Global Change Biology

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Global Change Biology

RESEARCH ARTICLE OPEN ACCESS

Coexistence, Extinction and Survival—The Evolutionary History of *Bison* Species in Western Eurasia

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Received: 16 February 2025 | **Revised:** 4 June 2025 | **Accepted:** 11 June 2025

Funding: This research was supported by Narodowe Centrum Nauki.

Keywords: ancient DNA | European bison | mitochondrial genomes | palaeoclimate | palaeodiet

ABSTRACT

European bison (*Bison bonasus*) are one of the few European megafaunal species to survive the Late Pleistocene mass megafaunal extinction. Current conservation management efforts have proceeded in the absence of information about their evolutionary history, which has been obscured by recent severe population bottlenecks. We characterized mitochondrial genomes from 135 ancient bison samples spanning > 50,000 years (> 50 ka) across the Eurasian continent and detected three distinct phylogenetic groups: two distinct clades of European bison and the extinct steppe bison (*Bison priscus*). The geographical distributions and dietary/ecological signatures of the three groups overlapped during the Late Pleistocene climate cycles and tracked environmental changes including vegetation cover and human impacts. The abundance of European bison specimens responded negatively to the extent of forest cover, including Holocene cycles of abrupt atmospheric-ocean circulation changes originating in the North Atlantic. European bison remain preferentially adapted to an open environment, but with today's anthropogenic landscapes, are now largely restricted to forest habitats with negative implications under scenarios of future environmental change.

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Rafał Kowalczyk and Alan Cooper have contributed equally to this work.

1 | Introduction

European bison (*Bison bonasus*), or wisent, are one of the only megafaunal species to survive the Late Pleistocene megafaunal extinctions in Europe, and remain the largest endemic vertebrate (Bocherens et al. 2015; Cooper et al. 2015). They were broadly distributed across the continent and (now submerged) North Sea during the Late Pleistocene (120,000–12,000 years ago; ka) and Holocene (12 ka to present day) (Hofman-Kamińska et al. 2019; Soubrier et al. 2016), along with the now extinct steppe bison (*Bison priscus*). However, by the end of the 19th Century, human persecution had restricted their distribution to two small populations (recognized as subspecies) in the Białowieża Forest between Poland and Belarus (*Bison bonasus bonasus*) and the Caucasus Mountains (*Bison bonasus caucasicus*) (Krasińska and Krasiński 2013; Pucek et al. 2004; Węcek et al. 2017). By the 1920s, European bison were extinct in the wild with just 54 individuals remaining in captivity, and these in turn were effectively descended from just 12 individuals (Krasińska and Krasiński 2013; Pucek et al. 2004; Slatis 1960).

The severe historical genetic bottleneck (Massilani et al. 2016; Soubrier et al. 2016; Tokarska et al. 2009, 2011; Węcek et al. 2017; Wójcik et al. 2009) and resulting extremely limited genetic diversity of modern European bison has obscured the genetic records of their evolutionary history, while range restriction to just two refugial locations has also obscured their ecological history and potential adaptive capacity, and as a result, they have been described as a refugee species (Kerley et al. 2012). However, the evolutionary history of European bison (Kerley et al. 2012), craniodental morphology (Mendoza and Palmqvist 2008), and the isotopic signatures of historical populations (Bocherens et al. 2015; Hofman-Kamińska et al. 2019) indicate adaptation to foraging in open habitats. This evidence is at odds with the re-introduction of European bison in non-optimal habitats such as forests (Kerley et al. 2012), where current populations are highly dependent on human care, leading to potentially serious impacts on genetic fitness. The pressing conservation needs of this species make the issue of potential resilience to environmental and anthropogenic challenges critically important, along with the increasing interest in restoring the ecological role of megaherbivores as habitat engineers and umbrella species in ecosystems such as Eurasia and North America (Svenning 2020; Svenning et al. 2024; Trepel et al. 2024). The European situation also provides important insights for current conservation efforts in North America, where the evolutionary history of North American bison (*Bison bison*) similarly revolves around two putative subspecies, the woods and plains bison (*B. b. athabascae* and *B. b. bison*), which have also experienced recent genetic bottlenecks and are currently managed separately in small populations (Cronin et al. 2013; Hartway et al. 2020).

Several ancient DNA (aDNA) studies have investigated the evolutionary history of bison in Western Eurasia (Grange et al. 2018; Massilani et al. 2016; Onar et al. 2017; Soubrier et al. 2016) using specimens principally from the North Sea, Ural Mountains, and France. Most of the data generated has been mitochondrial control region sequences, with smaller amounts of full mitochondrial genomes. The amount of ancient genomic data currently remains limited due to the sub-optimal preservation conditions of many sites, but has revealed that Late Pleistocene *Bison bonasus* mitochondrial lineages derive from a major admixture event with aurochs (*Bos primigenius*) around 120 ka, during the last (Eemian) interglacial (Massilani et al. 2016; Soubrier et al. 2016). Although geographically and temporally restricted, the ancient DNA data support the division of ancient European bison into two distinct clades, only one of which survives. An extinct Pleistocene clade was first termed Clade X, or colloquially "the Higgs Bison," to distinguish it from contemporary European bison (Massilani et al. 2016; Soubrier et al. 2016). Subsequent studies have termed these two groups *Bison bonasus* clades 1 and 2 (Bb1 and Bb2) (Grange et al. 2018; Massilani et al. 2016). We use this terminology for consistency, where Bb1 refers to the extinct Late Pleistocene clade and Bb2 refers to the clade including present-day European bison. Initial results suggest that Bb1, Bb2, and steppe bison might potentially reflect distinct groups adapted to specific ecological niches found in the Ural mountains/eastern Europe, western Europe, and western Siberia respectively (Massilani et al. 2016; Soubrier et al. 2016), but this hypothesis cannot be examined without a continental scale geographic and temporal sampling of genetically typed individuals.

In this paper, we generate 75 ancient mitochondrial genome sequences from bison specimens across Eurasia, spanning over 50 thousand years, substantially increasing the spatial and temporal coverage of previous studies. Together with published sequences, the total dataset comprises 135 bison mitochondrial genomes. In addition, radiocarbon dates and stable isotope information were generated for each of the new specimens. The resulting dataset allows the evolutionary history of European bison to be reconstructed in some detail, permitting comparison with contemporaneous environmental and climatic changes.

2 | Materials and Methods

2.1 | Sample Collection and Processing

Samples of bison specimens were collected from museums and private collections across Europe (Table S1) to complement an existing collection at the Australian Centre for Ancient DNA (ACAD), University of Adelaide. The final sample set includes specimens ranging from over 50 ka to present, across regions from Scandinavia and Siberia (Russia) to Italy and Turkey (Figure 1A,D). All ancient DNA work was performed in the purpose-built ancient DNA facilities at ACAD. Ancient DNA was extracted from bone fragments following a silica-based extraction protocol (Brotherton et al. 2013) with modifications as previously described (Soubrier et al. 2016). Double-stranded Illumina sequencing libraries were built from 25 μL of DNA extract following a partial uracil-DNA-glycosylase (UDG) treatment protocol (Rohland et al. 2015) modified to include dual 7-mer internal barcode sequences as previously described (Soubrier et al. 2016). We used RNA baits targeting bison mitochondrial genome sequences and in-solution hybridization capture followed by high-throughput sequencing as previously described (Soubrier et al. 2016) to generate complete or

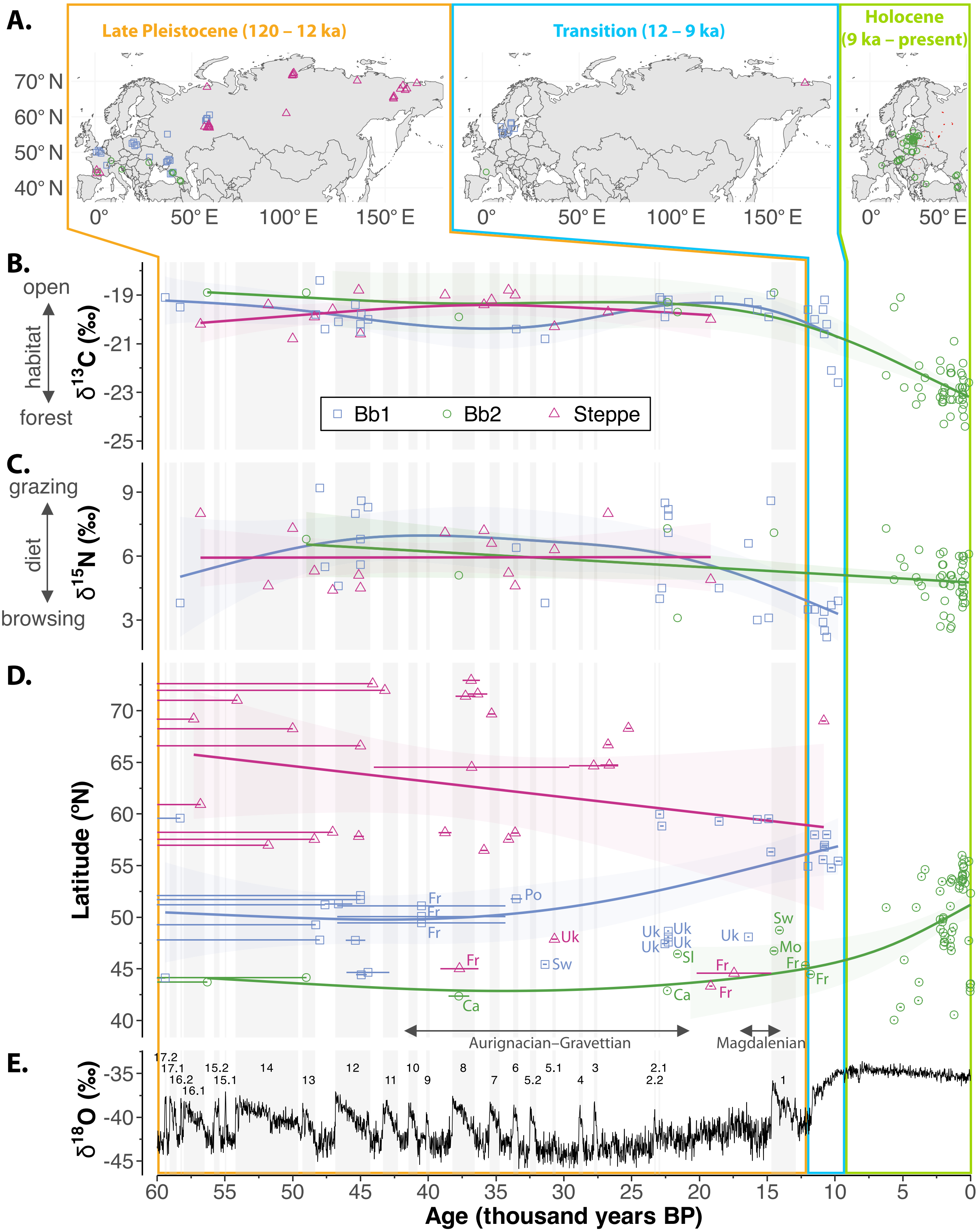

FIGURE 1 | Legend on next page.

FIGURE 1 | (A) Geographical distribution of bison samples during three time periods of interest in Eurasia; Bb1: Blue squares, Bb2: Green circles, and steppe bison: Purple triangles. Current distribution of European bison is represented in red (from Kowalczyk and Plumb 2022). Map lines delineate study areas and do not necessarily depict accepted national boundaries (B) $\delta^{13}C$ isotope values as a function of sample age. (C) $\delta^{15}N$ isotope values as a function of sample age. (D) Latitude as a function of sample age. The Aurignacian–Gravettian and Magdalenian periods are represented, with corresponding Western Eurasian specimens annotated with Ca (Caucasus), Fr (France), Mo (Moldova), Po (Poland), Sl (Slovenia), Sw (Switzerland), Uk (Ukraine). (E) NGRIP $\delta^{18}O$ record (Andersen et al. 2004) with Greenland interstadials numbered and highlighted in gray. Fitting lines in panels B–D are generalized additive models with integrated smoothness; colors correspond to bison clades and confidence intervals are highlighted.

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near-complete mitochondrial genomes from 81 bison samples, although we retained only 75 samples after data quality filtering (see below).

Sequencing reads were mapped to published mitochondrial genomes of European bison (chrM from NC014044) or Steppe bison (chrM from NC012346) according to the morphological assignment of bones (unidentified bones were mapped to steppe bison). We used the programs AdapterRemoval v2.2.1 (Lindgreen 2012), BWA v0.7.15 (Li and Durbin 2009), Picard Tools (broadinstitute.github.io/picard), Genome Analysis ToolKit (McKenna et al. 2010), SAMtools v1.3.1 (Li 2011; Li et al. 2009), and mapDamage v2.0.6 (Jónsson et al. 2013), as implemented in the pipeline PALEOMIX v1.2.9 (Schubert et al. 2014) and as previously described (Soubrier et al. 2016). The sequencing summary statistics for each specimen were compiled from the PALEOMIX pipeline output (Table S2). A mitochondrial genome consensus sequence was generated in Geneious v9.0.5 (geneious.com) with a minimum read depth of 3 and a consensus call threshold of 75%. Any ambiguities were visually inspected and manually realigned if necessary, and the resulting 75 consensus sequences were aligned to previously published bovid mitochondrial sequences using MAFFT7 (Katoh et al. 2002) to create a multiple sequence alignment (MSA). A Maximum-Likelihood (ML) tree was generated in PhyML v3.0 (Guindon et al. 2010) from the MSA (substitution model: HKY+G6, data type: nucleotide, tree improvement: BEST) to classify specimens as Steppe bison, Bb1, or Bb2. In cases where the ML tree species assignment did not match the initial morphological assignment, sequencing reads from that specimen were re-mapped to the morphologically appropriate reference genome and variants called as described above.

Sequence data from the 75 new specimens were combined with 17 mitochondrial genomes re-sequenced from enriched DNA libraries generated in (Soubrier et al. 2016) and (Onar et al. 2017), plus 43 published European bison mitochondrial genomes available on NCBI and ENA databases (i.e., specimens from western Eurasia identified as either Bb1, Bb2, *B. bonasus*, or *B. priscus*). We did not include two previously published mitogenome sequences, as one was chimeric (KY055664) (Węcek et al. 2017), and the other was sourced from a hyena coprolite (GAO1/KU886087) (Palacio et al. 2017) and has an unclear taxonomic origin (Grange et al. 2018). The resulting final dataset included 135 bison mitochondrial genomes from across western Eurasia (Table S1).

2.2 | Phylogenetic Analyses

Median-joining haplotype networks were generated in PopART (Bandelt et al. 1999; Leigh and Bryant 2015) from the alignment of all mitochondrial genomes, masking sites with more than 5% missing data from the analysis. Bayesian phylogenetic analyses were performed on the MSA of all complete mitochondrial genomes of European bison using BEAST v1.8.4 (Drummond and Rambaut 2007; Suchard and Rambaut 2009) through the CIPRES Science Gateway v3.1 (Miller et al. 2010) (HKY+G6 nucleotide substitution model, GMRF skyride, uncorrelated relaxed clock). Specimens were assigned to clades of European bison according to their position in an initial ML tree (Figure S3) for subsequent analyses. The radiocarbon dates of specimens were used as tip dates to calibrate the tree and calculate rates of molecular evolution. The ages of specimens with infinite radiocarbon error margins were treated as parameters to be estimated in the model. Steppe bison mitochondrial genomes were not included in the Bayesian phylogenetic analyses as the GMRF skyride coalescent tree prior implemented in BEAST requires all samples to be drawn from a single species. Three Markov Chain Monte Carlo (MCMC) chains were run for 100 million generations, sampling every 10,000 generations. The first 10% of generations were discarded as burn-in, and the remaining 90% combined using LogCombiner v1.8.3, with all parameters showing convergence when inspected in Tracer v1.7 (Rambaut et al. 2018). The resulting maximum clade credibility (MCC) tree was annotated in TreeAnnotator v1.8.4 and visualized in FigTree v1.4.2 (Drummond and Rambaut 2007). It is clear that Bb1 and Bb2 populations have relatively distinct geographical distributions, and it is likely that they were not randomly mating, which violates the assumptions of the Bayesian skyride (Ho and Shapiro 2011). As a result, we ran two separate skyride analyses, first with all Bb1 specimens and then with just Bb2 specimens. Both skyride plots were generated under the GMRF skyride analysis option in Tracer v1.5 (Ho and Shapiro 2011; Minin et al. 2008). For two samples with low coverage (A17406 and A17482), the BEAST maximum credibility tree was used as a backbone constraint in RAxML v8 (Stamatakis 2014) and the position of both samples estimated via ML.

2.3 | Stable Isotope Data

Stable isotope data ($\delta^{13}C$ and $\delta^{15}N$) were generated for the new samples at the Department of Geosciences at the University of Tübingen (Germany) using an Elemental Analyzer NC 2500 connected to a Thermo Quest Delta+XL mass spectrometer as previously described (Hofman-Kamińska et al. 2018). Previously published isotope data for Holocene European bison (Bocherens et al. 2015; Hofman-Kamińska et al. 2019; Soubrier et al. 2016) were collated and included in the analyses. In total, values for both $\delta^{13}C$ and $\delta^{15}N$ were available for 96 sequenced bison specimens. We used a Kruskal–Wallis test by ranks to identify differences in stable carbon and nitrogen isotope values ($\delta^{13}C$ and

$\delta^{15}N$, respectively) for the 3 bison clades in the Late Pleistocene (120–12 ka). Using a Mann–Whitney *U*-test, we tested for pairwise temporal differences in $\delta^{13}C$ and $\delta^{15}N$ signals between the Pleistocene and Holocene for Bb1 (120–12 ka and 12–9 ka) and Bb2 (120–12 ka and 9 ka–present). Analyses were performed using Statistica v.9.1 (StatSoft).

2.4 | Radiocarbon Dating and Kernel Density Estimation

Samples were AMS radiocarbon dated at the Laboratory of Ion Beam Physics, Eidgenössische Technische Hochschule Zürich, Switzerland (Swiss Federal Institute of Technology Zürich) (ETH). ^{14}C ages were calibrated with OxCal v4 using the IntCal20 curve (Table S1) (Ramsey 2009; Reimer et al. 2020). The quality of bone collagen was tested (collagen yield, proportion of C:N and collagen %N and %C) and samples with parameters different from fresh bones were excluded to avoid the need for extensive pre-treatment and ultra-filtration steps (Hofman-Kamińska et al. 2018). The distribution of Holocene radiocarbon ages exhibited an unusual degree of discrete clusters, so a kernel density estimation (KDE) was generated in OxCal v4.4 to provide a more robust estimate with less high-frequency noise, as an alternative to summed probability modeling (Turney et al. 2006). The standardized normal kernel was used to model the measurement and sampling error, where $K(\mathrm{u}) \sim N(0,1)$, with Silverman's rule (Silverman 1986) providing the optimal choice of bandwidth (Ramsey 2017). Ensembles of possible KDE distributions from the MCMC sampler (using the Bayesian likelihood and model priors) were then combined.

2.5 | Spectral Analysis

To investigate the potential relationship between climate changes and the temporal distribution of Holocene bison populations reflected by the KDE of the calibrated radiocarbon ages, we explored two key datasets. Whilst the North Atlantic Holocene record of iceberg-rafted debris (IRD) provides a continuous record of changes in ocean circulation with 8 recognized "Bond" events (Bond et al. 2001), unfortunately, the slow sedimentation rate and changing marine reservoir impacts prevents precise alignment with the calibrated ages of the bison samples. As a result, we compared the bison KDE data (Figure 2C–E) with an annually resolved population dataset of Irish bog oaks that provides a measure of westerly airflow over Eurasia (Figure 2A,B). The continuous Irish bog oak chronology of > 750 trees extends back to 7468 years ago and has been obtained from marginal environments across northern Ireland, and linked to the IRD records in the North Atlantic (Pilcher et al. 1984; Turney et al. 2005). Peaks in tree numbers have been interpreted as representing episodes of surface drying conducive for oak colonization (Turney et al. 2005, 2016). Previous studies have demonstrated that bog oaks are sensitive to precipitation/water table levels (García-Suárez et al. 2009; Scharnweber et al. 2015; Turney et al. 2016).

To explore the relationship between climate and European bison population over century to millennium timescales we undertook wavelet and cross-wavelet analysis that allows the identification of changing periodicities. Continuous wavelet transforms can be used for analysing data that may contain non-stationary power to identify periodicities in both the time and frequency domains (Torrence and Compo 1998). Wavelet and cross-wavelet analysis was undertaken on the KDE using the wt() and xwt() functions respectively in the R package "Biwavelet" (github.com/tgouhier/biwavelet). The cross-wavelet analysis allows the testing of relationships in time frequency space between two time series (Grinsted et al. 2004). A Morlet continuous wavelet transform was applied, and the data were padded with zeros at each end to reduce wraparound effects (Torrence and Webster 1999). The "cone of influence" represents the region where zero padding affects the estimation of the wavelet spectrum; the spectral peaks within this region are likely to be reduced in magnitude, and therefore may represent an artifact from the padding. To test the robustness of the obtained periodicities, the Lomb–Scargle algorithm was employed, a spectral decomposition method that computes the spectral properties of time series. This technique was undertaken using the lsp() function within the R package "lomb" (Ruf 1999).

3 | Results

3.1 | Sequencing Results

Of the 92 samples that were sequenced for the first time or re-sequenced, 75 had more than 70% coverage of the mitochondrial genome and an average depth-of-coverage ≥ 3. The remaining 17 samples had poor preservation but yielded sufficient data to determine their taxonomic affiliation. Sequencing and mapping summary statistics are presented in Table S2, and damage patterns for all specimens were consistent with ancient DNA (Table S3). Out of the 135 Eurasian bison included in this study, 107 specimens were directly radiocarbon dated and ranged in age from greater than 50,000 cal BP (calibrated radiocarbon years before present) to modern day (Table S1). For the remaining 28 specimens, 3 were undated, but 25 were dated indirectly or came from well-characterized archeological layers that provided an associated date range for the specimens (Hofman-Kamińska et al. 2019).

3.2 | Phylogenetic Analysis

The median-joining haplotype network (Figure S1) clearly shows three distinct groups of mitochondrial genomic diversity: two clades of European bison (Bb1 and Bb2), and steppe bison. There was no obvious genetic structure within either of the European bison clades, which is important as Bb2 includes the Holocene specimens previously classified as *B. b. bonasus* or *B. b. caucasicus* (Figures S3 and S4). The pronounced genetic divergence of the Bb1 and Bb2 mitochondrial clades (81 base changes, 0.5%) matches the genomic phylogeny (Soubrier et al. 2016), and is comparable to mitochondrial diversity seen within steppe bison (Froese et al. 2017). This indicates that Bb1 and Bb2 should be considered two clades of the same species. The Maximum Clade Credibility (MCC) tree generated in BEAST, calibrated with the sample radiocarbon dates, shows that the Bb1 and Bb2 clades diverged ~97 ka (95% Highest Posterior Density, HPD: 113–79 ka), centred around the warm

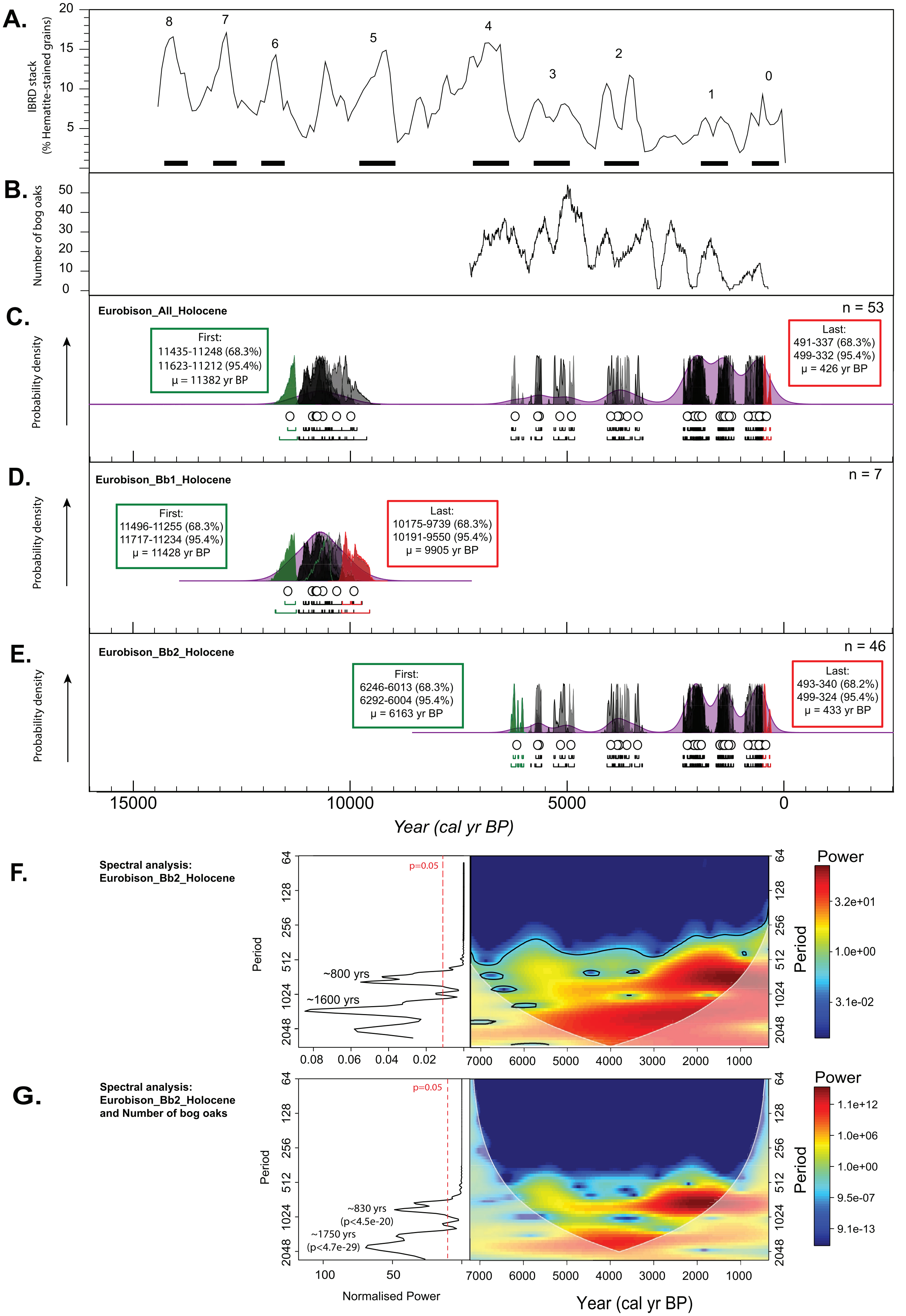

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FIGURE 2 | KDE model of Holocene specimens based on OxCal v4.4.4 with key climate records. (A) Iceberg-rafted debris (IRD) stack with "Bond" numbers and events indicated by the numbers and black bars (Bond et al. 2001); (B) Number of Irish bog oaks as a proxy of surface wetness over northern Ireland (Turney et al. 2016); (C–E) Holocene Kernel density estimation (KDE) for all Bison, population Bb1 and Bb2 respectively. Colored boxes labeled "First" and "Last" population cohorts and "Span" provide the range and median age estimates. (F) Spectral analysis for the KDE of the Holocene specimens (Bb2), including a Morlet wavelet transform (right) and a Lomb–Scargle periodogram (left). (G) Cross-wavelet power spectrum between KDE of the Holocene specimens (Bb2) and Irish bog oak number (right), and periodogram (left). Wavelet power spectra illustrate the change in concentration of spectral power with time with areas of red denoting strongest expression of the periodicity at ~800 and ~1600 years (F) and ~830 and ~1750 years (G). Solid black line in wavelet denotes 95% confidence in periodicity; white dashed line and shaded area denotes cone of influence where signal interpretation can be unreliable. Note, the peaks in bison numbers coincide with decreases in Irish bog oaks associated with wetter conditions over western Europe, consistent with the preference of these megafauna for relatively open landscapes.

phase of Marine Isotope Stage 5c. This date estimate is more precise and slightly younger than that obtained by Soubrier and colleagues (Soubrier et al. 2016) (~120 ka; 95% HPD: 152–92 ka) (Figure S2B), due to the additional radiocarbon date calibrations and mitochondrial data. Previous estimates for the divergence of Bb1 and Bb2 have been considerably earlier but were generated using less robust temporal calibrations (Massilani et al. 2016) (~246 ka; 95% HPD: 283–212 ka) and (Grange et al. 2018) (~395 ka; 95% HPD: 445–343 ka).

3.3 | Global Distribution and Ecology

All three groups (steppe bison, Bb1 and Bb2) were present in Europe until the end of the Pleistocene, but after the Pleistocene–Holocene transition (~12–9 ka) there was a major reduction in bison diversity, with only Bb2 surviving into the Holocene and modern day (Figure 1D). Reconstructed population sizes using GMRF (Gaussian Markov Random Field) Bayesian skyride plots revealed no signs of marked decreases prior to the extinction of Bb1 around the early Holocene, suggesting the decline was rapid. In contrast, Bb2 specimens showed a population decline starting ~10 ka (Figure S2A).

The geographical distributions of steppe, Bb1, and Bb2 bison overlapped across the European continent throughout the Late Pleistocene (Figure 1A,D,E). The steppe bison specimens appear more common during the cold Greenland stadials. The distribution of Bb1 specimens is concentrated across northern Europe and western Eurasia, while in contrast, the records of Bb2 individuals appear limited to southern and southwestern Europe. The European extirpation of steppe bison appears to occur during the end-Pleistocene, with the last individuals observed in Spain and southern France during the Last Glacial Maximum. Around the same time, the Bb1 clade also appears to contract in range and shift northwards into Scandinavia, prior to disappearing in the early Holocene, with the last identified specimen in Sweden at ~9900 cal BP (Figure 1A,D). The fossil record of the Bb2 clade disappears around the Pleistocene–Holocene transition, before reappearing by the mid-Holocene, initially in Poland ~6242 cal BP, and then quickly re-emerging across the rest of the European continent (Figure 1A,D). Despite this expansion, by the late Holocene, Bb2 specimens were restricted predominantly to the Caucasus Mountains and the Białowieża Forest (Poland), until their extinction in the wild by the 1920s (Figure 1A,D, Table S1).

During the Late Pleistocene there are no clear differences in the dietary δ^{13}C and δ^{15}N stable isotope values between Steppe bison, Bb1, and Bb2 (Table S4) with all three groups showing signals of graze and mixed diet (higher proportions of δ^{15}N) and utilization of open habitats (higher proportions of δ^{13}C) (Figure 1B,C). The dietary isotopic signals changed during the rapid climatic shifts at the Pleistocene–Holocene transition (12–9 ka), with a decrease in δ^{15}N levels observed in Bb1 specimens ($Z = 3.54$, $p = 0.0004$, $N = 30$) (Table S1). During this period, δ^{15}N in Bb1 appears to have remained low, but δ^{13}C changed from higher between ~12,000 and ~10,650 cal BP to lower between ~10,250 and ~9850 cal BP (δ^{13}C: −22.15‰ and −22.61‰) (Figure 1B,C) prior to becoming extinct. A parallel significant decrease in δ^{13}C signals can also be observed in Bb2 between the Late Pleistocene and Holocene ($Z = 3.48$, $p = 0.0005$, $N = 52$) (Table S4) suggesting a shift from more open Late Pleistocene habitats to more forested sites in the Middle Holocene. Two exceptions were from high-altitude meadows (1900 m.a.s.l.) around Sevan Lake in Armenia.

3.4 | Periodicity of Climate Changes and Bison Numbers

Analysis of the KDE plots with both wavelet and Lomb–Scargle spectral analyses demonstrates that the clumping pattern of the dates of the Holocene Bb2 specimens exhibits two pervasive periodicities at ~800 years and ~1600 years over the last 6000 years (within the cone of influence) (Figure 2F). Further exploration of the KDE plots with Holocene climate changes suggests a close relationship. The cross-wavelet plot between the Bb2 KDE and Irish bog oak number (Figure 2G) indicates a strong and significant correlation between the time series centered on two frequencies, corresponding to ~830 years and ~1750 years. The high power, represented by the red color, suggests that variations in one time series are strongly related to those in the other time series over the last 6000 years.

4 | Discussion

The broad geographic and temporal range of the sampled specimens provides the first comprehensive overview of bison paleoecology across Europe during the Late Pleistocene and the Holocene, and the genetic impacts of the megafaunal extinctions. The phylogenetic analyses illustrate a dramatic loss in bison genetic diversity across the Eurasian continent at the end of the Pleistocene, as exemplified by the extinction of both steppe and Bb1 bison (Figures S1 and S2B). For Bb2, the apparent loss of genetic diversity during the Late Pleistocene–Holocene transition might be due to extirpation or a lack of sampling in regions where they persisted. Either way, the loss of bison genetic diversity continued during the Holocene, where Bb2 mitochondrial diversity declined from 11 haplotypes during the early-to-mid Holocene to just four in the late Holocene (Figures S1 and S2).

The geographically broad dataset reveals the pre-Holocene Bb2 clade bison was more widespread across southern Europe than the previously reported isolated occurrence in the Caucasus region (Soubrier et al. 2016). Indeed, the Bb1 and Bb2 clades appear to have been present in roughly comparable numbers during the Late Pleistocene (Figure S2A), as shown by the overlapping highest posterior densities of the Bayesian skyride analyses. While more Bb1 individuals were found in Late Pleistocene sites, this may reflect a sampling bias due to geographic distribution or sample preservation. For example, Bb1 specimens were located across northern Europe and western Eurasia where cold conditions lead to good fossil bone preservation conditions. In contrast, late Pleistocene Bb2 specimens were found in southern and southwestern Europe where warmer conditions are less conducive to DNA preservation, except in mountainous areas. Interestingly, the distributions of Bb1 and Bb2 appear to match a species distribution modeling study of *Bison bonasus* (Pilowsky et al. 2023), which predicted separate late Pleistocene populations in western Russia and southwestern Europe. A longer-term geographic separation of *Bison bonasus* populations as suggested by this model could explain the genetic divergence between Bb1 and Bb2 mitochondrial and genomic data, estimated here at ~100 ka, MIS 5c. It would also match the Late Pleistocene geographic distributions observed in this dataset, with the Bb1 group distributed across the colder environments of western Russia and northern Europe.

The two currently recognized lineages or subspecies of European bison are the Lowland and Lowland-Caucasian bison (Pucek et al. 2004; Tokarska et al. 2011), and captive and wild populations of the two groups are currently kept artificially separated. This has important consequences for the genetic diversity of the species, which is very low in both lines (Tokarska et al. 2011). The Lowland-Caucasian bison is a hybrid of *B. b. bonasus* and *B. b. caucasicus*, as only one pure Caucasian male was available during restoration efforts (Tokarska et al. 2015, 2011). The ancient DNA records show that these two groups have shared the same mitochondrial clade (Bb2) throughout the Holocene and appear to be two descendant groups of the Late Pleistocene southern European distribution of *Bison bonasus*. The limited amounts of genetic differentiation currently observed between these groups appear to be an effect of geographic structure and differing numbers of founders during population restoration rather than distinct evolutionary paths (Tokarska et al. 2015, 2011), and do not justify separate management plans. This has important ramifications for the conservation management of modern European bison populations, where limited genetic diversity and the risk of disease (Didkowska et al. 2023; Kołodziej-Sobocińska et al. 2016) or environmental events on isolated herds are existential threats. Similar patterns are potentially likely for a number of other currently threatened megafaunal groups globally that have survived environmental and human-caused population bottlenecks. For example, the separation of Holocene populations of North American bison into separate plains and wood bison appears very similar, and the genetic evidence used to support the current strategy of separate management has been contested (Cronin et al. 2013).

Dietary isotopic signatures suggest that Late Pleistocene steppe, Bb1, and Bb2 bison all foraged in non-forested habitats and had broad but overlapping diets. Similar niche and dietary overlap of Late Pleistocene steppe bison and aurochs (*Bos primigenius*) has also been observed in Belgian and French sites (Bocherens et al. 2015). Late Pleistocene Eurasia supported a rich community of megaherbivores on a productive tundra steppe habitat that has no close modern analog (Guthrie 2013). While the steppe bison appears to have dominated cold dry steppe environments (Figure 1A,D), European bison specimens appear more common in the southern latitudes. Within that, however, the Bb1 clade appears to have been more common in colder, more open northern environments, while Bb2 was largely present in warmer southern and southwestern European sites. Steppe bison disappeared in Europe before the end of the Pleistocene, while the Bb1 group survived until the early Holocene by moving northwards into Scandinavia as temperatures increased and following the retreat of the Fennoscandanavian ice sheet. This movement suggests Bb1 was utilizing the shrinking open habitats as forests expanded (Figure 1A,D). The decreases in Bb1 dietary δ^{15}N signals during this period likely reflect the low nitrogen levels in the early stages of soil formation and vegetation following deglaciation and the dominance of shrubs and bushes in northern Europe preceding forest expansion (Hofman-Kamińska et al. 2019) (Figure 1C).

The stark contrast between dietary isotope signatures in Late Pleistocene and early Holocene bison individuals is consistent with the predicted forest cover across their respective geographical ranges (Figure 1B,C). During the Late Pleistocene, repeated glaciation events restricted cold-sensitive plant species (such as deciduous trees) to glacial refugia throughout Europe, resulting in a "mosaic" forest-steppe environment. Following deglaciation of the European Ice Sheet Complex (EISC) at the end of the Younger Dryas and the onset of the Holocene (~11.6 ka), continuing cold temperatures and seasonal dryness restricted forest expansion for the next two millennia. After this point, deciduous forest species rapidly expanded to cover the continent from ~10 ka onwards until reaching a maximum extent ~6.7 ka (Binney et al. 2017; Birks and Tinner 2016; Leuschner and Ellenberg 2017; Marquer et al. 2014; Patton et al. 2017; Theuerkauf et al. 2014). As the EISC retreated there were migrations of human populations into Scandinavia, northwards through Germany and Denmark, and southwards down the ice-free Norwegian Atlantic coast (Günther et al. 2018). As a result, the remaining Bb1 populations in early Holocene Scandinavia would have been restricted to a region between rapidly expanding dense forest to the south, newly arriving human populations, and the remaining ice sheets. This confluence of factors perhaps ultimately resulted in the extinction of the Bb1 clade.

Recent modeling studies suggest that a combination of climatic change and human pressure interacted with key ecological processes to cause the abrupt decline of European bison, similarly to steppe bison in Siberia (Pilowsky et al. 2023, 2022). Humans are likely to have exacerbated regional extinctions of these species by interrupting critical metapopulation processes (e.g., dispersal and recolonisation) that are critical in providing long-term demographic stability in the face of frequent and abrupt climate and environmental change, as has been suggested for other Late Pleistocene megafauna (Cooper et al. 2015; Fordham et al. 2024). Environmental change and habitat fragmentation are also recognized as major risk factors for extirpation or extinction of contemporary megafaunal herbivores due to their body size, low population density, larger spatial requirements, and disproportionate exploitation by humans (Cardillo et al. 2005). In this regard, it is interesting that the distributions of Bb1 and Bb2 appear differentiated by latitude, although it is important to note that the oldest non-Caucasus Bb2 specimen is 21.6 ka in Slovenia (Figure 1D; Table S1). Assuming Bb1 had a long-term presence in southern Europe during the late Pleistocene, this may have provided important adaptations to periodic warmer and more forested conditions, such as environmental and foraging plasticity (Gautier et al. 2016; Hofman-Kamińska et al. 2019). Such adaptations may have provided a key advantage to surviving the Holocene transition and rapid increase in forested habitats. The occupation of naturally open areas within forests such as river valleys and gaps after windfalls or insect/disease outbreaks is also likely to have provided important habitats for foraging and shelter from human pressure in the early Holocene, until the expansion of pastoralism and domestic animals (cattle, sheep, and subsequently pigs) into central European forest sites from around 6 ka (Zampirolo et al. 2023). It is notable that the reappearance of Bb2 in mid-Holocene Polish sites occurs at the same time as the expanding human pressure in central European forests, potentially competing for habitat. This suggests that increased human activity may have prevented populations of European bison from rebounding during the Holocene, despite seemingly improved environmental conditions following deforestation by humans (Pilowsky et al. 2023).

We hypothesize that European bison partly avoided human pressure by using dense forests as refuge habitats during the Holocene (Kerley et al. 2012) as indicated by isotopic signatures (Hofman-Kamińska et al. 2019) (Figure 1B,C), but ultimately faced gradual extirpation from the European continent at the end of the Holocene (Benecke 2005), potentially accelerated by the development of firearms (Pilowsky et al. 2023). Although the modern-day Bb2 bison forage under dense forest cover in a typically warm/humid environment (Hofman-Kamińska et al. 2018), during the Middle to Late Holocene this group was successfully occupying high-altitude (1900 m above sea level) meadows in the Caucasus. They survived in those sites in the wild until the beginning of the 20th century, considerably longer than in Polish-Lithuanian forests where bison have been under royal protection since the 15th century (Samojlik 2005). Together with the paleorecords, this supports the conclusion that dense forest environments are not optimal habitat for European bison, because they do not offer enough food for large herbivores in winter—the animals are given supplementary feed during winter months to compensate for the lack of dietary resources and avoid bison incursions into farmed lands (Hofman-Kamińska and Kowalczyk 2012; Samojlik et al. 2019). Open environments are far more suitable as they are much more productive, with longer periods with green grasses and large amounts of dry vegetation available in winter (Hurtado-Uria et al. 2013; Kowalczyk et al. 2019). However, not all open habitats provide the optimal conditions for the existence and survival of European bison. An average winter temperature below −5°C is a key climatic factor in current habitat use (Kuemmerle et al. 2011), so for example, while steppe bison inhabited the Iberian Peninsula during the Pleistocene, current and projected climatic conditions would not support the presence of European bison (Pilowsky et al. 2023). Milder winters mean temperatures only reach 0°C in the coldest month at the highest mountain peaks, while warmer conditions are projected for the future (IUCN/SSC, 2013), so the region would likely be unsuitable for re-introduction (Paniagua et al. 2019).

Bison comprise around 20% of the species depicted in European cave art, and the diversity of bison horn and body shapes is thought to reflect variants of the steppe bison and European bison, which were the only two bison groups known to be present in Europe during the Late Pleistocene (Soubrier et al. 2016). The cave artists accurately recorded morphological details, and the presence of both Bb1 and Bb2 clades in Late Pleistocene central and southern Europe raises the potential that both might be represented in the cave art. The genetic separation between Bb1 and Bb2 is greater than between American wood and plains bison, which have distinct morphologies and profiles, potentially related to ecological adaptations for forest and plains niches (Guthrie 2013). European cave art images include a distinctive steppe bison outline (pronounced humped back and downswept horns), a European bison-like profile (Bb1; upswept horns and flatter profile) (Soubrier et al. 2016), and also a number of unidentified forms (Soubrier et al. 2016). Many of the latter artworks were painted in southern French caves during the Magdalenian (17–14 ka) and in central France during the Aurignacian and Gravettian periods (~42–21 ka), when Bb2 does not appear to have been present (Figure 1D; Table S1). The only recorded Bb2 specimens in Western Eurasia during these periods were in Switzerland, Moldova, Slovenia, and the Caucasus (Figure 1D; Table S1). It is therefore unclear if Bb2 is part of the unidentified forms depicted in Paleolithic French cave art.

The apparent periodicity in the radiocarbon dates of the Holocene bison specimens suggests some form of multi-centennial- to millennial-scale forcing, with periodicities around ~800 and ~1600 years (Figure 2). The peaks in bison numbers also coincide with decreases in Irish bog oaks associated with wetter conditions (Figure 2A,B,G) (Turney et al. 2016). Climate changes with similar variability have previously been identified in Holocene Western Europe, including the content of ice-rafted debris in marine sediments and reconstructed atmospheric circulation over the North Atlantic (Bakke et al. 2008; Bond et al. 2001; Darby et al. 2012; Jonsson et al. 2010; Turney et al. 2005). These fluctuations appear to have had substantial impacts on climate and vegetation across the wider region, most notably Eurasia, where changes in glacier limits, lake levels, vegetation, and human populations have been reported (Balascio et al. 2015; Bevan et al. 2017; Daley and Barber 2012; Dobrowolski et al. 2016; Magny 2004; McDermott et al. 2001; Smith et al. 2016; Turney et al. 2006; Zielhofer et al. 2017). In the North Atlantic, peaks in ice-rafted debris are generally associated with wetter conditions (higher lake levels), a shorter growing season over western Europe, changes in vegetation cover, and a decrease in human population numbers (Bevan et al. 2017; Pèlachs et al. 2011; Poska et al. 2022).

The wetter conditions are related to a pervasive phase state in the North Atlantic Oscillation (NAO), characterized by surface sea-level pressure differences between the Icelandic (subpolar) Low and the Azores (subtropical) High pressure systems, which modulate the route and intensity of the North Atlantic jet stream and storm track (Smith et al. 2016). In the negative phase of the NAO, below-normal pressure over western Europe and high pressure in the high latitudes of the North Atlantic results in a weakening of the winter storm tracks, which migrate southwards, resulting in cooler air masses over northwestern Europe and wetter conditions across southern Europe (Trouet et al. 2009). The opposite pattern is observed during a positive phase of the NAO. Here, we show a synchronicity of climate changes and variations in dated bison specimens that is consistent with known 800-year cycles in westerly airflow and increased discharge of IRD into the North Atlantic resulting in colder and relatively short growing seasons across Europe. A number of scenarios may explain this observation. For instance, associated cooler conditions would have reduced vegetation growth, favoring open habitat spaces suitable for European bison, and potentially increasing bone deposition through larger population sizes and ranges, or enhanced bone preservation rates (e.g., alluvial sites, open areas). Alternatively, a similar impact may have resulted from reduced human activity in the landscape including a smaller agricultural footprint (Turney et al. 2006) so the driver remains somewhat unclear. Nevertheless, it is intriguing that short-term European climate cycles can be observed so clearly within Holocene megaherbivore skeletal remains.

5 | Conclusion

The extinction of steppe bison and the Bb1 European bison group was associated with human pressure but also the development of dense forest coverage across most of Europe around the Late Pleistocene–Holocene transition. This prompted the potentially more northerly distributed Bb1 group to follow shrinking open habitats into Scandinavia before eventually becoming extinct. The Bb2 lineage potentially survived due to having a Late Pleistocene distribution in southern and southwestern Europe, where warmer climates and forested habitats periodically occurred. This group appears to have survived in open areas of forests during the early Holocene before recolonizing the continent in the mid-Holocene. This geographic expansion coincides with human population growth during the Neolithic, including the increasing use of forest habitats for pastoralism. The observation that forests provide sub-optimal European bison habitat has important implications for long-term bison conservation under projected climate warming (Kerley et al. 2012). In particular, the combination of our genetic, ecological, and climate findings strongly challenges the proposed rewilding initiative to use extant European bison as a megaherbivore replacement for extinct steppe bison in Spain (Nores et al. 2024).

Although modern European bison populations occur in an increasing number of locations and are becoming more numerous (Kowalczyk and Plumb 2022), isolation and low genetic variability pose a threat to their survival and restoration of their role as environmental engineers in ecosystems. The current artificial isolation of genetic lineages based solely on several hundred years of geographic isolation, rather than their genetic history, appears unwarranted and increases threats in multiple ways, including disease susceptibility and reproductive issues. It is critical that the evolutionary history of species is used to inform current conservation management of these last remnants of the European megafaunal ecosystem.

Author Contributions

Bastien Llamas: conceptualization, formal analysis, methodology, supervision, visualization, writing – review and editing. **Ayla L. van Loenen:** data curation, formal analysis, methodology, writing – original draft, writing – review and editing. **Kieren J. Mitchell:** methodology, supervision, writing – review and editing. **Emilia Hofman-Kamińska:** data curation, formal analysis, writing – review and editing. **Hervé Bocherens:** resources, writing – review and editing. **Holly Heiniger:** methodology, writing – review and editing. **Martina Pacher:** resources, writing – review and editing. **Daniel Makowiecki:** resources, writing – review and editing. **Giedrė Piličiauskienė:** resources, writing – review and editing. **Dorothée G. Drucker:** resources, writing – review and editing. **David Brown:** resources, writing – review and editing. **Zoe A. Thomas:** formal analysis, methodology, visualization, writing – review and editing. **Chris S. M. Turney:** methodology, resources, writing – review and editing. **Rafał Kowalczyk:** conceptualization, funding acquisition, resources, writing – review and editing. **Alan Cooper:** conceptualization, funding acquisition, methodology, resources, supervision, writing – review and editing.

Acknowledgements

This research was supported by the Australian Research Council, the National Science Centre, Poland projects no: DEC-2013/11/B/NZ8/00914; N N304 301940 and 2015/17/N/ST10/01707 and the European Commission's project No. PIRSES-GA-2009-247652, BIOGEAST within the Seventh Framework Programme. We thank for providing access to specimens: D. Anatolie, N. Spassov, M. S. Arakelyan, N. H. Manaseryan, M. Križnar, V. Rusu, T. V. Krakhmalnaya, L. Chaix, C. Cupillard, Dr. M. Nussbaumer, E. Keczyńska- Moroz, L. Wickström, K. Rauscher, A. A. Krotova, A. T. Halamski, J. Kobylińska, A. Stępień, H. Długoszewska-Nadratowska, M. Krajcarz, B. Stachowiak, B. Studencka, I. Lorek, A. Archacka, M. Szymkiewicz, J. Jastrzębski, J. Deptuła, A. D. Ya, B. Antoniuk, A. Sepioł, N. Czeremnyh, D. O. Klimishin, V. Boretskiy, P. Kjellander, A. Hultman, V. Gedminas, U. B. Göhlich, F. E. Zachos, K. Berggren, and K. M. Gregersen, W. Litwińczuk, A.-M. Hultman, L. Golovanova, V. Doronichev. We thank T. Kamiński for assistance with map preparation. A.L.L. was supported by an Australian Government Research Training Program (RTP) Scholarship. K.J.M., B.L., and A.C. were supported by the Australian Research Council. Open access publishing facilitated by The University of Adelaide, as part of the Wiley - The University of Adelaide agreement via the Council of Australian University Librarians.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Aligned DNA sequences for the new mitogenomes are available in the Sequence Read Archive under BioProject PRJNA1279061. Dietary isotope data and radiocarbon dates are available in Figshare (DOI: 10.25909/29253974; CC BY-NC 4.0).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.