrian Alps and on few mountaintops in Switzerland, where its taxonomic and conservation status remains unclear.
3. We aimed to clarify the relationships among geographically isolated populations of *E. nivalis* using whole-genome resequencing data to reconstruct population connectivity and phylogenetic relationships. We inferred current and historical effective population sizes and combined these with tests for ecological differentiation based on available occurrence data to re-evaluate the conservation status of *E. nivalis*.
4. Our results confirm that Swiss and Austrian *E. nivalis* should be considered different evolutionarily significant units for conservation purposes, as they are marked by moderate genomic differentiation, distinct demographic histories and a difference in the abiotic conditions of their habitats.
5. In both Austria and Switzerland, we found rapid uphill range shifts over the last decades, little population connectivity, low current effective population sizes and low genetic diversity. The above, combined with the low dispersal capacity of this 2-year lifecycle species and its need to overwinter under snow, which is rapidly retreating from several regions of the Alps, leads us to suggest that the status of *E. nivalis* should be updated to 'Vulnerable' or 'Endangered' in the European, Swiss and Austrian Red Lists.

## KEYWORDS

Alpine butterflies, biodiversity genomics, demographic history, insect conservation, niche divergence, taxonomy

## INTRODUCTION

Global climate change, together with habitat loss, is predicted to adversely affect the majority of the extant biodiversity during the

21st century (Habibullah et al., 2022; Hoegh-Guldberg et al., 2018). Alpine environments are particularly vulnerable to rapid climate change, owing in part to the narrow climatic niches and limited dispersal capacity of many cold-adapted species (Ernakovich et al., 2014;

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Grabherr et al., 2010; Guisan et al., 2019). In addition, temperatures increase more rapidly in high mountain environments compared with the global average (Keiler et al., 2010; Pepin et al., 2015; Pepin et al., 2022). Responses to changing conditions include shifts in phenology (Renner & Zohner, 2018; Richardson et al., 2013) or range shifts, often towards increasing elevation or latitude, which is a common phenomenon among cold-adapted species (Lenoir & Svenning, 2015; Parmesan et al., 1999). However, for high-elevation species, the potential to mitigate climate change effects by shifting distributions upwards may be limited because they cannot adapt to higher altitudes (Shah et al., 2020). They may also lack the ability to shift in elevation if they are already on mountain summits or at the edge of suitable habitat conditions (Dirnböck et al., 2011). Persistence in such cases may then depend on the adaptive potential of a species or population (Hoffmann & Sgrò, 2011; Razgour et al., 2019), as well as conservation efforts.

The consequences of climate change are predicted to vary among taxonomic groups (Rödger et al., 2021). Small ectotherms such as insects, and butterflies in particular, are at a very high risk of habitat loss and extinction (Sánchez-Bayo & Wyckhuys, 2019, but see Mupepele et al., 2019, Simmons et al., 2019 and Wagner et al., 2021). For butterflies, species adapted to cold, mountainous environments are expected to decline most rapidly since their distributions often span few, disjunct mountain regions (Kudrna et al., 2015; Minter et al., 2020; Sistri et al., 2022). Climatic changes may then result in even smaller and more fragmented habitat areas (Habel et al., 2011; Romo et al., 2023). The loss of even a few populations of such a geographically disjunct species may reduce the overall adaptive potential of this species (Razgour et al., 2019) and increase its chance of extinction (Brooks et al., 2015; Pauls et al., 2013).

Genetic data have enhanced our ability to study conservation-related aspects of vulnerable species and populations (DeSalle & Amato, 2004). As a result of the increasing abundance of whole-genome data and the availability of high-quality reference genomes, even for non-model organisms, genomic tools are increasingly used to study biodiversity (Theissinger et al., 2023). When extensive sampling efforts are impractical, for example, when species are particularly rare or threatened, whole-genome sequence data of even a few individuals can already provide information on demographic histories, phylogenetic relationships and hybridization, which is vital for the implementation of conservation measures (Hohenlohe et al., 2021). It can also be used to delineate populations and define units of conservation, that is, the intraspecific population units that conservation efforts are ideally geared towards, such as evolutionarily significant units (ESUs) and management units (Funk et al., 2012; Hohenlohe et al., 2021). While conservation genomic studies are not yet common for insects, they show great potential to detect gene flow and cryptic taxonomic variation and to secure legal protections for unique butterfly populations (Sucháčková Bartoňová et al., 2023).

The butterfly genus *Erebia* Dalman, 1816 (Lepidoptera: Nymphalidae, and Satyrinae) comprises approximately 100 described species that occur across the Northern Hemisphere, with a hotspot

in central Europe (Peña et al., 2015). The diversity of *Erebia* is a result of repeated range shifts during glacial cycles, associated with isolation in different refugia (Peña et al., 2015), ecological differentiation (Klečková et al., 2014) and chromosomal rearrangements that likely promoted reproductive isolation (Augustijn et al., 2023). As *Erebia* species are often restricted to mountainous or otherwise cold environments and often have geographically isolated populations, they have been considered model species to study biodiversity loss in mountain butterflies (e.g., De Groot et al., 2009; Minter et al., 2020; Romo et al., 2023; Scalercio et al., 2014; Sistri et al., 2022). However, the impacts of climate change and other stressors are often species-specific. For example, the south-eastern Alps endemic *Erebia calcaria* (Lorkovic, 1949) is threatened by habitat fragmentation (De Groot et al., 2009), while *Erebia cassioides* (Reiner & Hohenwarth, 1792) in the southern Apennines has undergone a range contraction but at the same time increased in population size (Scalercio et al., 2014). Other studies indicate that the genetic diversity of *Erebia epiphron* (Knoch, 1783; Minter et al., 2020) and *Erebia pandrose* (Borkhausen, 1788; Sistri et al., 2022) will likely decline due to climate change as a result of habitat loss. Most drastically, all *Erebia* of the Iberian Peninsula are predicted to lose about 95% of their suitable habitats or go entirely extinct by 2070 (Romo et al., 2023), further highlighting the need for conservation measures for this flagship genus.

The genetic diversity of *Erebia* is often lower than that for other co-occurring butterflies, with little gene flow even between closely related species (Schmitt et al., 2016). Furthermore, the niches of *Erebia* are often narrow and conserved to their local optima and tend to become narrower with increasingly specialised living conditions (Klečková et al., 2023). The larval performance of several *Erebia* is similarly reduced when thermal conditions shift too far from their usual range, though some species seem to perform better under warmer conditions (Zografou et al., 2022). An exception to the study above was *Erebia nivalis* (Lorkovic & De Lesse, 1954), which primarily occurs at elevations above 2100 m a.s.l. (Kudrna et al., 2015) only in the Swiss and Austrian Alps (with a few populations in adjoining Italy). This species shows little capacity to tolerate different temperature conditions (Zografou et al., 2022). In addition, *E. nivalis* is one of the few *Erebia* species known to have a 2-year generation time, thus likely requiring overwintering under snow for two consecutive winters, which indicates that this species could be especially vulnerable to temperature changes that affect snow cover (Gratton et al., 2016; Sonderegger, 2005). Similar to *E. pronoe* (Esper, 1780) (Wendt et al., 2021), *E. nivalis* is likely an opportunistic species using a multitude of flower species, with individual butterflies rapidly specialising in the most locally abundant nectar sources (Ehl et al., 2018).

While *E. nivalis* is relatively widespread in the Austrian Alps, in Switzerland, only few populations exist on separate mountains (Kudrna et al., 2015; Sonderegger, 2005). The Swiss *E. nivalis* has been described as a distinct subspecies: *E. nivalis warreniana* (De Lesse, 1957; Albre et al., 2008), but its taxonomic status and relationship to Austrian populations nevertheless have remained uncertain (Tschudin

et al., 2017). In Austria, two subspecies were described as *E. nivalis nivalis* (Lorkovic & De Lesse, 1954), covering the Austrian range from the Grossglockner region westwards, and *E. nivalis campestris* (Warren, 1955), covering the rest of the Austrian species' range to the East (Albre et al., 2008; Kudrna et al., 2015). The Swiss populations already inhabit the highest available locations in the local massifs (summits at 2681 and 2970 m a.s.l., respectively; Sonderegger, 2005). The limited migration capacity of *Erebia* (Polic et al., 2014), combined with the vast distance between the Austrian and Swiss populations (> 200 km), indicates that gene flow should be nearly impossible. The life history of *E. nivalis* may lead to an increased local vulnerability; indeed, the species was recorded as declining by van Swaay et al. (2011). However, genomic insight into *E. nivalis* is lacking, making it an ideal candidate to study genetic diversity changes in a disjunctly distributed species.

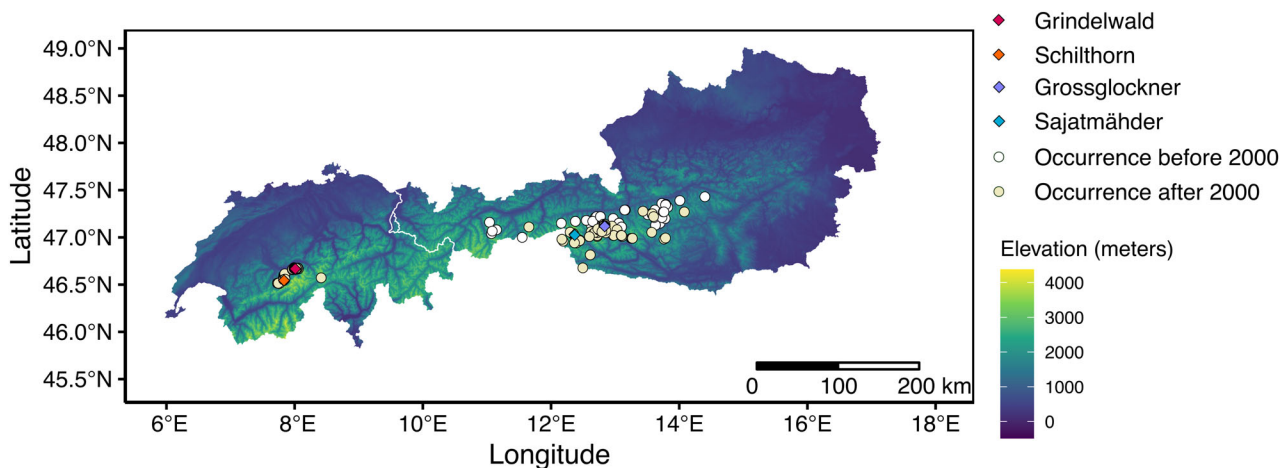
In line with previous conservation studies on butterflies reviewed by Sucháčková Bartoňová et al. (2023), we use genomic data to assess the genetic diversity of populations and to infer demographic trends, allowing us to identify populations in decline (Sherpa et al., 2022). We combine genomic and environmental data to clarify the relationship between Swiss and Austrian *E. nivalis* and to identify populations requiring conservation efforts. As such, we generated whole-genome data of 33 individuals from two populations in Switzerland and two in Austria that were previously studied (Schmitt et al., 2016). We (i) assessed gene flow and the degree of genomic differentiation between populations, (ii) dated the divergence between Swiss and Austrian subspecies and (iii) reconstructed current and historical population sizes ( $N_e$ ) to determine whether populations arose from different glacial refugia, as suggested by Schmitt et al. (2016), and whether they were stable through time. Using occurrence data for *E. nivalis*, we (iv) traced potential shifts in elevation due to changing climate as found in other butterflies (Sistrri et al., 2022) and (v) assessed whether Swiss and Austrian populations differ in their abiotic environmental niche.

## MATERIALS AND METHODS

### Data collection

We collected 13 Swiss *E. nivalis* individuals from Grindelwald (Figure 1) in 2017 and 2019 (Table S1). In 2019, we sampled two individuals from the nearby Schilthorn. All individuals were caught by hand net, euthanized with an overdose of ethyl acetate and stored in paper bags at  $-20^{\circ}\text{C}$ . From Austria, we used 18 individuals from two populations that were previously studied (Schmitt et al., 2016) and sampled in 2006 (Sajatmähder,  $n = 8$ ) and 2007 (Grossglockner,  $n = 10$ ). These samples were preserved in ethanol at  $-20^{\circ}\text{C}$ . We recorded individual-based geographic coordinates for the Swiss individuals and took the population-wide coordinates of Schmitt et al. (2016) for the remaining samples (Table S1).

We retrieved occurrence data of *E. nivalis* from GBIF (Table S2; GBIF.org, accessed on 4 January 2023, <https://doi.org/10.15468/dl.dt87xc>) and curated the data to include only data points from Austria and Switzerland, that is, the two countries the species primarily occurs (Albre et al., 2008; Kudrna et al., 2015; Sonderegger, 2005). We further excluded data with missing country codes ( $n = 38$ ), missing dates ( $n = 24$ ), missing coordinates ( $n = 23$ ) or that lacked taxonomic certainty ( $n = 34$ ). We further removed 236 occurrences because these records were considered unreliable. We limited the dataset to occurrences that were recorded after 1954, when *E. nivalis* was initially described. We removed occurrences with coordinate uncertainty  $\geq 1000$  m ( $n = 131$ ). When absent, the record's elevation was extracted from the European Digital Elevation Model, version 1.1 (25 m resolution). Because only seven occurrences from Switzerland remained due to low coordinate accuracy, we included all occurrences of *E. nivalis* in Switzerland from InfoFauna (<http://www.cscf.ch/>) at the highest possible resolution. This resulted in 178 additional occurrences, of which we discarded six due to coordinate uncertainty and seven for being collected prior to the description of *E. n. warreniana* in 1957. We found no



**FIGURE 1** Elevation maps of Switzerland and Austria, with study samples. The populations for which whole-genome resequencing data were generated are indicated. Curated occurrence data points are depicted in light yellow if they were gathered before 2000, and in white if later. Country boundaries were extracted from GeoBoundaries (Runfola et al., 2020).

duplicates between the GBIF and InfoFauna datasets. As described above, we extracted elevation when it was not recorded ( $n = 72$ ). Including our genotyped specimens, the final dataset represented 442 occurrence points of *E. nivalis*, 255 from Austria and 187 from Switzerland (Figure 1 and Table S2). Austria encompasses both putative subspecies (*E. n. nivalis* and *E. n. campestris*), as the GBIF data do not distinguish between them. Further analyses based on these occurrence data, therefore, aim to examine whether there is an overall differentiation between Swiss and Austrian *E. nivalis* for conservation purposes, and cannot delineate subspecies within Austria.

## Genomics

We extracted DNA from thorax tissue using the manufacturer's standard protocol of the Qiagen Blood & Tissue Kit (Qiagen AG, Hombrechtikon, Switzerland). The DNA of each of the 33 individuals was standardised to 20 ng/ $\mu$ L in a total volume of 30  $\mu$ L. The construction of paired-end sequencing libraries and the subsequent sequencing via Illumina Nova-Seq 6000 was outsourced to the Department of Biosystems Science and Engineering of ETH Zürich in Basel. Raw sequence reads were deposited to NCBI under the bio project accession number PRJNA1068509.

Using fastp (Chen et al., 2018), we demultiplexed the raw sequence reads and trimmed poly-G tails. Retained reads were mapped to an *E. cassioides* reference assembly (NCBI PRJNA941023) using bwa v0.7.17 (Li & Durbin, 2010), achieving an overall 14.3 $\times$  mean coverage (max: 20.0 $\times$ , min: 6.4 $\times$ ) with less than 20% missing data per sample on average (max: 30.5% and min: 15.3%). We employed SAMtools v.1.13 (Li et al., 2009) to remove reads that did not map, were unpaired or were duplicated. We generated a *pileup* file for each individual with *mpileup* in BCFtools v.1.12 (Li, 2011) and called variants with BCFtools *call* (Danecek et al., 2021). The merged VCF file was then filtered using VCFtools v. 0.1.16 (Danecek et al., 2011) to remove (a) non-biallelic single nucleotide polymorphisms (SNPs), (b) SNPs with a Phred quality score <25, (c) SNPs with a depth <10 or >30, (d) SNPs with minor allele frequencies (maf) of less than 0.03 and (e) SNPs for which less than 80% of individuals had sequence data available. In addition, we removed SNPs that fell within repetitive parts of the reference genome as determined by Repeat-Masker 4.0.9 (Smit et al., 2015). The resulting dataset contained 2,447,395 SNPs for 33 specimens.

## Phylogenetic inference of relationships between *E. nivalis* populations

To investigate the evolutionary history and relationships between *E. nivalis* populations, we generated a maximum-likelihood-based phylogeny using IQTREE (Nguyen et al., 2015) with 1000 ultrafast bootstraps. The substitution model was set to gamma-time reversible with ascertainment bias correction, as suggested for SNP data (Nguyen et al., 2015). We used *E. cassioides* as an outgroup, filtered out sites with more than 10% missingness and removed non-binary and

invariant SNP sites with the R package *Phyromics* (Banbury & Leache, 2014) to retain ~650k SNPs. Excluding the outgroup, we also ran Splitstree v. 4.0 (Huson et al., 2008) on the same dataset to visualise the phylogeny as a network and to assess the degree to which alleles may be shared among individuals.

In order to reconstruct the demographic history of *E. nivalis* evolution in relation to glacial cycles in the Alps, we estimated divergence times between populations using the SNAPP package implemented in BEAST2 (Bouckaert et al., 2014), which uses a multi-species-coalescent model based on SNP data to infer and date phylogenies. We used the same dataset as above but pruned SNPs in linkage with  $r^2 > 0.2$  in windows of 50 kb with a step size of 10 kb using PLINK v. 2 (Chang et al., 2015) using a publicly available script from (<https://github.com/joanam/scripts/blob/master/ldPruning.sh>). We selected two individuals per population for our inference given the computational limitations and the limited number of individuals from the Schilthorn population ( $N = 2$ ). We excluded monomorphic sites and sites with missing data for a resulting dataset containing about 33k SNPs. We set up SNAPP as in Stange et al. (2018) with a strict clock model and used the divergence between *E. cassioides* and *E. nivalis* in Augustijnen et al. (2023) as a dating constraint. The age of this node was previously estimated to be 1.4783 Mya (95% highest posterior density interval (HPD): 0.8606–2.6302), which was approximated in our new inference by a lognormal distribution with offset 0, mean 1.4783 and standard deviation 0.1: lognormal(0,1.4783,0.1). We ran SNAPP in BEAST2 with a chain length of 2,000,000 Markov Chain Monte Carlo (MCMC) generations, and confirmed that the effective sample size (ESS) of all parameters was at least 200 in Tracer v.1.7.2 (Rambaut et al., 2018).

## Population genomics

We first estimated the degree of genomic differentiation ( $F_{ST}$ ) between populations and between countries with VCFtools (Danecek et al., 2011) in non-overlapping windows of 100 kb. We visualised population structure using principal component analysis (PCA) using PLINK (Chang et al., 2015). To infer population structure and individual ancestry of samples, we pruned our dataset for linkage disequilibrium in windows of 100 kb along the genome, with a step size of 25 kb and a threshold of  $r^2 = 0.2$ , retaining 108,155 SNPs. We then ran ADMIXTURE (Alexander et al., 2009), assuming 1–6 genetic clusters (K) and standard parameters. We determined the optimal clustering by the lowest cross-validation error. We then evaluated the genetic diversity of each population in terms of average per-site nucleotide diversity ( $\pi$ ) and estimated  $d_{xy}$  between population pairs to calculate net mean divergence  $d_a = d_{xy} - \pi$  (Nei & Li, 1979). We annotated the *E. cassioides* reference genome with BRAKER (Hoff et al., 2019) based on the Arthropoda OrthoDB v. 10 (Kriventseva et al., 2019) and identified 9542 putative genes that were fully supported. We retained only SNPs within these coding regions and applied the same VCFtools filter as above without the *maf* filter to retain monomorphic sites, which is required for the correct calculation



of  $d_{xy}$  and  $\pi$  (Korunes & Samuk, 2021). Using publicly available custom scripts ([https://github.com/simonhmartin/genomics\\_general](https://github.com/simonhmartin/genomics_general), accessed on 4 March 2023), we then calculated  $\pi$  for each population and  $d_{xy}$  between each population pair in non-overlapping windows of 100 kb across the genome. We compared  $\pi$  between populations using a linear mixed effects model in R (v. 3.6.3; R core team, 2021), with genome scaffolds and genomic windows as nested random effects. Using the full dataset of 2.4 million SNPs, we likewise compared the calculated values of Tajima's D in windows of 100 kb along the genome with a similar linear mixed effects model. For  $\pi$  and Tajima's D, we calculated Tukey post hoc contrasts with the package *multcomp*, applying a false discovery rate (FDR) correction (Benjamini & Hochberg, 1995). We report the 95% confidence intervals of the mean of our estimates of  $F_{ST}$ ,  $\pi$ ,  $d_{xy}$  and Tajima's D; these were calculated based on the standard error generated with the R package *plotrix* v. 3.8-1 (Lemon, 2006). We further assessed individual-based genetic diversity ( $\theta$ ) calculated in *mlrho* (Haubold et al., 2010). Using *VCftools*, we established the inbreeding coefficient  $F$  for each individual, where  $F = (O_{(hom)} - E_{(hom)}) / (N_{sites} - E_{(hom)})$ , with  $O_{(hom)}$  the observed number of homozygote sites,  $E_{(hom)}$  the expected number of homozygote sites and  $N_{sites}$  being the total number of genotyped sites. We compared these variables between populations and between countries using analysis of variance (ANOVA) in R. We used country as a proxy for region, given the disjunct distribution of Swiss and Austrian *E. nivalis*. Normality was assessed, and when needed, the data were log<sub>10</sub>-transformed prior to analysis.

## Estimating current and historical effective population sizes ( $N_e$ )

As our genomic data had an appropriately high coverage and given our reference assembly, we were able to establish long-term trends in effective population size ( $N_e$ ), which relates directly to genetic diversity (Hohenlohe et al., 2021). We estimated  $N_e$  across the last 1,000,000 years using Pairwise Sequentially Markovian Coalescent (PSMC) model; Li & Durbin, 2011). We first generated diploid consensus files for each genotyped individual using *vcf2fq*, which is part of the *vcfutils.pl* package of *BCFtools*, making sure to exclude sites for which the mapping coverage was  $<1/3$  or more than  $2\times$  of that individual's average coverage, as recommended (Nadachowska-Brzyska et al., 2016). We generated the input files for PSMC with the *fq2psmcfa* script that is included in the PSMC package with a minimum quality threshold of 20. We ran PSMC with 100 bootstraps and the following parameters:  $4 + 30 \times 2 + 4 + 10$ , suitable for butterflies (García-Berro et al., 2022). Prior to bootstrapping, the genome was first split into shorter sequences with the *splitfa* function. Each bootstrap consisted of an independent PSMC run based on selected short genome sequences randomly sampled with replacement. We then scaled and visualised the output of PSMC with a generation time of 2 years for *E. nivalis* (Sonderegger, 2005) and with a mutation rate of  $0.29 \times 10^{-8}$ , derived from studies on the model butterfly species *Heliconius melpomene* (Linnaeus, 1758) (Keightley et al., 2015).

Since the estimates of  $N_e$  provided by PSMC become less robust towards the very recent past (Li & Durbin, 2011), we complemented this analysis with a model that particularly aims to reconstruct more recent trends in  $N_e$ . We employed SNeP (Barbato et al., 2015), which, in contrast to PSMC, uses a population-based approach rather than a calculation based on individual genomes. We used standard parameters with exception of the minimum distance allowed between SNP pairs, which was set to 500 bp to account for the smaller size of our scaffolds compared with most fully resolved chromosomes. For all populations except Grindelwald, the maximum number of SNPs per scaffold to be included in the analysis was constrained to 1000 due to computational limitations. We further set the number of bins to 40 and the threshold for a scaffold to be included in the analysis at 30 SNP pairs. We did not filter again for minor allele frequency as this was done prior to SNeP. We accounted for our relatively small sample sizes with the *-samplesize* setting implemented in SNeP (Grindelwald  $n = 13$ , Sajatmähnder  $n = 8$ , Grossglockner  $n = 10$ ) and excluded the Schilthorn population ( $n = 2$ ) from this analysis. We further constrained the analysis to the 680 largest scaffolds, accounting for  $>25\%$  of the genome, since for Grindelwald, the smaller ones did not contain enough SNP pairs at the allotted distance to be included in the analysis.

Finally, in order to investigate whether trends in  $N_e$  across the recent past might explain current population sizes and to assess whether each population meets conservation guidelines for population size (Palstra & Ruzzante, 2008, Pérez-Pereira et al., 2022), we complemented our SNeP analysis with an analysis of current  $N_e$  using *NeEstimator* v. 2 (Do et al., 2014). To ensure that the  $N_e$  estimates reflect current demographic history rather than past, we first selected 20,000 random SNPs (0.008% of the genome) to minimise linkage (Saura et al., 2015). We then ran *NeEstimator* with the bias-corrected linkage disequilibrium-based model at three thresholds of allele frequency: 0.05, 0.02 and 0.01. We also ran a model that did not allow for singleton alleles in the dataset, which led to the best model fit ( $R^2$ ) for most populations.

## Shifts in elevation of *E. nivalis* occurrence

We performed a least-square regression between observation year and elevation to determine whether shifts in elevation have occurred over the last 68 years in R. We also included the country of origin as a fixed effect and allowed for an interaction between observation year and country to assess whether the magnitude of shifts in elevation differed between the geographically disjunct Swiss and Austrian populations. We also carried out the regression for each country separately to determine the per-country elevation shift in meters per year.

## Niche differentiation between Austrian and Swiss populations

Given the disjunct distribution of Swiss and Austrian *E. nivalis* populations, we examined which extant *E. nivalis* varies in its abiotic niche

conditions across its range. We extracted abiotic environmental parameters for each occurrence data point from the CHELSA V2.1 dataset at a resolution of 1 km<sup>2</sup>, resolution as well as additional bioclimatic variables (Brun et al., 2022; Karger et al., 2017; Table S2). For environmental variables for which monthly data were available, we focused on the months during which *E. nivalis* occurrence data were collected (June–September), taking the average across these months for each variable. To test whether the environment for Swiss and Austrian individuals differed overall, we ran a multivariate ANOVA (MANOVA) and individual ANOVAs in R with an FDR using all variables.

We also analysed the environmental data, focusing on the least correlated variables (correlation coefficients <−0.6 and >0.6) and testing for collinear structures in the data using the variance inflation factor (Beck, 2017; Guisan et al., 2017). The eight least correlated environmental variables included the following: average maximum temperature for June–September (tmax\_mean\_6\_9), mean monthly potential evapotranspiration for June–September (pet\_mean\_6\_9), mean monthly surface downwelling shortwave flux in air for June–September (rsds\_mean\_6\_9), mean monthly near-surface wind speed for June–September (sfcWind\_mean\_6\_9), precipitation amount of the wettest month (bio13), precipitation seasonality (bio15), snow water equivalent (swe) and frost change frequency (fcf). We employed a PCA in R and determined whether Swiss and Austrian *E. nivalis* differ in their abiotic environment through a linear mixed effect model, with the scores of the first principal component (PC) axis as the dependent variable, the country of origin and the elevation as the explanatory variables, and year of occurrence as a random effect.

We calculated niche overlap and tested for niche similarity (Broennimann et al., 2012) between *E. nivalis* in Switzerland and Austria with the *ecospat* package in R (Di Cola et al., 2017), using the eight least correlated environmental variables for all occurrence data points. We set the occurrence density grid resolution at a sufficient distance to ensure no autocorrelation based on a Mantel correlogram (i.e., aggregated the cells by factor  $R = 50$ ) and calculated the niche density grid for all occurrences. The niche overlap test was calculated, once correcting for the occurrence density of the species by the prevalence of the environments in their range and once without the correction. For the similarity tests, we used null model tests with 1000 permutations and a 10 km radius around the occurrences as the background area to test the niche divergence hypothesis, that is, that the niche of Austrian and Swiss *E. nivalis* is less similar than randomly expected (Broennimann et al., 2012). To identify the specific niche variables for which the niche of Austrian and Swiss *E. nivalis* diverges most, we repeated the calculation for niche overlap and tested similarity for each variable separately. The output was the overlap values of Schoener's overlap metric  $D$  (Schoener, 1968) and  $I$ , a modified Hellinger metric (Warren et al., 2008). Additionally, we extracted the niche dynamic indices for niche stability, unfilling and expansion that were measured across the distribution of both populations. Niche stability represents the shared niche space by both populations. The niche unfilling represents the proportion of the niche space of Swiss *E. nivalis* that does not overlap with the niche space of Austrian *E. nivalis*. Similarly, niche expansion is the proportion of the niche

space of Austrian *E. nivalis* that is non-overlapping with the niche space of Swiss *E. nivalis*.

## RESULTS

### Phylogenetic inference of relationships between *E. nivalis* populations

Our maximum likelihood phylogeny recovered the divergence of Swiss and Austrian populations as the first split in the *E. nivalis* tree with very high support (Figure 2a). The estimated divergence time between Swiss and Austrian *E. nivalis* was 0.1513 Mya (95% HPD: 0.1211–0.1842 Mya; Figure 2c), that is, during the Penultimate Glacial Period. The Splitstree phylogeny network revealed some degree of gene flow within each population, but little allele sharing among populations or countries (Figure S1).

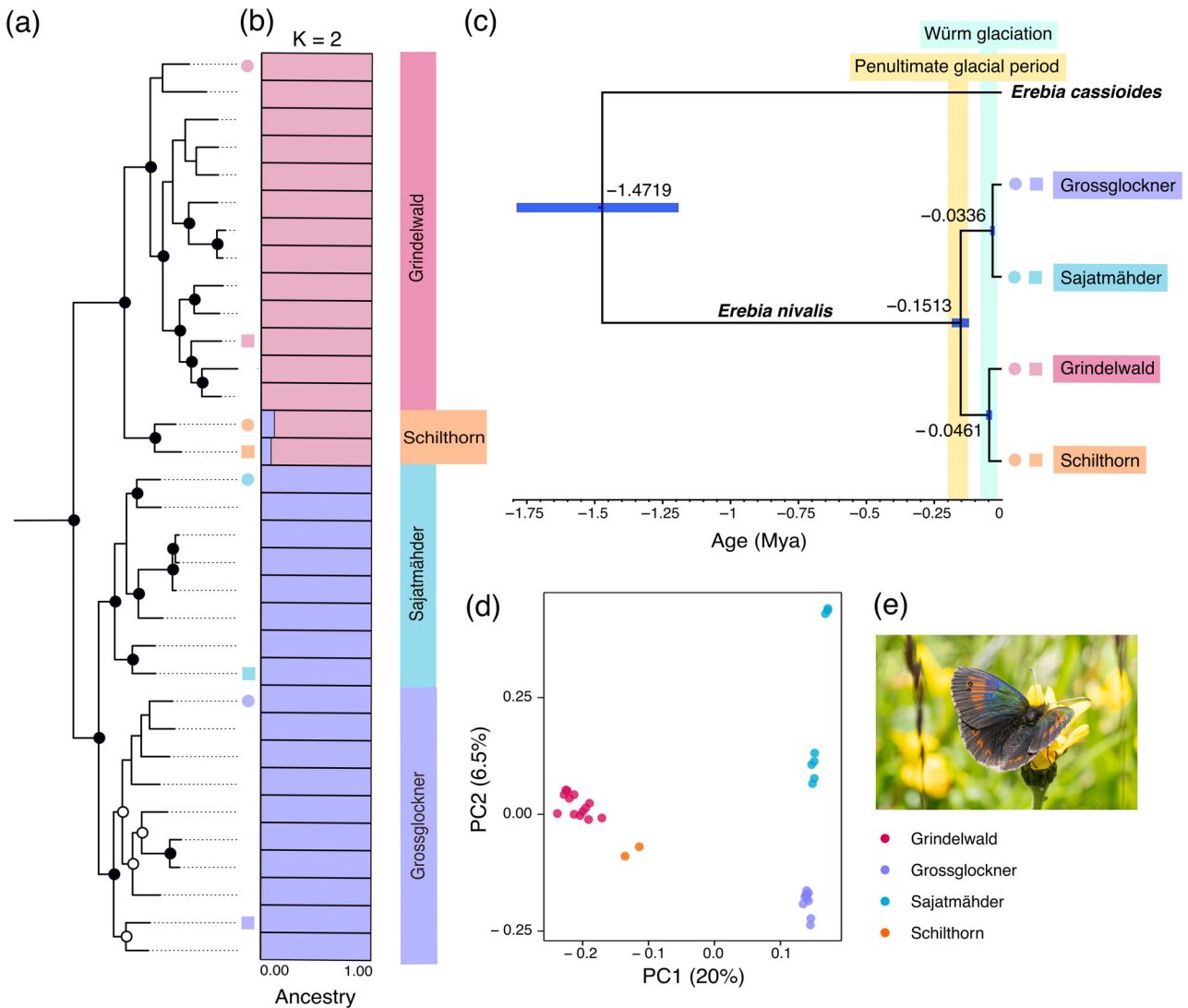
### Population genomics

The average genome-wide degree of differentiation ( $F_{ST}$ ) between Swiss and Austrian *E. nivalis* was 0.141 (Table S3). While populations within Austria showed some degree of differentiation (average  $F_{ST}$  0.05), which is similar to estimates from allozyme data (Schmitt et al., 2016),  $F_{ST}$  was lower among Swiss populations (Table S3), albeit sample sizes were limited. ADMIXTURE separated the Swiss and Austrian individuals, with  $K = 2$  as the best supported number of clusters (Figure 2b), though some substructure occurred in Austrian and Swiss *E. nivalis* at higher  $K$  (Figure S2). A PCA likewise divided *E. nivalis* first by countries along PC1, which accounted for 20.0% of the total variation (Figure 2d).

Genome-wide nucleotide diversity ( $\pi$ ) in windows across the genome differed significantly among populations (Figure 3a;  $\chi^2_{3,21,480} = 89.36$ ,  $p < 0.001$ ): The Schilthorn population showed the lowest average per-window  $\pi$  (0.0296 [0.0284–0.0308]; Table S4). Average Tajima's  $D$  was >0.0 in all populations (Figures 3c and S3), which could indicate a population contraction or balancing selection, leading to the loss of rare alleles (but see Korneliusen et al., 2013). This effect is more pronounced in Swiss populations (mean Tajima's  $D$  of Switzerland: 0.790; Austria: 0.635) and differed among populations (Figure 3c;  $\chi^2_{3,24,793} = 902.27$ ,  $p < 0.001$ ). Individual-based genetic diversity  $\theta$  is lower in the Schilthorn population compared with that in all other populations (Figure 3a; among population comparison:  $F_{3,29} = 5.30$ ,  $p = 0.005$ ; Table S5). Observed and expected homozygosities did not differ among populations (Figure 3d;  $F_{3,29} = 1.60$ ,  $p = 0.211$ ; Table S6).

### Effective population sizes ( $N_e$ ) through time

We estimated effective population sizes with PSMC based on genome-wide heterozygosity and coalescent modelling for each



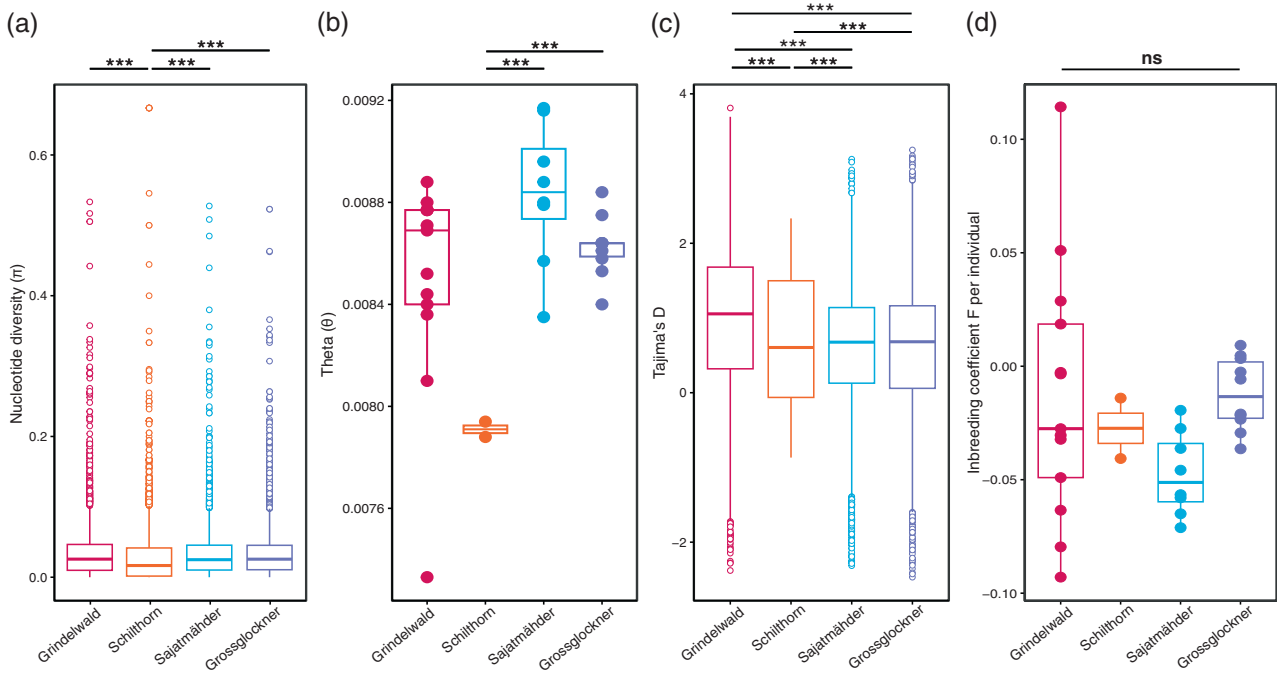
**FIGURE 2** Phylogenomic overview of *Erebia nivalis* (a) Phylogenetic relationships between and within *E. nivalis* populations calculated using IQTREE with 1000 bootstrap rounds. Full black circles at the nodes represent a bootstrap score of 100%, while blank circles represent 90%–99% bootstrap support. The outgroup *E. cassioides* was pruned. (b) Inferred ancestry of *E. nivalis* samples calculated using ADMIXTURE for  $K = 2$ . (c) Dated phylogeny using two individuals per *E. nivalis* population with *E. cassioides* as outgroup. The individuals included in this analysis are denoted in the phylogeny in (a) with either a circle or square. Blue bars denote the 95% highest density probabilities (HDP) of the node ages. (d) Biplot of the first principal component axes based on 2,477,000 SNPs. Swiss populations are Schilthorn (orange) and Grindelwald (pink), Austrian populations Sajatmähder (blue) and Grossglockner (purple). (e) Picture of *E. nivalis* warreniana by Manuel Schweizer.

genotyped individual and found similar declines in  $N_e$  prior to the split between Swiss and Austrian populations at about 150,000 years ago (Figures 4a and S4 and Table S7). The Austrian populations experienced an increase in population size around 100,000 years ago and a subsequent decline about 50,000 years ago. The Swiss populations reached a relatively more stable  $N_e$  after their split from the Austrian populations but continued to gradually decline, and their  $N_e$  remained consistently smaller than that of the Austrian populations. All populations seem to have experienced an increase in  $N_e$  around the end of the last glacial maximum (LGM: 26,000–20,000 Mya, Figure 4a), with Austrian populations starting their population expansion earlier and reaching higher  $N_e$  than Swiss ones, particularly the Grossglockner population (Figure S4f).

For the Swiss Schilthorn, population increase may not have taken place until after the LGM.

We used an SNeP analysis to determine effective population sizes in the more recent past. The Schilthorn population was excluded given the limited sample size. All other populations showed a steep decline in  $N_e$  across the last 500 years, with the Grindelwald population initially declining less rapidly until a population bottleneck about 80 years ago (Figure 4b). The elevated Tajima's D of all populations, particularly in Grindelwald (Figure 3c), may, therefore, reflect population contractions rather than balancing selection.

Consistent with the historical trends for the decline, current effective population sizes were estimated to be small (Table S8), well below the conservation guideline that should ensure the



**FIGURE 3** Population genomic estimates of diversity for *Erebia nivalis*. (a) Nucleotide diversity ( $\pi$ ) calculated in non-overlapping windows of 100 kb across the genome for each population. (b) Genetic diversity in terms of the average per-site  $\theta$  for each individual, as represented by full dots, grouped by population (c) Population-wide Tajima's D calculated in non-overlapping windows of 100 kb across the genome. (d) Inbreeding coefficient F for each individual. Bars indicate between-population comparisons for which post hoc tests after ANOVA indicate a significant difference. For (a) and (c) a false discovery rate correction was applied to these post-hoc tests to account for multiple testing across windows. Significance levels: ns, not significant; \* $p < 0.01$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$ .

maintenance of sufficient adaptive potential to persist long term ( $N_e = 500$ – $1000$ ; Palstra & Ruzzante, 2008; Pérez-Pereira et al., 2022). The Grossglockner population remains the largest by our estimates, with  $N_e = 200.1$  under the best supported model and  $N_e$  as high as 13,111.4 under alternative models using a different allele frequency cutoff (Table S8). Under the best supported model, the Swiss Grindelwald population is estimated to have a  $N_e$  of only 53.2 (45.0–60.9 under alternative models). Remarkably, the effective population size of the Austrian Sajatmähdler population is estimated to be low ( $N_e = 11.3$ ; up to  $N_e = 15.9$  under alternative models) despite showing a higher genetic diversity than other populations (Figure 3a,b).

### Shifts in elevation of *E. nivalis* occurrence

The average elevation of *E. nivalis* occurrences increased significantly since its description in 1954; by 3.3 m per year in Austria (SD = 0.574;  $t_{1,253} = 36.34$ ,  $p < 0.001$ ), and by 1.2 m per year in Switzerland (SD = 0.431;  $t_{1,185} = 7.39$ ,  $p = 0.007$ ; Figure 5a). Overall, the difference in elevation between years was significant ( $F_{1,440} = 55.01$ ,  $p < 0.001$ ), as was the difference in elevation between countries ( $F_{1,440} = 136.88$ ,  $p < 0.001$ ), with *E. nivalis* occurring at higher elevations in Switzerland (Figure 5a). The significant interaction between country and collection year ( $F_{1,440} = 6.14$ ,  $p = 0.014$ ) suggests that the slopes of the elevation shifts differ significantly between Switzerland and Austria.

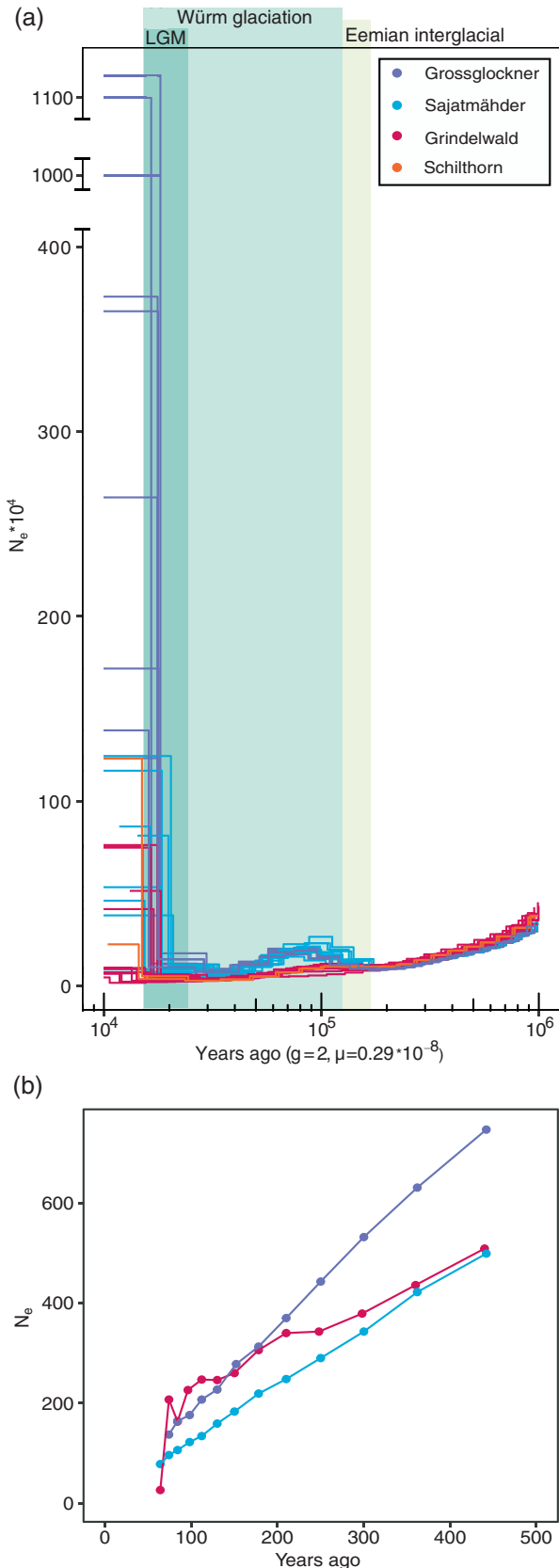
### Environmental difference between Austrian and Swiss populations

Using all available variables, the overall MANOVA supports significant environmental differences between the two countries ( $F_{1,440} = 46.64$ ,  $p < 0.001$ ); all single variables differed significantly in ANOVAs, except for frost frequency change (fcf,  $F_{1,440} = 0.01$ ,  $p = 0.921$ ) and isothermality (bio3,  $F_{1,440} = 0.01$ ,  $p = 0.921$ ; Table S9). The PCA based on the eight least correlated environmental variables (Table S10) revealed that *E. nivalis* experience different abiotic environments in the two countries (Figure 5b). The trait loadings of the two leading PC axes (Table S11), which explain 46% and 25%, respectively, suggest that Swiss *E. nivalis* experience lower average temperatures, lower precipitation seasonality but more precipitation in the wettest month and a far higher snow water equivalent, indicating a higher volume of snow (Table S12). The linear mixed effect model on the scores of the first PC axis suggested a difference between Switzerland and Austria ( $\chi^2_{1,411} = 214.30$ ,  $p < 0.001$ ), as well as by elevation ( $\chi^2_{1,411} = 176.26$ ,  $p < 0.001$ ). No further significant interaction existed between country and elevation ( $\chi^2_{1,411} = 1.02$ ,  $p = 0.313$ ; Figure 5a).

### Niche differentiation between Austrian and Swiss populations

The niche similarity test showed that the niche space the two populations occupy does not differ significantly from random in the given





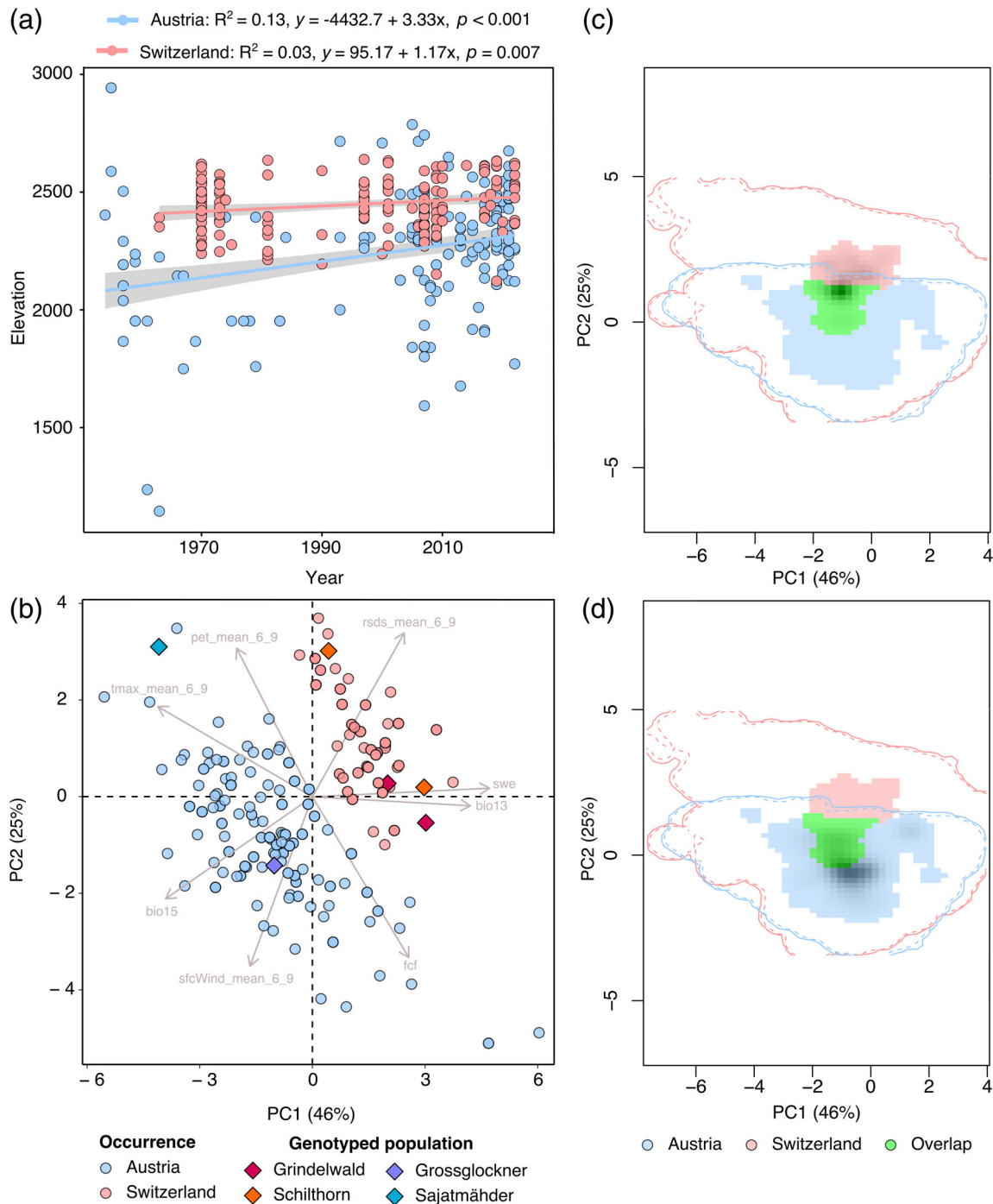
0.38 when the analysis was corrected for environmental prevalence (uncorrected: Schoener's  $D = 0.13$ ; Warren's  $I = 0.25$ ), revealing differences in the multivariate niche space of *E. nivalis* between the two countries (Figure 5c,d). Austrian *E. nivalis* had a larger multivariate niche space, while the realised niche of *E. nivalis* in Switzerland was smaller. However, the 95% confidence interval of the background area of the latter largely overlaps with the realised niche space of Austrian populations (Figure 5c,d). This indicates that Swiss *E. nivalis* could potentially occupy a niche space nearly as diverse as the one of its Austrian counterparts, but they do not actually do so. In the univariate space, we observed the highest niche overlap for frost change frequency (68%), followed by potential evapotranspiration (65%) and maximum temperature (52%; Figure 6 and Table S13). Moderate overlap existed for surface downwelling shortwave flux in air during the flying period (43%), precipitation of the wettest month (37%) and near-surface wind speed (37%; Figure 6 and Table S13). We observed the least overlap for snow water equivalent (26%), while no niche overlap was found for precipitation seasonality (0%; higher for the Austrian populations; Figure 6 and Table S12 and S13).

### DISCUSSION

Disjunct distributions are commonly observed in Alpine species (e.g., Dixon et al., 2007; Huemer & Mutanen, 2012; Schwönbetter et al., 2002; Watts et al., 2022) and often result from glacial or preglacial divergence (Schmitt et al., 2010; e.g., Sistri et al., 2022). Each disjunct population may hold a significant part of the overall genetic diversity of each species as a consequence of local adaptation and random allele shifts (Brooks et al., 2015; Razgour et al., 2019). This also applies to *E. nivalis*, with Swiss and Austrian populations representing genetically distinct lineages that split before the last glaciation (Figure 2a,b,d), with no current gene flow between countries, and very little even between nearby populations (Figure S1). Adaptation to cold environments and a generation cycle likely requiring 2 years of good winter conditions in a row (Sonderegger, 2005) may render *E. nivalis* particularly vulnerable to the impact of climate change, which already threatens other *Erebria* species that live at comparatively low altitudes (Minter et al., 2020; Romo et al., 2023). In addition, the Swiss

**FIGURE 4** Historic and recent effective population sizes of different *Erebria nivalis* populations. (a) Historic effective population sizes ( $N_e$ ) calculated from individual sequence analysis by pairwise sequentially Markovian coalescent models (PSMC). The cuts in the y-axis were made to accommodate the relatively higher  $N_e$  values for Grossglockner individuals, these same individuals are shown with their original axes in Figure S4f. The rectangles represent the Eemian interglacial in light green, the Würm glaciation in light turquoise and the last glacial maximum (LGM) in turquoise. The population sizes were estimated using a generation time ( $g$ ) of 2 years and a mutation rate ( $\mu$ ) of  $0.29 \times 10^{-8}$ , derived from Keightley et al. (2015). (b) Recent estimates of effective populations sizes ( $N_e$ ) calculated based on linkage disequilibrium between SNP pairs. Only bins based on >500 pairwise SNP comparisons are shown.

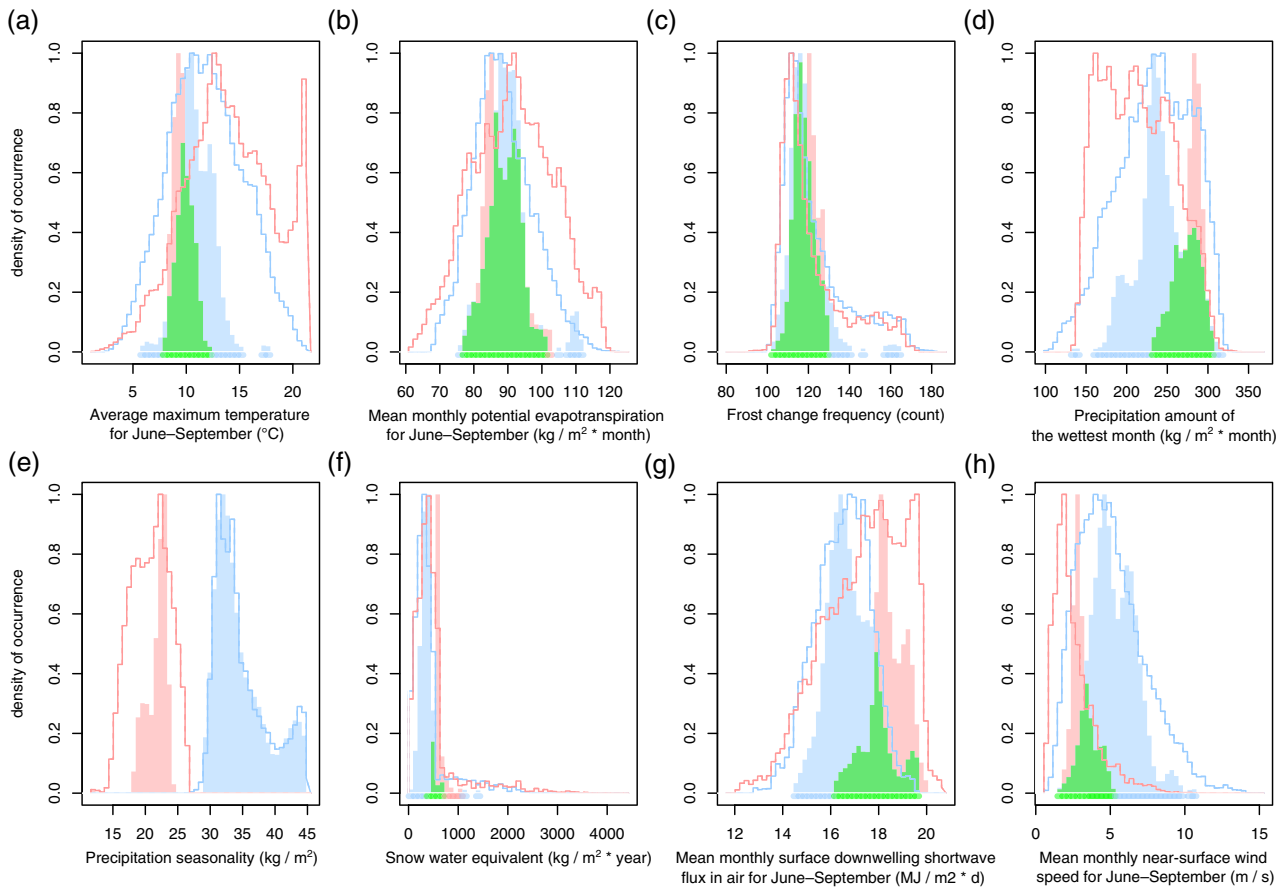
environmental background area (all  $p$ -values > 0.05). However, Austrian and Swiss *E. nivalis* show little overlap in their multivariate niche space (Figure 5c,d). Schoener's  $D$  was 0.28 and Warren's  $I$  was



**FIGURE 5** Occurrence data of *E. nivalis* (a) Elevation versus collection year for occurrence data of *E. nivalis* in Switzerland and Austria. The grey shaded areas represent the 95% confidence intervals of the regression lines for each country. The per-country  $R^2$ , regression intercepts, slopes and  $p$ -values are given. (b) Biplot of the first and second leading axes of a principal component analysis (PCA) based on eight scaled least-correlated environmental traits extracted from CHELSA V.2.1. The trait loadings of each variable are shown by grey arrows. Niche dynamics of *E. nivalis* in Switzerland (pink) and Austria (blue), with focus on the niche density of (c) Swiss and (d) Austrian *E. nivalis*. Niche overlap is shown in green. The pink continuous lines show the available niche space around Swiss populations with dashed lines delineating the most prevalent climates in the region (95% of the distribution). Similarly, blue continuous lines show the same for the available niche space around Austrian populations.

populations already occupy the highest points in their respective mountain habitats, with little opportunity for upward shifts (Figure 5a). However, their status as a distinct and potentially vulnerable Swiss lineage had not yet been evaluated.

The estimated split time between Swiss and Austrian *E. nivalis* about 150,000 years ago (Figure 2c) falls within the Penultimate Glacial Period (200,000–140,000 years ago), whereas splits between individual populations fall within the Würm ice age



**FIGURE 6** Niche dynamics at the univariate space for the *Erebia nivalis* in Switzerland (pink) and Austria (blue). Common niche space is coloured in green. The pink continuous lines show the available niche space around Swiss and the blue continuous lines show the same for the available niche space around Austrian individuals. (a) Average maximum temperature for June–September (tmax\_mean\_6\_9). (b) Mean monthly potential evapotranspiration for June–September (pet\_mean\_6\_9). (c) Frost change frequency (fcf). (d) Precipitation amount of the wettest month (bio13). (e) Precipitation seasonality (bio15). (f) Snow water equivalent (swe). (g) Mean monthly surface downwelling shortwave flux in air for June–September (rsds\_mean\_6\_9). (h) Mean monthly near-surface wind speed for June–September (sfcWind\_mean\_6\_9).

(70,000–11,700 years ago) during which the Alps became largely covered by ice. It is likely that the *E. nivalis* in Switzerland and Austria survived at least the Würm ice age and maybe even Riss II, in different refugia and differentiated there. This is a common phenomenon for *Erebia* in the Alps (Haubrich & Schmitt, 2007; Minter et al., 2020; Schmitt et al., 2006, 2014; Wendt et al., 2021), with repeated range expansion and contraction aiding in their diversification (Schmitt et al., 2016). The refugia of *E. nivalis* may be similar to those of *E. epiphron*, that is, at the foothills of the Alps near the Po Valley and at the southern foothills of the central and eastern Alpine margin, which similarly promoted the divergence of *E. epiphron* into a distinct Swiss and an Austrian lineage (Schmitt et al., 2006). Alternatively, the Swiss *E. nivalis* lineage could have survived in a northern refugium near the Black Forest (Haubrich & Schmitt, 2007; Schmitt et al., 2016), which might have limited its ability to reach major parts of the Alps as the ice retreated from South to North, resulting in its currently limited distribution compared with Austria (Figure 1). This pattern is also found in the sister species complex *E. melampus/momos/sudetica* (Haubrich & Schmitt, 2007), where

*E. sudetica inalpina* is also restricted to the Grindelwald region (Sonderegger, 2005).

The taxonomic status of Swiss *E. nivalis* has been a long-standing debate (Sonderegger, 2005; Tschudin et al., 2017). Although the split between the two distribution ranges predates the last glaciation, the level of genetic differentiation is comparable with that found between glacial lineages in other butterflies (e.g., Sherpa et al., 2022), including other *Erebia* (Jospin et al., 2023), that have not been elevated to an independent taxonomic rank. However, Swiss and Austrian lineages of *E. nivalis* form distinct phylogenetic clusters (Figure 2a), with a little to no gene flow between them (Figure S1). In addition to their differences in elevation (Figure 5a), Swiss and Austrian *E. nivalis* occur in different abiotic environments (Figure 5b) and show limited overlap in their multivariate niche space (Figure 5c,d), and especially in snow water equivalent and precipitation amount of the wettest month (Figure 6 and Table S13). The niches of the Swiss and Austrian *E. nivalis* further differ completely in precipitation seasonality (Figure 6 and Table S13), with a much higher degree of precipitation seasonality in Austria (Table S12). Consequently, given their likely

different biogeographic histories, phylogenetic distinctiveness and ecological differentiation, Swiss and Austrian *E. nivalis* should be recognised as distinct ESU from a management perspective (Casacci et al., 2014; Funk et al., 2012). While there is some debate about the criteria that define an ESU, that is, a conservation unit below the taxonomic level of a species (Fraser & Bernatchez, 2001), the Swiss and Austrian populations of *E. nivalis* fit the concept well, and similar arguments for the establishment of ESUs in butterflies have been made (e.g., Escuer et al., 2022).

In *E. nivalis*, gene flow is minimal even between nearby locations (Figures 2d and S1), perhaps as a result of the limited dispersal capacity of *Erebia* (Polic et al., 2014). However, Ehl et al. (2018) estimate that *E. nivalis* may be capable of some more long-distance flights. Still, distances longer than 5 km should be overcome by less than 0.6% of the female population, based on a mark release recapture in the Grossglockner area (Ehl et al., 2018). The average genetic diversity of the studied *E. nivalis* populations (Figure 3a,b) was within the range of other European butterflies (Ebdon et al., 2021) but low compared with other arthropods (Leffler et al., 2012). These low values are in line with our assumption of recent changes in effective population size, showing steep population declines over the last 500 years and a likely rapid bottleneck event in Grindelwald about 80 years ago (Figure 4a). Other studies on demographic trends in butterflies report similar gradual population declines as for *E. nivalis* during historical ages (Figure 4b; Sherpa et al., 2022) and sharp bottlenecks in more recent years (Després et al., 2019). The Swiss bottleneck event also fits within an inferred window of general butterfly decline around the 1960s that may be related to the intensification of agricultural and/or touristic practices (Habel et al., 2022). We must note, however, that inferences on effective population sizes from relatively few samples may underestimate  $N_e$  if the actual population sizes are large (Waples, 2016), which could explain the discordance between  $N_e$  and the genetic diversity of the Sajatmähder population, as may the random sampling of family structures in this population (Figure 2a). For the other Austrian population, our  $N_e$  estimate is indeed lower under the best fit model (Table S8) than the one estimated by a capture-recapture study on *E. nivalis* in the same national park (Ehl et al., 2018) but remains in the same order of magnitude.

## Implications for conservation

Given the negative scenarios for other, similarly isolated *Erebia* species (e.g., Romo et al., 2023; Sistri et al., 2022), the loss of at least some *E. nivalis* mountaintop populations over the next decades seems likely. *E. nivalis* shows little ability to tolerate thermal shifts at the larval stage (Zografou et al., 2022) and, like other Alpine *Erebia* species, is considered freeze-avoidant due to its overwintering under snow (Vrba et al., 2012). Abiotic changes in mountainous environments have already been documented in terms of loss of snow cover (Huss et al., 2017; Rumpf et al., 2022), which may especially impact Swiss *E. nivalis*, given the higher snow water equivalent in its abiotic environment (Figure 6 and Tables S12 and S13). In Austria, such climate

change effects might be mitigated by upward migration, which has been taking place over the past 80 years by about 3.3 m per year, for a total of 260 m (Figure 5a). Austrian *E. nivalis* therefore have moved considerably faster uphill than the average for terrestrial species (1.78 m/year) but within the range of other insects (Lenoir et al., 2020).

For Swiss populations, further uphill migration may be limited since they already occupy the upper sections of their respective mountain ranges. Their relatively slower elevation shift of 1.2 m per year may reflect this. Alternatively, this could indicate that *E. nivalis* in Switzerland might experience comparatively fewer environmental changes and, therefore, does not need to shift upwards as quickly. However, this is less likely given the apparent population declines in Switzerland (Figure 4). Furthermore, the 95% confidence interval of niche density for Swiss *E. nivalis* is much broader than its currently realised niche (Figure 5c). This interval encompasses the realised niche space of Austrian *E. nivalis*, suggesting that Swiss *E. nivalis* could potentially occupy a more diverse niche space than it currently does. This might result from habitat use differences between the Swiss and Austrian Alps at high elevations through niche conservatism, or from environmental constraints. The availability of microhabitats also may differ across the range of this species so that habitat heterogeneity might contribute to persistence, as has been shown for other *Erebia* species (Klečková et al., 2023; Klečková & Klečka, 2016).

While changes in abiotic factors due to climate change are driving butterfly decline in Switzerland, other anthropogenic factors, such as regional land-use changes, may have played a significant role in the past and might still trigger biodiversity loss at small scales (Neff et al., 2022). For *E. nivalis*, factors that might negatively impact extant populations include livestock grazing, which is commonplace in Switzerland, even at high altitudes (Fragniere et al., 2022). Consequently, changes in grazing management could reduce the removal of high Alpine meadow plants and benefit population growth for *E. nivalis* and other Alpine species. For example, changes to a light grazing regime have been suggested to be beneficial to maintain the microhabitats of *E. calcaria*, a closely related species of lower elevations; however, this was not empirically tested (De Groot et al., 2009). Another option might be the installation of snow fences to promote a longer time of snow cover for the larvae to overwinter (Sistri et al., 2022). Finally, translocation to nearby mountains remains a possibility, though one that requires more extensive research on *E. nivalis*' microhabitat requirements.

Measures are required to preserve *E. nivalis* across its entire range, particularly in Switzerland, where the entire lineage is likely threatened by extinction. Given (a) the disjunct distribution of *E. nivalis* with a lack of gene flow, (b) the rarity of populations and recent declines in effective population sizes and (c) the low genetic diversity remaining in each population, we suggest updating the assessment of *E. nivalis warreniana* in the IUCN Red List of Switzerland from Near Threatened (Wermeille et al., 2014) to Vulnerable or Endangered. The status of *E. nivalis* at the European level (Least Concern; van Swaay et al., 2011) may be similarly critical given the decline in population sizes that we observed across the species' entire range (Figure 4).

The results of this study have important implications for future conservation efforts for *E. nivalis*, also demonstrating the potential of whole-genome data for resolving taxonomic delineations with a view to the conservation of vulnerable and endemic species (see Sucháčková Bartoňová et al., 2023). Finally, we wish to highlight that disjunct distributions such as those of *E. nivalis* are common in mountain systems and that this study could serve as a template for such cases, combining whole-genome and occurrence data to detect obscure endemic lineages, assess their connectivity and guide future conservation efforts.

## AUTHOR CONTRIBUTIONS

**Hannah Augustijnen:** Conceptualization; writing – original draft; writing – review and editing; visualization; methodology; formal analysis; resources. **Theofania Patsiou:** Formal analysis; writing – review and editing. **Thomas Schmitt:** Resources; writing – review and editing. **Kay Lucek:** Conceptualization; writing – review and editing; funding acquisition; supervision; resources.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Raw sequence reads were deposited on NCBI (BioProject PRJNA 1068509), all code was deposited on Zenodo: 10.5281/zenodo.10606549.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Table S1.** Information on the genotyped *E. nivalis* individuals from Switzerland and Austria.

**Table S2.** Curated occurrence data, its locations, elevation, and collection time, as well as and its environmental variables.

**Table S3.** Mean weir-and-cockerham  $F_{st}$  between populations of *E. nivalis*, calculated in windows of 100 kb across the genome.

**Table S4.** Per-population average of  $\pi$  and  $d_{xy}$  in non-overlapping windows of 100 kb across the genome, and net mean divergence  $d_a$  calculated from  $d_a = d_{xy} - \pi$ .

**Table S5.** Individual-based average per-site genetic diversity ( $\theta$ ) calculated in  $mlr_{ho}$ .

**Table S6.** homozygosity and inbreeding coefficient  $F$  per individual.

**Table S7.** Per-individual historic effective population sizes ( $N_e$ ) calculated from individual sequence analysis by pairwise sequentially Markovian coalescent models (PSMC).

**Table S8.** Current effective population size ( $N_e$ ) at different allele frequency thresholds as estimated by NeEstimator.

**Table S9.** Manova responses for individual environmental variables, with a false discovery rate correction applied to all P-values.

**Table S10.** Pearson's correlation coefficient for environmental variables.

**Table S11.** Trait loadings of the PC axes of a principal component analysis based on 8 scaled, least-correlated environmental variables (see Table S2).

**Table S12.** Average values of the 8 least-correlated environmental variables for each population.

**Table S13.** Niche overlap and dynamics indices at the univariate space.

**Figure S1.** Splittree phylogenetic network based on 650 k SNPs. Lines represent genetic divergence between samples.

**Figure S2.** Inferred ancestry of *Erebia nivalis* samples calculated using ADMIXTURE for  $K = 2$  to  $K = 4$ .

**Figure S3.** Tajima's  $D$  in windows of 100 kb across the genomes of *Erebia nivalis* from the Grindelwald, Sajatmähder and Grossglockner populations, and the inbreeding coefficient  $F$  per individual of those populations. The bold horizontal line denotes the zero value for the inbreeding coefficient  $F$ .

**Figure S4.** Historic effective population sizes ( $N_e$ ) calculated from individual sequence analysis by pairwise sequentially Markovian coalescent models (PSMC). Grey lines denote the 100 bootstrap repeats for each individual. In all cases, effective population sizes were estimated using a generation time ( $g$ ) of 2 years and a mutation rate ( $\mu$ ) of  $0.29 \times 10^{-8}$ , derived from Keightley et al. (2015). (a) Historic  $N_e$  of the Swiss Schilthorn population. (b) Historic  $N_e$  of the Austrian Sajatmähder population, capped at  $N_e = 600$  to allow for comparison of

deep time patterns across all *E. nivalis* populations. The original estimates of the Sajatmähder population, and their bootstraps, are given in (c), please note the different scale. (d) Historic  $N_e$  of the Swiss Grindelwald population. (e) Historic  $N_e$  of the Austrian Grossglockner population, capped at  $N_e = 600$  to allow for comparison of deep time patterns across all *E. nivalis* populations. The original estimates of the Grossglockner population, and their bootstraps, are given in (f), please note the different scale.

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