Contrasting population-level responses to Pleistocene climatic oscillations in an alpine bat revealed by complete mitochondrial genomes and evolutionary history inference

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ABSTRACT

Aim We used an integrative approach to reconstruct the evolutionary history of the alpine long-eared bat, Plecotus macrobullaris, to test whether the variable effects of Pleistocene climatic oscillations across geographical regions led to contrasting population-level demographic histories within a single species.

Location The Western Palaeartic.

Methods We sequenced the complete mitochondrial genomes of 57 individuals from across the distribution of the species. The analysis integrated ecological niche modelling (ENM), approximate Bayesian computation (ABC), measures of genetic diversity and Bayesian phylogenetic methods.

Results We identified two deep lineages: a western lineage, restricted to the Pyrenees and the Alps, and an eastern lineage, which expanded across the mountain ranges east of the Dinarides (Croatia). ENM projections of past conditions predicted that climatic suitability was reduced during cold stages in the areas inhabited by the western lineage, while the opposite trend was observed in the mountains inhabited by the eastern lineage. The palaeodemographic scenario that best fitted our data is consistent with the western lineage population size having shrunk repeatedly because of the extensive glaciation events that occurred in the Alps and Pyrenees during the Pleistocene. In contrast, the eastern lineage maintained a constant population size as is consistent with more limited glaciation in the mountains of south-eastern Europe and the Middle East.

Main conclusions This study shows that the demographic response of populations to Pleistocene climatic oscillations depended on their geographical location, offering an example of population-level variations in the effects and long-term consequences of climate change.

Keywords Alpine long-eared bat, approximate Bayesian computation, Bayesian phylogenetics, Chiroptera, ecological niche modelling, Europe, historical biogeography, phylogeography, Plecotus macrobullaris.
and multiple rearrangements of communities (Dynesius & Jansson, 2000; Williams & Jackson, 2007; Stewart, 2009). However, while species-level responses to Pleistocene climatic variations have been studied extensively (Schmitt, 2007; Stewart, 2008; Stewart et al., 2010), population-level variations within species have received less attention (Bennett & Provan, 2008).

The response of species to climate change has commonly been depicted as a homogeneous process, with all populations within a species following a similar pattern (Stewart et al., 2010). For example, populations of cold-adapted species take refuge in boreal or alpine areas during interglacial periods (Shapiro et al., 2004; Dalén et al., 2005), while populations of warm-adapted species retreat to southern refugia during glacial periods (Hewitt, 2000). However, the periodical cooling and warming during the Pleistocene was not the same across the Palaearctic, and its effect varied considerably across geographical regions (Hughes & Woodward, 2008). Thus populations within a species might have responded individualistically according to the particular conditions they experienced (Stewart, 2009; Stewart et al., 2010), especially in widely distributed species. Alpine species with large distributions could provide an illustrative example of such population-level responses to climatic variations during the Pleistocene because large parts of their distribution were covered by ice sheets for extended periods while other parts were not (Hughes & Woodward, 2008).

The recently discovered alpine long-eared bat, Plecotus macrobullaris (Kuzjakin, 1965), extends across multiple mountain ranges with diverse climatic conditions in southern Europe and the Middle East (Spitzenberger et al., 2003; Alberdi et al., 2013, 2014). Genetic studies have identified two main lineages with distinct genetic structures: a western lineage with low genetic diversity and a more diverse eastern lineage, separated in the Balkans (Spitzenberger et al., 2003, 2006). However, these studies focused on the genus-level and the resolution of the molecular markers was limited. As a result they were unable to draw robust conclusions about the species’ evolutionary history. The two potential P. macrobullaris lineages are separated geographically into two broad mountainous regions that experienced contrasting climatic conditions during the Pleistocene. While the Pyrenees and the Alps were largely covered by ice for extended and multiple periods (Hughes et al., 2006; Ivy-Ochs et al., 2008), glacial processes were more limited on eastern Mediterranean and Middle Eastern mountain ranges (Çiner, 2004; Kehl, 2009).

Its geographical, ecological and molecular characteristics make P. macrobullaris an appropriate model species for studying whether geographical differences in the effects of Pleistocene climatic oscillations led to contrasting responses at a population level. To test this hypothesis, we first generated ecological niche models (ENMs) for multiple past climatic conditions, to estimate how climatic oscillations could have affected the environmental suitability for this species in different epochs and geographical areas. Second, based on the modelling information and preliminary data on the genetic structure of P. macrobullaris, we generated one null and three alternative scenarios of demographic history that were compared using the approximate Bayesian computation (ABC) model-based inference approach. The genetic structure and timing of vicariance events were reconstructed using Bayesian phylogenetic and haplotype network analyses. Molecular analyses were based on complete mitochondrial genomes obtained using newly developed DNA handling methods and next-generation sequencing, which enabled a much finer phylogenetic resolution than possible in previous studies.

**MATERIALS AND METHODS**

**Field sampling and data gathering**

Genetic samples in the form of wing biopsies were obtained from 66 bats captured across the species’ range. Bats were trapped between 2008 and 2012 in alpine habitats in the Western and Central Palaearctic (Fig. 1) using the mist-netting technique described by Alberdi et al. (2013). Samples

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**Figure 1** The current distribution of Plecotus macrobullaris (white area) and the number of individuals sampled from each geographical region for molecular analyses.
from Turkey, Syria and Iran were provided by J. Juste, and P. Georgiakakis provided the samples from Crete. The ENM analysis was based on 74 precise and molecularly confirmed presence location records obtained from across the species’ distribution (see Appendix S1 in Supporting Information).

**Complete mitochondrial genomes**

We used two methods to prepare the DNA extracts for high-throughput sequencing: long-range polymerase chain reaction (PCR) coupled to the Illumina sequencing protocol modified from Morin et al. (2010), and target enrichment hybridization capture, following Maricic et al. (2010). The molecular methodologies are explained in detail in Appendix S1.

**Ecological niche modelling**

To determine how changes in environmental suitability during the Pleistocene could have affected the demographic history of *P. macrobullaris* populations, we used Maxent 3.3.3 (Phillips et al., 2004) to generate geographical suitability maps for the Western Palaearctic region under different climatic conditions. The reference model was selected from the 45 candidate models generated by Alberdi et al. (2014) with different combinations of climatic and topographic variables and regularization parameters. It included six climatic variables (BIO4, temperature seasonality; BIO8, mean temperature of the wettest quarter; BIO10, mean temperature of the warmest quarter; BIO12, annual precipitation; BIO15, precipitation seasonality; BIO17, precipitation of the warmest quarter; Hijmans et al., 2005) and a single topographic variable (ABR, abruptness of landscape, defined as the maximum elevational difference within a 5-km buffer around each cell; Alberdi et al., 2014). Models were also generated separately for each lineage. Detailed information about the modelling procedures and model selection can be found in Appendix S1.

The reference model was projected onto nine climatic scenarios: present climatic conditions; the Last Glacial Maximum (LGM; c. 21 ka); the Last Interglacial period (LIG; c. 130 ka); and six additional intermediate scenarios. Climatic maps for the present, LGM and LIG were downloaded from WorldClim (http://www.worldclim.org) and were used to generate the intermediate scenarios by averaging climatic layers from different time periods (present and LGM, or present and LIG; see Appendix S1 for detailed methods). We used this approach because present, LGM and LIG conditions represent static climatic situations that occurred at certain moments in the Pleistocene. The LGM and LIG reflect two climatic extremes, very cold and dry (LGM) and very hot and humid (LIG). However, the climate during most of the Pleistocene epoch was characterized by intermediate conditions (Kohler et al., 2010). Therefore, using this approach, we could estimate the effect of a wide range of climatic conditions on the suitability of different geographical areas for *P. macrobullaris* and assess the resilience of these areas to climatic variations.

The mean suitability value (the averaged suitability value of all the cells in the study area) and the fraction of suitable area (the percentage of suitable area relative to the study area) were measured for the whole of the Western Palaearctic and for each mountain range, under different climatic conditions. The response of these areas to temperature variations was calculated using linear regression. The values of the two most important climatic variables (BIO10 and BIO12) were plotted for each location record to observe climatic niche differences between the two lineages. Raster modifications, calculations and visualizations were performed using the R 3.0.2 packages raster and dismo, and ArcGIS 9.3 (ESRI, Redlands, CA, USA).

**Conflicting palaeodemographic scenarios**

Based on the ENMs generated for different palaeoclimatic conditions and the preliminary molecular information, we formed one null and three alternative palaeodemographic scenarios that could explain the current genetic structure of *P. macrobullaris* populations (Fig. 2).

- **S0**: Split from a large ancestral population: both populations split from a large ancestral population before the last glaciation (> 100 ka), and have sustained identical and stable population sizes since then. This is the null scenario, where the demographic history of both populations is identical and unchanged through time.
- **S1**: Split from a large ancestral population + small population size in the west with post-LGM population expansion.
- **S2**: Split from a large ancestral population + initial bottleneck in the western population.
- **S3**: Recent colonization of the west. LGM, Last Glacial Maximum; W, western population; E, eastern population.

![Figure 2](https://example.com/figure2.png)

**Figure 2** Graphical representations of the four demographic history scenarios of *Plecotus macrobullaris* in the Western Palaearctic, compared using approximate Bayesian computation (ABC) and posterior probabilities. (a) S0: split from a large ancestral population and even population size; (b) S1: split from a large ancestral population + small population size in the west with post-LGM population expansion; (c) S2: split from a large ancestral population + initial bottleneck in the western population; (d) S3: recent colonization of the west. LGM, Last Glacial Maximum; W, western population; E, eastern population.
S1: Split from a large ancestral population + small population size in the west with post-LGM population expansion: both populations split from a large ancestral population before the last glaciation (> 100 ka) and the size of the western population remained small until after the LGM.

S2: Split from a large ancestral population + initial bottleneck in the western population: both populations split from a large ancestral population before the last glaciation (> 100 ka) and the western population underwent an initial short bottleneck.

S3: Recent colonization of the west: the western population split from the eastern population after the last glaciation (< 20 ka).

The conflicting palaeodemographic scenarios were compared using the ABC approach implemented in DIYABC 2.0.4 (Cornuet et al., 2014); see Appendix S1 for details.

Phylogenetic relationships

Because of the absence of a reliable and informative fossil record for plecotine bats (Spitzenberger et al., 2006), we estimated when the ancestral split of the two main lineages occurred by building Bayesian phylogenetic trees. We used complete mitochondrial genomes of nine vespertilionid species and a representative mitogenome from each P. macrobullaris lineage, and tested different molecular clock frameworks and data partitioning. The posterior distributions for divergence times between the main P. macrobullaris lineages were used as prior tree-root calibration to generate the intraspecific phylogeny of P. macrobullaris (Appendix S1).

Genetic structure analysis

We performed an analysis of molecular variance (AMOVA) using Arlequin 3.5 (Excoffier et al., 2005). Several metrics of genetic diversity (haplotype diversity, nucleotide diversity, theta-W per sequence and average number of nucleotide differences) were computed within and between the two main lineages identified in this study using DnaSP 5.10 (Librado & Rozas, 2009). Further detailed information about the relationship between the P. macrobullaris haplotypes was obtained by constructing a median-joining network using the Greedy FHP distance calculation method implemented in NETWORK 4.610 (Fluxus Technology, Clare, UK).

RESULTS

Complete mitochondrial genomes

Of the 66 samples from across the species’ distribution (Fig. 1), nine (including the two specimens from Crete) were discarded because of either low read number or recombination issues. Consequently, we successfully sequenced and assembled the complete mitochondrial genome of 57 individuals. The mean coverage of sequences ranged from 85 to 186 reads, and 100% of sites were covered by > 5 reads in all cases. The length of the mitochondrial genome of P. macrobullaris was c. 16,830 bp (16,829–16,835 bp).

Ecological niche modelling

The selected model had a high predictive ability [area under the receiver operating characteristic curve for the tested model (AUCtest) = 0.911, Akaïke’s information criterion corrected for small sample size (AICc) = 2409.327, minimal predicted area (MPA) = 0.237, percentage of records within the suitable area (RWSA) = 89.4%] and the suitability threshold was set at 0.224. Habitat suitability values differed across the nine climatic ENMs. The most suitable conditions (highest mean suitability and largest suitable area) were predicted for current climatic conditions, and suitability values decreased under other climatic conditions (Fig. 3; see Table S1 in Appendix S2). The loss of suitable areas was more pronounced when global temperatures rose (–1.13% for 1 °C variation) than when temperatures dropped (–0.03%), as was the loss of mean suitability (–0.73 for a 1 °C increase; –0.23 for a 1 °C decrease). However, the specific patterns of suitability variation for each mountain system differed considerably (Fig. 4). Mountain ranges in western Europe (the Pyrenees and Alps) maintained suitability under warm conditions better than cold conditions, while the opposite trend was observed in eastern mountain ranges, such as the Taurus and Zagros mountains. The highest overall resilience was shown by the Caucasus, which retained suitable areas under the entire estimated range of temperature variations, while the Dinarides was the mountain range with the narrowest climatic resilience (Fig. 4). There was no clear niche separation between the two lineages (see Fig. S1 in Appendix S3) but the eastern lineage covered a wider range of environmental conditions than the western lineage, including warmer and drier conditions. The separated models for the two lineages showed results similar to the main models (see Fig. S2 in Appendix S3).

Demographic history

The ABC analysis identified scenario 1 as the demographic history scenario with the highest posterior probability (Fig. 2, and see Fig. S3 in Appendix S3). It was supported by a posterior probability of 0.992, while the other scenarios received around zero support (P10 = 0.005; P12 = 0.003; P13 = 0.000). Confidence in scenario choice was high [low type I (0.028) and type II (0.031) errors]. ABC model-based inference indicated that the two P. macrobullaris lineages split long before the LGM, and that the western population size remained small until it expanded substantially (about 700-fold expansion) after the LGM, while the eastern population size remained stable.
**Phylogenetic relationships**

The best-supported phylogenetic analysis of vespertilionids included five partitions ([ribosomal (r)RNA, codon position 1, codon position 2, codon position 3 and transfer (t)RNA] and a Yule process tree prior. The split of the two main P. macrobullaris lineages was estimated to have occurred 1.67 Ma [95% highest posterior density (HPD), 1.18–2.15 Ma] (see Fig. S4 in Appendix S3). This information was used to calibrate the intraspecific phylogenetic analysis. The model selected for the intraspecific analysis contained six partitions (rRNA, codon position 1, codon position 2, codon position 3, tRNA and the control region) and a constant population size coalescent tree prior. The results indicated that the diversification of the eastern lineage began around 899 ka (95% HPD, 727–1093 ka) with the split of the Iranian clade, and continued around 583 ka (95% HPD, 472–711 ka) with the divergence of the Caucasus and Anatolia–Balkan clades. Conversely, the diversification of extant lineages in the western lineage occurred around 68 ka (95% HPD, 45–95 ka) (Fig. 5a, and see Fig. S5 in Appendix S3). The haplotype-network analysis supported the more recent diversification of the western lineage, whereby haplotypes were separated by only a few mutational steps, as opposed to the much deeper divergence across the eastern lineage (Fig. 5c).

**Population structure analysis**

AMOVA depicted a genetic structure with a higher differentiation between populations from different mountain ranges than within population variance. The analysis showed that 79.5% of the genetic variation occurred between the two main clades, while inter- and intra-population variation explained 13.8% and 6.7% of the genetic variation, respectively (see Table S2 in Appendix S2). Genetic differentiation between the lineages was 3.4 ± 0.07%. The structure and diversity of the lineages differed considerably, and all diversity indices (Table 1) indicated a lower genetic diversity in the western lineage compared with the eastern lineage.

**DISCUSSION**

The use of complete mitochondrial genomes and the implementation of different molecular and ecological modelling approaches have allowed us to decode the genetic structure and evolutionary history of a widely distributed alpine
species. We show that geographical variation in Pleistocene climatic oscillations led to contrasting demographic histories of two geographically distinct *P. macrobullaris* lineages. These findings are in accordance with recent studies that have uncovered the complexity of species’ responses to Pleistocene climatic oscillations (Schmitt, 2007; Provan & Bennett, 2008; Schmitt et al., 2009).

The use of a single molecular marker has its limitations and associated biases (Ballard & Whitlock, 2004). However, the validity of molecular markers depends largely on the structure of populations, and the high philopatry and limited long-distance dispersal ability of long-eared bats means the analysis of maternally inherited markers is a suitable approach for studying the evolutionary history of *P. macrobullaris*. The breeding unit of long-eared bats is the maternity colony, where female descendants remain linked to their female ancestors (Burland et al., 1999). Therefore, the demography and genetic diversity of females is what determines the long-term population reproductive success and survival. The analysis of complete mitochondrial genomes does not provide the entire detailed demographic and evolutionary history of a species, but it has proven to be a very useful tool for shedding light on closely related populations (Gilbert et al., 2008; Morin et al., 2010; Keis et al., 2012; Hirata et al., 2013).

**Demographic and evolutionary history**

Our phylogenetic analysis identified two main groups across the range of *P. macrobullaris*, in line with previous studies. Inconsistencies between preliminary molecular information and morphometric traits have provoked discussions regarding the geographical boundary separating the groups (Spitzenberger et al., 2003, 2006; Benda et al., 2004). This uncertainty has occurred because the partial mitochondrial sequences employed in previous studies were unable to provide sufficient phylogenetic inference resolution to resolve the evolutionary history of *P. macrobullaris* (Juste et al., 2004; Spitzenberger et al., 2006). In contrast, the complete mitochondrial genomes provided results robust enough to identify a complex sublineage structure, as well as the dating of lineage split and diversification events. There is discordance, however, between our molecular data and the previously published morphological information. Craniodontic analysis linked the bats from the Caucasus with the specimens in the Alps (Spitzenberger et al., 2006), while our results depict a deep split between a highly homogeneous western lineage restricted to the Pyrenees and the Alps, and a diverse eastern lineage that extended across the mountain ranges east of the Dinarides. The northern Dinarides were identified as the friction zone between the lineages.

The best supported demographic history scenario for *P. macrobullaris* based on the ABC analysis of the complete mitochondrial genomes indicates that the two main lineages split long before the LGM, and the current lower diversity of the western lineage is the result of a small population size until a recent population expansion after the LGM. The demographic scenario of recent post-glacial colonization of the western range, suggested by Juste et al. (2004) based on partial mitochondrial sequences, was strongly rejected in our analysis, and should therefore be considered as improbable.

The interspecific Bayesian phylogenetic analysis supported split times estimated with the ABC approach, dating the split between the main *P. macrobullaris* lineages at around 1.6 Ma. Such a deep divergence between lineages could be considered to indicate an incipient allopatric speciation process, with two speciation centres, one located in the western European mountains and the other in the Middle Eastern or Central Asian mountains. The mitochondrial dissimilarity between specimens of the two lineages is 3.4%, which is identical to the dissimilarity level between the Burchell’s zebra and Grevy’s zebra (Vilstrup et al., 2013), and larger than the 3.1% dissimilarity between the Siberian weasel and the European polecat (Yu et al., 2011). However, further molecular analyses including nuclear markers would be needed to confirm this plausible speciation process and evaluate the taxonomic status of the two *P. macrobullaris* lineages.

**Figure 4** Changes in the suitability of mountain massifs in the Western Palaearctic for *Plecotus macrobullaris* under different climatic conditions. The dark colour indicates the absolute percentage of suitable area. The light colour indicates the percentage of suitable area relative to the maximum suitable area estimated for the mountain massif. The Pyrenees and the Central Alp host populations belong to the western lineage, while the Taurus, Caucasus and Zagros mountains hold eastern lineage populations. The Dinarides provide a bridge between the two lineages. LGM, Last Glacial Maximum; LIG, Last Interglacial.
Figure 5 The phylogeny and haplotypes of *Plecotus macrobullaris* in the Western Palaearctic, and temporal temperature variation. (a) Time-calibrated phylogenetic tree of *P. macrobullaris*. Node bars show the 95% bound of the highest posterior density (HPD) and the colours indicate the posterior probability: green, = 1; yellow, > 0.9; red < 0.9 (exact posterior probabilities are provided in Fig. S5 in Appendix S3). (b) Approximate global temperature variations relative to current conditions (ΔT), modified from Lisiecki & Raymo (2005) and Huybers (2009). (c) Haplotype network of the sampled individuals. The haplotypes are shown as circles, with the size indicating the frequency in the sampled populations and the colour showing the geographical location. Black points indicate missing intermediate haplotypes and grey numbers indicate the number of mutations between different haplotypes.
The split between the two lineages was estimated to be before the mid-Pleistocene transition (MPT) (Clark et al., 2006), when the climate was more stable and gliation events were less intense. At that period it is likely that the species was able to expand through mountain environments in southern Europe. However, the apparent genetic isolation between the populations suggests that at least female-mediated gene flow was suddenly stopped between the western and eastern ranges. The climate following the MPT was characterized by high-amplitude oscillations that were the origin of the glacial cycles that have spanned the last million years (Williams, 1998). These drastic climatic oscillations probably played a key role in the isolation and subsequent population differentiation in P. macrobullaris. Today both lineages are linked by the ecologically suitable Dinarides bridge between the Alps and the mountain ranges in the Balkan Peninsula. Individuals from both lineages are present in that region (Spitzenberger et al., 2003), suggesting that the populations are connected under current conditions. However, niche models show that the Dinarides is the mountain range with the lowest resilience to climatic variations. Subtle temperature differences in both directions produce drastic drops in suitability, suggesting that the current situation may be an exception. We did not analyse nuclear markers, and therefore we cannot discount the possibility of rare admixture events between the lineages. We expect the lineages may have been in contact during short periods when environmental conditions in the Dinarides became suitable. However, the incidence of contact events was probably too low to result in the exchange of mitochondrial haplotypes between lineages because the bridge connecting their respective geographical ranges remained ecologically unsuitable for most of the time.

The intraspecific phylogenetic tree revealed patterns of genetic diversity and diversification in the two lineages. The eastern lineage began to diversify around 900 ka, continuing throughout the whole middle and late Pleistocene. Strongly structured clades, previously undetected using partial mitochondrial sequences (Juste et al., 2004; Spitzenberger et al., 2006), were identified in the eastern Mediterranean (Montenegro, Greece, Turkey and Syria), the Caucasus and Iran. In contrast, in the western lineage, the time to the most recent common female ancestor was estimated to be 68 ka, and no clear separation between the Alps and the Pyrenees was identified. Bats from the Pyrenees appear to be nested within the P. macrobullaris population in the Alps. This pattern has been observed in other alpine organisms (Pușçaș et al., 2008), supporting the strong biogeographical link between these mountain massifs (Schmitt, 2009). However, we were unable to resolve the post-glacial demographic dynamics of the western lineage because of the low support (posterior probability, PP < 0.90) for recent nodes, which suggests that faster evolving markers (e.g. microsatellites) are more suitable for resolving recent events.

**Contrasting effect of Pleistocene climatic oscillations**

Ecological needs and the importance of abrupt landscapes for roosting and foraging (Alberdi et al., 2014) may be the main driver behind the current geographical restriction of P. macrobullaris to mountain areas, rather than physiological thermal tolerance. Hence, assuming an invariable environmental niche, P. macrobullaris would have remained associated with mountain areas even during the coldest stages. This pattern is shown by the distribution model extrapolations to ancient climatic situations, and is supported by the high molecular differentiation between mountain massifs depicted by the AMOVA. This contradicts the traditional view of cold-adapted alpine species expanding across the lowlands during cold stages while retreating to high mountain areas during warmer stages, like present conditions (de Lattin, 1967). In fact, recent studies have demonstrated that these interpretations are oversimplified, and several different responses to climatic variations have been reported (Schönswetter et al., 2002; Stehlik, 2002; Schmitt & Hewitt, 2004).

Both the complete dataset and lineage-specific models show that the influence of Pleistocene climatic oscillations on the suitability of mountains across the Palaearctic was inconsistent, affecting differently, sometimes in opposing ways, the ranges of the two main lineages of P. macrobullaris.

The extent of environmentally suitable areas within the region inhabited by the eastern lineage contracted when temperatures became warmer, while suitable areas remained stable or expanded during cold stages. Variability in the environmental suitability in regions inhabited by the western lineage followed an opposite pattern, whereby suitability dropped during cold stages but warm conditions did not result in substantial suitability losses. This phenomenon probably occurred in part because the climatic conditions in the western massifs (the Pyrenees and Alps) are closer to the coldest limits of the wide climatic niche of P. macrobullaris (Alberdi et al., 2014), and therefore slight cooling substantially reduces their suitability. In contrast, the massifs in south-eastern Europe and the Middle East mainly represent the warmest limits of the species’ climatic niche, and as such were less suitable when conditions became warmer. But above all, the intensity and incidence of Pleistocene glaciation events varied among mountain ranges. The extent of glaciation was substantially lower in mountain massifs in

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**Table 1** Summary statistics for the genetic diversity of Plecotus macrobullaris lineages from the Western Palaearctic based on complete mitochondrial genomes excluding the control region.

<table>
<thead>
<tr>
<th></th>
<th>Western lineage</th>
<th>Eastern lineage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>31</td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td>Haplotype diversity</td>
<td>0.940</td>
<td>0.969</td>
<td>0.976</td>
</tr>
<tr>
<td>Nucleotide diversity</td>
<td>$6.5 \times 10^{-3}$</td>
<td>$9.63 \times 10^{-3}$</td>
<td>$17.84 \times 10^{-3}$</td>
</tr>
<tr>
<td>Theta-W (per sequence)</td>
<td>20.0</td>
<td>191.8</td>
<td>232.4</td>
</tr>
<tr>
<td>Average number of nucleotide differences</td>
<td>10.0</td>
<td>148.3</td>
<td>274.6</td>
</tr>
</tbody>
</table>
south-eastern Europe and the Middle East than in the Alps and the Pyrenees (Hughes et al., 2006; Hughes & Woodward, 2008), producing considerable differences in the Quaternary histories of different *P. macrobullaris* lineages.

ENMs suggest that the splits between the different clades of the eastern lineage probably occurred during the short, warm interglacial periods. Reduced suitability of the eastern mountain regions could have led to a more disjunct distribution and population contractions and vicariance events. However, temperatures during most interglacial periods were lower than during the LIG (LIG or marine isotope stage 5 was used as the warmest reference for our ENMs) (Henderson-Sellers & McGuffie, 2012), when an almost complete loss of suitability occurred according to our palaeo-ENMs. Moreover, long interglacial periods were uncommon, and the generally short duration of these periods may have allowed populations to survive in separate high mountain refugia, conserving their genetic diversity and sustaining relatively stable effective population sizes, as indicated by the ABC demographic history analysis.

The western lineage experienced an opposite fate, as climatic conditions colder than current conditions substantially reduced suitable areas. Overall the temperature during the last million years has been colder than current conditions (Fig. 5b), which would explain the lower diversity of the western lineage compared with the eastern lineage. ENMs suggest that populations from the Alps might have retreated to the Apennines, which remained climatically suitable during cold stages, such as the latest Würm glaciation. Similarly, the importance of climatically suitable mountains on Corsica as glacial refugia cannot be ruled out because the island was connected to the mainland during the LGM. In contrast, the ancient Pyrenean populations (if they existed) would have been unable to move to contiguous suitable areas, and as a result became extinct, as occurred with other populations undergoing processes of demographic retreat (Dalén et al., 2007). The large temporal variations in habitat suitability suggest that these local extinction events were probably the rule in the western lineage, resulting in the small population size depicted by the ABC analysis. Similar demographic contractions have driven certain lineages to extinction during the last glaciation (Gilbert et al., 2008), suggesting that the survival of the western lineage might have hung by a thread during the last cold period and probably also in previous glaciation events. The retreat of ice shields and the recovery of suitable areas in the Pyrenees and the Alps enabled the western lineage to expand its range and population size post-LGM.

**CONCLUSIONS**

By applying a combination of newly developed molecular and environmental niche modelling techniques, we have been able to reconstruct the Pleistocene demographic history of *P. macrobullaris*, which had remained unresolved to date principally because of the limited resolution of previously employed molecular markers. We tested a specific hypothesis with complete mitochondrial genome data, analysed with techniques drawn from the fields of population genetics, phylogenetics and model-based inference, and ENMs projected to past climatic conditions. Such multi-methodological approaches are being used increasingly for inferring the evolutionary history of species (Carstens & Richards, 2007; Chan et al., 2011; Alvarado-Serrano & Knowles, 2013; Razgour et al., 2013) because they allow evolutionary and palaeodemographic processes to be tackled from different perspectives. Our approach considerably increased the evolutionary history inference capability, enabling the testing of questions and hypotheses that were impossible to solve using traditional and/or single-approach methods.

The evolutionary history of *P. macrobullaris* was shaped by the differential effect of glaciation events in different mountain systems, which yielded complex population dynamics, with contrasting patterns of expansion and retreat depending on the geographical location. Species-specific differences in responses to climate changes are widely studied and accepted (Williams & Jackson, 2007) and, although the empirical evidence is still poor, some authors have proposed that species’ responses might also vary at a population level (Bennett & Provan, 2008; Stewart, 2008). This study provides evidence of population-level variation and demonstrates that the demographic responses of species to events of climate variation are not geographically and temporally homogeneous, but complex processes that can be studied successfully by integrating multiple newly developed techniques.

**ACKNOWLEDGEMENTS**

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Details of the methodologies used.

**Appendix S2** Supporting tables (Tables S1–S5).

**Appendix S3** Supporting figures (Figs S1–S5).

**DATA ACCESSIBILITY**

All complete mitochondrial sequences have been deposited in GenBank (accession numbers KR134353–KR134409).

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**BIOSKETCH**

Antton Alberdi is a researcher at the University of the Basque Country with interests in ecology, biogeography and evolutionary biology, with a primary focus on bats.

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