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1 Past, current and potential future distributions of unique genetic diversity in a cold-adapted
2 mountain butterfly

3 **Short running title less than 40 characters:** Climate change and insect genetic diversity

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14 **Biosketch**

15 **Melissa Minter** is a PhD researcher in the Biology Department at the University of York, UK. Her
16 research examines the genetic diversity of retracting northern and montane butterflies under climate
17 change.

18 **Author contributions**

19 M.M., K.K.D., C.D.T., M.D.M., A.T., T.S. and J.K.H. conceived the ideas; M.M., S.S. and T.S.
20 collected the data; M.M. analysed the data; M.M. drafted the manuscript; all authors contributed to the
21 final manuscript.

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33 Heritage for providing permission to sample.

34

35 **Abstract** (300 words)

36 **Aim** Climatic changes throughout the Pleistocene have strongly modified species distributions. We
37 examine how these range shifts have affected the genetic diversity of a montane butterfly species, and
38 whether the genetic diversity in the extant populations is threatened by future climate change.

39 **Location** Europe.

40 **Taxon** *Erebia epiphron* Lepidoptera: Nymphalidae.

41 **Methods** We analysed mtDNA to map current genetic diversity and differentiation of *E. epiphron*
42 across Europe to identify population refugia and post-glacial range shifts. We used species
43 distribution modelling (SDMs) to hindcast distributions over the last 21,000 years to identify source
44 locations of extant populations, and to project distributions into the future (2070) to predict potential
45 losses in genetic diversity.

46 **Results** We found substantial genetic diversity unique to specific regions within Europe (total number
47 of haplotypes = 31, number of unique haplotypes = 27, $H_d = 0.9$). Genetic data and SDM hindcasting
48 suggest long-term separation and survival of discrete populations. Particularly high rates of unique
49 diversity in post-glacially colonised sites in England ($H_d = 0.64$), suggests this population was
50 colonised from a now extinct cryptic refugium. Under future climate change, SDMs predict loss of
51 climate suitability for *E. epiphron*, particularly at lower elevations (< 1000 metres above sea level)
52 equating to 1 to 12 unique haplotypes being at risk under climate scenarios projecting 1 °C and 2-3 °C
53 increases respectively in global temperature by 2070.

54 **Main conclusions** Our results suggest that historical range expansion and retraction processes by a
55 cold-adapted mountain species caused diversification between populations, resulting in unique genetic
56 diversity which may be at risk if distributions of cold-adapted species shrink in future. Assisted
57 colonisations of individuals from at-risk populations into climatically-suitable unoccupied habitat
58 might help conserve unique genetic diversity, and translocations into remaining populations might
59 increase their genetic diversity and hence their ability to adapt to future climate change.

60

61 **Keywords**

62 Refugia, climate change, Last Glacial Maximum, mountain systems, butterfly, genetic diversity

63 **Data availability statement**

64 Genbank accession numbers for each mtDNA COI sequence can be found in supporting information
65 Appendix S1 in Supporting Information. (All sequences will be uploaded to Genbank upon
66 acceptance.)

67

68

69

70 **1. Introduction**

71 Projecting the future geographic distribution of genetic variation within species' ranges, and the
72 potential loss of genetic variation from anthropogenic climate change, requires understanding of the
73 past, present and future distributions of species (Wroblewska & Mirski, 2018). Geographic variation
74 in the distribution of genes across a species' range results from a combination of historical and current
75 conditions, which influence patterns of genetic differentiation among populations that are, or have
76 been, geographically isolated, and from colonisation bottlenecks during range shifts (Hewitt, 2004).
77 These range shifts and their genetic consequences have primarily been driven by the fundamental
78 niche of a species, or their 'climate-envelope', and species' ranges shift to track environmental
79 changes, altering the location of populations and their genetic structure (Hewitt, 2004; McCallum,
80 Guerin, Breed, & Lowe, 2014; Thomas, 2010). The Earth has gone through many climate
81 fluctuations, including glaciations in the Pleistocene and human-induced climate change in the current
82 Anthropocene (Hewitt, 2004; Santer et al., 2019). Future anthropogenic climate warming may further
83 impact species through distribution changes, genetic erosion and extinctions (Botkin et al., 2007).
84 Cold-adapted/mountain species may be especially vulnerable to future climate changes as they are
85 already restricted to mountain ecosystems where suitable climate space is limited, and loss of genetic
86 diversity within these range-restricted cold-adapted species may reduce their ability to adapt to future
87 changes (Elsen & Tingley, 2015). Understanding how past climatic changes have impacted current
88 genetic structure may allow us to make predictions for the likely extent of genetic loss under future
89 climate change, and thereby prioritise at-risk populations for conservation management (McCallum et
90 al., 2014; Wroblewska & Mirski, 2018).

91 During the last ice age, ice sheets were at their greatest extension 20,000-21,000 years ago, during the
92 last glacial maximum (LGM) (Crowley & North, 1991; Ray & Adams, 2001). During the LGM,
93 species were thought to persist where climatic conditions were buffered, at lower elevations or in
94 more southerly regions (Dapporto et al., 2019; Morelli et al., 2016), however some studies have
95 shown evidence of species surviving in northern isolated refugia (Provan & Bennett, 2008; Schmitt &
96 Varga, 2012; Stewart & Lister, 2001). Cool-adapted species which currently occur in mountain
97 ecosystems were probably more widespread during the LGM and only became isolated in their
98 current interglacial populations after climate-induced range retraction, although some cold-adapted
99 species were already restricted to isolated glacial refugia during the LGM (Schmitt, 2009; Schmitt,
100 Hewitt, & Muller, 2006). The consequences of past distribution changes will be reflected in current
101 genetic diversity, because contractions and expansions from long-term refugia leave a genetic
102 signature of high diversity in refugia compared to lower diversity in recently-colonised populations
103 (Hewitt, 2000; Keppel et al., 2012; Morelli et al., 2016). Thus understanding historical interactions of
104 cold-adapted species with climate can help us understand current genetic structure and diversity of
105 populations.

106 Lepidoptera are poikilothermic and therefore sensitive to changes in climate, and those species which
107 are cold-adapted are particularly vulnerable to warmer conditions (Deutsch et al., 2008; Elsen &
108 Tingley, 2015). Some cold-adapted Lepidoptera are experiencing extinctions at their low
109 latitude/elevation margins as the climate deteriorates for these species (Franco et al., 2006; Wilson,
110 Gutierrez, Gutierrez, & Monserrat, 2007). The Mountain Ringlet *Erebia ephron* is a butterfly found

111 in the mountains of continental Europe and Britain, and its distribution has retracted 130-150 m uphill
112 in Britain over the past five decades due to climate warming (Franco et al., 2006). Therefore *E.*
113 *epiphron* represents a good model organism to understand how past climate-induced changes have
114 impacted current genetic structures of populations, and whether genetic diversity may be lost with
115 further climate-induced local extinctions.

116 Species distribution models (SDMs) are commonly used to project future distributions of species
117 under climate change scenarios (Guo et al., 2017; Urban, 2015), and to develop climate adaptation
118 conservation strategies. These modelling approaches have also been used with palaeoclimate data to
119 hindcast past distributions and to understand how they shape current population structures (Smith,
120 Gregory, Anderson, & Thomas, 2013). Phylogeography with genetic techniques can be used to
121 identify divergence between populations and to infer historical distribution patterns and colonization
122 routes (Luquet et al., 2019). Previous studies have shown how a combination of species distribution
123 modelling and phylogeography can provide better understanding of past, present and future
124 distributions of species, and predict the potential loss of genetic diversity resulting from climatic
125 warming (Schmitt, Habel, Rodder, & Louy, 2014; Wroblewska & Mirski, 2018; Yannic et al., 2014).

126 In this study, we use mtDNA sequencing to map the current distribution of genetic diversity of the
127 cold-adapted butterfly, *E. epiphron*, and also use species distribution modelling to project current,
128 past, and future distributions of the species. We use this genetic and modelling information to
129 determine the distribution of *E. epiphron* in continental Europe during the last glacial maximum, the
130 locations of glacial refugia, and patterns of subsequent postglacial expansion into northerly latitudes
131 in Britain. We identify populations with unique genetic diversity and examine potential loss of genetic
132 diversity under future climate change scenarios in order to prioritise populations for protection.

133 **2. Methods**

134 *2.1 Genetic analyses to map current haplotype diversity*

135 We sampled 146 adults of *E. epiphron* from 13 mountain regions across continental Europe and
136 Britain. European populations (76 adults) were sampled between July - August 2002-2014,
137 populations in England and Scotland (74 adults) were sampled in June-July 2016-2019, and adults
138 preserved in 100% ethanol at -20°C. All relevant fieldwork permissions were obtained. DNA was
139 extracted from 111 individuals with Omega bio-tek E.Z.N.A.® DNA Isolation Kit following the
140 manufacturer's protocol. For each individual, the head and antennae were removed and placed in 1.5
141 ml tubes with CLT buffer and Proteinase K and homogenised with pellet pestles. A 658-bp fragment
142 of the mitochondrial cytochrome oxidase-I (COI) gene was amplified using the primers LepF (5'-
143 ATCAACCAATCATAAAGATATTGG-3') and LepR (5'-
144 TAAACTTCTGGATGTCCAAAAATCA-3') (Hajibabaei, Janzen, Burns, Hallwachs, & Hebert,
145 2006). PCR amplification of individual DNA samples was carried out in 20 µl reactions which
146 included 1.8 µl of template DNA, 1x PCR reaction buffer (Promega), 1.5 mM MgCl₂, 0.2 mM of
147 dNTPs and 1U of *Taq* DNA polymerase (Promega GoTaq®). PCR conditions used the following
148 profile: 94°C for 2 minutes (one cycle), 2 minute at 94°C, 58°C for 45s and 72°C for 1 minute (35
149 cycles), followed by a final extension step of 75°C for 5 minutes. PCR products were purified and

150 Sanger sequenced with forward and reverse primers using © Eurofins Scientific PlateSeq service and
151 LightRun Tube service. Chromatograms were checked visually using SeqTrace (Stucky, 2012).
152 Additional COI sequences were obtained from a panel of 39 samples collected in England in June
153 2016 as a part of a whole genome resequencing project (NERC Highlight project NE/N015797/1).
154 Briefly, the complete mitochondrial genome was assembled for each individual sample using the
155 MitoZ toolkit (Meng et al. 2019) and annotated using the mitos2 webserver (Bernt et al. 2013). Low
156 coverage regions (<10) were masked to avoid introducing low quality SNPs and the COI region was
157 extracted for further analyses.

158 These 150 sequences along with 65 existing COI sequences from Genbank were combined to create a
159 data set of 215 COI sequences from 13 mountain regions across the species' European range (for
160 sample information see Appendix S1 and map of mountain regions see Appendix S2 in Supporting
161 Information). These sequences were aligned with ClustalX implemented in MEGA-X (Kumar,
162 Stecher, Li, Knyaz, & Tamura, 2018) and the alignment checked by eye and cropped to the same
163 length (649 bp). Haplotypes were identified and genetic diversity measures were determined using
164 DnaSP6 (Rozas et al., 2017). Genetic diversity measures included number of haplotypes (H_n), number
165 of unique haplotypes (H_u), haplotype diversity (H_d , the probability that two randomly sampled alleles
166 are different) and nucleotide diversity (π , the average number of nucleotide differences per site
167 between sequences (Nei, 1987). A TCS network (Templeton, Crandall, & Sing, 1992) of all
168 haplotypes was constructed using PopArt (Leigh & Bryant, 2015). A COI phylogenetic tree was
169 constructed in BEAST (Suchard et al., 2018) of the *Erebia* genus, outgroups and the *E. epiphron*
170 populations. The same methods and COI sequences were used from (Pena et al., 2015) using a log-
171 normal relaxed molecular clock, with a birth-death incomplete speciation model for the randomly
172 generated tree prior, and then an uncorrelated log-normal relaxed molecular clock and all the
173 programs other default settings to model the rate of evolution. The age between *Erebia* and its sister
174 taxa was set at 37.4 ± 2 Myr, (Pena et al., 2015) to estimate age in divergence between *E. epiphron*
175 subpopulations.

176 2.2 Using species distribution modelling (SDMs) to map current distribution of *E. epiphron*

177 Current distribution data for *E. epiphron* (50 × 50 km grid resolution) were obtained from the
178 Distribution Atlas of European butterflies ([http://www.ufz.de/european-](http://www.ufz.de/european-butterflies/index.php?en=42605)
179 [butterflies/index.php?en=42605](http://www.ufz.de/european-butterflies/index.php?en=42605)). Current (1970-2000) climate data were downloaded from
180 WorldClim (<http://www.worldclim.org/>) at a resolution of 2.5 arc minutes (~4.5 km grid cell
181 resolution). Climate variables for inclusion in SDMs were selected to reflect climate limitations and
182 extremes of cool-adapted species, which are likely to be most limited by climatic conditions during
183 the coldest and hottest times of the year. We therefore included climate data on annual mean
184 temperature and mean precipitation of the coldest quarter (December to February) and warmest
185 quarter (June to August) of the year (Smith et al., 2013). Spatial autocorrelation was tested using
186 Moran's I in R. The butterfly distribution data were at 50 km grid resolution, but the species is likely
187 to be restricted by local climate conditions in each grid square (Smith et al., 2013). Thus, we included
188 in models only the coldest/warmest and wettest/driest cells (4.5 km resolution) within each 50 km
189 grid, resulting in a total of eight climatic variables being incorporated into our SDMs (see Appendix

190 S3 in Supporting Information). 50 x 50 km grid cell resolution data are appropriate for our model
191 building to address biogeographic questions at regional scales, because we are interested in changes in
192 the distribution of the study species over long periods of time (i.e. millennia), rather than shorter-term
193 changes at individual sites. This 50km spatial resolution also ensures that the pseudo-absences (i.e.
194 locations where *E. epiphron* is assumed to be absent) are more accurate representations of true
195 absences, because these grids have been visited by butterfly recorders but *E. epiphron* was not
196 recorded as present. In addition, 50km data for presences cover the entire global distribution of *E.*
197 *epiphron* at this spatial resolution. Butterfly distributions were modelled using an ensemble approach
198 (R package BIOMOD2; (Thuiller, Lafourcade, Engler, & Araujo, 2009), combining outputs from the
199 models; Generalised Linear Models (GLM), Multiple Adaptive Regression Splines (MARS),
200 Maximum Entropy (MAXENT.Phillips), Generalized Additive Model (GAM), Boosted Regression
201 Trees (GBM), Classification Tree Analysis (CTA), Artificial Neural Network (ANN), Surface Range
202 Envelope (SRE), Flexible Discriminant Analysis (FDA) and Random Forest (RF). We used the mean
203 Receiver Operating Characteristic (ROC) value to evaluate each model, with a threshold of ROC >
204 0.85 for inclusion of models within the ensemble model. We restricted pseudo-absences to locations
205 within a buffer of 250 km around presence data points to avoid placing absences in mountain systems
206 with potentially suitable climate space that are not currently occupied by the species (e.g.
207 Scandinavia) (Akçakaya & Atwood, 1997; Hirzel, Helfer, & Metral, 2001). Models were generated
208 using 70% training data and 30% testing data (Franklin, 2010; Huberty, 1994).

209 *2.3 Hindcasting past distributions and identifying glacial refugia*

210 We incorporated paleoclimate data into our ensemble SDM for the eight climate variables
211 representing the coldest/hottest and driest/wettest locations within each 50 km grid square. Data for
212 climate projections over the last 21,000 years were downloaded from Paleoview (2.5 × 2.5°
213 latitude/longitude) grid (Fordham et al., 2017), and downscaled to match the resolution of the
214 current climate data (2.5 arc minutes), using established methods (Mitasova & Mitas, 1993; Platts,
215 Omeny, & Marchant, 2015; Ramirez-Villegas & Jarvis, 2010). We projected climate suitability for *E.*
216 *epiphron* every 1,000 years from the LGM to 1,000 years before present, generating 21 outputs, which
217 were each clipped using Eurasian ice sheet data (Hughes, Gyllencreutz, Lohne, Mangerud, &
218 Svendsen, 2016). Long-term climate suitability of 50 km grid squares was calculated by overlaying
219 the 22 output maps and summing the climate-suitability probability values of each grid, and then
220 designating the top 30% of grids with highest probability values as areas of highest long-term climate
221 stability for the study species (Chan, Brown, & Yoder, 2011).

222 *2.4 Projecting future distributions and loss of genetic diversity*

223 Future climate projections for 2070 were obtained from IPCC 5th Assessment Report (Complete
224 Coupled System Model, CCSM4 global climate models) from WorldClim
225 (<http://www.worldclim.org/>; 2.5 arc minutes resolution) for high (RCP 8.5, ~2-3°C warming) and low
226 (RCP 2.6, ~1°C warming) future climate scenarios. Unique haplotypes were assumed to be at risk if
227 all 50 km grid squares in one of the 13 mountain regions were predicted to become climatically
228 unsuitable in the future (based on binary presence or absence threshold probability values from the
229 ensemble SDM output). We set the threshold value as the probability value associated with the low

230 elevation climatic range edge *E. epiphron* in its current range (low elevation range boundary in
231 England; threshold probability = 0.49). Using this threshold, model probabilities were converted into
232 presence/absence to show grid squares with no change over time (i.e., population persistence), grids
233 predicted to become climatically unsuitable (i.e., extinction), and grids predicted to become
234 climatically suitable (i.e., colonisation). Haplotype risk (H_r) was calculated as the number of unique
235 haplotypes at risk in each of the 13 mountain regions (Figure 1a) due to projected loss of all
236 climatically suitable areas within a region in the future.

237 **3 Results**

238 *3.1 Current haplotype diversity across 13 mountain regions in Europe*

239 From our 215 mtDNA samples, we identified 31 mtDNA haplotypes across Europe, including 27
240 haplotypes unique to a specific mountain region (Figure 1a, Table 1). The high frequency of unique
241 haplotypes across Europe suggests low levels of allele-sharing. There was also high genetic
242 differentiation between populations (AMOVA, $\phi = 0.76$, $p < 0.001$) and the divergence between some
243 of these populations is dated before the last glacial maximum (phylogenetic tree: see Appendix S5 in
244 Supporting Information). The mountain regions containing the highest haplotype diversity include the
245 Pyrenees ($H_d = 0.63$) the western Alps ($H_d = 0.91$) and England ($H_d = 0.64$) (Table 1). The mountain
246 regions containing only unique haplotypes include the Carpathians ($H_u = 2$) and the Tatras ($H_u = 2$).
247 Populations in England ($H_u = 6$) and the western Alps ($H_u = 6$) not only had the highest number of
248 unique haplotypes but also contained some shared haplotypes with other regions (Figure 1a). There
249 are six unique haplotypes in England which diverged from haplotype 8 (Figure 1b), which is present
250 in England, Scotland, Vosges and the western Alps. None of the 6 unique haplotypes in England were
251 found anywhere else, although the presence of the shared haplotype 8 suggests historical allele
252 sharing with the western Alps. Scotland, in addition to the shared haplotype 8, contains one unique
253 haplotype (haplotype 30), which has diverged from haplotype 8 by 1 substitution and shares haplotype
254 10 with the Apennines (Figure 1). Despite evidence that regions are differentiated, shared haplotypes
255 also provide evidence of historical gene flow across Pyrenees and Cantabrians, and between the Alps
256 and Balkans (Figure 1). The Massif Central population shares one haplotype (haplotype 16) with the
257 Pyrenees and Cantabrian Mountains, and has one unique haplotype (haplotype 29) which diverged
258 from haplotype 16 by one substitution (Figure 1b).

259 *3.2 Modelling the current distribution of E. epiphron*

260 Our ensemble SDM was a good fit to the current distribution of *E. epiphron* (95.4% of presences
261 predicted correctly, 76.3% of pseudo-absences predicted correctly (based on the total presence data),
262 ROC = 0.9) (Figure 2a). Areas predicted to be climatically suitable but currently uninhabited by *E.*
263 *epiphron* include Wales, Scandinavian mountains and eastern Balkans, the latter of which is currently
264 occupied by *Erebia orientalis*. The model rated the minimum temperature of the warmest quarter of
265 the year (June – August) as the most important variable for predicting climate suitability for the
266 species (average importance of this variable across models = 0.73; importance rated from 0-1),
267 probably because this is an important variable in identifying high elevation areas within a 50 km grid
268 square.

269 3.3 Hindcasting past distributions of *E. epiphron* and identifying areas of long-term survival

270 Climate suitability in the LGM (21,000 years before present) showed overlap of climatically suitable
271 areas (at 50 km grid resolution) with many locations currently occupied by *E. epiphron*, as well as
272 some southerly locations (Figure 2e). This overlap was confirmed when all 21 SDM outputs for each
273 1000-year time period up to the present day were combined to show long-term climatic stability since
274 the LGM (Figure 2f). These climate stability maps provided evidence that the locations of glacial
275 refugia were in areas of high topographic variation within the species' current distribution in
276 continental Europe.

277 3.4 Projecting future distributions and loss of genetic diversity

278 As expected for a cold-adapted species, SDM outputs from both high and low future climate change
279 scenarios project that many extant *E. epiphron* areas will have reduced climate suitability in the future
280 (38-64% loss of 7,000 km² occupied grids across Europe) (Figure 3, Table 1). The loss of climate
281 suitability is most severe in lower elevation sites, as shown by significant linear regressions between
282 change in probability over time and average elevation of the 50 km grid square (low scenario: $p <$
283 0.001 , $R^2: 0.27$, $F_{150} = 56.51$, high scenario: $p < 0.001$, $R^2: 0.13$, $F_{150} = 22.86$). The mountain regions
284 predicted to experience the greatest reduction in range size are the Vosges (100% loss of grid squares
285 under both scenarios) and Apennines (100% loss of grid squares under both scenarios), followed by
286 the Balkans (75-100% loss), Carpathians (70-100% loss), England (50-100% lost) and Cantabrians
287 (63-81% loss) (Figure 3, Table 1). These range retractions result in the potential loss of 1 haplotype
288 under the low climate change scenario (RCP 2.6); and the total loss of 12 unique haplotypes under the
289 high climate change scenario (Figure 3, Table 1). Many of the haplotypes predicted to be lost are a
290 single substitution from their nearest haplotype, however the haplotypes in the Carpathians are more
291 genetically distinct (Figure 1b). By contrast, range sizes in the Alps and Scotland are projected to
292 remain relatively stable, assuming the species colonises sites at higher elevations that are predicted to
293 become climatically suitable in these regions. Under both scenarios, areas north of Scotland and
294 England become suitable in the future. Although *E. epiphron* does not currently occur in
295 Scandinavia, our models predict that this area will remain stable in climate suitability in the future.

296 **4. Discussion**

297 By using species distribution modelling and mtDNA analyses, we explore the past, present and
298 potential future distributions of genetic diversity in the cold-adapted species *E. epiphron*. We identify
299 high levels of genetic differentiation across Europe, and found evidence of long-term climate
300 suitability in many of these regions since the LGM, which suggests these climatically stable regions
301 were refugial areas of long-term survival by our study species over the last 21,000 years, and
302 potentially longer-term areas of persistence over previous glacial-interglacial cycles. Our study
303 focuses on a single mountain species but our findings are likely to be widely applicable to other
304 mountain species where populations contain unique genetic diversity as a consequence of past climate
305 fluctuations, and which may be at risk under future climate warming. These areas of long-term
306 survival are within topographically heterogeneous landscapes, allowing populations to shift to the
307 foothills during glacial periods. Our analyses also revealed that populations in the Massif Central,

308 Vosges and Britain are presumed postglacial colonisations (Figure 1, Figure 2f) due to low climate
309 suitability over time, shared haplotypes and the fact that Britain was under an ice sheet during the
310 LGM. Britain was apparently colonised via two different routes, with the Scottish populations likely
311 originating from populations in Vosges/Alps mountain regions due to the high prevalence of shared
312 haplotype 8. By contrast, the English population has high levels of unique genetic diversity, and no
313 evidence that any of the six unique haplotypes are shared with other extant populations (although
314 there is one shared haplotype present), suggesting the English population has separated from the
315 western Alps before the last glacial maximum (given the large number of nucleotide substitutions;
316 Figure 1b), and colonised Britain via a different route, from a cryptic refugium in an area where the
317 study species survived during the glacial period but where it no longer exists. Under future climate
318 change scenarios, we predict 30-56% loss of range size, which equate to 1 unique haplotype to 12
319 unique haplotypes being at risk of loss under climate scenarios projecting 1 °C and 2-3 °C increases
320 respectfully.

321 4.1 Limitations

322 This study has potential limitations, which are inherent in species distribution modelling, especially
323 when projecting into different climates (Buisson, Thuiller, Casajus, Lek, & Grenouillet, 2010). We
324 did not have suitable data to include sampling effort formally into our models and so the areas outside
325 of the current *E. epiphron* distribution are considered ‘pseudo-absences’ rather than ‘true’ absences.
326 However, other butterfly species have been recorded in these squares (Lepidopterists have visited
327 these squares) without recording *E. epiphron* as present, and hence the proportion of false absences in
328 the data is likely to be very low at the spatial (50 km across the whole of Europe) and temporal
329 (accumulation of Lepidoptera records over 3 decades) scales considered here. We consider that our
330 modelling approach robustly describes the bioclimatic conditions occupied by *E. epiphron* at a
331 continental scale (the species’ global distribution). Future work could use sampling effort to account
332 for imperfect species detection, with standardised sampling and occupancy modelling providing
333 additional insight into (especially) within-region distributions and dynamics.

334 For future projections, the loss of populations and consequently genetic diversity was based on a
335 probability threshold to define butterfly presence or absence. This threshold was based on the
336 probability value for English populations, given that this region represented the lowest elevational
337 range edge for the study species. However, currently realised and fundamental niche characteristics
338 may differ among regions (i.e. thresholds may differ), and hence caution should be taken with our
339 predictions. The difference between using two different thresholds (either the lowest elevation versus
340 a threshold calculated by the Biomod2 program), affects whether or not the entire English and
341 Apennines regions are lost, and hence there is some uncertainty about the level of genetic diversity at
342 risk. Nonetheless, the relatively low probability of future persistence in both of these regions suggests
343 that these populations are at the climatic range limit for the species and therefore at risk. While
344 regional adaptations may differ, we have no evidence that haplotypes are individually adaptive to
345 climate variables and hence we use them as markers of colonisation rather than as adaptive traits. For
346 the same reason, we did not model the specific niches of individual haplotypes when considering the
347 potential future loss of genetic variation (Breiner, Nobis, Bergamini & Guisan et al., 2018). Future

348 work could use next generation sequencing to further test our hypotheses; and to model specific
349 genetic-climatic relationships in the future (see Bay et al., 2018).

350 Our analyses suggest that entire mountain regions of the butterfly's distribution could be lost under
351 future climatic change, but it is possible that isolated populations could survive in particular
352 microhabitats, at least temporarily. However, these localised populations may not contain all of the
353 genetic variation currently present in the wider region, and overtime these refugial populations may
354 gradually lose genetic variation and viability (e.g., through inbreeding), and so they may not persist in
355 the longer term due to their isolation (metapopulation failure). A variety of processes may lead to the
356 loss of genetic diversity following isolation, and there can sometimes be a delay in genetic loss
357 following population decline (Kadlec, Vrba, Kepka, Schmitt, & Konvicka, 2010). For example, the
358 sister species of *E. epiphron*, *Erebia orientalis*, is very localised and currently occurs only in the
359 Eastern Balkans and is genetically homogeneous, potentially putting it at risk of inbreeding
360 depression (Hinojosa, Monasterio, Escobes, Dinca, & Vila, 2019). Therefore, our model projections
361 should be seen as representing much longer-term regional-scale expectations, rather than short-term
362 predictions at the local population or microhabitat scale. We believe that our conclusions about the
363 long-term (LGM to present) continental-scale dynamics of *E. epiphron* are robust, and that this
364 knowledge of the past helps frame future risks and provides information for conservation
365 management.

366 *4.2 Long-term survival resulting in unique genetic diversity in cool-adapted species*

367 SDM outputs provide evidence that our exemplar cold-adapted study species occurred in disjunct
368 regions throughout the period from the LGM to the present day, based on the distribution of suitable
369 climate; the genetic data confirm likely separation not only since the LGM, but most probably over
370 much longer periods and successive glacial-interglacial cycles. For mountain species, limited gene
371 flow between the disjunctive parts of their range during glacial and interglacial periods results in
372 divergence and unique haplotypes, unlike lowland European species which colonised northwards
373 from their glacial refugia, and where large parts of the current geographic ranges often share
374 haplotypes (Hewitt, 2004). Only limited areas of postglacial expansions and retractions are evident in
375 *E. epiphron*, and the British populations would be susceptible to extinction if the climate was to return
376 to LGM conditions at some time in the future. Similarly, our SDM outputs suggest that additional
377 populations of *E. epiphron* could have existed further south in southern Europe at the LGM (Figure
378 2E) but as they no longer exist a northwards translocation of the range might have taken place under
379 interglacial conditions. If cold-adapted species such as *E. epiphron* were more widespread during
380 glacial periods, then the current divergence could be associated with subsequent losses of genetic
381 diversity (e.g., due to selection, or random drift during population bottlenecks), or a failure of our
382 analyses to detect localised or rare haplotype variation. However, this alternative hypothesis seems
383 unlikely because our estimates of times of genetic divergence (phylogenetic tree: see Appendix S5)
384 imply that most splits occurred before the LGM. However, other divergence dates between *E.*
385 *epiphron* and *E. orientalis* have been reported (e.g. 1.53 (± 0.65) Mya (Hinojosa et al., 2019)).
386 However they still reported strong mtDNA divergence and long term separation (Hinojosa et al.,
387 2019) and therefore different assumptions of divergence dates do not impact the interpretation of our

388 results. Hence we conclude that populations of *E. ephron* survived as allopatric populations in
389 mainland Europe during the LGM, with postglacial colonisations from these regions into the Massif
390 Central, Vosges, Scotland and England.

391 High genetic differentiation is observed among populations of other mountain *Erebia* species,
392 supporting the hypothesis that they also survived as allopatric populations during the LGM (Haubrich
393 & Schmitt, 2007; Louy, Habel, Abadjiev, et al., 2014; Louy, Habel, Ulrich, & Schmitt, 2014; Martin,
394 Gilles, Lortscher, & Descimon, 2002; Schmitt et al., 2014; Schmitt, Louy, Zimmermann, & Habel,
395 2016; Schmitt & Seitz, 2001). LGM separation of populations has also been identified in mountain
396 plants and other invertebrates (Bettin, Cornejo, Edwards, & Holderegger, 2007; Huck, Budel, &
397 Schmitt, 2012; Margraf, Verdon, Rahier, & Naisbit, 2007; Pauls, Lumbsch, & Haase, 2006). The
398 numbers of glacial-interglacial cycles over which populations have remained disjunct remains
399 unclear, but some studies have indicated divergence dates covering several glacial-interglacial cycles
400 or even predating the Pleistocene (Hewitt, 2000). The reality is likely to be more complex with areas
401 of persistent separation, but with occasional links between them (i.e. rare gene flow or brief periods of
402 connection), as indicated by the distributions and relatedness of haplotypes in Figure 1.

403 *4.3 Unique haplotypes in populations derived from northern cryptic refugia*

404 Following the LGM, the ice retreated in northern Europe and many species colonised northwards, for
405 example via the land bridge between continental Europe and Britain, which was present until sea level
406 rise ~7,000 years before present (Sturt, Garrow, & Bradley, 2013). The locations of southerly glacial
407 refugia, which are thought to be the main sources of colonisations, have been debated extensively,
408 with proposed glacial refugia in the Iberian Peninsula, Italy and the Balkans (Hewitt, 2000) and this
409 has recently been reinforced in European butterflies (Dapporto et al., 2019). However, there is also
410 evidence for more northern cryptic refugia based on fossil, pollen and genetic evidence (Birks &
411 Willis, 2008; Provan and Bennett, 2008; Stewart and Lister, 2001), where species apparently persisted
412 at higher latitudes in sheltered locations with suitable microclimates (Stewart, Lister, Barnes, &
413 Dalen, 2010). However, most cryptic refugia described to date have been for relatively warm-adapted
414 species. Here, we present evidence for the existence of northern cryptic population(s) for cold-adapted
415 species during the LGM, based on high unique genetic diversity of the present-day *E. ephron*
416 populations in England, an area that was beneath an ice sheet at the LGM (Hughes et al., 2016). The
417 high genetic uniqueness of populations in England, together with a single shared haplotype with
418 Scotland/Vosges/Alps (haplotype 8; Figure 1b), is consistent with northern colonisations from the
419 Alps, but distinct separate colonisation of Britain via two routes, although there are alternative
420 explanations. For example, the 6 unique haplotypes in populations in England might occur elsewhere
421 but were not detected in this study. Alternatively, the six unique haplotypes identified in England
422 could have diverged from the shared haplotype in Scotland, Vosges and Alps populations (haplotype
423 8; Figure 1b) since the LGM, although this seems highly unlikely given the short time period for one
424 to three mutations to occur (Figure 1b). It is possible that these LGM populations were situated on
425 land that is currently below sea level, at an edge of the glacier, or in sheltered low elevation
426 microclimates on land. Multiple colonisation events have also been shown in other taxa in the UK
427 (Piertney et al., 2005), and the locations of cryptic refugia during the LGM are assumed to be ice free

428 areas in southern England (Bocherens et al., 1995, Lister, 1984), northern Scotland (Bennett, 1984)
429 and southern Ireland (Montgomery et al., 2014). Evidence for cryptic refugia for insects in Britain
430 also comes from cold-adapted beetles (see Appendix S6 in Supporting Information; (Buckland &
431 Buckland, 2006)), which currently have mountain or northern distributions in the UK, but were found
432 as sub-fossil remains in southern England 18,000-26,000 years BP, providing evidence of cold-
433 adapted insects surviving in ice-free locations in Britain in the LGM. It is, therefore, possible that the
434 current population of *E. epiphron* in England survived elsewhere in Britain during the LGM as
435 populations which no longer exists.

436 *4.4 Future loss of unique genetic diversity in cold-adapted species*

437 High levels of genetic diversity are important in relation to the capacity for populations and species to
438 adapt to changing environmental conditions, including climate change (Balint et al., 2011; Hoffmann
439 & Sgro, 2011). Cold-adapted species that have been shaped by diversification across mountain
440 systems during the Pleistocene contain high levels of genetic diversity and unique populations, and
441 are under threat from climate warming. Populations with unique genetic diversity may have evolved
442 independently to be adapted to their local environment (Weeks, Stoklosa, & Hoffmann, 2016) and
443 thus may be particularly vulnerable to future climatic changes. Our SDMs project loss of suitable
444 climate for *E. epiphron* in many locations in Europe, especially in regions with predominantly low
445 elevation populations and few opportunities to shift uphill to high elevation, which could result in loss
446 of genetic diversity. However, our projections of range retraction do not take into account any
447 potential of populations to adapt to warmer temperatures *in situ* (Franks & Hoffmann, 2012). Future
448 loss of genetic diversity has also been predicted in other species (Alsos et al., 2012; Beatty & Provan,
449 2011; Yannic et al., 2014), and rates of loss of genetic diversity in wild populations since the
450 industrial revolution (Leigh, Hendry, Vázquez-Domínguez, & Friesen, 2019) are consistent with our
451 projections.

452 *4.5 Conservation interventions to mitigate climate-driven genetic erosion*

453 Conservation management and adaptation could protect cold-adapted populations and safeguard
454 unique genetic diversity from climate change (Mawdsley, O'Malley, & Ojima, 2009). Options include
455 translocation or assisted colonisation to areas that have, or are predicted to have, suitable climate and
456 habitat in the future (Hoegh-Guldberg et al., 2008). Translocations are a controversial topic due to the
457 fear that translocated species may become 'invasive' in their new ranges, posing threats to ecosystems
458 including disturbance, disrupting ecological interactions, disease spread, competition and extinctions
459 (Ricciardi & Simberloff, 2009). However, others argue that the arrival of new species is typical of
460 ecosystem changes in the Anthropocene, and that translocations mirror colonisations occurring as a
461 consequence of current environmental change (Thomas, 2011). Translocations of *E. epiphron* and
462 other butterflies into unoccupied but climatically-suitable areas have been successful (Cizek,
463 Bakesova, Kuras, Benes, & Konvicka, 2003; Willis et al., 2009), and cold-adapted insects may
464 represent good targets for translocations given that the climate is rapidly deteriorating for them in
465 many parts of their range, and they may find it difficult to colonise new areas across inhospitable
466 landscapes (Thomas, 2011). For *E. epiphron*, our SDMs reveal areas in Scandinavia to be climatically
467 suitable, although the species does not occur there, and climate is predicted to increase in suitability in

468 future in Scandinavia for *E. epiphron* (Fig 3) and for other *Erebia* species (Settele et al., 2008).
469 However, although Scandinavia may have suitable climate, it may not have the required habitat for *E.*
470 *epiphron*. Local translocations within mountain systems that are currently occupied by *E. epiphron*
471 could also be implemented, for example moving individuals to areas of colder climate at higher
472 elevation, or neighbouring mountains which are too isolated for the species to colonise naturally.
473 However there may be very few areas of unoccupied but climatically-suitable habitats within some
474 mountain systems occupied by *E. epiphron*, particularly if the species already occurs at high
475 elevations in these regions. Future work could include finer scale country specific SDMs with
476 additional land use and genetic data on habitat availability could be used to locate areas for potential
477 translocations.

478 As well as translocating individuals to new sites, it might be possible to consider translocating genes
479 or ‘genetic rescue’ by moving individuals among existing populations. Not only might this conserve
480 unique genetic diversity at risk from local extinction of populations, but might increase the adaptive
481 capacity of populations by increasing their genetic diversity (Aitken & Whitlock, 2013). This could
482 involve moving warm-adapted individuals into cooler populations to increase their adaptive capacity
483 as the climate warms (Weeks et al., 2011). However, moving locally-adapted populations may result
484 in outbreeding depression and maladaptation, negatively impacting populations (Weeks et al., 2011),
485 although some genetic rescue interventions have resulted in increases in populations, and alleles
486 associated with local adaptation were not lost following gene flow (Fitzpatrick et al., 2020). Genetic
487 conservation interventions for insects, and specifically butterflies, has been rarely implemented,
488 although increasing habitat connectivity has led to genetic rescue of populations (Jangjoo, Matter,
489 Roland, & Keyghobadi, 2016) and genetic data have been used to inform on reintroductions (Dinca et
490 al., 2018). There is no evidence of attempted genetic rescue via translocations of butterflies, although
491 translocating individuals is a genetic conservation strategy which may be important in ensuring future
492 survival and adaptability of populations under climate change. As with translocations, these
493 conservation options may also be controversial, but could remove the need for on-going intervention
494 and management at sites with declining populations (Weeks et al., 2011). We recommend that before
495 the implementation of any climate adaptation strategy, populations are closely monitored to determine
496 if populations are retracting and likely to become extinct in areas that are becoming too warm for the
497 species. In addition, individual species’ assessments are required to assess the genetic diversity of
498 populations and any local adaptation, which would determine the most appropriate conservation
499 strategy.

500 **5. Conclusions**

501 The genetic diversification of cool-adapted mountain species, as demonstrated in our study species *E.*
502 *epiphron*, has been shaped by Pleistocene glaciations, the locations of long-term survival of
503 populations, and colonisation patterns after the LGM, resulting in unique genetic diversity in isolated
504 populations. Mountain and cold-adapted species are vulnerable to future climate warming, and we
505 predict *E. epiphron* will lose 30-55% of its range in the future, especially at low elevations. The
506 uniqueness of genetic diversity contained in these populations could be at risk depending on the
507 severity of future climate change. Conservation strategies such as translocation could ensure the

508 survival of these cold-adapted species, but more research is needed on the likely effectiveness of such
509 approaches.

510

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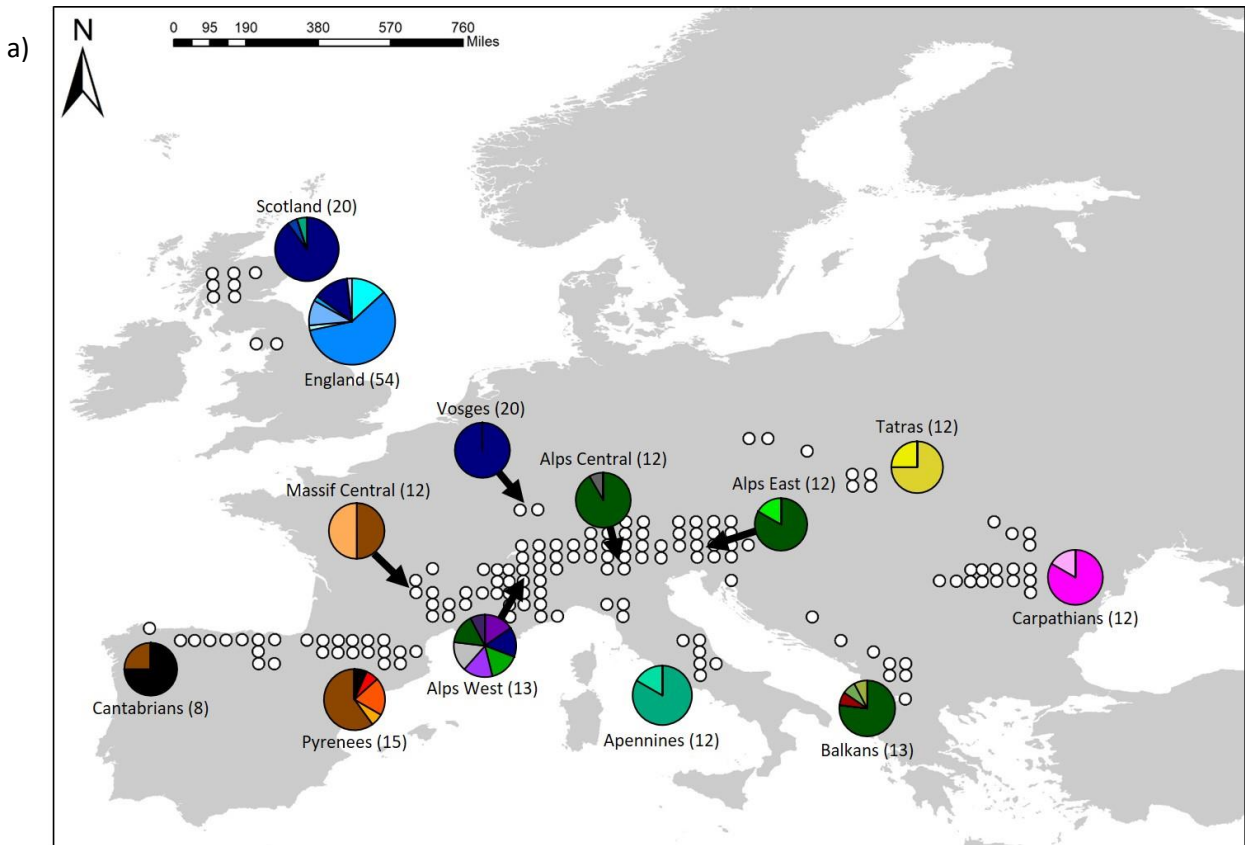
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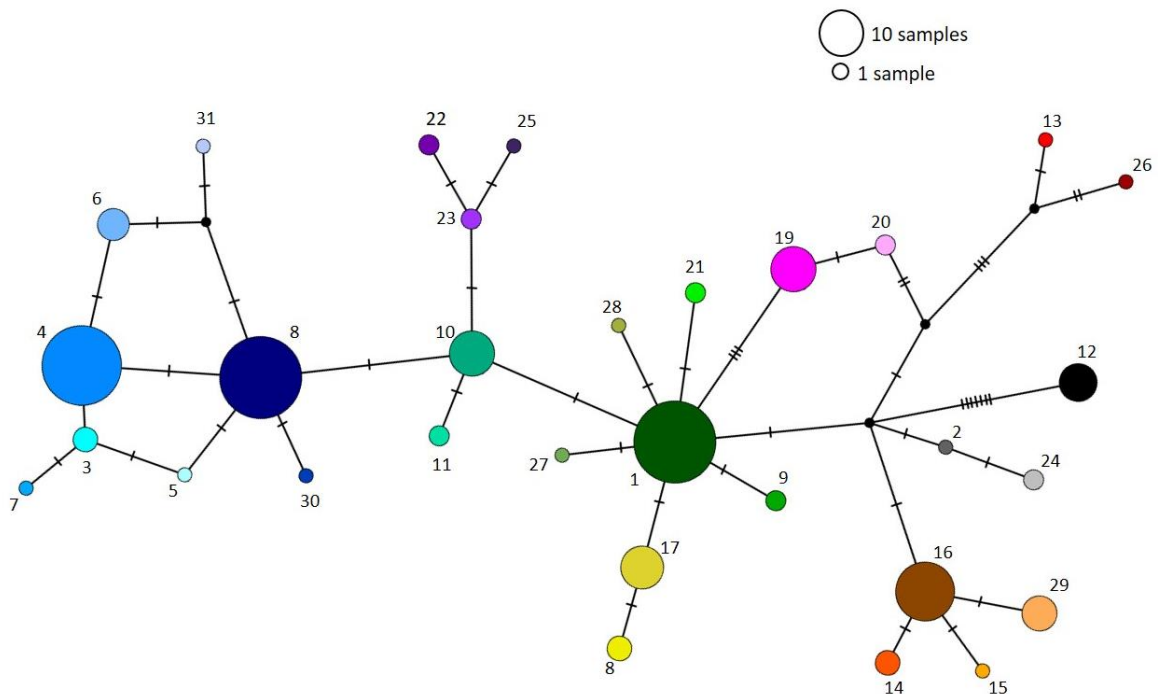
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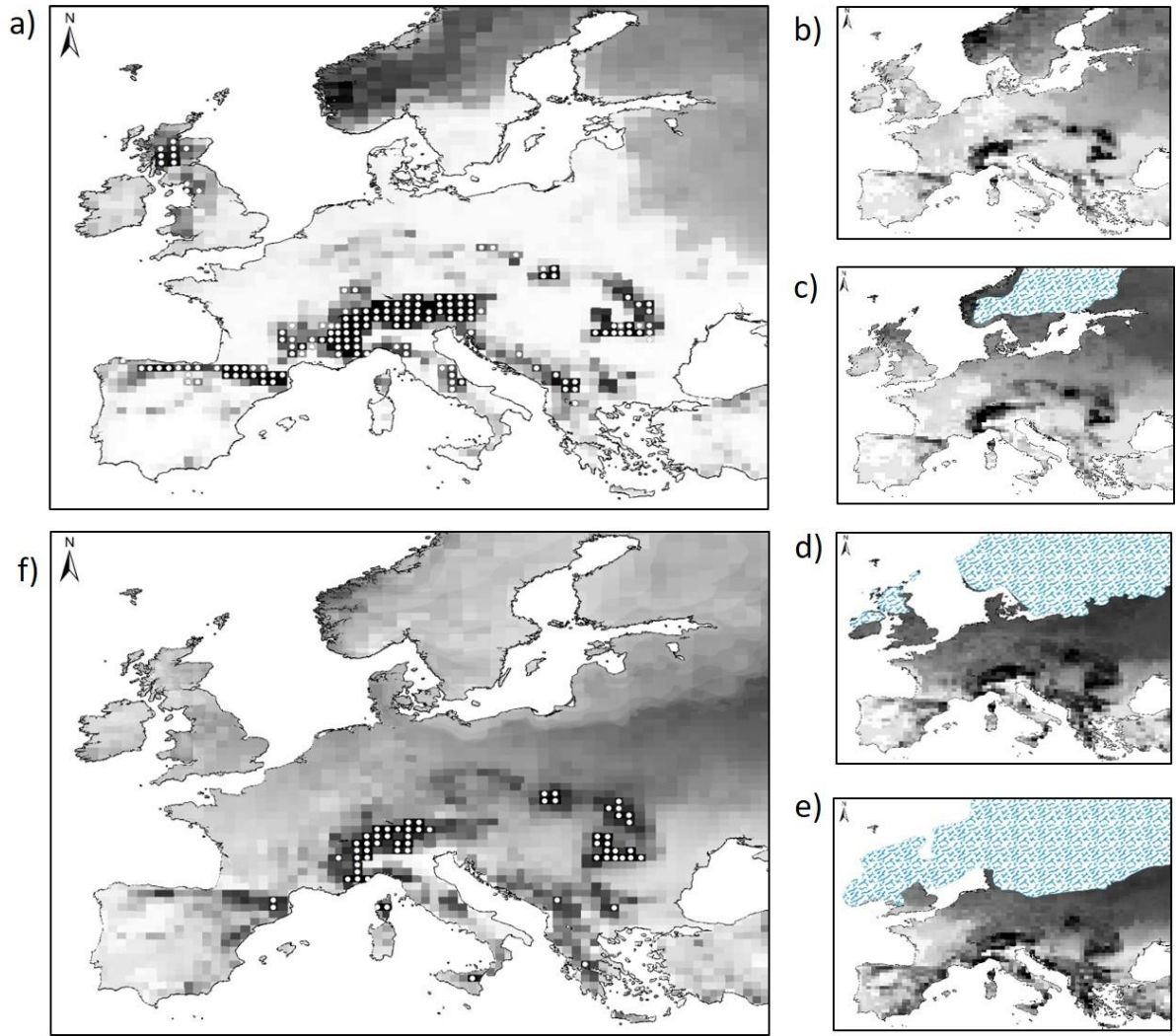
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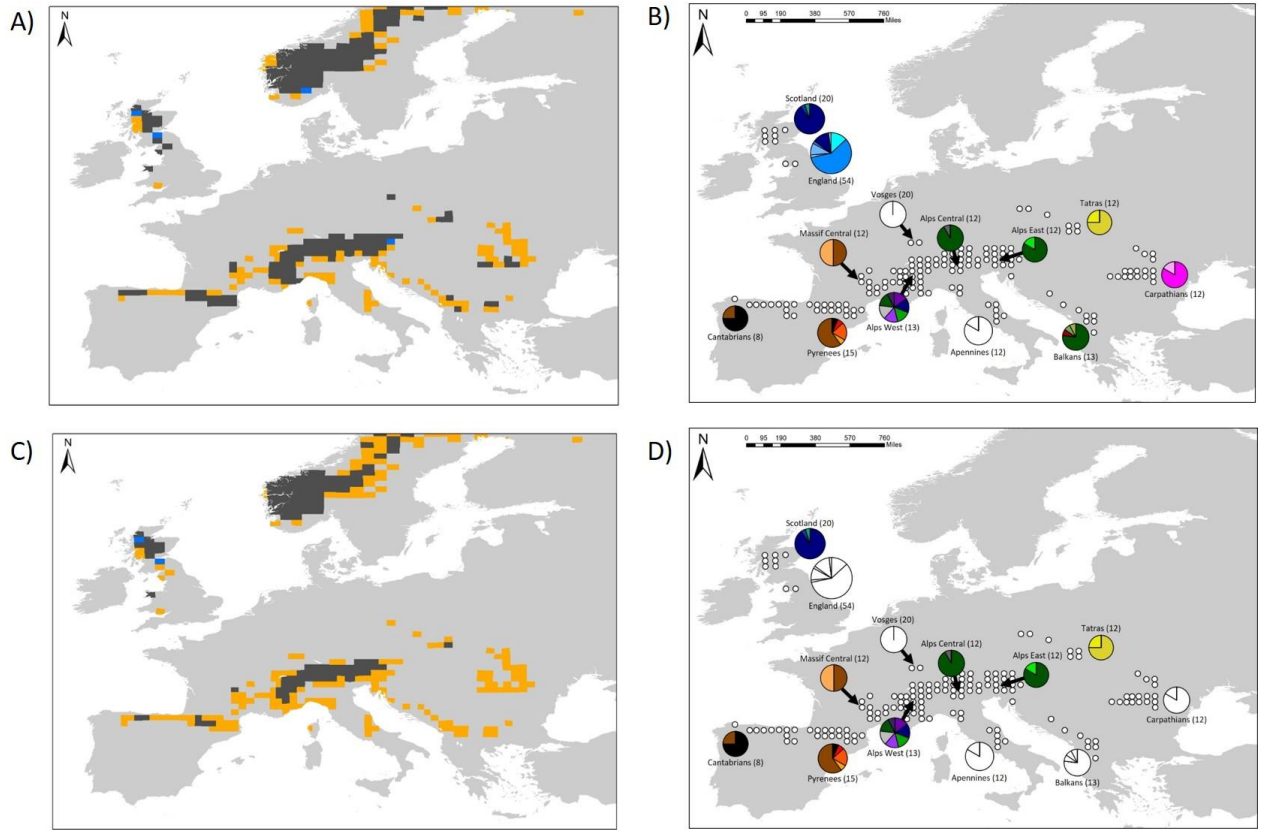
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772 **Figure 1**



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774 **Figure 2**



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776 **Figure 3**

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793 **Table 1**

Region	Current genetic diversity				% Range change (low)	% Range change (high)	<i>Haplotypes at risk</i>	
	H_n	H_u	H_d	π			H_r (low)	H_r (high)
All	31	27	0.89	0.0055	-38.6%	-64.3%	1	12
Vosges	1	0	0	0	-100%	-100%		
Scotland	3	1	0.194	0.0003	-37.5%	-25%		
Pyrenees	5	3	0.629	0.004	-20%	-73.3%		
Massif Central	2	1	0.545	0.0008	No change	-50%		
England	7	6	0.638	0.0015	-50%	-100%		6
Carpathians	2	2	0.303	0.0005	-70.6%	-100%		2
Tatras	2	2	0.409	0.0006	-25%	-75%		
Cantabrians	2	0	0.429	0.0059	-63.6%	-81.8%		
Balkans West	4	3	0.423	0.0024	-75%	-100%		3
Apennines	2	1	0.303	0.0005	-100%	-100%	1	1
Alps West	7	5	0.912	0.0043	-14.3% (all Alps)	-41.3% (all Alps)		
Alps East	2	1	0.303	0.0005				
Alps Central	2	1	0.182	0.0006				

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813 **Figure 1:** Current distribution of genetic diversity of *E. ephron* and historical divergence. a)
814 Frequency pie charts of haplotypes across the species' European range, including the current observed
815 distribution of *E. ephron* (white circles; 50 km resolution) in 13 mountain regions, with number of
816 samples (individuals) in brackets. b) TCS network of all 31 identified haplotypes. Size of circle
817 represents number of individuals containing that haplotype and tick marks represent a nucleotide
818 substitution.

819 **Figure 2:** Current and past projected distributions of *E. ephron*, a) current probability of climate
820 suitability and current distribution records (white circles). Past climate suitability b) 6,000 years ago,
821 c) 11,000 years ago, d) 16,000 years ago e) 21,000 years ago (i.e. LGM; blue shading shows the
822 extent of the ice sheet (from (Hughes et al., 2016)). Probability values of occurrence for b-e scaled
823 from 0 (unsuitable, white) to 1 (suitable, black). Panel f shows climate stability over time since the
824 LGM produced by summing 22 outputs from SDMs for the last 21,000 years, plus the output for the
825 present (summed probability values scaled from 0.73 (white) to 20 (black), with the top 30% of grids
826 shown as white circles. See Appendix S4 in Supporting Information for all output maps.

827 **Figure 3:** Projecting future climate suitability for *E. ephron* in 2070 under two RCP climate change
828 scenarios, and associated projected loss of genetic diversity. A) low RCP 2.6 climate scenario (~1°C
829 increase by 2070), and C) high RCP 8.5 scenario (~2-3°C increase by 2070) showing grids projected
830 to remain climatically suitable (black), become unsuitable (orange), and become suitable (blue). B)
831 low RCP 2.6 scenario haplotype map with predicted lost haplotypes coloured in white (2 regions lost,
832 1 unique haplotype lost), and D) high RCP 8.5 haplotype map with predicted lost haplotypes coloured
833 in white (5 regions lost, 12 unique haplotypes lost).

834 **Table 1:** Current genetic diversity, and projected loss of climate suitability and haplotype loss in the
835 future (2070). H_n = number of haplotypes; H_u = number of unique haplotypes; π = Nei nucleotide
836 diversity (P_i); % range change = % change in range size (number of occupied 50km grid squares) in
837 the future compared with current distribution, and H_r = number of unique haplotypes at risk in the
838 future, under RCP 2.6 (low) and 8.5 (high) climate scenarios.

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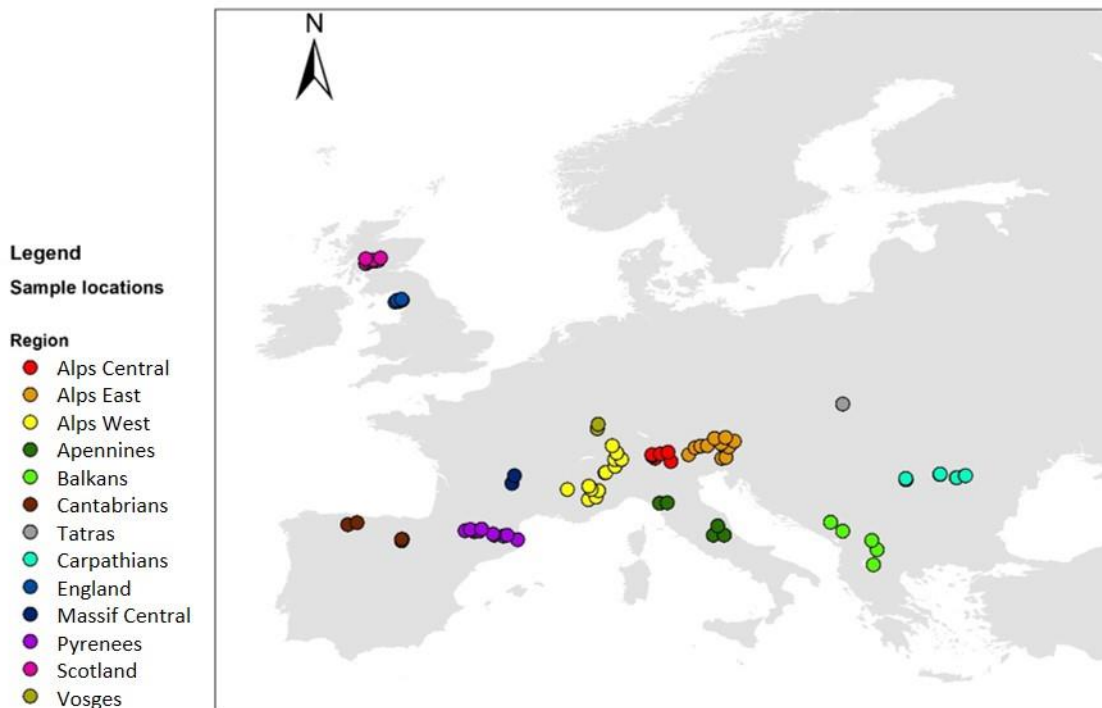
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849 **Appendix S2:** Population locations for all individuals used in mtDNA analysis from 13 mountain
 850 regions.

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853 **Appendix S3:** Bioclimatic variables used in SDMs to predict climate suitability for *E. epiphron*,
 854 extracted from mean temperature and precipitation data between 1970 and 2000
 855 (<http://www.worldclim.org/>). 'Cells' are 2.5 arc minute (~4.5 km) resolution data extracted from
 856 within a 50km grid at the same spatial extent as distribution data. 'Season' refers to mean data from
 857 summer (June, July, August) and winter (December, January, February).

Climate Variable	Location in 50km grid	Season
Mean Temperature	Coldest cell	Winter (Coldest quarter)
Mean Temperature	Coldest cell	Summer (Warmest quarter)
Mean Temperature	Warmest cell	Winter (Coldest quarter)
Mean Temperature	Warmest cell	Summer (Warmest quarter)
Mean Precipitation	Wettest cell	Winter (Coldest quarter)
Mean Precipitation	Wettest cell	Summer (Warmest quarter)
Mean Precipitation	Driest cell	Winter (Coldest quarter)
Mean Precipitation	Driest cell	Summer (Warmest quarter)

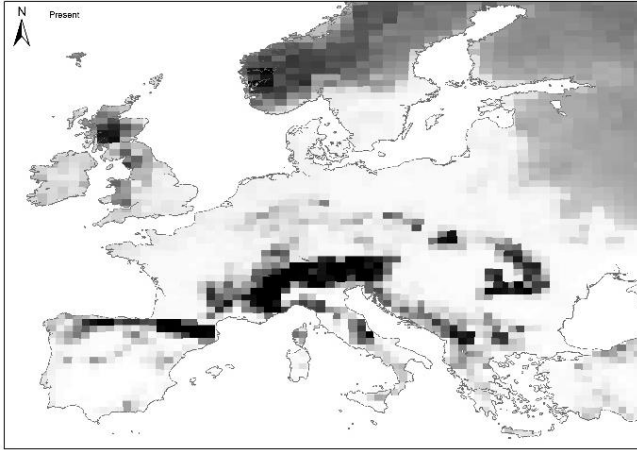
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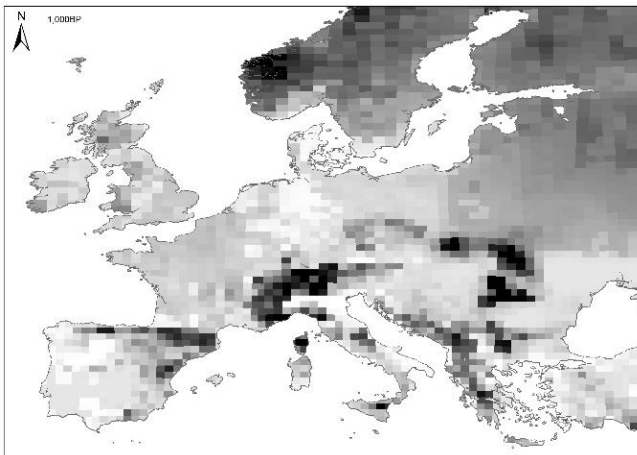
861 **Appendix S4:** All SDM outputs showing probability of climate suitability from present-day to 21,000
862 years ago (22 outputs in total). Probability values of occurrence for all panels are scaled from 0
863 (unsuitable, white) to 1 (suitable, black). Ice sheets (from (Hughes et al., 2016), blue shading) are
864 present from 21,000 years BP to 10,000 years BP.

865 Present



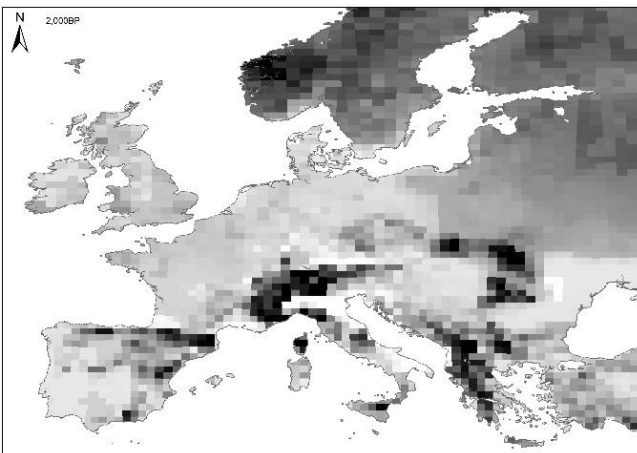
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867 1,000 years BP



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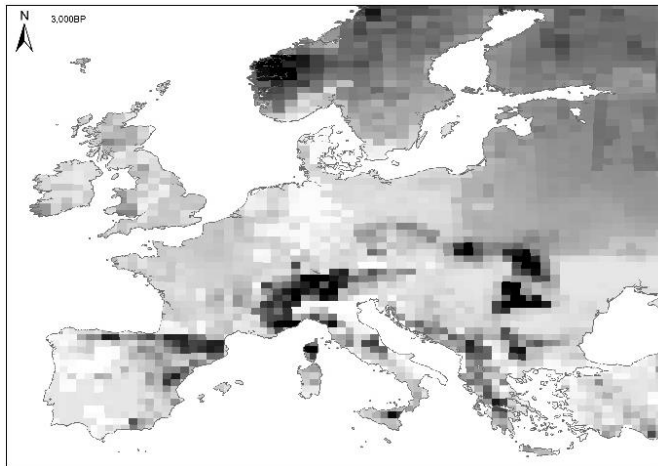
869 2,000 years BP



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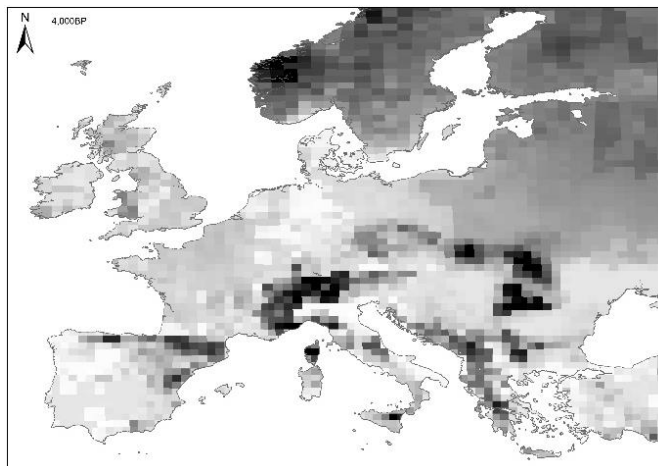
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872 3,000 years BP



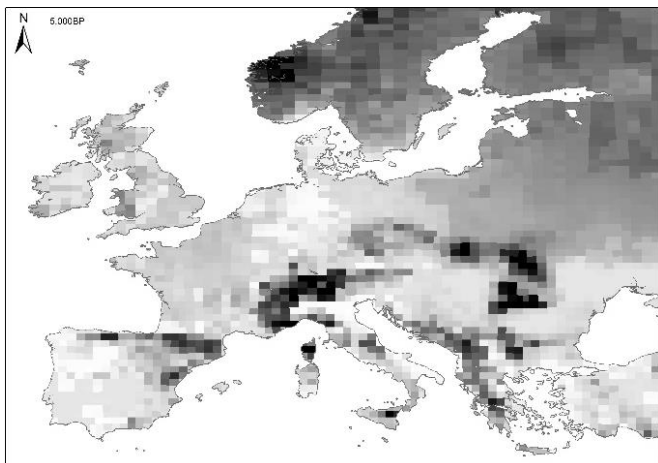
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874 4,000 years BP



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876 5,000 years BP



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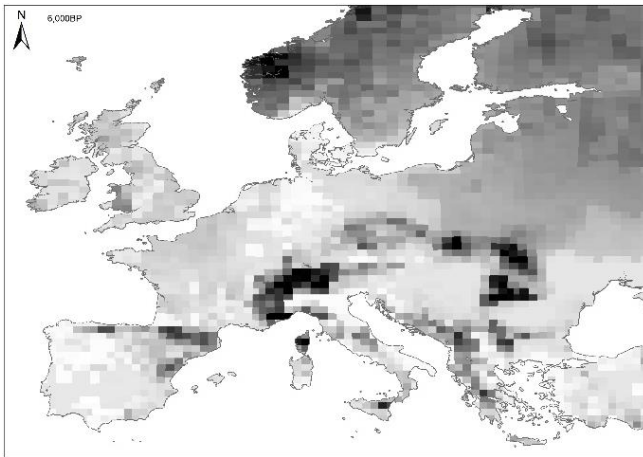
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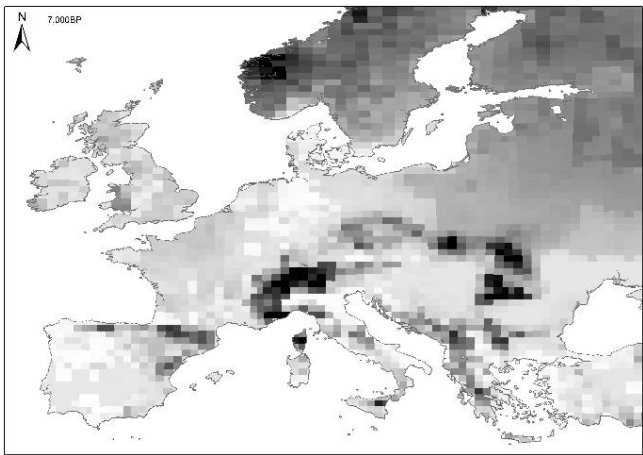
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882 6,000 years BP



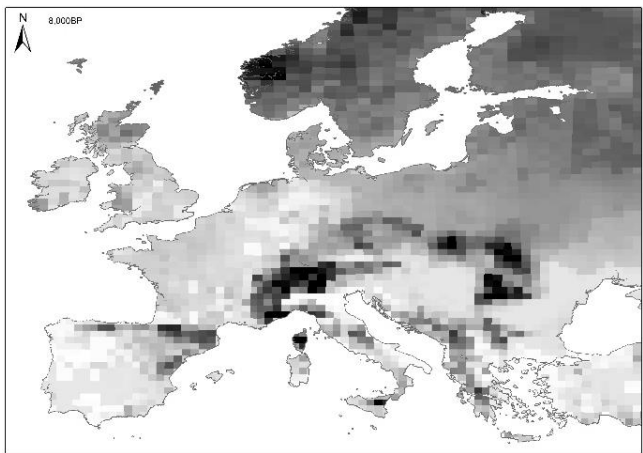
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884 7,000 years BP



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886 8,000 years BP



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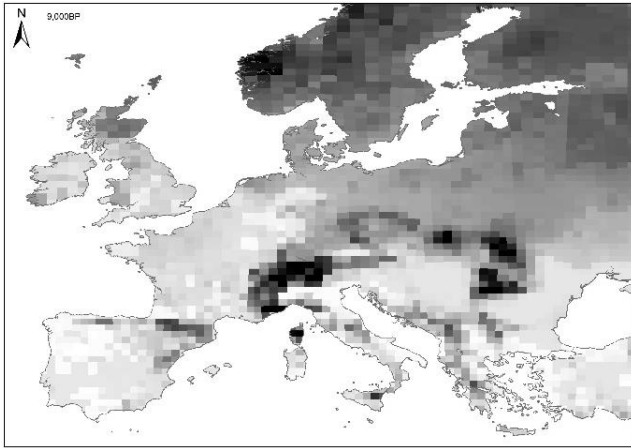
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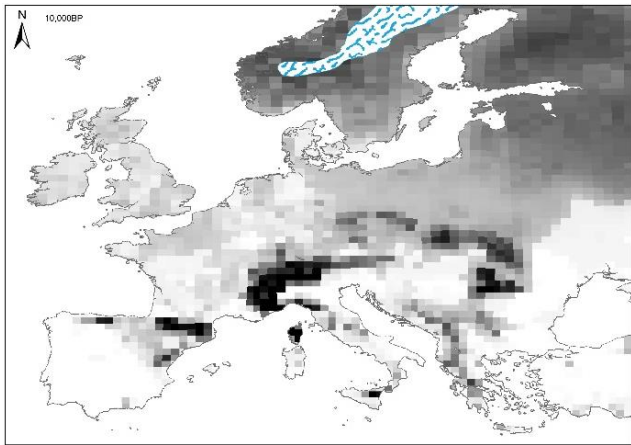
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892 9,000 years BP



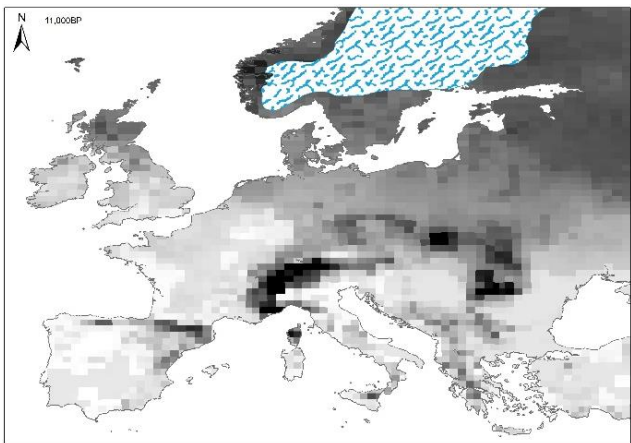
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896 11,000 years BP



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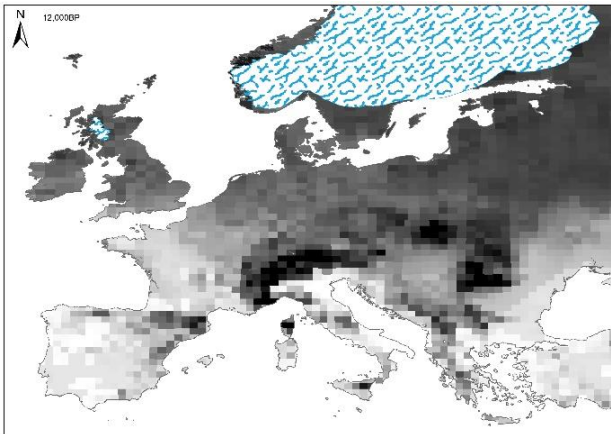
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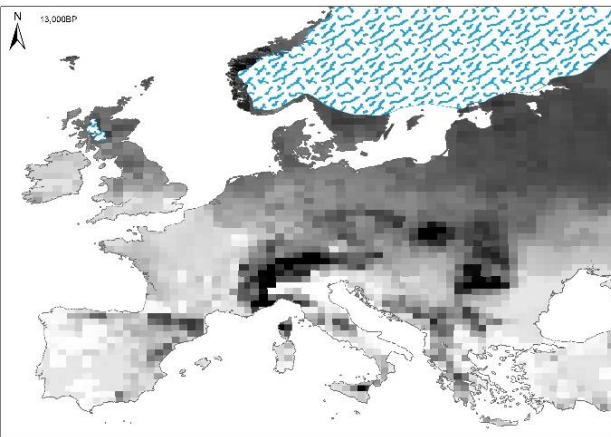
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903 12,000 years BP



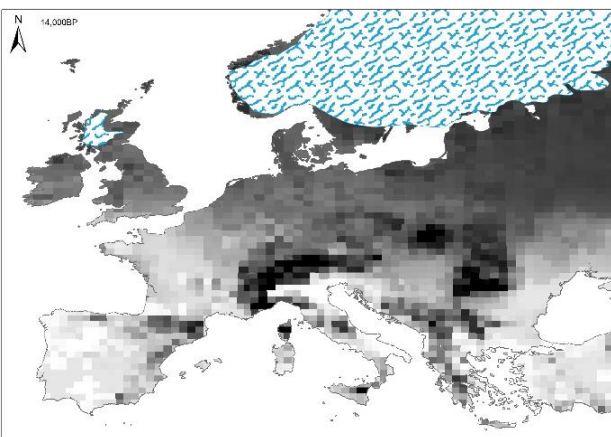
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905 13,000 years BP



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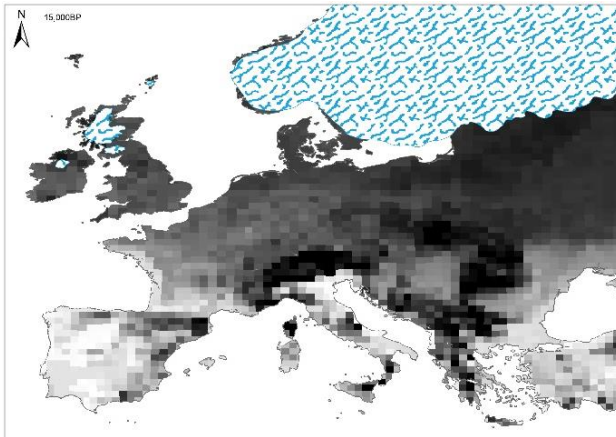
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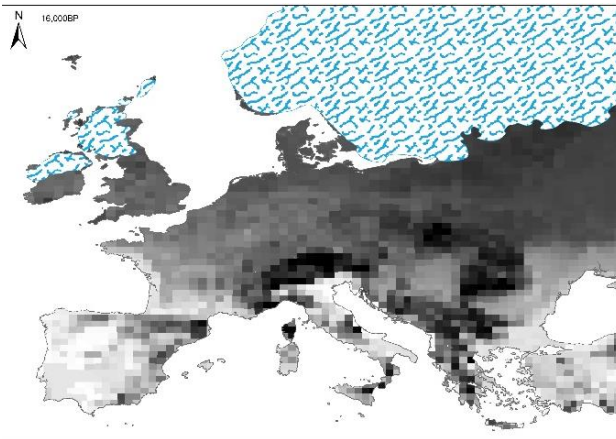
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914 15,000 years BP



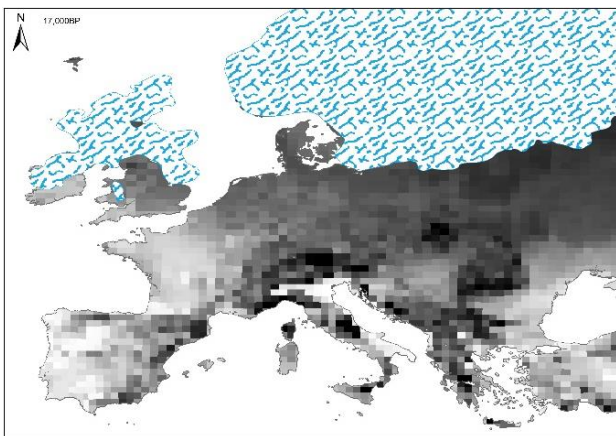
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916 16,000 years BP



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918 17,000 years BP



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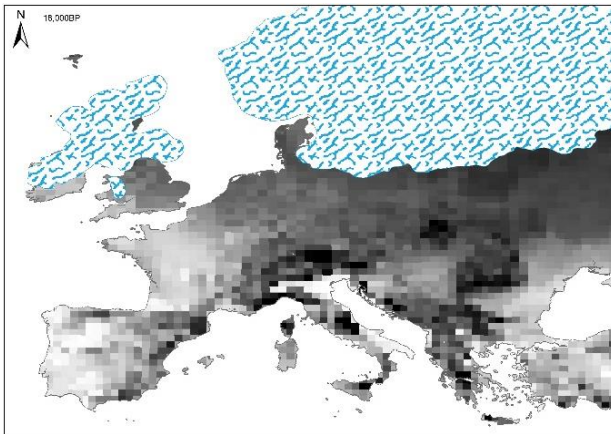
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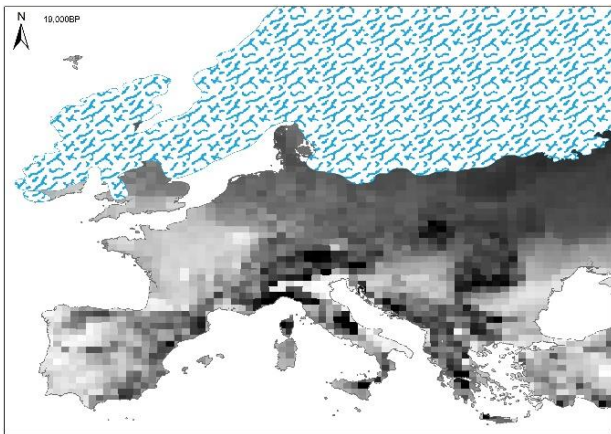
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925 18,000 years BP



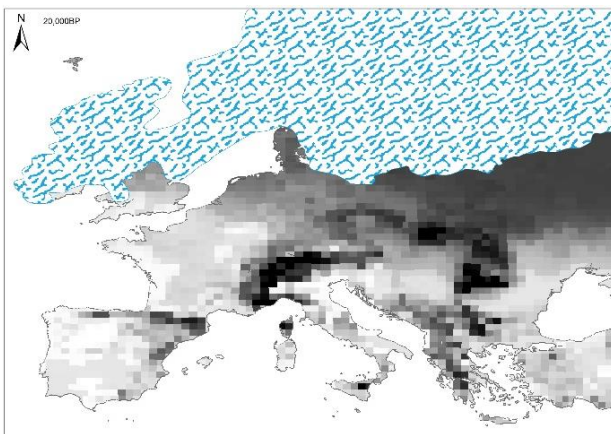
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927 19,000 years BP



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929 20,000 years BP



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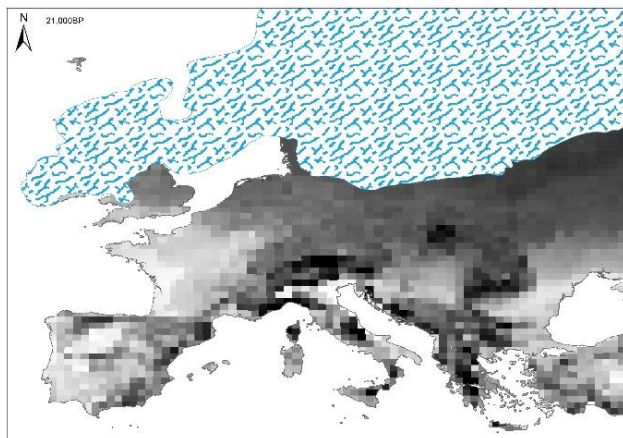
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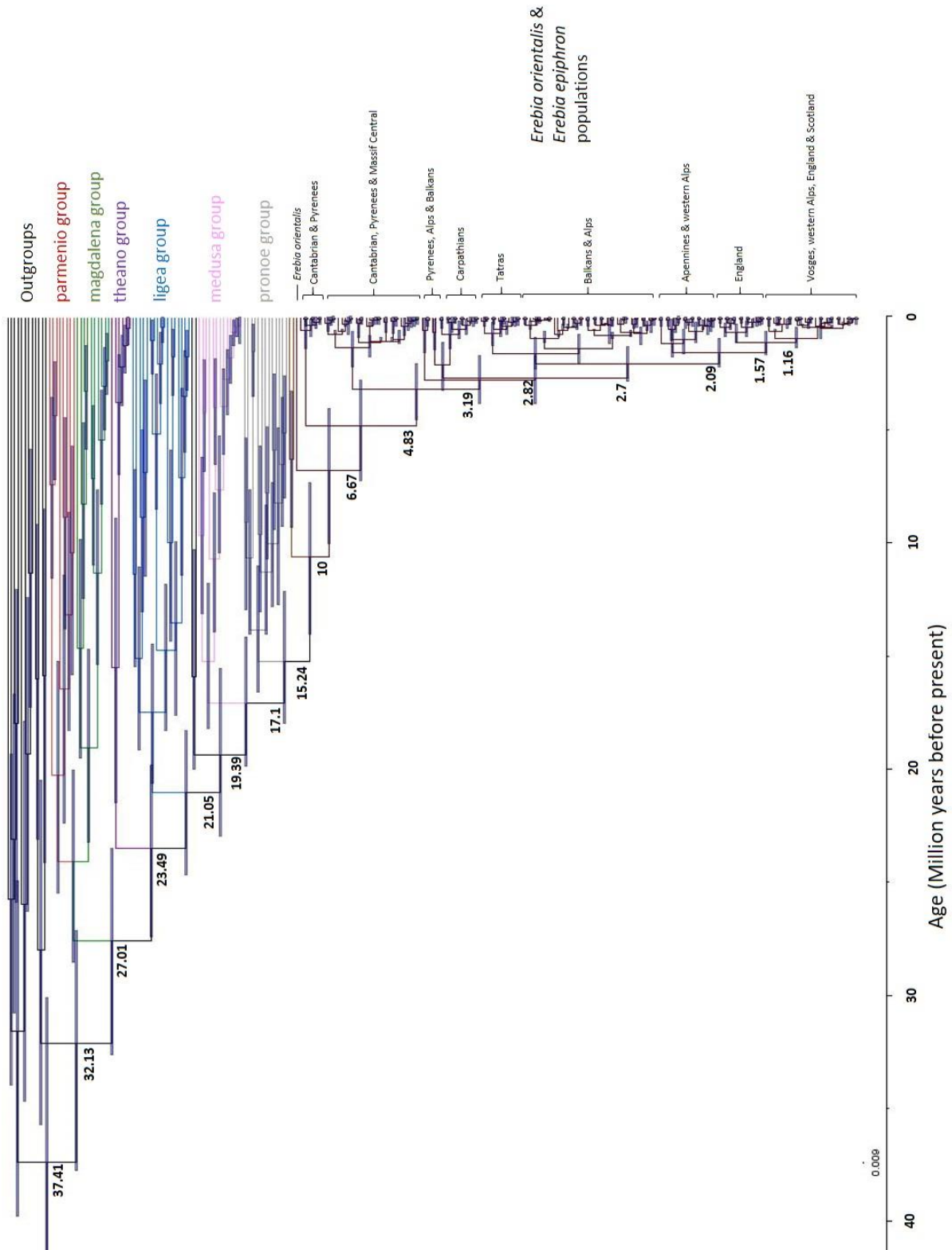
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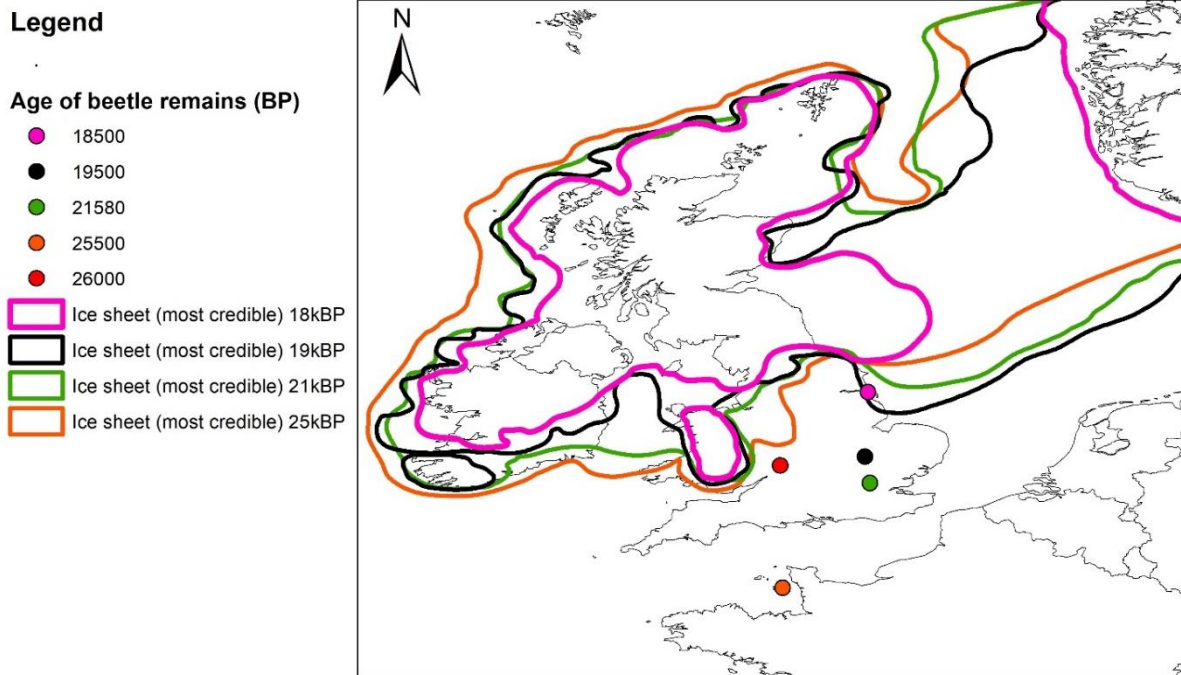
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960 **Appendix S5:** CO1 phylogenetic tree of the *Erebia* genus, outgroups and *E. epiphron* populations.
 961 Phylogenetic tree analyses were performed in Beast using methods described by Pena, Witthauer,
 962 Kleckova, Fric, & Wahlberg, (2015). Outgroup and *Erebia* genus data were accessed from Genbank
 963 using accession numbers in Pena et al., (2015). Age of split between *Erebia* and sister taxa of 37.41
 964 Myr (Pena et al., 2015) was used to calibrate the age split between *Erebia epiphron* and *E. orientalis*.
 965 Scale bar represents age of tree in million years before present. Node number represent estimated age
 966 of node with blue error bars.



968 **Appendix S6:** Locations and ages of remains in the UK of the cool-adapted beetle species *Partobus*
 969 *septentrionis*, *Amara alpina*, *Amara quenseli* and *Notaris aethiops*, with corresponding most credible
 970 ice sheet extent. Beetle fossil data acquired from BugsCEP (Buckland & Buckland, 2006), ice sheet
 971 data from (Hughes, Gyllencreutz, Lohne, Mangerud, & Svendsen, 2016).



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993 **Appendix S1:** Sample locations and accession numbers

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Area	Region	Code	Locality	LATITUDE	LONGITUDE	Haplotype	Genbank Accession No.	BOLD Process ID
Europe	Alps Central	10-1_AlpsC_4	Sellajoch, Italy	46.50	9.87	1		
UK	Scotland	10-1_Scot_6	Ben Lawers, Perth and Kinross, UK	56.53	-4.25	8		
Europe	Alps Central	10-2_AlpsC_5	Sellajoch, Italy	46.50	9.87	1		
Europe	Alps Central	10-3_AlpsC_8	Sellajoch, Italy	46.50	9.87	1		
UK	Lake District	1-1_Lakes_1	Irton fell, Cumbria, UK	54.41	-3.32	4		
UK	Scotland	11-1_Scot_7	Beinn Odhar, Tyndrum, UK	56.46	-4.69	8		
Europe	Alps East	11-4_AlpsE_1	Rein in Taufers, Italy	46.95	12.07	1		
UK	Lake District	1-2_Lakes_2	Irton fell, Cumbria, UK	54.41	-3.32	4		
Europe	Alps East	12-1_AlpsE_3	Hochköng, Austria	47.42	13.05	1		
UK	Scotland	12-1_Scot_8	Ben Lawers, Perth and Kinross, UK	56.56	-4.17	8		
Europe	Apennines	13-1_Apen_5	Prati di Tivo, Italy	42.47	13.55	10		
UK	Scotland	13-1_Scot_9	Schiehallion, Perth and Kinross, UK	56.67	-4.07	8		
Europe	Apennines	13-2_Apen_6	Prati di Tivo, Italy	42.47	13.55	10		
Europe	Apennines	13-3_Apen_7	Prati di Tivo, Italy	42.47	13.55	10		
Europe	Apennines	13-4_Apen_8	Prati di Tivo, Italy	42.47	13.55	10		
Europe	Apennines	14-1_Apen_1	Terminillo, Italy	42.47	13.00	10		
Europe	Apennines	14-2_Apen_2	Terminillo, Italy	42.47	13.00	10		
Europe	Apennines	14-3_Apen_3	Terminillo, Italy	42.47	13.00	10		
Europe	Apennines	14-4_Apen_4	Terminillo, Italy	42.47	13.00	10		
Europe	Pyrenees	15-1_Pyr_2	Candanchu, Spain	42.75	0.53	14		
Europe	Alps East	16-1_AlpsE_4	Sölkpass, Austria	47.27	14.07	1		
Europe	Alps Central	17-1_AlpsC_6	Thanai, Italy	46.72	10.67	1		
Europe	Alps Central	17-2_AlpsC_7	Thanai, Italy	46.72	10.67	1		
Europe	Alps East	18-1_AlpsE_5	Schönfeld, Austria	46.98	13.78	1		
Europe	Alps East	19-1_AlpsE_2	Sajatmähder, Ajustria	47.03	12.35	1		
Europe	Alps Central	2-1_AlpsC_1	Berninapass, Switzerland	46.40	10.02	1		
UK	Lake District	2-1_Lakes_3	Grisedale, Cumbria, UK	54.52	-2.95	3		
Europe	Carpathians	21-1_CarpS_2	Valea Caprei, Romania	45.58	24.62	19		
Europe	Alps Central	2-2_AlpsC_2	Berninapass, Switzerland	46.40	10.02	1		
UK	Lake District	2-2_Lakes_4	Grisedale, Cumbria, UK	54.52	-2.95	3		
Europe	Alps West	22-1_AlpsW_3	Passo del Monte Moro, Italy	45.98	7.97	1		
Europe	Alps Central	2-3_AlpsC_3	Berninapass, Switzerland	46.40	10.02	1		
Europe	Pyrenees	23-1_Pyr_1	Panticosa, Spain	42.68	0.27	13		
Europe	Pyrenees	24-1_Pyr_4	Canigou, France	42.47	2.42	16		
Europe	Vosges	26-1_Vosg_7	Markstein, France	47.92	7.04	8		

Europe	Vosges	26-2_Vosg_8	Markstein, France	47.92	7.04	8		
Europe	Vosges	26-3_Vosg_9	Markstein, France	47.92	7.04	8		
Europe	Vosges	26-4_Vosg_10	Markstein, France	47.92	7.04	8		
Europe	Vosges	26-5_Vosg_11	Markstein, France	47.92	7.04	8		
Europe	Vosges	26-6_Vosg_12	Markstein, France	47.92	7.04	8		
Europe	Vosges	27-1_Vosg_1	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	27-2_Vosg_2	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	27-3_Vosg_3	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	27-4_Vosg_4	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	27-5_Vosg_5	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	27-6_Vosg_6	Col du Calvaire, France	48.14	7.10	8		
Europe	Tatras	28-1_CarpN_1	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-10_CarpN_10	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-11_CarpN_11	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-12_CarpN_12	Babky, Tatra Mts, Slovakia	49.18	19.63	18		
Europe	Tatras	28-2_CarpN_2	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-3_CarpN_3	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-4_CarpN_4	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-5_CarpN_5	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-6_CarpN_6	Babky, Tatra Mts, Slovakia	49.18	19.63	18		
Europe	Tatras	28-7_CarpN_7	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Tatras	28-8_CarpN_8	Babky, Tatra Mts, Slovakia	49.18	19.63	18		
Europe	Tatras	28-9_CarpN_9	Babky, Tatra Mts, Slovakia	49.18	19.63	17		
Europe	Carpathians	29-4_CarpS_1	Retezat, Romania	45.37	22.87	19		
Europe	Balkans	30-1_Balk_1	Kom vasjeviak, Montenegro	42.68	19.63	1		
Europe	Balkans	30-2_Balk_2	Kom vasjeviak, Montenegro	42.68	19.63	1		
Europe	Balkans	30-3_Balk_3	Kom vasjeviak, Montenegro	42.68	19.63	1		
Europe	Balkans	30-4_Balk_4	Kom vasjeviak, Montenegro	42.68	19.63	1		
Europe	Alps West	3-1_AlpsW_2	Täschalp, Switzerland	47.05	7.82	1		
UK	Lake District	3-1_Lakes_5	Raise, Cumbria, UK	54.55	-3.00	7		
Europe	Balkans	31-1_Balk_5	Vjetrena brda, Durmitar, Montenegro	43.12	19.02	1		
Europe	Balkans	31-2_Balk_6	Vjetrena brda, Durmitar, Montenegro	43.12	19.02	1		
Europe	Balkans	31-3_Balk_7	Vjetrena brda, Durmitar, Montenegro	43.12	19.02	1		
Europe	Balkans	31-4_Balk_8	Vjetrena brda, Durmitar, Montenegro	43.12	19.02	1		
Europe	Balkans	31-5_Balk_9	Vjetrena brda, Durmitar, Montenegro	43.12	19.02	1		
UK	Lake District	3-2_Lakes_6	Raise, Cumbria, UK	54.55	-3.00	3		
UK	Lake District	4-1_Lakes_7	Langdale, Cumbria, UK	54.46	-3.10	4		
Europe	Pyrenees	4-1_Pyr_3	Étang d'Areau, France	42.77	1.12	15		
UK	Lake District	4-2_Lakes_8	Langdale, Cumbria, UK	54.46	-3.10	5		
Europe	Alps West	5-1_AlpsW_1	Grindelwald, Switzerland	46.67	8.03	8		

UK	Lake District	5-1_Lakes_9	Wynrose, Cumbria, UK	54.42	-3.13	6		
UK	Lake District	5-2_Lakes_10	Wynrose, Cumbria, UK	54.42	-3.13	6		
Europe	Massif Central	6-1_MasC_7	Puy Mary, France	45.52	2.80	16		
UK	Scotland	6-1_Scot_1	Glen Lyon, Perth and Kinross, UK	56.58	-4.44	8		
Europe	Massif Central	6-2_MasC_8	Puy Mary, France	45.52	2.80	16		
Europe	Massif Central	6-3_MasC_9	Puy Mary, France	45.52	2.80	16		
Europe	Massif Central	6-4_MasC_10	Puy Mary, France	45.52	2.80	16		
Europe	Massif Central	6-5_MasC_11	Puy Mary, France	45.52	2.80	16		
Europe	Massif Central	7-1_MasC_1	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
UK	Scotland	7-1_Scot_2	Ben Lui, Tyndrum, UK	56.39	-4.83	8		
Europe	Massif Central	7-2_MasC_2	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
Europe	Massif Central	7-3_MasC_3	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
Europe	Massif Central	7-4_MasC_4	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
Europe	Massif Central	7-5_MasC_5	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
Europe	Massif Central	7-6_MasC_6	Puy de Sancy/Chastraix-sancy, France	45.52	2.80	29		
UK	Scotland	8-1_Scot_3	Ben Lui, Tyndrum, UK	56.38	-4.81	8		
Europe	Alps East	9-1_AlpsE_6	Mangart, Slovenia	46.45	13.65	9		
UK	Scotland	9-1_Scot_4	Glencoe, Argyll, UK	56.63	-4.85	8		
Europe	Alps East	9-2_AlpsE_7	Mangart, Slovenia	46.45	13.65	9		
UK	Scotland	9-2_Scot_5	Glencoe, Argyll, UK	56.63	-4.85	8		
Europe	Carpathians	RVcoll06M974	Săcele, Braşov, Romania	45.52	25.92	19	HQ004371	EZROM149-08
Europe	Carpathians	RVcoll06M985	Măneciu, Prahova, Romania	45.52	25.93	19	HQ004369	EZROM672-08
Europe	Carpathians	RVcoll06M987	Măneciu, Prahova, Romania	45.52	25.93	19	HQ004373	EZROM914-08
Europe	Carpathians	RVcoll06V683	Râu de Mori, Hunedoara, Romania	45.30	22.87	19	HQ004372	EZROM150-08
Europe	Carpathians	RVcoll06V706	Uricani, Hunedoara, Romania	45.31	22.88	19	GU669667	EZROM1037-09
Europe	Carpathians	RVcoll07D631	Buşteni, Prahova, Romania	45.40	25.48	20	HQ004374	EZROM915-08
Europe	Carpathians	RVcoll07E456	Moroeni, Dâmboviţa, Romania	45.40	25.47	20	HQ004370	EZROM151-08
Europe	Carpathians	RVcoll07E495	Uricani, Hunedoara, Romania	45.30	22.88	19	HQ004375	EZROM916-08
Europe	Pyrenees	RVcoll07W121	Vielha e Mijaran, Lleida, Spain	42.66	0.75	14	GU669854	EZSPC381-09
Europe	Carpathians	RVcoll08M607	Arefu, Argeş, Romania	45.59	24.63	19	HQ004377	EZROM917-08
Europe	Carpathians	RVcoll08M614	Arefu, Argeş, Romania	45.59	24.63	19	HQ004376	EZROM918-08
Europe	Pyrenees	RVcoll08M994	El Pas de la Casa, Encamp, Andorra	42.54	1.70	16	HM901314	EZSPC1113-10
Europe	Pyrenees	RVcoll08M995	El Pas de la Casa, Encamp, Andorra	42.54	1.70	16	HM901315	EZSPC1114-10
Europe	Pyrenees	RVcoll08R174	Setcases, Girona, Spain	42.43	2.24	16	GU669853	EZSPC380-09
Europe	Pyrenees	RVcoll08R257	Alt Àneu, Lleida, Spain	42.67	0.99	16	HM901357	EZSPC1157-10
Europe	Pyrenees	RVcoll08R259	Alt Àneu, Lleida, Spain	42.67	0.99	16	GU669855	EZSPC382-09

Europe	Pyrenees	RVcoll08R260	Alt Àneu, Lleida, Spain	42.67	0.99	16	GU669848	EZSPC383-09
Europe	Pyrenees	RVcoll08R261	Alt Àneu, Lleida, Spain	42.67	0.99	16	GU669849	EZSPC384-09
Europe	Cantabrian	RVcoll08R410	Lena, Asturias, Spain	43.00	-5.76	16	GU675818	EZSPM221-09
Europe	Cantabrian	RVcoll08R413	Caso, Asturias, Spain	43.11	-5.27	16	GU675815	EZSPM223-09
Europe	Pyrenees	RVcoll09T080	Vielha e Mijaran, Lleida	42.67	0.73	14	JF847985	EZSPN036-09
Europe	Pyrenees	RVcoll09X029	Meranges, Girona	42.47	1.76	16	HM901499	EZSPC1365-10
Europe	Alps West	RVcoll10B939	Uvernet-Fours, Alpes-de-Haute-Provence	44.29	6.59	23	DQ338778	WMB2684-13
Europe	Alps West	RVcoll10C021	Arvioux, Hautes-Alpes, France	44.82	6.74	25	KR138782	WMB2693-13
Europe	Alps West	RVcoll11I916	Chichilianne, Isère, France	44.81	5.52	8	KP870625	EULEP170-14
Europe	Alps Central	RVcoll11J460	Bever, Grisons, Switzerland	46.55	9.85	1	KP870445	EULEP183-14
UK	Lake District	RVcoll12R462	Cockermouth, Cumbria, UK	54.50	-3.21	4	KP870916	EULEP261-14
UK	Lake District	RVcoll12R463	Cockermouth, Cumbria, UK	54.50	-3.21	4	KP870577	EULEP261-14
UK	Lake District	RVcoll12R464	Cockermouth, Cumbria, UK	54.50	-3.21	4	KP870587	EULEP262-14
UK	Lake District	RVcoll12R465	Cockermouth, Cumbria, UK	54.50	-3.21	4	KP870931	EULEP263-14
UK	Lake District	RVcoll12R466	Cockermouth, Cumbria, UK	54.50	-3.21	4	MK155216	EULEP264-14
UK	Scotland	RVcoll12R468	Killin, Stirling, UK	56.51	-4.50	8	KP870980	EULEP265-14
UK	Scotland	RVcoll12R469	Killin, Stirling, UK	56.51	-4.50	8	KP870580	EULEP266-14
UK	Scotland	RVcoll12R471	Killin, Stirling, UK	56.51	-4.50	8	KP870616	EULEP267-14
Europe	Apennines	RVcoll14A259	Ussita, Macerata, Italy	42.94	13.22	10	MK155192	EULEP1875-15
Europe	Apennines	RVcoll14A260	Ussita, Macerata, Italy	42.94	13.22	10	KR138751	WMB5256-14
Europe	Apennines	RVcoll14A446	Abetone, Pistoia, Italy	44.13	10.64	11	MK155190	EULEP1878-15
Europe	Apennines	RVcoll14A619	Massa, Lucca, Italy	44.10	10.23	11	KR138798	WMB5276-14
Europe	Alps West	RVcoll14D994	Villar Pellice, Turin, Italy	44.75	7.11	22	MK155199	BIBSA206-15
Europe	Alps West	RVcoll14E100	Acciglio, Cuneo, Italy	44.43	6.98	23	MK155180	BIBSA298-15
Europe	Alps West	RVcoll14I053	Saint-Marcel, Aosta, Italy	45.66	7.44	21	MK155204	BIBSA385-15
Europe	Alps West	RVcoll14I060	Saint-Marcel, Aosta, Italy	45.69	7.48	21	MK155214	BIBSA392-15
Europe	Massif Central	RVcoll14J771	Le Falgoux, Cantal, France	45.11	2.66	16	MK155198	EULEP2115-15
Europe	Alps West	RVcoll14N049	Parco Veglia Devero, Italy	46.34	8.28	24		
Europe	Cantabrian	RVcoll14N230	Villavelayo, La Rioja, Spain	42.18	-3.00	12	HE614683	WMB5455-14
Europe	Balkans	RVcoll14N877	Ljuboten	42.20	21.13	27	MK155194	EULEP2793-15
Europe	Alps Central	RVcoll14O005	Pradalago, Trentino, Italy	46.25	10.81	2		
Europe	Cantabrian	RVcoll15D777	Ezcaray, La Rioja, Spain	42.26	-2.98	12	MK155181	EULEP5633-17
Europe	Cantabrian	RVcoll15D778	Ezcaray, La Rioja, Spain	42.26	-2.98	12	MK155185	EULEP5634-17
Europe	Cantabrian	RVcoll15D779	Pazuengos, La Rioja, Spain	42.25	-2.95	12	MK155187	EULEP5635-17
Europe	Cantabrian	RVcoll15D780	San Millán de Cogolla, La Rioja, Spain	42.25	-2.94	12	MK155210	EULEP5636-17
Europe	Cantabrian	RVcoll15D781	Pazuengos, La Rioja, Spain	42.25	-2.95	12	MK155217	EULEP5637-17

Europe	Pyrenees	RVcoll15D782	Fresneda de la Sierra Tirón, Burgos, Spain	42.24	2.97	12	MK155191	EULEP5638-17
Europe	Alps West	RVcoll15H312	Mund, Valais, Switzerland	46.33	7.94	24	MK155202	EULEP5649-17
Europe	Alps East	RVcoll15I016	Chiusaforte, Udine, Italy	46.41	13.44	1	MK155179	EULEP5650-17
Europe	Alps East	RVcoll15I330	Kals am Großglockner, Tyrol, Austria	47.04	12.69	1	MK155215	EULEP5651-17
Europe	Alps East	RVcoll15I602	Ramsau am Dachstein, Styria, Austria	47.46	13.62	1	MK155211	EULEP5652-17
Europe	Alps East	RVcoll15I860	Muhr, Salzburg, Austria	47.15	13.38	1	MK155178	EULEP3794-16
Europe	Alps Central	RVcoll15I957	La Punt-Chamues-ch, Grisons, Switzerland	46.58	9.84	1	MK155212	EULEP3795-16
Europe	Alps Central	RVcoll15J040	Tschierv, Grisons, Switzerland	46.63	10.29	1	MK155213	EULEP3796-16
Europe	Alps West	RVcoll15J516	Val-des-Prés, Hautes- Alpes	44.97	6.61	22	MK155197	EULEP3797-16
Europe	Alps East	RVcoll15K528	Santa Cristina Gherdëina, Bolzano, Italy	46.60	11.74	1	MK155205	BIBSA1077-15
Europe	Balkans	RVcoll15P093	Studeničani, Skopje	41.73	21.40	28	MK155193	EULEP3798-16
Europe	Balkans	RVcoll15P094	Pelister Mt. (Gol.Ez.- Or.Bar.)	40.96	21.20	1		
Europe	Balkans	RVcoll15Q015	Shar Mts. (prema vrv Ljuboten)	42.20	21.13	26		
UK	Lake District	01_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	02_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	03_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	04_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	14_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	11_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	13_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	15_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	17_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	16_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	18_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	21_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	22_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	12_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	4		
UK	Lake District	24_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	26_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	25_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	29_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	28_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	27_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	23_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		

UK	Lake District	30_EE_snps	Irton fell, Cumbria, UK	54.41	-3.33	4		
UK	Lake District	38_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	4		
UK	Lake District	36_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	4		
UK	Lake District	39_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	4		
UK	Lake District	35_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	6		
UK	Lake District	40_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	6		
UK	Lake District	37_EE_snps	Fleetwith (Wasdale Screes), Cumbria, UK	54.51	-3.22	6		
UK	Lake District	05_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	06_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	08_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	09_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	20_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	8		
UK	Lake District	10_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	31		
UK	Lake District	19_EE_snps	Kidsty Pike/High Raise, Cumbria, UK	54.51	-2.87	31		
UK	Lake District	31_EE_snps	Kirkstone Pass (Troutbeck), Cumbria, UK	54.47	-2.91	3		
UK	Lake District	32_EE_snps	Kirkstone Pass (Troutbeck), Cumbria, UK	54.47	-2.91	3		
UK	Lake District	33_EE_snps	Kirkstone Pass (Troutbeck), Cumbria, UK	54.47	-2.91	3		
UK	Lake District	34_EE_snps	Kirkstone Pass (Troutbeck), Cumbria, UK	54.47	-2.91	3		
UK	Scotland	Scot_6-2	Glen Lyon, Perth and Kinross, UK	56.58	-4.44	10		
UK	Scotland	Scot_6-3	Glen Lyon, Perth and Kinross, UK	56.58	-4.44	30		
UK	Scotland	Scot_7-2	Ben Lui, Tyndrum, UK	56.39	-4.83	8		
UK	Scotland	Scot_7-3	Ben Lui, Tyndrum, UK	56.39	-4.83	8		
UK	Scotland	Scot_18-1	Beinn Chaorach, Stirling, UK	56.45	-4.68	8		
UK	Scotland	Scot_18-2	Beinn Chaorach, Stirling, UK	56.45	-4.68	8		
UK	Scotland	Scot_19-1	Stob Mhic Mhartuim, Kinlochleven, UK	56.67	-4.94	8		
UK	Scotland	Scot_19-2	Stob Mhic Mhartuim, Kinlochleven, UK	56.67	-4.94	8		
Europe	Vosges	Vosg_26-7	Markstein, France	47.92	7.04	8		
Europe	Vosges	Vosg_26-8	Markstein, France	47.92	7.04	8		
Europe	Vosges	Vosg_26-9	Markstein, France	47.92	7.04	8		
Europe	Vosges	Vosg_26-10	Markstein, France	47.92	7.04	8		
Europe	Vosges	Vosg_27-7	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	Vosg_27-8	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	Vosg_27-9	Col du Calvaire, France	48.14	7.10	8		
Europe	Vosges	Vosg_27-10	Col du Calvaire, France	48.14	7.10	8		

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