The isolated *Erebia pandrose* Apennine population is genetically unique and endangered by climate change

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Abstract. 1. Climate change is causing shifts in the distribution of many species and populations inhabiting mountain tops are particularly vulnerable to these threats because they are constrained in altitudinal shifts. Apennines are a relatively narrow and low mountain chain located in Southern Europe, which hosts many isolated populations of mountain species. The butterfly *Erebia pandrose* was recorded for the last time in the Apennines in 1977, on the top of a single massif (Monti della Laga).

2. We confirmed the presence of a small, isolated population of *E. pandrose* in the Apennines, at a distance of more than 400 km to any other known populations. Then, we examined the cytochrome c oxidase subunit 1 mitochondrial DNA marker of this species across the Palaearctic area and estimated the potential decline over the Alps and the Apennines due to future climatic changes.

3. The Apennine population represents an endemic lineage characterised by eight mutations over the 658 bp analysed (1.2%). In the Alps and Apennines, this species has shifted uphill more than 3 m per year since the end of the 19th century and more than 22 m per year since 1995. Species distribution models suggested that these mountain populations will experience a generalised loss of climatic suitability, which, according to our projections, could lead to the extinction of the Apennine population in a few decades.

4. *Erebia pandrose* has the potential to become a flagship species for advertising the risk of losing unique fractions of genetic diversity for mountain species.

Key words. apennines, butterflies, climate warming, COI, endemicity, *Erebia pandrose*, species distribution model.

Introduction

Butterflies of the genus *Erebia* Dalman, 1816 (Lepidoptera: Nymphalidae, Satyrinae) are represented mainly by orophilous and/or chionophilous species (i.e., species associated with mountain areas and/or thriving in cold winter conditions),

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generally limited to the boreo-alpine regions (Tennent, 2008). *Erebia* shows a particularly high diversity in Europe, due to an adaptive radiation that occurred during the Pleistocene (Martin *et al*., 2000). During glaciations, *Erebia* populations occupied refugial areas represented by humid climate environments around wetter margins of glaciated high mountain systems and coniferous mountain forests (Peña *et al*., 2015). *Erebia* species inhabiting the European mountains typically include highly isolated, small populations strongly threatened by the ongoing increases in temperatures (Minter *et al*., 2020; Konvicka *et al*., 2021). Although elevational shifts allow species to persist in areas within their physiological range, insect populations shifting to higher elevations can run into various problems, such as anoxia, reduced resources, and negative interactions with the species of the recipient communities (Cerasoli *et al*., 2020; Hülber *et al*., 2020; Shah *et al*., 2020). The consequence of displacement might be dramatic for populations of medium-high mountain ranges, located at the southern (warmer) margins of their distribution [Minter *et al*., 2020 for *Erebia epiphron* (Knoch, 1783)] because they have little to no possibilities to shift upward. Moreover, because of the isolation of mountain tops and conical mountain shape, elevational range shifts can transform previously large and continuous ranges into smaller and fragmented distributions (Fattorini *et al*., 2020a). When the distribution is scattered in small areas, even the extinction of a single population can result in the loss of a high proportion of the total genetic richness (Brooks *et al*., 2015) with possible loss of genetic variants that may reduce the adaptive potential of the species (Sgrò *et al*., 2011). In the case of *Erebia*, a recent study on *E. epiphron* showed that climatic changes are expected to erode the genetic diversity of this species considerably (Minter *et al*., 2020). This is likely to happen in the southern margins of its distribution (Minter *et al*., 2020) where genetic richness is higher due to the long-term persistence of this genus during Pleistocene climatic fluctuations (Dincă *et al*., 2021).

Although most *Erebia* species in Europe occur on the highest massifs such as the Alps, the Pyrenees and the Balkans, 12 species also occur along the Italian Peninsula on the Apennine chain. A striking difference between Apennines and Alps is that the former are substantially lower in elevation than the latter and high elevation areas are smaller in extent. As a result, the Apennines populations of cold-adapted species cannot support large upward movements and are inevitably more vulnerable to climate change than their Alpine counterparts. Nevertheless, there is potential for Apennine populations to represent endemic lineages because the Alps and the Apennines are two distinct endemicity centres; in many cases, populations assigned to the same species represent highly diverging endemic lineages, even to the level of quasi-species (GMYC entities) (Menchetti *et al*., 2021).

In this article, we focused on the Apennine population of *Erebia pandrose* (Borkhausen, 1788). This species is widespread in the Alps, the Pyrenees, the Carpathians, the Balkans, and parts of Scandinavia but all records in the Apennines are from a single location above 2000 m on the Monti della Laga massif (more precisely in meadows near the top of Monte Gorzano), with an area of occupancy presumably smaller than 10 km^2 (Supplementary Material, Figs. S1 and S2). In the larval stage,

this species feeds on several genera of Poaceae (*Festuca, Poa, Sesleria*) (Tolman & Lewington, 2008), which are widespread in the area, thus providing *E. pandrose* with abundant food. This population occurs in an area that is difficult to reach and located in a national park, so the persistence of this population does not appear to be potentially threatened by land-use changes, whereas climate change may represent a serious threat. The last literature record in the Apennines dates back to 1977 (Balletto *et al*., 1977). As such, it was possible that this population had already gone extinct at the time of this study, as it had happened with several local populations of *Erebia* in the Apennines (Menchetti *et al*., 2021). In fact, this population inhabits the highest available region of the massif (the maximum elevation of the highest peak is 2500 m), thus limiting possibilities of further uphill movements. The distance between the Apennine and the Alpine populations (more than 400 km apart) makes any genetic flow and population rescue highly unlikely. Due to its extreme isolation, the Apennine population could represent an endemic lineage encompassing a considerable fraction of the global genetic diversity of this species, making it an important evolutionary significant unit deserving conservation attention (Casacci *et al*., 2014; Sgrò *et al*., 2011; Brooks *et al*., 2015).

In this study, we: (i) organised a citizen science field initiative (Butterfly Week 2019) that allowed us to ascertain the persistence of this population after more than 40 years since the last published record; (ii) obtained sequences of the cytochrome c oxidase subunit 1 (COI) marker from collected specimens to evaluate the genetic uniqueness of this population and compared them with those available for Europe and Asia; and (iii) applied a species distribution modelling approach to evaluate the potential future loss of climatic suitability for the Monti della Laga population (Habel *et al*., 2010; 2011; Minter *et al*., 2020; Talavera *et al*., 2015). We expected that the highly isolated population of *E. pandrose* can be genetically unique and particularly at risk due to climate change.

Materials and methods

Data collection

We surveyed the mountain top of the Monti della Laga massif searching for *E. pandrose* on 11 July 2019, during the sixth Butterfly Week (www.butterflyweek.com) organised by the ZEN lab (University of Florence) (Supplementary Material, Figs. S1 and S2). To keep the impact on the population to a minimum, we collected only three individuals for genetic analysis. However, we recorded the geographic coordinates and the elevation (10 m precision) of the sites where the species was observed.

We collected occurrence data of *E. pandrose* over the Alps and the Apennines as defined by Menchetti *et al*. (2021) (Fig. 1). Data belong to the Italian CKmap project (Balletto *et al*., 2007, constantly updated by EB with published and unpublished data), to collection data stored in Roger Vila's laboratory at Institut de Biologia Evolutiva CSIC-UPF (Barcelona, Spain), as well as to data deposited in iNaturalist. org and in Observations.org platforms. Only data with high-resolution coordinates (error lower than 1000 m) were used.
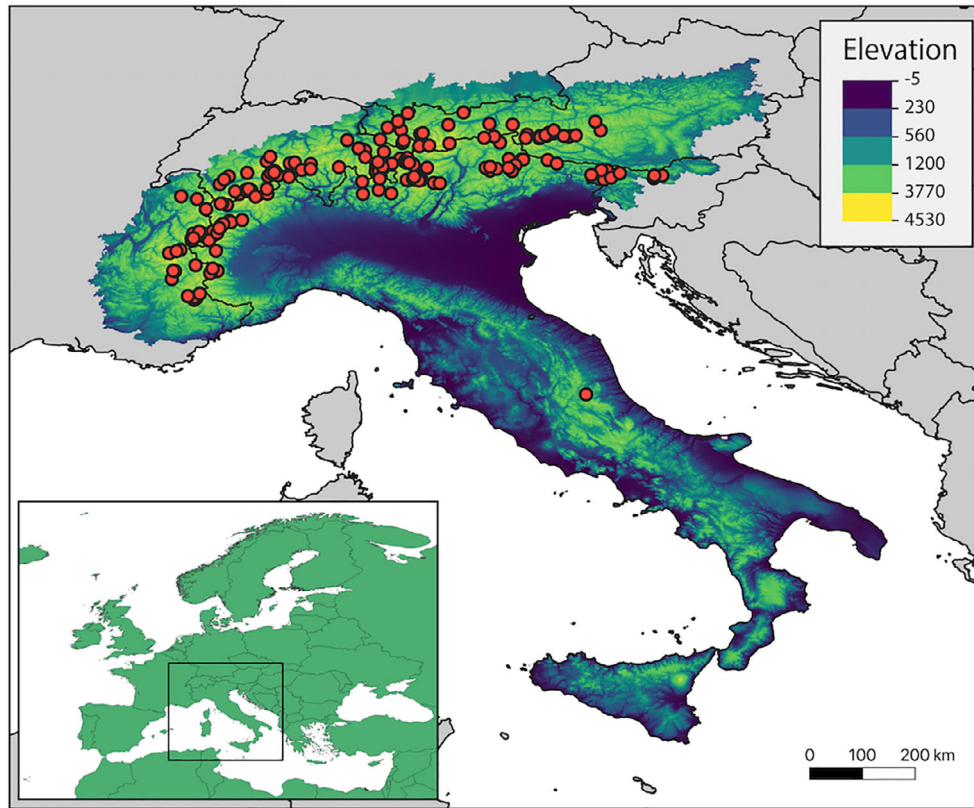
Fig 1. The study area plotted with elevation. Thinned occurrences, represented by red dots, for *Erebia pandrose* used for the species distribution modelling.

A subset of occurrences was obtained using only the specimens with year and elevation available (see below). The iNaturalist.org and Observation.org records were included in the dataset only if accompanied by a good quality photograph and if the attribution to *E. pandrose* of each of those records was ascertained by our research group. Overall, we collated 316 occurrence points. To reduce clustered occurrences due to unbalanced sampling, we filtered the occurrence dataset by selecting points at a minimum distance of 2 kilometres to each other with the 'thin' function of the 'spThin' R package (Aiello-Lammens *et al.*, 2015) in R v. 4.0.3 (R Core Team, 2020). We chose the minimum distance of 2 km taking into account the limited dispersal propensity of *Erebia* species (Kuras *et al.*, 2003; Grill *et al.*, 2020). This reduced the final dataset for species distribution modelling to 166 records (Fig. 1).

We gathered COI sequences for this species from across its distribution (Eurasia) by downloading data publicly available in GenBank and in BOLD and by adding a series of 11 unpublished sequences specifically analysed for this study (three of which from the Monti della Laga massif) (Supplementary Material, Table S1). COI sequences were generated by the International Barcode of Life project (iBOL) with standard procedures at the Canadian Centre for DNA Barcoding (University of Guelph, Canada) using the primers LepF1 and LepR1 (de Waard *et al.*, 2008).

COI analyses

We aligned all the sequences with MegaX. We removed potential contaminant sequences as well as sequences shorter than 597 bp to include the highest number of reliable sequences. This resulted in a final alignment of 68 specimens (Supplementary Material, Table S1). We constructed maximum parsimony haplotype networks using the COI alignments and TCS 1.21 (Clement *et al.*, 2000) by imposing a 95% connection limit (11 steps). The haplotype network was then edited with tcsBU (dos Santos *et al.*, 2016) and Adobe Illustrator CC 2019. Haplotypes were assigned with colours based on p-distances projected after Principal Coordinates Analysis (PCoA) on the RGB space using the recluster.col function of the R package 'recluster'. Subsequently, the colours were plotted on a map using the recluster.plot.pie function of the same package.

Species distribution modelling

We modelled the distribution of *E. pandrose* in the study areas as one single entity, although Alps and Apennine populations may be differently adapted to local conditions. Recent studies (Razgour *et al.*, 2019) highlighted the importance of including adaptive genetic variation in climate change vulnerability

assessment. However, given the extremely limited distribution of *E. pandrose* in the Apennines (virtually a single raster cell without any variation) this was not possible.

Erebia populations living in high elevation areas are considered at risk because they rely on snow cover to resist the extremely cold mountain winter (Vrba *et al.*, 2012, 2017; Konvička *et al.*, 2021). Thus, according to the typical orophilous and chionophilous character of *Erebia*, we selected the following variables: temperature annual range between the coldest and the warmest month, mean temperature of the coldest quarter, annual precipitation, and precipitation of the coldest quarter. We obtained these climatic layers from CHELSA (www.chelsa-climate.org) with a resolution similar to that of the occurrence data (30 s, ~1 km^2) and clipped them for the study polygon. We checked for multicollinearity among the selected variable by applying the variance inflation factor (VIF) using the R package 'usdm' (Naimi *et al.*, 2014) and we verified that all values were <5 which indicates a low multicollinearity (Alin, 2010; Kock & Lynn, 2012) (Supplementary Materials, Table S2). We downloaded and clipped the same variables for future projections from CHELSA at the same resolution for the 2041–2060 period and the Representative Concentration Pathway (RPC) 4.5, described by IPCC as the intermediate scenario (Adger & Coauthors including Fischlin, 2007). For future climatic variables, we used the median of four global circulation models (GCMs): NorESM1, CM5A-MR, MPI-ESM-MR, HadGEM2-AO.

Species distribution modelling was performed using the R package 'biomod2' (Thuiller, 2014). No single algorithm performs best in all situations (Norberg *et al.*, 2019). In general, overall statistical models tend to be less prone to overfitting than machine learning models, but the latter usually achieve higher accuracy in present predictions (e.g., Merow *et al.*, 2013). As such, it is recommended to use multiple and diverse algorithms (Araújo & New, 2007; Norberg *et al.*, 2019) and validate them using a robust blocking approach, in order to estimate the uncertainty around predictions. Accordingly, we used four statistical models to forecast the current and future species distribution: random forests (RF), generalised additive model (GAM), maximum entropy (MAXENT), and generalised linear model (GLM). This set of models includes two statistical linear models (GLM and GAM) and two machine learning algorithms (MAXENT and RF), which cover a wide range of complexity in distribution models (Merow *et al.*, 2013) allowing appropriate estimates of uncertainty in model projections (Buisson *et al.*, 2010).

Thereafter, we generated 10 datasets of 1000 randomly selected background points, one dataset for each of 10 repetitions used for the model validation. To estimate the relative importance of the predictor variables, we correlated the predicted probabilities of presence using the full dataset with the predicted probabilities of presence obtained after permuting the variable of interest. The relative importance of each variable was quantified as one minus the Pearson rank correlation coefficient (Thuiller *et al.*, 2009). We validated the models using spatial block validation with the R package 'blockCV' (Valavi *et al.*, 2019). We split the study area into six spatial blocks and two folds (Supplementary Material, Fig. S3), iteratively fitted the model on all but one block, and tested against the left-out block. This procedure is known to provide a more objective assessment of model transferability for future projections (Bahn & McGill, 2013; Roberts *et al.*, 2017). We measured model performance using the true skill statistic (TSS) and the area under the curve (AUC). For both present and future projections, we obtained one occurrence probability raster for each statistical model by calculating the mean of all the projections of models with a TSS > 0.5 and an AUC > 0.7. To obtain a single projection for each scenario, we then averaged the four raster matrices and excluded cells with an occurrence probability <10%. In the results, the term 'cells' will thus indicate cells with an occurrence probability >10%. Then we calculated the difference between the two scenarios by subtracting the current average predictions from the future ones; raster cells with positive delta values indicate a predicted improvement of climatic conditions, whereas raster cells with negative delta values indicate a deterioration of climatic conditions. To estimate the uncertainty in the predictions due to disagreements among different algorithms, we assigned −1 to all cells with negative values, +1 to all cells with positive values (and 0 otherwise) of the average single-model predictions and assessed the consensus of model predictions by summing the four binarized maps. This resulted in a raster map with values ranging between −4 and +4, with extreme values indicating that all the four statistical models predicted a decrease (−4) or increase (+4) in the probability of occurrence, and intermediate values indicating partial (±2–3) or high disagreement (−1 to +1) among the predictions of the models. To ensure transparency and full replicability of the methods we provide an Overview, Data, Model, Assessment and Prediction (ODMAP) protocol as part of the supplementary material that details all methodological choices and parameters (Supporting Information Appendix 1; Zurell *et al.*, 2020). The 166 occurrence data used for species distribution modelling is available in the Supporting Information Appendix 2.

Elevational shifts of E. pandrose *records through time*

Changes in elevation in the last 147 years were obtained by regressing the elevation of each literature record against the year of collection. When explicitly reported, elevation values were obtained from literature and collections data. For occurrences without explicit report of elevation but with precise coordinates (error lower than 1000 m like most citizen science data), elevations were extracted using a high-resolution (1 km^2) elevation layer available at https://www.earthenv.org/topography (Amatulli *et al.*, 2018). The final dataset was composed of 688 records, ranging from 1873 to 2020. An ordinary least squares regression between time and elevation was used to test for a temporal trend in elevational shifts. An increase in occupancy elevation along more than a century could be exacerbated by some biases like the researcher perception in recent years that high elevation areas are of higher interest and the inaccessibility of high altitude areas in the past. The former is unlikely due to the wide diversity of sources in our dataset (collections by the authors and by amateurs, published data and citizen science records), which should not be affected

by the same bias. To reduce the first potential bias, we also performed analyses limited to the 50 years between 1970 and 2020 ($n = 553$) and to the 25 years between 1995 and 2020 ($n = 429$).

Results

We confirmed the existence of the Apennine population of *E. pandrose* on Monti della Laga (Central Italy) in a restricted area close to the summit of Monte Gorzano ranging from 2200 to 2300 m above sea level (a.s.l.) (coordinates in decimal degrees: 42.619, 13.388; Supplementary Material, Figs. S1 and S2). We observed several individuals (15–20), mostly worn-out females (Supplementary Material, Fig. S1b) indicating that the peak of adult activity probably occurs in early July.

COI structure

We obtained a total of 68 COI sequences with a length between 597 and 658 bp. The haplotype network analysis showed that the Central Asian and Apennine populations form two distinct haplogroups compared to the European one (Fig. 2a). The population from the Apennines is represented by an endemic lineage separated by a minimum of six mutations (0.9% of the 658 bp reference length) from the Asiatic haplogoup, and by 8 changes (1.2%) from the European one. The relative genetic distance among the sequences is shown in the distance plot (Fig. 2b) and in the haplotype network (Fig. 2c).

Climate change effects

The spatial-block validation of the statistical models indicated good predictive performances, with TSS = 0.60–0.89 and (AUC) = 0.82–0.96 (Supplementary Material, Fig. S4). The most important variable in predicting the occurrence of *E. pandrose* according to all the four statistical models was the mean temperature of the coldest quarter (values of the mean importance of all variables are given in Supplementary Material, Table S3). Accordingly, the smoothed response curves showed a strict dependence of *E. pandrose* occurrence probability on the mean temperature of the coldest quarter with a great concordance between different algorithms; conversely, the three other variables showed weaker relationships and lower concordance between the algorithms (Supplementary Material, Fig. S5).

The projections of each statistical model (Supplementary Material, Fig. S6) pictured slightly different results that were averaged in the ensemble model. The ensemble model projections for the present and the future indicated a high probability of occurrence across the Alps, and in the Apennine area where *E. pandrose* has been recorded (Fig. 3a,b). The predicted change in the probability of occurrence raster highlighted a widespread decline in climatic suitability across the study area, including the Monti della Laga site in the Apennines (Fig. 3c). The only exception on the Apennines could be the top of Gran Sasso massif, which is predicted to slightly increase climatic suitability in

small and restricted areas (for which, however, no records of *E. pandrose* exist). Similarly, many marginal areas of the pre-Alps are predicted to deteriorate their climatic suitability (Fig. 3c). We also found a high consensus among model predictions indicating a high probability of decline throughout the Alps and the Apennines (Fig. 3d). The climatic suitability of the 316 occurrence locations is predicted to change from 0.76 ± 0.14 (average $\pm$ s.d.) in the present to 0.45 ± 0.13 (average $\pm$ s.d) in the future (Fig. 3e). In particular, the cell of the Apennine population will decrease from a suitability value of 0.54–0.34 in the future. In the current scenario, only three occurrences over 316 show a suitability value lower than 0.34 which represents a 0.9% quantile. This is a value much lower than the 10% quantile threshold used in similar studies to predict future occurrence at a given site (e.g., Habel *et al.*, 2010) (Fig. 3e). Overall, 86.8% of the cells appear to decrease their suitability (Supplementary Material, Fig. S7) in the future. Most of the cells (73.7% + 9.0%) have a high consensus value (-4 -3 and $+3$ $+4$, Fig. 3f) and belong to high elevation areas.

Regression between elevation and year of records indicated an average increase of 3.2 m ($\pm$ 0.4 S.E) in elevation per year since 1873 ($t = 8.16$, $P < 0.001$, Fig. 4). A similar result is found by limiting the analysis to data from 1970 to 2020 [increase of 4.0 m ($\pm$ 0.9 S.E) in elevation per year, $t = 4.96$, $P < 0.001$]. When limiting data from 1995 to 2020 the elevation increase appears much stronger (22.1 m $\pm$ 2.7 S.E per year, $t = 8.07$, $P < 0.001$).

Discussion

We confirmed the presence of *Erebia pandrose* in the Apennines 42 years after the last dated literature record in 1977 (Balletto *et al.*, 1977). Analysis of the COI mitochondrial marker revealed that this population belongs to an endemic lineage, differentiated by eight and six mutations over 658 bp from the European and the Asiatic populations, respectively. This endemic lineage is restricted to a very small, high elevation area at the top of Monti della Laga. Historical changes in the altitudinal range and species distribution modelling across the Alps and the Apennines indicate that the Apennine *E. pandrose* population may disappear in a few decades. In the next sections, we (i) delineate the loss of genetic diversity produced by a putative future extinction of this population, (ii) assess the likelihood of such an extinction based on the current knowledge on climate change and, finally, (iii) highlight the potential for *E. pandrose* to become an iconic taxon for the conservation of butterfly diversity in the Apennines.

The population of Monte Gorzano

The Apennine population of *E. pandrose* likely represents a remnant of a formerly more widely distributed population occurring in the Italian Peninsula during colder periods of the Pleistocene, when Northern and Central European areas were covered with ice. As the climate warmed and the ice receded, cold adapted species shifted northwards presumably from the
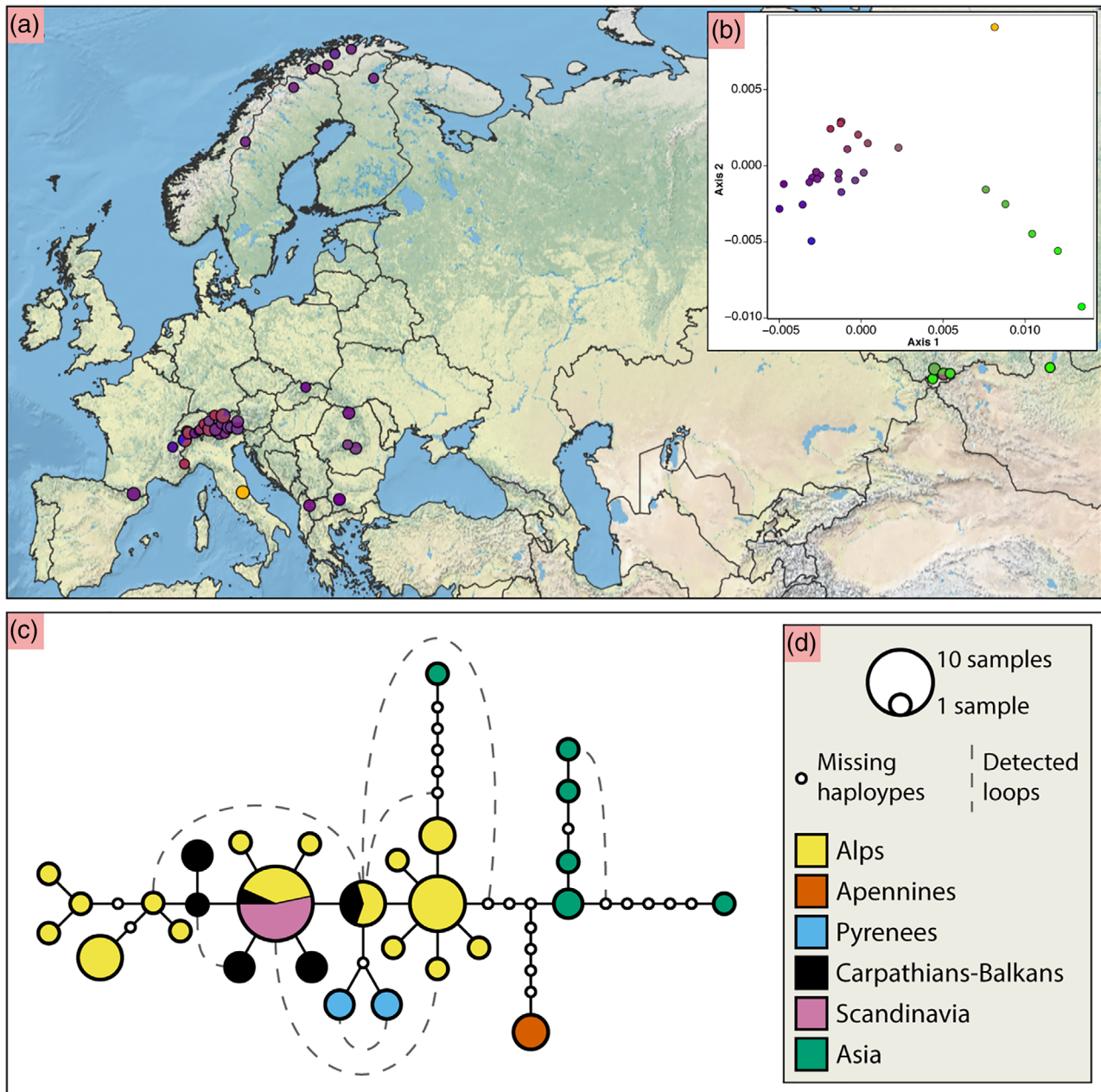
Fig 2. Genetic structure of *E. pandrose* based on COI sequences: (a) Map of *E. pandrose* locations (2° resolution) showing where the different COI haplotypes (coloured circles) were collected. Haplotype colours are attributed based on their relative genetic distances by using a PCoA configuration on p-distances projected in RGB space. (b) Relative genetic distance plot among the different *E. pandrose* haplotypes. Colours association corresponds to Fig. 2a. (c) Maximum parsimony COI haplotype network of *E. pandrose*. Each line segment represents a mutation between two different haplotypes (coloured circles). According to the legend (d) white dots represent missing haplotypes, dashed lines represent detected loops and circle area is proportional to the number of sequenced specimens possessing a given haplotype. Haplotype colours have been assigned according to the geographic origin of the COI sequences.

northern border of their distribution, leaving isolated populations on southern mountain areas (Schmitt *et al*., 2010; Fattorini, 2014; Schmitt, 2017; Menchetti *et al*., 2021). The Pyrenees, the Alps, and the Apennines are rich in such 'glacial relics' among plants and arthropods, which are now particularly threatened by global warming (Hodd & Sheehy Skeffington, 2011; Cowie, 2012; Fattorini *et al*., 2020b). Accordingly, the population of the Monti della Laga massif appeared to be genetically differentiated (1.2%) from the main haplogroup distributed all over Europe (Fig. 2). Although we
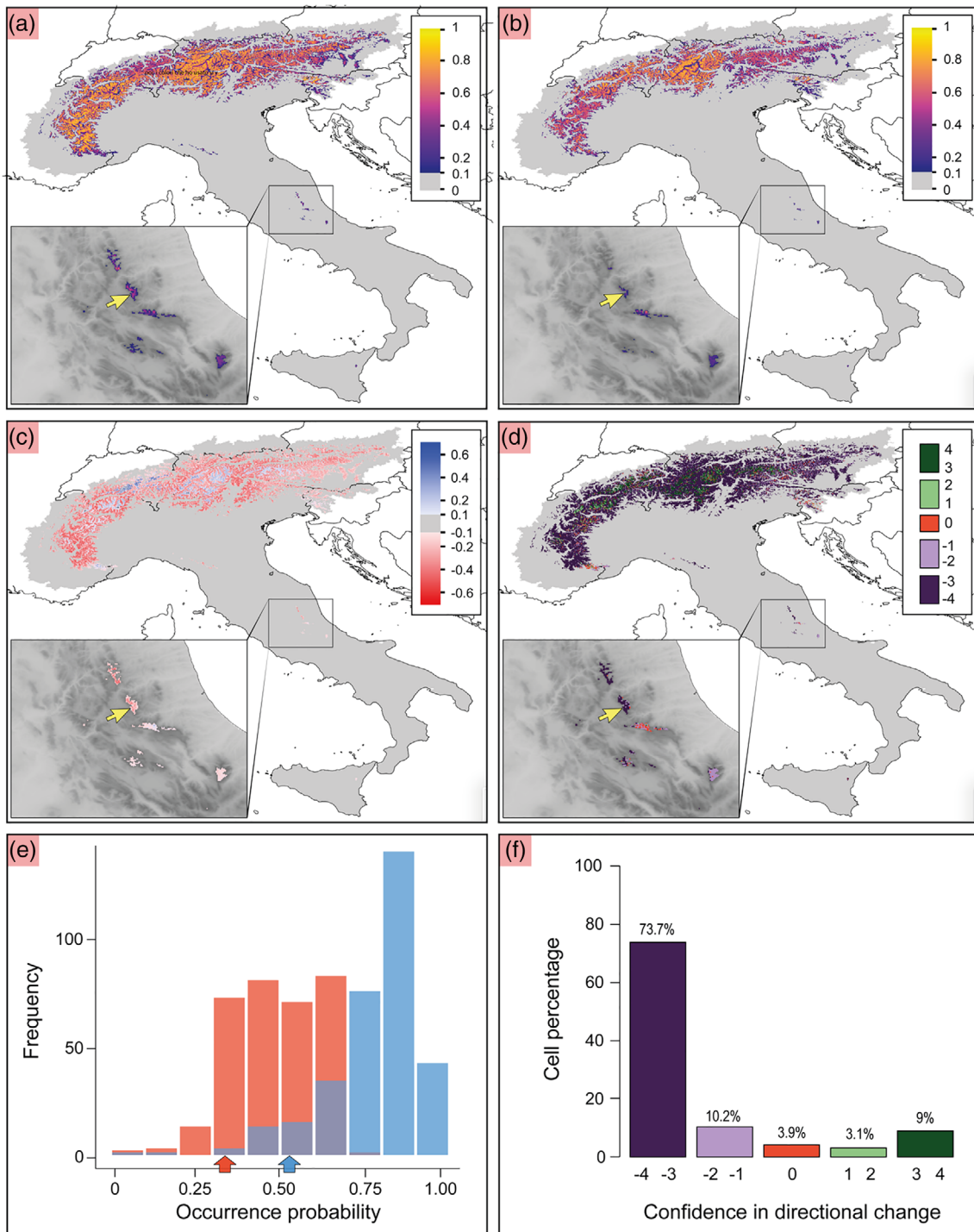
Fig 3. Species distribution modelling of *Erebia pandrose* (cells with an occurrence probability <10% were excluded). The panels (a–d) are accompanied by insets showing a detail of the central Apennine area and a yellow arrow indicates the exact position of the Laga population. Panels (a) and (b) show the current and future (2041–2060) distribution of the species, respectively. They are based on ensemble models, ensemble present (a) and ensemble future (b), and the colours represent the species occurrence probability (in a range between 0 and 1). (c) Ensemble delta: this panel shows the differences between the future and current occurrence probability of the species. Negative values indicate loss of suitability, while positive values indicate increase of suitability in the future (the values between −0.1 and 0.1 were excluded). Panel (d) shows the confidence in directional change of the four statistical models (consensus change). The colours represent the number of statistical models which agree in the future occurrence loss (purple) or gain (green): +4 and −4 values indicate full agreement among all the models, while the values +2, 0 and −2 indicate an intermediate situation in which different models predict
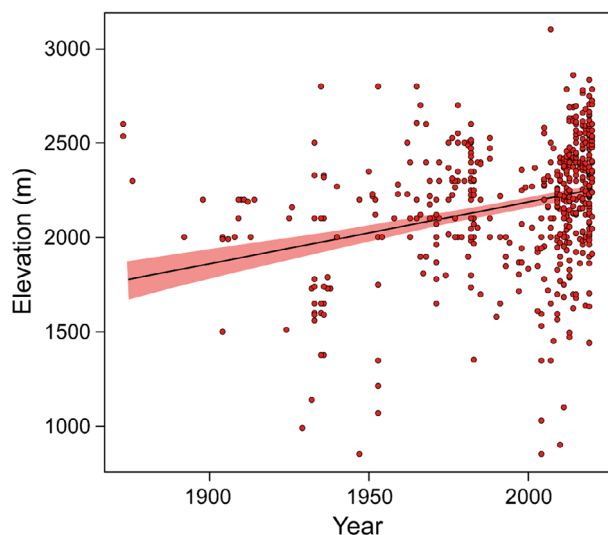
Fig 4. The relationship between year of collection and elevation for records of *E. pandrose* in Europe. The shaded area represents the 95% confidence interval of the regression line.

dot-like distribution on the Laga mountaintop since the onset of the last interglacial.

The level of mtDNA differentiation falls within the intraspecific range; nevertheless, the Apennine population encompasses a large fraction of the global diversity of this species, representing one of three main haplogroups. There is also evidence that the Apennine population is characterised by a different external phenotype, which led to the description of the *E. pandrose sevoensis* subspecies (Willien & Rachel, 1975), as well as a peculiar genital morphology characterised by a lower number of spines per valva (Cupedo, 2007). Our COI data indicate that *E. pandrose sevoensis* is the only European subspecies of this taxon that can be identified based on mtDNA sequences. It should be noted that, among the eight mutations differentiating the Apennine and Alpine populations, three are non-synonymous with the potential to induce a different respiratory performance (Pichaud *et al.*, 2012; Toews *et al.*, 2014). Thus, we cannot exclude that the Apennine population also evolved physiological adaptations to local climate, with important implications for the reliability of future survival forecasts of the Laga population (Razgour *et al.*, 2019).

only sampled three specimens from the Apennines, they all showed the same haplotype, which did not occur in the comprehensive sampling from other European and Asiatic regions. This strongly suggests that the Apennine population is represented by an endemic haplotype with a low intra-population variation compared to the high variation between populations, a pattern which is commonly found in mtDNA of butterflies and other organisms (Hewitt, 1999; Dapporto *et al.*, 2019). Assuming mutation rates ranging between 1.5% (Quek *et al.*, 2004) and 2.3% (Brower, 1994), as commonly done for butterfly mtDNA, the Laga population has likely diverged in allopatry from about 500 ka to 1 Ma. This indicates that the populations from the Apennines remained separated from the populations from the sub-Alpine area and the Balkan refugia along some glacial maxima, at least in the Mindel, Riss and Würm, (Schmitt, 2007; Dapporto *et al.*, 2019), with no postglacial northern dispersal events.

This contrasts with the wide, postglacial northern expansion of the main European haplogroup, which now occupies the Alps, the Pyrenees, the Carpathians, and the Balkans to the northernmost areas of Scandinavia formerly covered by ice sheets (Ehlers *et al.*, 2011). The high richness of haplotypes found in the Alps and the lower genetic diversity occurring in Scandinavia, the Carpathians and the Balkans (Fig. 2) suggest a recent colonisation characterised by gene surfing events and loss of genetic diversity, due to an expansion of populations from the Alpine region (Dincă *et al.*, 2021 for European butterflies). In contrast with this scenario, the high genetic differentiation of the Apennine population suggests that it has likely shrunk to its current

What is the future for E. pandrose *on the Apennines?*

Being poorly accessible to humans, mountain areas protected several species from the strongest forms of anthropogenic pressures that affect lowlands (Fattorini *et al.*, 2020a). So far, such inaccessibility and the consequent lack of grazing have probably contributed to the preservation of *E. pandrose* on the Apennines. However, historical data provided evidence of uphill shifts of *E. pandrose* populations of several metres per year, thus indicating that this species is suffering due to temperature increases. Italy did not experience butterfly species loss in the last decades (Bonelli *et al.*, ; Cerrato *et al.*, 2019), but several populations went extinct mostly among mountain specialists (Bonelli *et al.*, 2011), which are limited in their potential to expand towards higher elevations (Cerrato *et al.*, 2019). *E. pandrose* on the Apennines is an exemplary case of this situation, since the Monti della Laga are among the southernmost areas where this species occurs, and the mountain summit is only 300 m above the lowest elevation where we recorded the species. While in the 1970s, *E. pandrose* was recorded around 2000 m along the area we walked in 2019 (Balletto *et al.*, 2007), in our sampling we only observed it at 2200 m. If the absence of *E. pandrose* at lower elevations will be confirmed (see below), we could conclude that the Apennine population has shifted uphill for about 200 m in 40 years (i.e., 5 m/year), which is an even higher value than the generalised trend recorded along the study area in the last 147 and 50 years. An uphill shift of 3–5 m/year is higher than that recorded for tropical moth assemblages (1.6 m year^{-1}; Chen *et al.*, 2009) but similar to that observed in tropical and

different results. (e) Barplot showing frequency of present (blue) and future (red) relative occurrence probabilities for the 316 locations where *E. pandrose* was recorded. The blue and red arrows indicate, respectively, present and estimated future values corresponding to the cell where the Apennine population of *E. pandrose* has been found. (f) Frequency of the confidence in directional changes among the four statistical models represented as the percentage of cells for which models agreed. On the *x*-axis, the consensus values and on the y-axis their percentages (the colours are associated with Fig. 3d).

Alpine butterflies (6 m year^{-1}; Molina-Martínez et al., 2016; Habel et al., 2021). In the last 25 years, the shift is even more marked, with an increase in elevation that is higher than 20 m per year. Doubtless, the Monti della Laga massif cannot support substantial further upward movements of E. pandrose, as the habitat for this species will strongly narrow around the peaks, thus compromising the resilience of the population.

A series of studies showed that hibernating larvae of high elevation *Erebia* species are less tolerant to cold (freeze-avoidant) because they are adapted to survive the winter under a snow cover, while low elevation species exposed to unpredictable snow cover are freeze-tolerant (Vrba et al., 2012, 2017; Konvicka et al., 2021). *Erebia pandrose* can be considered as a high elevation *Erebia* species since it tends to occur above 2000 m (Fig. 4). The annual presence of the snow cover on the Alps and the Apennines has been already reduced by several days because of the ongoing climate change (Valt & Cianfarra, 2010).

Species distribution modelling predicts a strong reduction in climatic suitability for E. pandrose in the Apennines between 2040 and 2060. This prediction is based on an intermediate scenario, thus, according to the emission trend and to the unpredictable climatic fluctuations, the predicted lowering of climatic suitability and the consequent population decline could be delayed or advanced. It seems unavoidable that, in a few decades, the areas where this species is now present will be characterised by climatic conditions, which are currently recorded in lower elevation areas where the species rarely occurs (Fig. 3e). It must be noted that the alpine areas often provide a complex mosaic of different microhabitats, microclimates and thermal conditions (Scherrer & Körner, 2010). In areas characterised by a variety of suitable microhabitats, alpine species may survive longer than suspected based on modelling analysis (Scherrer & Körner, 2011). On the other hand, extreme meteorological events, which are predicted to increase with climate change (Thompson et al., 2013), can also produce strong fluctuations and local extinctions in mountain butterfly populations (Ehrlich et al., 1972, 1980; McDermott Long et al., 2017). Thus, even a single event might wipe out the small population of E. pandrose on the Monti della Laga massif.

Erebia pandrose *in the context of preserving Apennine butterflies*

It is hard to imagine conservation actions that can safeguard the Apennine population of E. pandrose by climate change. Among the solutions proposed by Sgrò et al. (2011) to sustain the evolutionary resilience at population level, the possibility of increasing population size and maintaining adaptive genes and traits seems very difficult to pursue. The area inhabited by this small population is already part of a National Park and human disturbance is minimal. It is also hard to hypothesise any action to locally mitigate the effects of climatic changes such as prolonging the permanence of the snow cover (e.g., by using snow fences). Translocation to other Apennine areas is also challenging, since no large areas appear as suitable in the future (Fig. 3b) and no positive trend is envisaged anywhere in the

region (Fig. 3c,d). Moreover, the Monti della Laga massif is characterised by a marly arenaceous geologic substrate instead of the calcareous type that is widespread in the central Apennines. This peculiar geological condition allows the maintenance of a more humid soil, which is probably fundamental for the occurrence of E. pandrose. In this respect, any attempt to translocate E. pandrose to other (higher) Apennine areas might be worthless.

The Italian Environment Ministry has recently committed the Italian National Parks to monitor pollinators by establishing Monitoring Scheme transects (Pollard & Yates, 1994). By using E. pandrose as a main target species, the Gran Sasso and Monti della Laga National Park has designed one of these transects along the eastern slope of Monte Gorzano (Supplementary Material, Fig. S1a). The semi-quantitative information produced by the transect count will allow monitoring population trends and phenology. This will be crucial to assess whether a reduction in climatic suitability is accompanied by a reduction in population consistency as shown by Scalercio et al. (2014) for *Erebia cassioides* (Reiner & Hochenwarth, 1792). Furthermore, taking advantage of the standardised data collected along the transect, it will be possible to assess trends over the entire butterfly community along a relatively wide altitudinal range. Finally, since microhabitat choice seems to be a major buffer for many butterfly species (included *Erebia* spp.) in front of climate change (Kleckova et al., 2014), it would be important to obtain fine resolution meteorological data (using data loggers) that can shed light on the influence of local conditions.

Erebia pandrose is a candidate to serve as an iconic flagship species to raise awareness regarding the ongoing loss of species and their genetic diversity in the Apennines. Indeed, it has the potential to become the object of specific citizen science initiatives and disseminating actions, mainly because of its genetic uniqueness and differentiation rather than its aesthetic value. The Italian Peninsula is one of the main centres of diversification in Europe, (Hewitt, 1999; Schmitt, 2007; Dapporto, 2010; Husemann et al., 2014; Dapporto et al., 2019; Scalercio et al., 2020; Menchetti et al., 2021) but, most butterfly endemics for Apennine are represented by deeply diverged intraspecific genetic lineages, which include an important fraction of European genetic diversity, but are not considered in most conservation measures (Menchetti et al., 2021). Recent reviews suggest that conservation plans should include 'threatened genetic diversity' represented by small and isolated populations encompassing a large fraction of genetic diversity of a given species (Brooks et al., 2015), a definition that perfectly fits with the Apennine E. pandrose and many other species from this region (Menchetti et al., 2021). Thus, it is necessary to raise awareness on the conservation of the entire butterfly community over the Apennines. This task is complicated by the fact that the IUCN Red List, the main tool to identify species under extinction risk, is typically assessed at the global (not assessed), regional (Europe: LC, van Swaay et al., 2011) or country level (Italy: LC, Bonelli et al., 2018). Again, E. pandrose occurs in 22 European countries and for 19 of them a national assessment is available; by integrating all the assessments according to Maes et al. (2019) the weighted Red List value for the species in Europe still appears quite low (11.14).

Apennine populations of *E. pandrose* and other *Erebia* species, *E. gorge* (Hübner, 1804), *E. pluto* (de Prunner, 1798) and *E. montana* (de Prunner, 1798) are declining and disappearing on the Apennines (Balletto *et al.*, 2007; Menchetti *et al.*, 2021; Piazzini & Favilli, 2020), but they are all considered as 'least concern' species for Italy because of the large distribution and relatively stable populations in the Alps (Bonelli *et al.*, 2018). The creation of a regional Red List (Gärdenfors *et al.*, 2001) for the Apennines would solve the issue and avoid that the conservation attention towards isolated and genetically unique populations in this region relies merely on species delimitation decisions. We believe that this proposal is justified by the specific evolutionary histories of the endemic taxa of Apennines (Menchetti *et al.*, 2021) and by the peculiar impact of climate change on populations of these relatively low and southern mountains which together call for the application of completely different strategies to protect butterfly diversity.

Conclusions

Climate change is renowned as one of the major factors responsible for the dramatic decline of biodiversity in the last decades. This is particularly true for organisms adapted to specific environments (e.g., high mountains), as is the case of the *Erebia* butterflies adapted to cold climates. We showed that *E. pandrose* in central Italy has an endemic mitochondrial lineage, which is the result of differentiation along several glacial maxima and is now restricted to a single mountain. Because of its very narrow distribution and distinct environmental requirements, this population is extremely vulnerable to the increasing temperatures. Thus, this population appears to be at risk of extinction in the next decades, which would represent an important loss of genetic and likely functional diversity at species level. A constant population monitoring will be crucial to figure out possible local actions and tackle the effects of global warming. Meanwhile, *E. pandrose* could represent an important flagship species to increase the interest in the conservation of endemic genetic lineages of the Italian peninsula, as well as other European areas of endemism with similar features.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

COI sequences are available on BOLD in the project DS-EPANDRO. Species distribution modelling protocol is provided as a ODMAP file in supplementary material.

Supporting information

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

Appendix S1: Supplementary Information.
Figure S1. A view of Monte Gorzano (Italy) from the North with the indication of the area (in red) where *E. pandrose* has been collected on 11th July 2019 and the transect (in yellow) designed in collaboration with the Gran Sasso e Monti della Laga National Park (a). A worn-out female of *E. pandrose* observed on Monte Gorzano (Photo by Emiliano Franci) (b) and the logo of the Butterfly Week event in 2019 having as the main goal the rediscovery and the study of this population (c).
Figure S2. The meadow in Monte Gorzano (Italy) where *E. pandrose* has been found on 11th July 2019.
Figure S3. Spatial blocking strategy, with a systematic fold assignment, used for the cross- validation of the models. The study area is plotted with the climatic variable bio_11 (Mean Temperature of Coldest Quarter).
Figure S4. Plot of the mean (dots) and the standard deviation (segments) among ten datasets of the model evaluation scores according to TSS and ROC (AUC). a) Model evaluation scores of the four statistical models; b) Model evaluation scores of the ten pseudo-absence datasets; c) Model evaluation scores of the three runs.
Figure S5. *Erebia pandrose* smoothed response curves among the four selected variables. The four model curves are evaluated together for each variable. Black lines represent the medians across model predictions, whereas shades of green represent different quantiles (0.25–0.75; 0.05–0.95; 0.025–0.975) of the distribution. a) response curves of bio7, Temperature Annual Range; b) response curves of bio11, Mean Temperature of Coldest Quarter; c) response curves of bio12, Annual Precipitation; d) response curves of bio19, Precipitation of Coldest Quarter.
Figure S6. Projections of the four models resampled by mean at 10 km to improve the visibility of the geographic pattern; a-c GLM results; d-f GAM results; g-i MaxEnt results; j-l Random Forest results. a) current occurrence probability predicted by GLM; b) future occurrence probability predicted by GLM; c) delta values resulted from GLM predictions; d) current occurrence probability predicted by GAM; e) future occurrence probability predicted by GAM; f) delta values resulted from GAM predictions; g) current occurrence probability predicted by MaxEnt; h) future occurrence probability predicted by MaxEnt; i)

delta values resulted from MaxEnt predictions; j) current occurrence probability predicted by Random Forest; k) future occurrence probability predicted by RandomForest; l) delta values resulted from Random Forest predictions.

Figure S7. Barplot showing frequency of differences between the averaged current and future distribution of *E. pandrose* among cells.

Table S1. *Erebia pandrose* COI sequences used. Coordinates are expressed in decimal degrees.

Table S2. Variance Inflation Factors of the four selected variables, rounded to three decimal numbers. All the values are <5 showing there is not a multicollinearity issue.

Table S3. Mean variable importance scores for each model, rounded to three decimal numbers.

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