



RESEARCH ARTICLE

Ancient DNA reveals interstadials as a driver of common vole population dynamics during the last glacial period

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Abstract

Aim: Many species experienced population turnover and local extinction during the Late Pleistocene. In the case of megafauna, it remains challenging to disentangle climate change and the activities of Palaeolithic hunter-gatherers as the main cause. In contrast, the impact of humans on rodent populations is likely to be negligible. This study investigated which climatic and/or environmental factors affect the population dynamics of the common vole. This temperate rodent is widespread across Europe and was one of the most abundant small mammal species throughout the Late Pleistocene.

Location: Europe.

Taxon: Common vole (*Microtus arvalis*).

Methods: We generated a dataset comprised of 4.2 kb long fragment of mitochondrial DNA (mtDNA) from 148 ancient and 51 modern specimens sampled from multiple localities across Europe and covering the last 60 thousand years (ka). We used Bayesian inference to reconstruct their phylogenetic relationships and to estimate the age of the specimens that were not directly dated.

Results: We estimated the time to the most recent common ancestor of all last glacial and extant common vole lineages to be 90 ka ago and the divergence of the main mtDNA lineages present in extant populations to between 55 and 40 ka ago, which is earlier than most previous estimates. We detected several lineage turnovers in Europe during the period of high climate variability at the end of Marine Isotope Stage 3 (MIS 3; 57–29 ka ago) in addition to those found previously around the Pleistocene/Holocene transition. In contrast, data from the Western Carpathians suggest continuity throughout the Last Glacial Maximum (LGM) even at high latitudes.

Main Conclusions: The main factor affecting the common vole populations during the last glacial period was the decrease in open habitat during the interstadials, whereas climate deterioration during the LGM had little impact on population dynamics. This suggests that the rapid environmental change rather than other factors was the major force shaping the histories of the Late Pleistocene faunas.

KEYWORDS

habitat, Late Pleistocene, *Microtus* sp., mitochondrial DNA, paleoclimate, small mammals

1 | INTRODUCTION

The climatic and environmental changes during the last glacial period (ca. 115–11.7ka ago) had a great impact on the evolutionary histories of most species. It has been suggested that species have responded to those changes according to their individual characteristics and there is no basis for considering them as complexes responding to climate and environmental changes in the same manner (Baca et al., 2017; Lorenzen et al., 2011; Stewart et al., 2010). However, species depend on a range of interactions at different trophic levels and across different ecological niches within the ecosystem (Walther, 2010). Thus, investigating species with different adaptations can reveal the spectrum of responses to the same climatic and environmental fluctuations and allow the identification of

the key factors driving ecosystem responses (Cooper et al., 2015). Small mammals are particularly well suited for such investigations as, in contrast to megafaunal species, they were little affected by the activities of Palaeolithic hunter-gatherers and their population dynamics were mainly driven by environmental changes.

The common vole, *Microtus arvalis* (Pallas, 1779), is a temperate rodent species that inhabits most of continental Europe (Figure 1b). This species feeds primarily on leaves and grasses and prefers well-drained grasslands, pastures and alpine meadows from lowlands to ca. 3000m a.s.l. At present common voles mainly utilise secondary habitats, such as agricultural fields, where they are often considered a pest (Jacob et al., 2014). The earliest remains of an ancestral common vole (*Microtus arvalis*-group) in Europe date to ca. 0.6–0.5Ma ago (Berto et al., 2021; Kučera et al., 2009; Maul & Markova, 2007). The

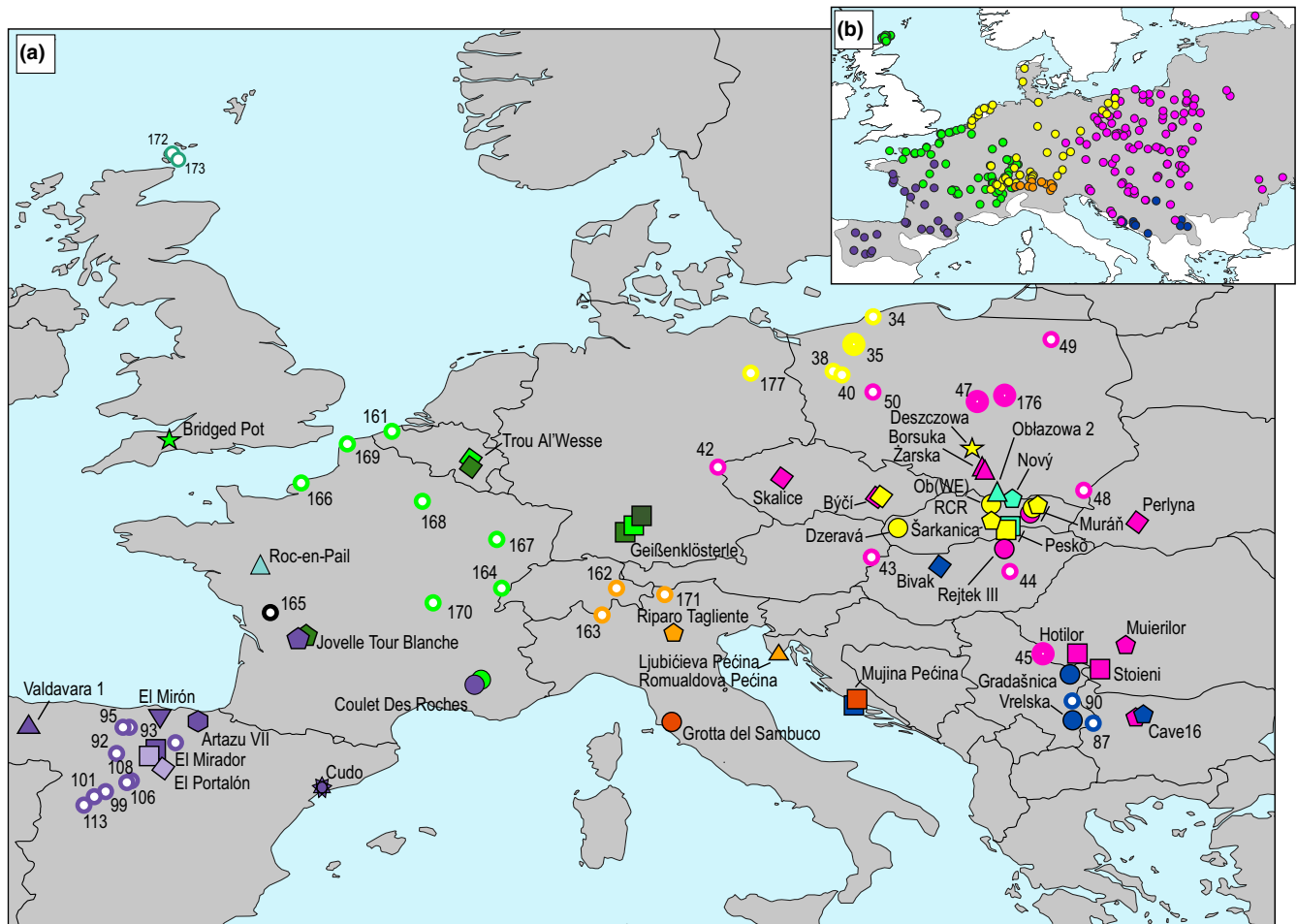


FIGURE 1 Sampling localities of the modern and ancient common voles across Europe (a). Filled symbols represent paleontological sites, while unfilled circles denote the localities of modern specimens. Numbers near modern localities correspond to specimen numbers (WM...) in Figure S2 and Table S2. Symbols and names are consistent with those in Figure 2 and are coloured according to the vole mtDNA lineage found at the site. If multiple mtDNA lineages were found at a site, two or more symbols are presented. (b) Distribution of the main mtDNA lineages in the extant populations. The grey area depicts the current range of the common vole in Europe. The coloured circles represent sampling localities of the common vole and mtDNA lineages compiled from previous studies: Pink—Eastern (E); yellow—Central (CEN); orange—Italian (ITA); green—Western-north (WN); violet—Western-south (WS); navy blue—Balkan (B).

fossil record from the Last Glacial Period (115–11.7 ka ago), attests to its continuous presence on most of the continent (Chaline, 1972; Horáček & Ložek, 1988; Jánossy, 1986; Nadachowski, 1989). In many localities on the European Plain, from France to Poland, the common vole was the most abundant small mammal, alongside the collared lemming (*Dicrostonyx torquatus*) and the European narrow-headed vole (*Lasiopodomys anglicus*; Royer et al., 2016; Socha, 2014).

The mitochondrial DNA (mtDNA) phylogeography of extant common vole populations has been intensively studied to reconstruct the post-glacial history of the species. The extant mtDNA diversity was partitioned into six divergent lineages with a parapatric distribution: Western-South (WS), Western-North (WN), Italian (ITA), Balkan (B), Central (CEN) and Eastern (E) (Bužan et al., 2010; Haynes et al., 2003; Heckel et al., 2005; Stojak et al., 2015; Figure 1b). Most previous studies estimated the time to the most recent common ancestor (tMRCA) of the extant common vole populations to be between 65 and 50 ka ago and subsequent divergence of main lineages to be between 50 and 20 ka ago (García et al., 2020;

Heckel et al., 2005; Stojak et al., 2016). In contrast, Fink et al. (2004) and Tougaard et al. (2008) used fossil calibration to suggest a much older diversification in the Middle Pleistocene. Analyses of nuclear DNA revealed very good correspondence with the spatial distributions of the mtDNA lineages (Fischer et al., 2014; Heckel et al., 2005) and detailed analyses of the contact areas between lineages demonstrated admixture only in narrow hybrid zones (Beysard & Heckel, 2014; Braaker & Heckel, 2009; Saxenhofer et al., 2022). However, the divergence time estimates of the evolutionary lineages based on the nuclear data are generally much more recent than those based on mtDNA and suggest that diversification of the common vole evolutionary lineages took place during or after the LGM (Heckel et al., 2005; Lischer et al., 2014).

The present-day distribution of the common vole mtDNA lineages (Figure 1b) has been interpreted as evidence for the survival of common vole populations during the LGM in both traditional southern glacial peninsular refugia, as well as at higher latitudes, particularly in central France (WN), north of the Alpine region (CEN) and

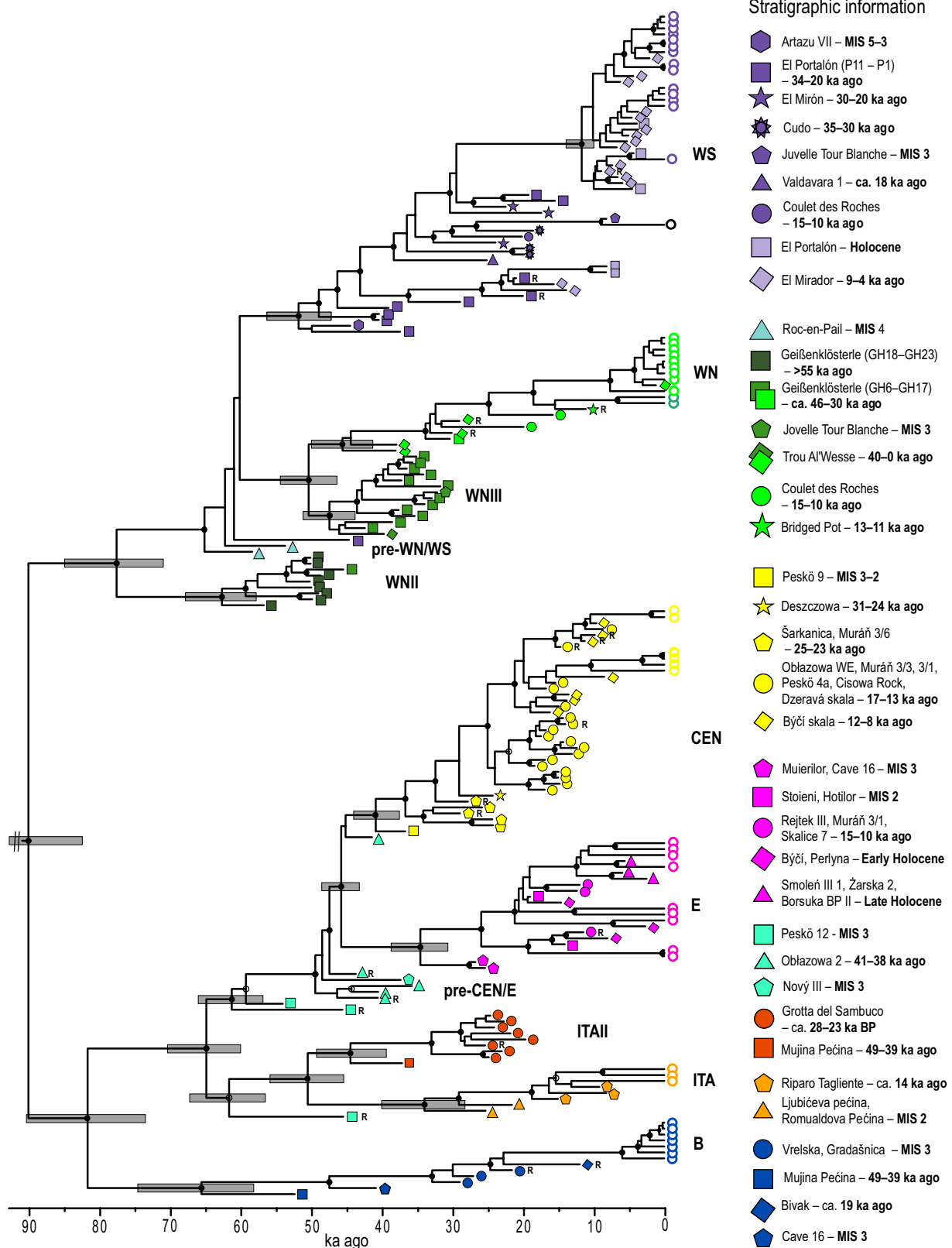


FIGURE 2 Maximum clade credibility tree of the European common vole mtDNA obtained in BEAST 1.10.4 and calibrated with radiocarbon dated specimens. Black dots indicate nodes with posterior probability above 0.95 and grey bars show the 95% HPD intervals of the node ages. The tips are annotated with colour symbols consistent with those in Figure 1. A tree annotated with sample names is presented as Figure S2. Directly radiocarbon dated specimens are marked with letter R. The empty circles represent modern specimens. The legend on the right side of the figure provides information on stratigraphy and the dating of the source sites and layers.

in the Carpathian area (E) (Heckel et al., 2005; Stojak et al., 2015; Tougard et al., 2008); however, the pre-LGM history of the species remains largely unknown.

A detailed study of the Eastern mtDNA lineage suggests that it originated in the Carpathian area and its current distribution is the result of an expansion that started after the LGM with a possible bottleneck during the Younger Dryas (12.8–11.7 ka ago) (Stojak et al., 2016). However, an ancient DNA investigation of common vole remains from the Western Carpathians has shown the replacement of the Central lineage by the Eastern around the Pleistocene/Holocene transition (Baca et al., 2020). This challenged the simple model of post-glacial recolonisation of the Eastern lineage from the Carpathian refugium and suggested that analyses based solely on extant genetic diversity may not recover the full complexity of the Late Pleistocene population dynamics. To refine the evolutionary history of the common vole populations in Europe and to compare it with the histories of coeval cold-adapted species, such as the collared lemming, we generated and analysed a new mitochondrial dataset consisting of nearly 200 sequences from Late Pleistocene and extant individuals.

2 | MATERIALS AND METHODS

2.1 | Ancient specimens

Isolated lower first molars or mandibular fragments with molars classified as *M. arvalis* or *Microtus* sp. based on the morphology of the occlusal surface were collected from various palaeontological sites across Europe (Table S1). Each tooth was photographed at the Institute of Systematics and Evolution of Animals, PAS.

2.2 | Modern specimens

The DNA of modern specimens from various locations across Europe was extracted previously (Table S2). A target 4.2 kb region of mtDNA spanning positions 12,000 to 16,247 according to the reference sequence (NC_038176; Folkertsma et al., 2018) was generated using various approaches. It was either polymerase chain reaction (PCR) amplified, sonicated, transformed into sequencing libraries and sequenced or the genomic DNA was sonicated and transformed into sequencing libraries. It was then either enriched for target fragment using in-solution hybridization and sequenced or the target region was extracted from the deep shotgun sequencing data (see Appendix A1.1 and Table S2 for more details).

2.3 | Ancient DNA extraction, target enrichment and sequencing

The DNA extraction and pre-PCR library preparation steps were performed in the dedicated ancient DNA laboratory at the Centre of New Technologies at the University of Warsaw. Each tooth

was thoroughly cleaned with ultra-pure water in a 2 ml tube and crushed with a pipette tip. DNA was extracted following a silica spin column-based protocol optimised to retrieve short DNA molecules (Dabney et al., 2013). A negative control without biological material was processed alongside each batch of 15 specimens. Double-stranded, double-indexed sequencing libraries were produced from half of the DNA extract (20 µl) following a previously established protocol (Meyer & Kircher, 2010) with minor modifications (Baca et al., 2019). For some specimens that yielded a low-quantity DNA additional double-indexed, single-stranded sequencing libraries were prepared following the protocol proposed by Gansauge et al. (2020) (see Appendix A1.3–A1.4 for more details).

The libraries were enriched for vole mitochondrial DNA using the in-solution hybridisation protocol described by Baca et al. (2019). Up to five libraries were pooled for each hybridisation reaction. We performed two rounds of hybridization at 65°C for 22–24 h each. After each round, library pools were washed and amplified in triplicate for 10 to 15 cycles. The enriched library pools were combined, quantified by qPCR and sequenced on Illumina NextSeq550 platform using MID output, 2×75 bp kit (see Appendix A1.2–A1.5 and Tables S4–S5 for more details).

2.4 | Sequence processing

The sequencing reads were demultiplexed using bcl2fastq v. 2.19 (Illumina). Overlapping reads were collapsed, adaptor and quality trimmed using AdapterRemoval v. 2.2.2 (Schubert et al., 2016). Then, reads were mapped to the common vole mtDNA genome using the *mem* algorithm in bwa v. 0.7.17 (Li & Durbin, 2010). Duplicates, short (<30 bp) and low mapping quality reads (mapq < 30) were removed using samtools v. 1.9. Variants and consensus sequences were called using bcftools v. 1.9 (Li et al., 2009). Read alignments and vcf files were inspected manually using Tablet v. 1.17 (Milne et al., 2013). Positions with coverage below three were masked with N. If a base was supported by less than 75% of the reads, an IUPAC symbol was inserted. MapDamage v. 2.08 (Jónsson et al., 2013) was used to assess the damage patterns and length distribution of the DNA molecules. See Appendix A1.6 for more details.

2.5 | Phylogenetic analyses and molecular dating of the specimens

We used a Bayesian approach, implemented in BEAST 1.10.4 (Suchard et al., 2018), to estimate the divergence times of the common vole lineages and the age of the specimens that were not directly dated. We used only ancient and modern sequences with at least 70% and 90% of the target mtDNA fragment (4.2 kb) recovered, respectively, for the phylogenetic inference. Sequences were aligned with MAFFT v. 7.407 (Katoh & Standley, 2013). The best substitution model selected by jModelTest2 (Darriba et al., 2012),

TIM2+F+I+G4, was not easily available in BEAST so we used the closest available one, which was GTR+I+G.

First, we used Bayesian evaluation of temporal signal (BETS) (Duchene et al., 2020) to determine whether there was sufficient temporal resolution within our dataset to calibrate the molecular clock. We used all directly radiocarbon dated ($n = 20$) and modern ($n = 51$) specimens and tested four alternative models. In two of them, we assigned real sampling times to the sequences (heterochronous analysis) and used either a strict clock or an uncorrelated relaxed lognormal clock. In the two other models, we used the same sampling time for all sequences (isochronous analysis) and applied either the strict clock or the uncorrelated relaxed lognormal clock (see Appendix A1.7 for more details). Then, we performed a leave-one-out analysis on the directly radiocarbon dated specimens to determine the accuracy of the age estimates produced using the available calibration dataset. In this analysis, we estimated the age of each directly radiocarbon dated specimen using all of the remaining radiocarbon dated and modern specimens to calibrate the molecular clock. Next, we estimated the age of each ancient, not directly dated, specimen ($n = 128$) in a separate BEAST run, using all directly dated and modern specimens to calibrate the molecular clock. Finally, we ran a joint analysis with all of the sequences. For the specimens that were not directly dated we set a lognormal prior with a mean equal to the mean age estimated in the individual analysis and the range covering the 95% highest posterior density (HPD) interval of the individual estimate (see Appendix A1.7, Tables S6–S9 for more details).

The demographic reconstructions using the Bayesian Skyline and Bayesian SkyGrid methods implemented in BEAST 1.10.4 were performed for the WN lineage specimens from Spain ($n = 58$). In the case of other localities either the number of available sequences was low or the assumptions of population continuity (i.e. lack of lineage turnovers) were violated (see Appendix A1.8 for details).

2.6 | Radiocarbon dating

Selected vole mandibles were pre-treated for radiocarbon dating in the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) following the protocol for <100mg bone samples described by Fewlass et al. (2019). The quality of the collagen extracts was assessed based on the collagen yield as a percentage of the original bone weight (minimum requirement 1%). The elemental and isotopic ratios of the extracts (~0.5 mg) were measured at the MPI-EVA on a Thermo Finnigan Flash elemental analyser coupled to a Thermo Delta Plus XP isotope ratio mass spectrometer. When sufficient collagen was extracted, the collagen was graphitised using automated graphitisation equipment (Wacker, Némec, & Bourquin, 2010) in the Ion Beam Physics Laboratory at ETH-Zurich (Switzerland) and dated on a MIni CARbon DAting System (MICADAS) accelerator mass spectrometer (AMS) (Wacker, Bonani, et al., 2010). Where the extracted collagen

yield was insufficient for graphitization, it was combusted to CO₂ and measured directly using a gas interface system coupled to the gas ion source of the MICADAS (Wacker et al., 2013) following the protocol described in Fewlass et al. (2019) (see Appendix A1.9 for more details).

To improve the stratigraphic information available for the sites from which the analysed specimens originated, we also obtained AMS radiocarbon dates from five palaeontological sites (Appendix A1.10, Table S10). The radiocarbon dates were calibrated in OxCal v4.4 (Bronk Ramsey, 2009) using the IntCal20 (Reimer et al., 2020) calibration curve.

3 | RESULTS

We generated a dataset of 4.2 kb long mtDNA sequences from 199 ancient and modern common vole specimens (148 ancient and 51 modern). The sequences of 82 ancient specimens are reported for the first time and either 4.2 kb or 1 kb fragments of mtDNA of the remaining 66 specimens were reported previously (Baca et al., 2020, 2021; Lemanik et al., 2020; Table S1). All 82 newly reported specimens yielded short inserts and elevated levels of cytosine deamination at the terminal nucleotides characteristic of ancient DNA (Table S1). The ancient specimens that yielded mtDNA sequences originated from 40 sites scattered across Europe and covered the period of the last ca. 60,000 years (Table S1, Figure 1a). The mtDNA cytochrome *b* sequences of most of the modern specimens were reported previously (García et al., 2020; Stojak et al., 2016) and here only the longer mtDNA fragment was generated to increase the phylogenetic resolution. Direct radiocarbon dating was undertaken for 12 vole mandibles and 10 yielded sufficient collagen quality for AMS dating (Table S3). The carbon to nitrogen ratio (C:N) of one specimen from Geißenklösterle (MI935) was at the limit (3.6) of accepted values for well-preserved collagen (2.9–3.6; Van Klinken, 1999) and yielded a relatively recent date with respect to the latest Electron Spin Resonance dates of the cave sediments (Richard et al., 2019), indicating that contamination with modern carbon and therefore, an under-estimate of the true age, was likely. Therefore, we discarded this radiocarbon date and estimated the age of this specimen using a molecular approach. As a result, we used sequences of 20 directly radiocarbon dated and 51 extant specimens to calibrate the molecular clock (Tables S2 and S3). The analysis of temporal signal (BETS) showed that the dated specimens included in our dataset were suitable for calibrating the molecular clock. The heterochronous model, with a strict clock and correct sampling times assigned to the specimens, was strongly supported over all of the other models (2lnBF > 9) (Table S4). The leave-one-out analysis revealed that the dated dataset enabled a relatively accurate estimate of the specimen ages, although the 95% HPD of the estimated ages did not overlap within the 2-sigma ranges of the radiocarbon dates for three specimens (MI074, MI1337 and MI1355; Figure S1; Appendix A). In addition, the estimated ages of most of the specimens agreed with their stratigraphic position, providing evidence for the accuracy of this approach (Table S1).

3.1 | Diversification of the common vole mtDNA lineages

The maximum clade credibility tree obtained from BEAST 1.10.4 (Figure 2) recovered, with high support, all six mtDNA lineages characterised previously in analyses of the mtDNA cytochrome *b* of modern individuals (Bužan et al., 2010; Haynes et al., 2003; Heckel et al., 2005). In addition, we identified three lineages that were only present in Late Pleistocene specimens: WNII and WNIII in western Europe and ITAII in Italy and Croatia (Figures 1a and 2). The tMRCA of all European common voles was estimated to be 90 ka ago (95% HPD: 98–83 ka ago). Divergence time estimates of the subsequent lineages ranged from 82 ka ago (95% HPD: 91–75 ka ago) for the split between B and ITA/CEN/E, through 77 ka ago (95% HPD: 84–70 ka ago) for the split between WNIII and WNII/WN/WS to ca. 50–45 ka ago for the splits between a number of lineages, including ITA and ITAII; CEN and E and WNII and WN (Figure 2).

3.2 | Temporal population structure and dynamics of the common vole

3.2.1 | Western and southwestern Europe

The oldest specimens from western Europe were classified into the WNIII lineage which was sister to the WS and WN/WNII lineages. The WNIII lineage contained mainly individuals from the lowermost layers of Geißenklösterle (GH23–18). The age of these individuals was estimated to be between 56 and 45 ka (Figures 1a and 2, Table S1). Three haplotypes of similar age as the latter (57–44 ka ago) from western France (Roc-en-Pail; MI122, MI123) and northern Spain (El Portalón P9; MI1279) were located at the base of the WS, WNII and WN lineages (pre-WN/WS; Figure 1a, Table S1). The WS lineage appeared in northern Spain and the WNII lineage appeared in France, Belgium and Germany about 42 ka ago. The latter, composed of specimens from the younger layers of Geißenklösterle, Trou Al'Wesse and Jovelle, disappeared about 32 ka ago. The age of the oldest specimens from the WN lineage, which came from Trou Al'Wesse, was estimated to be 37 ka ago. This lineage was found among Late Pleistocene specimens from western Germany, Belgium, France and the UK. The record from Spain suggests population continuity throughout the last 40 ka, although nearly all modern and Holocene specimens coalesced about 11.5 ka ago (Figure 2), suggesting a significant reduction in female effective population size (N_{ef}) around the Pleistocene/Holocene transition. This was further confirmed by the Bayesian demographic analysis, which suggested a five-fold reduction of N_{ef} around the Pleistocene/Holocene transition with the minimum around the Early Holocene (11.7–9 ka ago), followed by a slight recovery near the Middle Holocene (9–6 ka ago) (Appendix A1.8, Figures S2 and S3). The WS lineage was also detected in southern France at Jovelle and Coulet des Roches during MIS 2 (29–14 ka ago).

3.2.2 | Central and southeastern Europe

The oldest specimens from central and southeastern Europe came from the Western Carpathians. Their ages were estimated to be between 55 and 35 ka ago and they hold a basal position with respect to CEN and E lineages (pre-CEN/E). The two oldest specimens from Peskö 12 (MI299 and MI300) were noticeably more divergent than the others. Starting 35 ka ago, we found the CEN lineage in the Western Carpathians as well as to the north and the most recent specimens from this lineage were dated to the Early Holocene. The earliest specimens assigned to the E lineage were estimated to be ca. 27–25 ka ago and were found in central Romania (Mujina Cave; MI760) and northwestern Bulgaria (Cave 16; MI807). The more recent specimens from this lineage seem to represent a northern and northwestern expansion of this population, with the first appearance in the Ukrainian Carpathians in Perlyna about 14 ka ago and in the Western Carpathians about 12 ka ago (Rejtek III, Murán 3/1).

The Balkan lineage occupied the same area as its current distribution starting from at least 50 ka ago (Mujina pećina). A single specimen from Bivak cave in northern Hungary suggests its range extended further to the northeast before the Holocene.

The ITA and ITAII lineages were detected in the Italian and Balkan Peninsulas. ITAII specimens were found in central Italy (Grotta del Sambuco) and in middle Dalmatia (Mujina pećina) with ages estimated to be between 36.5 and 18.5 ka ago. These specimens were generally older than specimens from the ITA lineage, dated 22.6–0 ka ago and found in northern Italy (Riparo Tagliente) and Istria (Ljubičeva pećina). The single individual from layer 12 of Peskö cave (Western Carpathians; MI1701), directly radiocarbon dated to 45 ka cal BP, was located at the base of the ITA lineages.

4 | DISCUSSION

4.1 | The effects of climatic change on diversification of the common vole lineages

The estimated tMRCA of mtDNA for the European common vole (90 ka ago; 95% HPD: 98–83 ka ago) was substantially older than some previous estimates (García et al., 2020; Heckel et al., 2005; Stojak et al., 2015). It was similar to the recent estimates for the initial diversification of the mtDNA lineages of cold-adapted collared lemmings (100 ka ago, 95% HPD: 109–92 ka ago; E. Lord, personal communication). It has been suggested that this may be the effect of a bottleneck during the Eemian Interglacial (MIS 5e). It may also be related to the Brørup Interstadial (MIS 5c; ~GS-23; ca. 104–88 ka). Vegetation during the Brørup Interstadial was characterised by temperate deciduous forests in western Europe and boreal forests to the north and east (Guiter et al., 2003; Helmens, 2014), providing unfavourable habitats for the cold-adapted collared lemming and for the common vole, both of which rely on various open habitats.

The initial divergence of the common vole lineages may have been the result of survival in two distinct refugia in the Alpine and Carpathian regions causing a partition in the mitochondrial diversity of the species in the two geographical areas. The WNIII, WNII, WN and WS lineage occupied western Europe, and central and south-eastern Europe were occupied by the CEN, ITAI, ITA, B and E lineages. This geographic partitioning was maintained for at least 45 ka (Figure 3) and probably contributed to the patterns of restricted gene flow and partial reproductive isolation of the present-day populations of WN and CEN lineages (Beysard & Heckel, 2014).

The divergence of the main common vole mtDNA lineages estimated in this study was older than previously suggested. All mtDNA lineages present in the extant European populations (WS, WN, ITA, B, CEN and E) diverged before 40 ka ago and the earliest specimens from each of those lineages in our dataset pre-date 25 ka ago. This suggests, contrary to previous hypotheses (García et al., 2020; Heckel et al., 2005; Stojak et al., 2015), that climate deterioration during the LGM did not play a major role in the initial divergence of the main extant mtDNA lineages. However, the population decline and increased isolation during the period of the most inhospitable climatic conditions may have reinforced the previously existing divergence.

Most of the observed divergence events occurred between ca. 60 and 45 ka ago. These events may be related to a long interstadial period identified in the palynological records across Europe:

the Moershoofd Interstadial Complex in the Netherlands, the Pile Interstadial Complex at La Grande Pile pollen sequence (eastern France) and the Oerel and Glindé Interstadials at the Oerel pollen sequence (northern Germany) dated to ca. 58–48 ka uncal BP and is usually correlated with the Greenland Interstadials (GI) 16 and 14 (ca. 58–56.5 and 54–49.5 ka ago; Helmens, 2014).

4.2 | Phylogeography and demographic history of the common vole

4.2.1 | Western Europe and the Iberian Peninsula

We documented two consecutive mtDNA lineage turnovers at the end of MIS 3 (WNIII/WNII and WNII/WN) in western Europe. The first took place about 45 ka ago (Figure 3). There are a few specimens of similar age from other parts of Europe in our dataset; however, the divergent position of three specimens from the Western Carpathians (MI299, MI300 and MI1701; Figure 2) dated 53 and 45 ka ago suggests that synchronous lineage turnovers may have occurred in other parts of Europe. The second turnover occurred about 32 ka ago and appears to have been restricted to western Europe (Figure 3). The oxygen isotope record from the Greenland ice cores shows that the period between 45 and 29 ka ago was characterised by several short-term climatic oscillations (Rasmussen

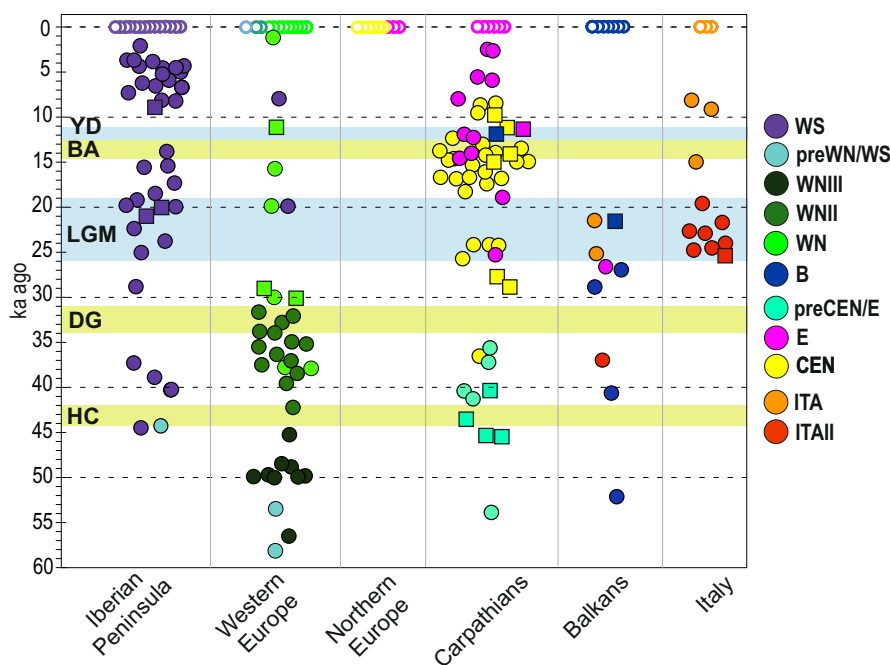


FIGURE 3 Temporal distribution of the mtDNA lineages divided by geographical region. Western Europe here includes France, southern Germany, Belgium and the United Kingdom; the northern Europe includes northern Germany and Poland (there were no ancient specimens from this region), the Carpathians include southern Poland, Czechia, Slovakia, Hungary, Ukraine and Romania and the Balkans include Bulgaria, Serbia and Croatia; circles denote medians of age estimated using the molecular approach while squares denote medians of calibrated radiocarbon ages. The green and blue strips indicate the main interstadials and stadials identified in the palynological records: H–C–Hengelo–Charbon; D–G–Denekamp–Grand Bois; LGM–Last Glacial Maximum, B–A–Bølling–Allerød; YD–Younger Dryas.

et al., 2014). The palynological records revealed two main interstadials that stand out during this period and have been identified in the most of sediment sequences across Europe (Helmens, 2014). The older one, Hengelo–Charbon, took place about 43–41 ka cal BP (38–36 ka uncal BP; Helmens, 2014; Vandenberghe & van der Plicht, 2016), while the younger one, Denekamp–Grand Bois, occurred about 34–33 ka cal BP (31–29 ka uncal BP Guiter et al., 2003; Helmens, 2014), approximately the same time as the recorded mtDNA lineage turnovers. The exact correlation between the palynological data and the Greenland ice-core records is problematic due to potential offsets and the wide error ranges involved. Hengelo–Charbon is usually associated with GI-11 (ca. 43.3–42.2 ka ago) or GI-10 (ca. 41.5–40.8 ka ago), although at times the earlier GI-12 (ca. 46.8–44.2 ka ago) as the longest and most pronounced interstadial around the end of MIS 3 and Denekamp–Grand Bois with GI-8 (38.2–36.6 ka ago; Helmens, 2014). Both interstadials were characterised by the emergence of *Betula* and *Pinus* forests in western Europe and of *Betula*, *Larix* and *Pinus* forests in central Europe, although it is assumed that the landscape remained relatively open as the duration of these interstadials was too short for the development of full forest cover (Guiter et al., 2003; Helmens, 2014). However, even partitioned landscapes limit dispersal and promote local extinction of common vole populations (Delattre et al., 1996); thus, fragmentation of primarily open, stadial habitats into patchy and mosaic interstadial landscapes may have led to large scale decreases in population density and local or regional extinctions. A similar explanation for common vole population dynamics was previously suggested by Tougaard et al. (2008). Martínková et al. (2013) showed partial replacement of mtDNA within the WN lineage in Late Holocene common vole populations from northern France and Belgium and suggested that the main factor driving this process was landscape reorganisation.

The record from northern Spain, occupied by the WS lineage, suggests population continuity throughout the last ca. 45 ka. The demographic reconstruction showed a drastic reduction in the effective population size around the Pleistocene/Holocene transition (Figures S2 and S3). This agrees with previous findings based on mtDNA cytochrome *b* and smaller sample size (Baca et al., 2020). Palynological records from the region from which both modern and ancient specimens originated suggest that during the Bølling–Allerød interstadial (14.7–12.8 ka ago) a high proportion of open landscapes persisted until the expansion of the deciduous woodlands started in the Early Holocene (Carrión et al., 2010).

4.2.2 | Central and southeastern Europe

The survival of common vole populations throughout the LGM at high latitudes, including the Carpathians, has been previously suggested based on several lines of evidence. The fossil record suggests the continuous presence of the common vole in the Pannonian Basin (Pazonyi, 2004), Bohemian Massif and Slovakia (Horáček & Ložek, 1988) and even north of the Carpathians (Sommer &

Nadachowski, 2006), although these findings were based only on the stratigraphic position of the specimens, rather than direct dates. The Carpathians was also suggested as a northern refugium based on the distribution of the E mtDNA haplogroup in modern populations (Stojak et al., 2015; Stojak et al., 2016) and ecological niche modelling (Stojak et al., 2019). Our data support the northern survival of the common vole throughout the LGM. In the Western Carpathians, the northernmost part of this mountain range, all individuals with ages estimated, or directly dated, between 36 and 10 ka ago to belong to the CEN mtDNA lineage. Two specimens from Šarkanica yielded pre-LGM, direct radiocarbon dates (28.9 and 27.8 ka cal BP; Table S3), while the age of the other four specimens was estimated to between 25.9 and 24.2 ka cal BP (Figure 2, Table S1). Lemanik et al. (2020) reported a signal of the rapid growth of the N_e of the common vole population from the Western Carpathians starting ca. 21 ka cal BP that continued until ca. 15 ka cal BP. Taken together, these results are consistent with population continuity through the LGM, although accompanied by a significant reduction in population size.

Most of central, eastern, and southeastern Europe is presently occupied by the E mtDNA lineage. Before the maximal extension of the Scandinavian Ice Sheet (23–19 ka ago), we detected the E mtDNA lineage only in southwestern Romania and Bulgaria. The presence of younger specimens bearing this mtDNA lineage in the same area, dated to 18.8 and 14 ka ago, suggests that this lineage may have survived the LGM in this part of the Carpathians. The expansion of the E mtDNA lineage from the southeastern Carpathian area is also consistent with the highest genetic diversity in the extant common vole populations in this area (Stojak et al., 2016). The colonisation of vast territories of central and eastern Europe, and replacement of CEN and B mtDNA lineages, occurred later during the Bølling–Allerød or the Younger Dryas. In contrast to previous suggestions (Baca et al., 2020), the similar ages of the CEN and E mtDNA lineage individuals from the Muráň 3 and Býčí sites estimated here, suggest that both lineages may have coexisted in the area for some time (Figure 2, Table S1), before the final extirpation of the CEN lineage during the Early Holocene. However, the limited resolution of molecular age estimates and inability to track admixture with mtDNA data does not allow for fine scale reconstruction of this process.

The record from the Italian peninsula suggests the replacement of the ITAlI lineage with the ITA lineage which has survived in Northern Italy and Switzerland until the present day. Although the data came from sites with a limited temporal span (Appendix A), our dated phylogeny suggests that extirpation of the ITAlI lineage occurred at some point after the LGM and the expansion of the ITA lineage occurred no later than the Bølling–Allerød warming. This turnover may be reflected in the significant decrease in the common vole remains for a short period after the LGM, which was observed in the southern Italian peninsula (Berto et al., 2019). This scenario is more consistent with repeated southward expansion of subsequent common vole populations rather than with their continuous presence in the region.

4.2.3 | Comparison with other European Late Pleistocene species

The common vole, along with the collared lemming and narrow-headed vole, is among the most numerous small mammals found in assemblages from the last glacial period in Europe and are assumed to have coexisted during much of this period. The explanation for this paradox, in which a temperate species coexists with a cold-adapted species, is based on the high tolerance of the common vole to low temperatures (Tougaard et al., 2008). Our study corroborates the continuous presence of this species in middle and high latitudes of Europe throughout the last at least 60 ka (Figure 3), although the evolutionary history of the common vole differed in some respects from other species inhabiting Europe during the Late Pleistocene.

The long-term regional continuity of the main lineages of common vole with limited evidence for migrations suggested by our mtDNA analysis is in contrast with the Late Pleistocene evolutionary histories of megafaunal species, such as cave bears and mammoths, both of which showed evidence for long-distance migrations and large-scale population replacements (Fellows Yates et al., 2017; Gretzinger et al., 2019). The collared lemming also shows a distinct pattern of mtDNA diversity consistent with multiple continent-wide population replacements (Palkopoulou et al., 2016). This is likely related to differences in the mobility of the two species, with the collared lemming being more capable of long-distance dispersal than the common vole (Ehrich et al., 2001). On the other hand, available data suggest that despite this different species-specific history, the common vole populations were affected by the same climatic and environmental changes as the cold-adapted taxa. The extirpation of the WNIII lineage in western Europe, and potentially of other common vole populations across Europe, occurred around the same time as the continent-wide disappearance of collared lemming populations represented by mtDNA lineages 1 and 2 (Palkopoulou et al., 2016). Similarly, the disappearance of the WNII lineage of common voles about 32 ka ago is at approximately the same time as the population replacement of cave bears recorded in the Ach Valley, Germany (Münzel et al., 2011) and of woolly mammoth populations across all of Europe (Fellows Yates et al., 2017; Palkopoulou et al., 2013). A new population of collared lemming (mtDNA lineage 3) appeared in Europe about 32 ka ago after a potential short-term extirpation (Palkopoulou et al., 2016). It has been suggested that the main driver affecting mammalian species throughout the last glaciation was the abrupt warming that occurred at the onset of the Greenland Interstadials (Cooper et al., 2015). Nevertheless, whether it was the abruptness of the climatic change, or the subsequent emergence of interstadial environment is unclear, although the high environmental instability during the period between 45 and 29 ka ago appears to have affected the entire spectrum of differently adapted species including the common vole.

The other period that has had a large effect on common vole populations is the Pleistocene/Holocene transition. The climatic warming and the emergence of forests during the Bølling-Allerød Interstadial (14.7–12.8 ka ago) and during the Early Holocene (11.7–9 ka ago) are considered the main causes of extirpation of many cold-adapted species from Europe including the collared lemming and narrow-headed vole (Berto et al., 2022; Royer et al., 2016). Our data confirm previous findings indicating a substantial population decline in the common vole of northern Spain and replacement of the mtDNA lineage in the Western Carpathians (Baca et al., 2020). In addition, we identified a potential lineage turnover on the Italian peninsula. Taken with the probable extirpation of the common vole from the British Isles during the Early Holocene (Baca et al., 2020), this suggests a continent-wide effect of interstadial environment on common vole populations.

5 | CONCLUSIONS

Our results suggest that the evolutionary history of common vole was distinct from typical cold-adapted species associated with steppe-tundra environments. However, the timing of common vole population dynamics suggests that the species responded to similar climatic and/or environmental changes and that habitat availability, rather than climatic variables themselves, was the primary factor affecting common vole populations. Recently, Kotlík et al. (2022) suggested that animal populations may carry adaptations to certain environmental or climatic conditions, which may be an important driver of modern phylogeographic patterns. Further examination of genome-wide data, from modern and fossil common voles, will provide a better understanding of actual population dynamics behind the observed mtDNA patterns. For example, the details of the process behind the expansion of the E mtDNA lineage from its southern Carpathian nucleus and replacement of other lineages over a vast area of central and eastern Europe at the Pleistocene/Holocene boundary may serve as a case study for testing the role of the local adaptation in the population dynamics associated with changing environments.

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CONFLICT OF INTEREST

We have no competing interests.

DATA AVAILABILITY STATEMENT

The consensus mtDNA sequences generated in this study have been deposited in GenBank under Accession numbers OL588336–OL588524. The alignment used for the reconstruction of phylogeny have been deposited in Dryad (<https://doi.org/10.5061/dryad.4j0zpc8d9>). Mitochondrial alignments generated in this study have been deposited in the European Nucleotide Archive under project number PRJEB53474.

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SUPPORTING INFORMATION

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