DR. ALEXANDER THEODORE SALIS (Orcid ID : 0000-0002-3205-3006)

Article type : From the Cover

Lions and brown bears colonised North America in multiple synchronous waves of dispersal across the Bering Land Bridge

Alexander T Salis[1,2#], Sarah C E Bray[1,3], Michael S Y Lee[4,5], Holly Heiniger[1], Ross Barnett[6], James A Burns[7], Vladimir Doronichev[8], Daryl Fedje[9], Liubov Golovanova[8], C Richard Harington[10†], Bryan Hockett[11], Pavel Kosintsev[12,13], Xulong Lai[14], Quentin Mackie[9], Sergei Vasiliev[15], Jacobo Weinstock[16], Nobuyuki Yamaguchi[17], Julie A Meachen[18], Alan Cooper[5#*], Kieren J Mitchell[1#*]

[1]Australian Centre for Ancient DNA (ACAD), School of Biological Sciences, University of Adelaide, South Australia, Australia, 5005

[2]Division of Vertebrate Zoology, American Museum of Natural History, New York, NY, USA

[3]Registry of Senior Australians (ROSA), South Australian Health and Medical Research Institute (SAHMRI), Adelaide, South Australia, 5000

[4]College of Science and Engineering, Flinders University, Bedford Park, South Australia, 5042

[5]South Australian Museum, Adelaide 5000, South Australia

[6]Natural History Museum of Denmark, University of Copenhagen, Copenhagen, Denmark

[7]Curator Emeritus, Royal Alberta Museum, Edmonton, Alberta, Canada, T5J 0G2

[8]ANO Laboratory of Prehistory, St Petersburg, Russia

[9]Department of Anthropology, University of Victoria, Victoria, B.C., Canada

[10]Curator Emeritus and Research Associate, Research Division (Paleobiology), Canadian Museum of Nature, Ottawa, Canada

[11]US Department of Interior, Bureau of Land Management, Nevada State Office, Reno, Nevada, USA

[12]Institute of Plant and Animal Ecology, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia

[13]Department of History, Ural Federal University, Yekaterinburg, Russia

[14]State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan, Hubei 430074, China

[15]Institute of Archaeology and Ethnography, Russian Academy of Sciences, Russia

[16]Faculty of Humanities (Archaeology), University of Southampton, UK

[17]Institute of Tropical Biodiversity and Sustainable Development, University Malaysia Terengganu, 21030 Kuala Nerus, Malaysia

[18]Anatomy Department, Des Moines University, Des Moines, IA, USA

[19]Otago Palaeogenetics Laboratory, Department of Zoology, University of Otago, Dunedin, New Zealand

[†]Deceased 8th September 2021

[#]Corresponding author(s): A.T.S. (alexander.t.salis@gmail.com), A.C. (alanjcooper42@gmail.com), K.J.M. (kieren.j.mitchell@gmail.com)

*These authors contributed equally

Abstract:

The Bering Land Bridge connecting North America and Eurasia was periodically exposed and inundated by oscillating sea levels during the Pleistocene glacial cycles. This land connection allowed the intermittent dispersal of animals, including humans, between Western Beringia (far north-east Asia) and Eastern Beringia (north-west North America), changing the faunal community composition of both continents. The Pleistocene glacial cycles also had profound impacts on temperature, precipitation, and vegetation, impacting faunal community structure and demography. While these palaeoenvironmental impacts have been studied in many large herbivores from Beringia (e.g., bison, mammoths, horses), the Pleistocene population dynamics of the diverse guild of carnivorans present in the region are less well understood, due to their lower abundances. In this study, we analyse mitochondrial genome data from ancient brown bears (*Ursus arctos;* n = 103) and lions (*Panthera* spp.; n = 39), two megafaunal carnivorans that dispersed into North America during the Pleistocene. Our results reveal striking synchronicity in the population dynamics of Beringian lions and brown bears, with multiple waves of dispersal across the Bering Land Bridge coinciding with glacial periods of low sea levels, as well as synchronous local extinctions in Eastern Beringia during Marine Isotope Stage 3. The evolutionary histories of these two taxa underscore the crucial biogeographic role of the Bering Land Bridge in the distribution, turnover, and maintenance of megafaunal populations in North America.

Keywords

Ancient DNA, brown bears, lions, phylogeography, Beringia

Introduction:

During the Pleistocene (2.58 million to 11,700 years ago), Eastern Beringia — the area comprising Alaska and parts of Yukon Territory — was inhabited by numerous species of megafauna (Harington, Naughton, Dalby, Rose, & Dawson, 2003). Many of these taxa belonged to endemic New World lineages, such as the giant short-faced bear (*Arctodus simus*), Jefferson's ground sloth (*Megalonyx jeffersonii*), and the stilt-legged horse (*Haringtonhippus francisci*) (Harington et al., 2003; Kurtén & Anderson, 1980). However, Eastern Beringian megafaunal diversity also included non-endemic species that dispersed from Western Beringia — the area of Russia east of the Lena River — during the Pleistocene (Elias & Crocker, 2008; Elias, Short, Nelson, & Birks, 1996; Harington et al., 2003). Some of these immigrant taxa, including moose (*Alces alces*) and elk/wapiti (*Cervus canadensis*), appear to have arrived during the Last Glacial Maximum (LGM) when the Bering Land Bridge connecting Western and Eastern Beringia was most recently exposed (Guthrie, 2006; Hundertmark et al., 2002; Meiri et al., 2014). Other taxa apparently invaded much earlier in the Pleistocene, including bison (*Bison* spp.) (Froese et al., 2017; Shapiro et al., 2004), and mammoth (*Mammuthus* spp.) (Enk et al., 2016; Lister & Sher, 2015). However, the exact timeline and processes underlying early Pleistocene dispersals are currently poorly characterised, and it remains uncertain whether the arrivals of individual species represented independent chance events or were temporally concentrated in waves of dispersal following changes in climate and environment.

Sea level records from the Northern Pacific indicate that the Bering Land Bridge opened and closed multiple times during the Pleistocene (Hopkins, 1973; Hu et al., 2010) during glacial and interglacial periods, respectively. During glacial Marine Isotope Stage 6 (MIS 6) ~185 thousand years ago (kya) to 135 kya, sea levels were low enough to allow the Bering Land Bridge to be uncovered (Colleoni, Wekerle, Näslund, Brandefelt, & Masina, 2016; Hopkins, 1973). In the subsequent MIS 5, interglacial sea levels increased to higher than present-day, flooding the Bering Land Bridge from approximately 135 to 70 kya before it re-emerged again ~70 to 60 kya during glacial MIS 4 (Hu et al., 2010). Intermittent connections may have occurred again during MIS 3, before the final emergence during MIS 2/LGM starting ~34 kya and finishing 11 kya (Hu et al., 2010; Jakobsson et al., 2017).

Repeated glacial cycles had profound effects on vegetation, which could also influence animal dispersal. For example, increased temperature during interstadials is likely to have resulted in the landscape becoming wetter, in turn facilitating the accumulation of organic matter ("paludification") and the expansion of peatlands (Mann et al., 2015; Treat et al., 2019). Paludification is thought to have lowered nutrient availability and favoured less palatable plant species, negatively impacting megafaunal herbivore populations. Indeed, Mann et al. (2015) observed that during interstadials in Alaska there was an initial increase in megafaunal herbivore abundance followed by a decrease coincident with peatland expansion. In addition, bone nitrogen isotopes demonstrate that the diet of horses in Alaska changed radically coincident with an increase in peatlands during Greenland Interstadial 1 (14.7-12.9 kya) (Mann et al., 2015). Changes in herbivore communities are likely to have impacted populations of megafaunal carnivores and omnivores, potentially affecting their ability to colonise or persist in Eastern Beringia through multiple glacial cycles. However, our understanding of fine-scale carnivore responses to environmental change in Eastern Beringia has been limited by their relative rarity in the fossil record. Although several studies have used ancient DNA to examine megafaunal carnivoran population dynamics (e.g., Barnes, Matheus, Shapiro, Jensen, & Cooper, 2002; Barnett et al., 2009), sample sizes have generally been small and resolution limited.

During the Late Pleistocene, a number of megafaunal carnivorans roamed Eastern Beringia, including the giant short-faced bear (*Arctodus simus*), grey wolves (*Canis lupus*), and scimitar-toothed cats (*Homotherium serum*) (Harington et al., 2003; Kurtén & Anderson, 1980). Lions (*Panthera* spp.) and brown bears (*Ursus arctos*) appear to have dispersed into northern North America from Eurasia via the Bering Land Bridge during the Pleistocene (Kurtén & Anderson, 1980), but genetic data from North American lion and brown bear subfossils (preserved non-mineralised animal remains) have revealed a complicated evolutionary history (Barnes et al., 2002; Barnett et al., 2009; Davison et al., 2011; Ersmark et al., 2015; Leonard, Wayne, & Cooper, 2000). North American brown bears appear to represent distinct mitochondrial lineages with diverse origins. Likewise, Pleistocene lions from North America have been grouped into two distinct lineages based on both fossil evidence and genetic data, potentially representing two species (or alternatively two subspecies of the extant lion, *Panthera leo*) with different origins.

The current consensus is that the cave lion — *Panthera (leo) spelaea* — was distributed across Eastern Beringia and Eurasia, while the American lion — *Panthera (leo) atrox* — was found exclusively south of the North American Cordilleran and Laurentide Ice Sheets (Barnett et al., 2009; Baryshnikov & Boeskorov, 2001; Kurtén, 1985). Some authors originally referred Eastern Beringian lions to *P. l. atrox* (Harington, 1969; Harington, 1996; Whitmore & Foster, 1967) while others referred the same specimens to either *P. l. spelaea* (Kurtén, 1985; Sotnikova & Nikolskiy, 2006) or a completely separate subspecies, *Panthera leo vereshchagini* (Baryshnikov & Boeskorov, 2001). More recently, it has become widely accepted that *atrox* lions were confined south of the North American ice sheets, distinct from

Beringian lions (Barnett et al., 2009; Christiansen & Harris, 2009; Stuart & Lister, 2011); the genetic divergence between the American lion and its relatives is estimated to have occurred ~340 kya (Barnett et al., 2009), suggesting that the ancestors of the American lion entered North America prior to MIS 6, consistent with fossil records in Eastern Beringia where lion remains are found in Illinoian deposits onwards (Kurtén & Anderson, 1980; Yamaguchi, Cooper, Werdelin, & Macdonald, 2004). In contrast, molecular data suggest that brown bears first colonised North America ~70 kya (around the MIS 5/MIS 4 transition), and subsequently appear to have become locally extinct in Eastern Beringia between ~35 kya and 21 kya (Barnes et al., 2002; Davison et al., 2011; Kurtén, 1985).

Genetic data from ancient lions and brown bears has so far been limited to only short fragments of mitochondrial DNA and a relatively small number of individuals. As a result, both the timeline for dispersal and the number of waves of dispersal of brown bears and lions into North America are still relatively uncertain. To better understand the dynamics and assembly of the Eastern Beringian megafaunal carnivoran guild and their responses to climatic and environmental change, we sequenced near-complete mitochondrial genomes from 39 Pleistocene lions and 103 Pleistocene/Holocene brown bears from North America and Eurasia. In combination with new radiocarbon dates and previously published genetic data this allowed us to refine the phylogenetic and temporal histories of both groups and identify common drivers of dispersal and turnover.

Materials and Methods:

Sample preparation, DNA extraction, library preparation, and mitochondrial enrichment

We sampled 120 brown bear subfossil bone and tooth specimens from northern Asia and North America, and 47 lion subfossils from Europe, northern Asia, and North America (Supplementary Tables S1 and S2). New radiocarbon ages for 26 samples were obtained from the Oxford Radiocarbon Accelerator Unit of the University of Oxford. These new radiocarbon data were combined with published dates from North American brown bears and lions, as well as *Arctodus simus* (Supplementary Table S3). All radiocarbon dates were calibrated with the IntCal13 curve (Reimer et al., 2013) using OxCal v4.4 (Ramsey, 2009).

Sample preparation, DNA extraction and library construction were conducted in purpose-built ancient DNA (aDNA) clean-room facilities at the University of Adelaide's Australian Centre for Ancient DNA (ACAD) or the Henry Wellcome Ancient Biomolecules Centre at the University of Oxford, and a number of precautions were taken to minimise contamination of samples with exogenous DNA (Cooper & Poinar, 2000).

DNA extraction was performed on bone or tooth powder using either an in-house silica-based extraction protocol adapted from Dabney et al. (2013) or a phenol-chloroform-based extraction protocol from Bray et al. (2013). Double-stranded Illumina libraries were constructed following the protocol of Meyer et al. (2012) with truncated Illumina adapters with unique dual 7-mer internal barcodes added to allow identification and exclusion of any downstream contamination. Further, partial uracil-DNA glycosylase (UDG) treatment (Rohland, Harney, Mallick, Nordenfelt, & Reich, 2015) was included to restrict cytosine deamination to terminal nucleotides.

Brown bear libraries were enriched with home-made RNA baits following Richards et al. (2019) produced from long-range PCR fragments amplified from modern brown bear DNA using primers from Hwang et al. (2008). For lion libraries, commercially synthesised biotinylated 80-mer RNA baits (Arbor Biosciences, MI, USA) were used to enrich for mammalian mitochondrial DNA (Mitchell et al., 2016). DNA-RNA hybridisation enrichment was performed according to manufacturer's recommendations (MYbaits protocol v3). Libraries were pooled and sequenced on an Illumina NextSeq using 2 x 75 bp PE (150 cycle) High Output chemistry. A more detailed description of the laboratory methods is available in the Supplementary Material.

Data processing

Sequenced reads were demultiplexed using SABRE (https://github.com/najoshi/sabre) and were then processed through Paleomix v1.2.12 (Schubert et al., 2014), with adapter sequences removed and pair end sequences merged using ADAPTER REMOVAL v2.1.7 (Schubert, Lindgreen, & Orlando, 2016), and merged reads mapped against either the mitochondrial genome of *Panthera spelaea* (KX258452) or *Ursus arctos* (EU497665) using BWA v0.7.15 (Li & Durbin, 2009). Reads with mapping Phred scores less than 25 were removed using SAMTOOLS 1.5 (Li et al., 2009) and PCR duplicates were removed using "paleomix rmdup_collapsed" and MARKDUPLICATES from the Picard package (http://broadinstitute.github.io/picard/). Data from our lion samples exhibited signals consistent with the presence of nuclear mitochondrial DNA segments (numts), which are known to be widespread in felid genomes (Kim et al., 2006). The numt sequence was identified and lion samples were remapped with the numt sequence included as an additional scaffold to allow separation of true mitochondrial sequences and numt sequences. Mapped reads were visualised in Geneious Prime v2019.0.4 (https://www.geneious.com) and we created a 75% majority consensus sequence, calling N at sites with less than 3x coverage. Subsequent analyses were restricted to specimens with greater than 70% of the mitochondrial genome covered, representing 103 and 39 of the brown bear and lion samples respectively. Published sequencing data from one modern brown bear (Liu et al., 2014) and two ancient cave lions (Barnett et al., 2016) were also processed through the pipeline described above (Supplementary Table S4). A more detailed description of the data processing methods is available in the Supplementary Material.

Phylogenetic analyses

Brown bear consensus sequences were aligned using MUSCLE v3.8.425 (Edgar, 2004) in Geneious Prime v2019.0.4 with an additional 46 brown bear and polar bear mitogenomes downloaded from GenBank (Supplementary Table S5). Lion sequences were aligned separately also using MUSCLE v3.8.425. PartitionFinder v2.1.1 (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016) was used to find the best-fitting partitioning scheme using the Bayesian information criterion, separating the data into five partitions for each alignment (Supplementary Table S6). Bayesian tip-dating analyses were then performed on each taxon using BEAST v2.6.1 (Bouckaert et al., 2019). The temporal signal in our dataset was evaluated using leave-one-out cross-validation (e.g., Stiller et al., 2014), using only the finite-dated specimens (Supplementary Fig. S1). The ages of undated specimens were then estimated one at a time using the dated specimens as calibration for the molecular clock (Supplementary Fig. S2). Once all samples were assigned an age (either based on radiocarbon dating or Bayesian date estimation), we conducted a date-randomisation test (Ramsden, Holmes, & Charleston, 2009; Stiller et al., 2014), to test for sufficient temporal signal within the datasets (Supplementary Fig. S3). Runs described above were performed with a strict clock with a uniform prior on rate (0–10^{-5} mutations per site per year), constant population coalescent tree prior with a 1/x distribution on population size, a uniform prior (0–500,000) on the age of the sequence being estimated (if required), and run for 30 million steps with sampling every 3,000 steps. Convergence was checked in Tracer v1.7.1 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018). Final BEAST analyses were conducted using a strict clock with a uniform prior on rate (0–10^{-5} mutations per site per year), and a Bayesian skyline coalescent tree prior. We ran three independent MCMC chains, each run for 50 million steps, sampling every 5,000 steps. Results from individual runs were combined using LogCombiner after discarding the first 10% of steps as burn-in. Maximum clade credibility trees were generated in TreeAnnotator using the median node age.

To test for the association of migrations between Eurasia and North America with glacial periods, phylogeographic model testing was performed in BEAST (Suchard et al., 2018). The same substitution model settings were used as described above, but the alignments were combined in a single analysis, with a separate tree estimated simultaneously for each taxon. Clade 2 brown bears were excluded from the analysis due to lack of sampling, and the introgressed nature with polar bears resulting in a complicated evolutionary history of the clade (Cahill et al., 2013; Cahill et al., 2018; Cahill et al., 2015; Edwards et al., 2011; Hailer, 2015; Hailer & Welch, 2016; Miller et al., 2012). Each tip was assigned a binary phylogeographic character (Eurasia vs North America), and the rate of evolution of this character was estimated directly from the data. Two models for the evolution of this character were tested: a strict clock, where rates of evolution were constant through time, and a two-epoch clock that had two separate rates (one for interglacial periods and one for glacial periods). Note, in this method, tree topology and dispersal times for the two clades are essentially estimated separately (unlinked trees), but dispersal rates for the two epochs (combined glacial and combined interglacial) are estimated based on the pooled data from both trees, and have identical priors. Bayes factors were estimated and compared using Akaike's Information Criterion for MCMC samples in Tracer v1.7.1 (Rambaut et al., 2018). Four independent MCMC chains were run for 20 million steps each, sampling every 2,000 steps. We checked for convergence and sufficient sampling of parameters in Tracer. A more detailed description of the phylogenetic analysis methods is available in the Supplementary Material.

Results:

Brown bears

We produced 103 new near-complete (i.e., >70% coverage) mitogenomes from Pleistocene/Holocene subfossil *Ursus arctos* specimens from North America (n=53) and Eurasia (n=50), which we analysed along with previously published data from 47 brown bears and polar bears (Hirata et al., 2013; Lindqvist et al., 2010; Liu et al., 2014; Miller et al., 2012; Rey-Iglesia et al., 2019), spanning 107 unique mitochondrial haplotypes. We used BEAST2 (Bouckaert et al., 2019) to create a time-calibrated phylogenetic tree (Fig. 1), which was largely concordant with previous studies in grouping Beringian brown bear mitochondrial diversity into four major spatio-temporally restricted clades: clade 2 (including clade 2a, 2b, and 2c, and also encompassing extant polar bears), clade 3 (including 3a, 3b, and 3c), clade 4, and clade 5 (Barnes et al., 2002; Davison et al., 2011; Hirata et al., 2013; Leonard et al., 2000; Talbot & Shields, 1996; Waits, Talbot, Ward, & Shields, 1998). The temporal and geographic distributions of the different clades appear to result from dispersals into Eastern Beringia at widely different points in time.

Within Eurasia we identified three ancient specimens (A155, A156, and A1945) with haplotypes closely related to North American clade 3b bears, and five deeply divergent Eurasian clade 3b bears (A138, A1944, A1946, A5889, and MH255807), including a published mitogenome previously assigned to clade 3c (28) (Fig. 1; Supplementary Fig. S4).

The addition of these specimens increased the estimate for the Time to Most Recent Common Ancestor (TMRCA) for Eurasian and North American clade 3b bears from 75 kya (Davison et al., 2011) to 114 kya (95% Highest Posterior Density [HPD]: 100.2–127.3 kya). We also identified a new haplotype that is sister-taxon to all clade 4 bears from an ancient specimen (A5883) from Da'an Cave in Northeast China, for which we estimated a median age of 103 kya (95% HPD: 66.7-140.6 kya).

Our time-calibrated Bayesian phylogenetic analysis returned median age estimates for five Eastern Beringian brown bear specimens that were older than the previous ~70 kya estimate for the initial colonisation of North America (Barnes et al., 2002; Davison et al., 2011; Kurtén & Anderson, 1980): A345 at 78.3 kya (95% HPD: 58.6–98.9 kya), A335 at 82.4 kya (95% HPD: 64.9–103.3 kya), A298 at 95.1 kya (95% HPD: 64.9–127.1 kya), A193 at 100 kya (95% HPD: 74.0–130.2 kya), and A318 at 111.4 kya (95% HPD: 79.0–148.8 kya) (Supplementary Fig. S2A). These older samples likely descend from the original wave of brown bears entering North America, and all belong to either mitochondrial clade 2c or 4 (Figs 1 and 2), neither of which is found in Eastern Beringia after 35 kya. Clade 4 bears are currently restricted to the contiguous USA and appear to have diverged from Eastern Beringian clade 4 bears ~83 kya (95% HPD: 73.4-93.8 kya), soon after the 92 kya TMRCA for all North American clade 4 brown bears (95% HPD: 83.2–101.6 kya). In turn, North American clade 4 brown bears appear to have diverged from Eurasian clade 4 bears (found today in Japan) much earlier, ~177 kya (95% HPD: 154.5–201.7 kya), during MIS 6. The other early bears, clade 2c, are currently represented by only six pre-35 kya samples from Eastern Beringia (related haplotypes have not been found in any modern bears) and have a TMRCA in early MIS 5, ~121 kya (95% HPD: 114.4–128.5 kya). An additional extinct clade, 3c, was also identified in Eastern Beringia between 40 and 35 kya, and these 15 specimens make up the majority of samples found during that time period. The TMRCA of the 15 clade 3c brown bears indicates that the clade arrived in Eastern Beringia during MIS 4 ~69 kya (95% HPD: 62.3–75.2 kya).

There is a marked absence of brown bears in the Eastern Beringian fossil record between 35 and 25 kya (Fig. 2) as previously noted (Barnes et al., 2002), and after this point all samples belonged to either clade 3b or 3a. Clade 3b is the dominant group through MIS 2, comprising 13 samples, and appears to have arrived during the LGM with a TMRCA ~25 kya (95% HPD: 22.9–28.1 kya) (Fig. 1). The upper limit of this dispersal is constrained by a 39 kya estimate for the TMRCA with the closely related Eurasian clade 3b brown bears (95% HPD: 31.9–46.4 kya). In contrast, clade 3a is represented by only a single Holocene specimen and two previously published modern bears, presumably constituting a terminal-Pleistocene dispersal into North America, as clade 3a bears arrive in Japan at a similar time (Hirata et al., 2013).

Lastly, we recovered mitochondrial data from ten ancient clade 2a bears from Haida Gwaii and Prince of Wales Island (Alexander Archipelago). Clade 2a is closely related to the polar bear mitochondrial clade 2b, and a divergent clade 2a specimen (A308) was also recovered from Engineer Creek Mine near Fairbanks, Alaska dating to 23.3 kya, the first record of clade 2a in interior Alaska. This specimen was previously reported as belonging to clade 2b based on control region sequences (Barnes et al., 2002; Davison et al., 2011), although doubts about species assignment (polar bear versus brown bear) and provenance have been raised (Barnes et al., 2002; Edwards et al., 2011). In any case, the TMRCA of all Haida Gwaii and Alexander Archipelago specimens dates to ~20 kya (95% HPD: 17–24 kya), while the TMRCA between the Engineer Creek sample and all other clade 2a bears is 41 kya (95% HPD: 32.7–28.7 kya).

Lions

We produced 39 new near-complete mitogenomes from lion subfossil material from North America (n=24) and Eurasia (n=15), and analysed these along with two mitogenomes reconstructed from previously published data (Barnett et al., 2016), represented by 35 unique haplotypes. The topological results of our phylogenetic analyses were in broad agreement with past studies, supporting the existence of two geographically restricted clades (Fig. 1B) corresponding to *Panthera* (*leo*) *spelaea* (Eastern Beringia and Eurasia) and *Panthera* (*leo*) *atrox* (all other North American specimens from Edmonton southwards). We observed one important exception to this pattern: a specimen from Sixtymile River in Yukon Territory (~64°N), A181, possessing an *atrox* (American lion) mitochondrial haplotype (Fig. 1;

Supplementary Fig. S5), the first genetically confirmed *atrox* specimen ever recorded from any locality farther north than Edmonton (~53°N). Radiocarbon dating of this specimen yielded an infinite radiocarbon age (>51,500 uncal. yBP), but our Bayesian phylogenetic analyses suggested a median age for the specimen of 67 kya (95% HPD: 51.5–84.5 kya). The TMRCA of all *atrox* lions, representing the split between the two older *atrox* specimens (>50 kya, including A181) and the younger specimens (< 35 kya), dates to MIS 5 ~81 kya (95% HPD: 74.7–87.6 kya).

Our Bayesian analysis indicated a split date between *Panthera* (*leo*) *spelaea* and *Panthera* (*leo*) *atrox* of approximately 165 kya (95% HPD: 145.0–185.2 kya). This MIS 6 divergence date is substantially younger than the previous estimate of 337 kya (95% HPD: 194.0–489.0 kya) based on short control region sequences (Barnett et al., 2009), which was likely an overestimate resulting from application of a fossil-based node-age constraint and the time-dependency of mitochondrial substitution rates (Subramanian & Lambert, 2011). By relying on radiocarbon-dated tips to calibrate our analysis we have minimised the impact of rate time-dependency, allowing more accurate dating of population splits and sample ages, as demonstrated by the results of our leave-one-out cross-validation (Supplementary Fig. S1).

Within Beringian lion diversity we were able to identify a genetically distinct pre-LGM mitochondrial clade of Eastern Beringian *Panthera* (*leo*) *spelaea* specimens with a TMRCA of 63 kya (95% HPD: 58.9–67.6 kya). These pre-LGM samples are genetically distinct from the two clades that include all younger Eastern Beringian lion specimens, which have TMRCAs of 23 kya (95% HPD: 22.1–24.5 kya) and 22 kya (95% HPD: 18.9–25.5 kya), and a combined TMRCA of 33 kya (95% HPD: 29.2–37.0 kya). This suggests that in addition to the original dispersal of the ancestors of *Panthera* (*leo*) *atrox*, lions appear to have dispersed into North America on at least two other occasions during the Late Pleistocene. It is notable that the hiatus in the fossil record between the pre- and post-LGM lion clades falls between 33 and 22 kya, closely mirroring the pattern of local extinction observed in brown bears (Fig. 2).

Phylogeography: Testing the influence of the land bridge.

The results of our separate phylogenetic analyses of brown bears and lions hinted at the existence of synchronous waves of dispersal and extinction tied to Pleistocene glacial cycles: in particular, most dispersal events seemed to occur during glacials, when the land bridge was present. To explicitly test whether the spatio-temporal distribution and parallel lineage turnover of lions and bears in Eastern Beringia were strongly affected by the presence or absence of the Bering Land Bridge, we performed a phylogeographic analysis in BEAST (Suchard et al., 2018). To overcome low power and over-parameterisation issues caused by the low number of dispersals in each clade, we used a novel approach uniting joint-tree (Sanmartin, Van der Mark, & Ronquist, 2008) and epoch-clock (Bielejec, Lemey, Baele, Rambaut, & Suchard, 2014) methods. We estimated both the bear and lion trees together in a single MCMC analysis (as separate unlinked trees); each tip in the trees (i.e., each specimen) was assigned an additional phylogeographic trait: Eurasia (Western Beringia) or North America (Eastern Beringia and South-of-the-Ice). We then estimated east-west dispersal rates (i.e., the rate of change of this phylogeographic trait) simultaneously across both the bear and lion phylogenies, along with all other parameters associated with the previous two separate analyses (i.e., clock models, substitution models, topology, branch lengths). By using a single shared biogeographic model, data from both brown bears and lions were pooled to estimate dispersal patterns and drivers (Sanmartin et al., 2008).

We compared two dispersal models using this method: (1) A simple null model, where a single dispersal rate across time was estimated, and (2) an epoch-based model where separate rates were estimated for two different groups of time slices — one rate for all periods when the Bering Land Bridge was likely emergent (i.e., glacials, even-numbered MISs) and another rate for all periods when the Bering Land Bridge was submerged (i.e., interglacials, odd-numbered MISs). Bayes factors (Kass & Raftery, 1995) provided moderate support for the epoch-based model over the single-rate null model (BF=3.038). The estimated dispersal rate for glacials was approximately 13 times higher than the dispersal rate during interglacials (1.56E-5 versus 1.22E-6 events per lineage per year). Figure 3 and Supplementary Figure S6 show the pattern driving this difference: branches along which dispersals were inferred are concentrated in glacials, yet the combined glacial epochs occupy less time and shorter tree length (compared to the combined interglacials).

Discussion:

Our new data regarding the age and genetic identity of brown bear and lion specimens from North America revealed previously unrecognised patterns of synchronous dispersal and extirpation in Eastern Beringia. For the first time we have detected a member of the *atrox* mitochondrial clade in Pleistocene lions from Eastern Beringia, and further revealed that Eastern Beringian lions from the *spelaea* clade comprise two temporally and genetically distinct sub-clades. This suggests that lions dispersed from Eurasia into Eastern Beringia in multiple waves that appear to have replaced earlier populations. Our results show that the timing of these dispersal and extinction events are mirrored by patterns observed in brown bears, and that Pleistocene glacial cycles were an important driver of population dynamics in both species. In particular, dispersal between Western and Eastern Beringia was heavily influenced by presence of the Bering Land Bridge, with inferred dispersal rates across both species being over an order of magnitude higher during colder periods (Fig. 3; Supplementary Fig. S6).

The respective origins of the American lion (*atrox*) mitochondrial lineage (~165 kya) and North American clade 4 brown bear lineage (~177 kya) — the earliest representatives of both species observed in North America (Fig. 2) — occurred during MIS 6, the Illinoian glaciation (Fig. 1), when the Bering Land Bridge was likely exposed (Fig. 4A). This is consistent with the first lion fossil records from Eastern Beringia in Illinoian deposits (MIS 6) (Kurtén & Anderson, 1980; Yamaguchi et al., 2004) followed by the first recorded lions occurring in Sangamonian (MIS 5) deposits in Kansas and Texas (Dalquest, 1962; Harington, 1969; Hibbard & Taylor, 1960). Notably, this also aligns with evidence that steppe bison (*Bison priscus*) and red foxes (*Vulpes vulpes*) arrived in North America during MIS 6 (Froese et al., 2017) or immediately prior (Kutschera et al., 2013; Statham et al., 2014), respectively. Further, a dispersal of caballine horses (*Equus* spp.) into North America from Eurasia likely occurred during MIS 6, after caballine horses initially expanded out of North America across the Bering Land Bridge into Eurasia earlier in the Pleistocene (Vershinina et al., 2021).

While our results suggest that clade 4 bears and *atrox* lions likely arrived in Eastern Beringia ~170 kya during MIS 6, they must have dispersed southwards soon afterwards, as individuals belonging to these lineages are never observed farther north than Edmonton (~53°N) following the end of MIS 3. The TMRCAs of the North American clade 4 brown bear clade at 92 kya and *atrox* lion clade (including all North American samples) at 81 kya both occurred during MIS 5, suggesting that both species dispersed southwards during this warmer period when ice sheets retreated and opened an ice-free north-south corridor (Fig. 4B). This movement coincides with the first southward dispersal of bison through the ice-free corridor between late MIS 6 and early MIS 5 (Froese et al., 2017; Heintzman et al., 2016; Shapiro et al., 2004). The dispersal and subsequent isolation of lions south of the ice was previously thought to have initiated the divergence between the American lion (*Panthera atrox*) and cave lion (*P. spelaea*) (Barnett et al., 2009). However, our discovery of a ~66.7 thousand-year-old *P. atrox* specimen north of the ice sheets in the Yukon Territory instead suggests that the formation of the endemic American lion lineage was more likely the result of their isolation in North America after the flooding of the Bering Land Bridge during MIS 5. Alternatively, this Yukon *atrox* sample could plausibly represent a migrant from south of the ice sheets, but we favour the former hypothesis as the timing of the split between *atrox* and *spelaea* coincides with the emergence of the Bering Land Bridge and there are no putative later examples of lions dispersing northwards.

Following MIS 6, the second wave of lion and brown bear dispersal into North America appears to have occurred during MIS 4 when lowered sea levels next exposed the Bering Land Bridge (Fig. 4C), corresponding with the respective TMRCAs of the North American endemic clade 3c bears and the clade comprising the four pre-LGM Eastern Beringian *spelaea* lions. However, during the interglacial period MIS 3, as the Bering Land Bridge was again submerged (Hu et al., 2010) (Fig. 4D), all lions (*atrox* and *spelaea*) and brown bears (clades 2c, 3c, and 4) appear to have become locally extinct in Eastern Beringia (Fig. 2), with *atrox* lions and clade 4 brown bears — descendants of the first wave of dispersal — surviving only in the contiguous USA and southern Canada. The absence of both brown bears and lions from the Eastern Beringian fossil record between 35 and 25 kya does not appear to be due to a taphonomic bias, as remains of the giant short-faced bear (*Arctodus simus*) are abundant during the same period (Fig. 2). Indeed, the reappearance of both lion and bear populations appears to be closely linked in time to the extinction of short-faced bears in the area, suggesting some form of competition (Barnes et al., 2002; Barnett et al., 2009; Davison et al., 2011; Ersmark et al., 2015; Leonard et al., 2000). Importantly, the timing of these carnivoran extinctions in Eastern Beringia coincides with evidence for widespread vegetation change in the region, namely expansion of peatlands caused by significant paludification (Mann et al., 2015; Reuther et al., 2020; Treat et al., 2019).

Populations of a number of megafaunal herbivores appear to have decreased during MIS 3, possibly related to the expansion of peatlands and restrictions on foraging and nutrition (Mann et al., 2015), which may have had reciprocal impacts on the megafaunal carnivores and omnivores that preyed upon them, plausibly causing the local extinction of both lions and brown bears. For example, musk-ox populations experienced a dramatic decrease in diversity and effective population size during MIS 3 (Campos et al., 2010), mammoth populations were steadily declining (Debruyne et al., 2008), and bison began to experience dramatic declines towards the end of MIS 3 into MIS 2 (Drummond, Rambaut, Shapiro, & Pybus, 2005; Lorenzen et al., 2011; Shapiro et al., 2004). In addition, it appears that non-caballine horses (i.e., *Haringtonhippus*) underwent a bottleneck during MIS 3 with only a single fossil specimen found in Eastern Beringia after ~31 kya (Guthrie, 2003; Heintzman et al., 2017) around the time that the brown bear and lion populations went extinct.

In contrast to lions and brown bears, the giant short-faced bear appears to have persisted in Eastern Beringia throughout MIS 3. It is possible that the mobility, large home range, and solitary behaviour that has been proposed for the giant short-faced bear (Matheus, 1995; Schubert & Wallace, 2009) may have allowed them to exploit food resources that were less available to lions or brown bears. Grey wolves also appear to be present in eastern Beringia throughout MIS 3, with no evidence of genetic turnovers (Leonard et al., 2007; Loog et al., 2020). Isotope analyses have suggested Beringian wolves had similar diets to that of Pleistocene lions and brown bears, consisting largely of large herbivores (Fox-Dobbs, Leonard, & Koch, 2008; Leonard et al., 2007; Pilot et al., 2010). However, dietary analysis of a mummified MIS 3 Beringian wolf indicated a diet with a significant proportion of aquatic resources (Meachen et al., 2020), possibly indicating wolves may have exploited resources that were less available to lions or brown bears in Beringia during MIS 3.

Following MIS 3, lions and brown bears do not reappear in the fossil record of Eastern Beringia until after 27 kya, at the height of the LGM (MIS 2), when the Bering Land Bridge once again connected Eurasia and North America. This coincides with the invasion of North America from Eurasia by wapiti and moose (Hundertmark et al., 2002; Meiri et al., 2014), and a secondary wave of bison dispersal across the Bering Land Bridge (Froese et al., 2017). The recolonising populations were genetically distinct from those present in Eastern Beringia pre-MIS 2 as well as those south of the ice sheets, confirming that they likely comprised a new wave of dispersal from Western Beringia (Fig. 4E). This wave of megafaunal dispersals associated with the re-emergence of the Bering Land Bridge in MIS 2 may also have included early Native American human populations, who are recorded shortly afterwards in the stratigraphic record of Chiquihuite Cave in Mexico, from approximately 26 kya (Ardelean et al., 2020).

The reappearance of lions and brown bears in Eastern Beringia during MIS 2 occurred at around the same time as the local extinction of *Arctodus*, which may relate to previously proposed competition between brown bears and *Arctodus* (Barnes et al., 2002; Steffen & Fulton, 2018). The apparent timing of the extinction of *Arctodus* in Eastern Beringia ~23 kya could be linked to the sharp climatic cooling associated with Heinrich Event 2 (24.3–23.3 ka BP), a period characterised by drastic climatic changes caused by large discharges of ice into the North Atlantic following the collapse of the Northern Hemisphere ice sheets (Heinrich, 1988; Hemming, 2004). In any case, the fact that Eastern Beringia was not instead recolonised by *atrox* lions and clade 4 bears from the contiguous USA may either reflect that conditions had not improved sufficiently to support lion and brown bear populations in

Eastern Beringia before the ice-free corridor closed during the LGM or suggest that some other geographical or biogeographical barrier prevented dispersal from south of the ice sheets. Concordantly, in bison there is little evidence for northward dispersal through the ice-free corridor until after the LGM when a pulse of south to north dispersal is observed (Heintzman et al., 2016). Further, it is possible that clade 4 bears and *atrox* lions did contribute to the new populations of brown bears and lions in Eastern Beringia but that we failed to observe any signal due to the strictly maternal inheritance of mitochondrial DNA or insufficient sampling.

All modern and ancient clade 2a brown bears from the Alexander and Haida Gwaii archipelagos coalesce at ~20 kya (95% HPD: 17.0–24.0 kya), comparable to the TMRCAs for Beringian clade 3b bears and *spelaea* lions. This supports the model proposed by Cahill et al. (2013) for the origin of clade 2a bears, under which the mitochondrial lineage was captured by brown bears following male-biased gene-flow into a population of polar bears stranded in the Alexander Archipelago after the retraction of ice sheets post-LGM. Assuming all our ancient Alexander and Haida Gwaii archipelago samples represent brown bears (or at least brown-polar hybrids), and that mtDNA diversity in the stranded polar bear population was low, the coalescence of our samples can be considered a proxy for the minimum age of hybridisation between polar and brown bears, and hence a minimum age for the arrival of brown bears in the Alexander and Haida Gwaii archipelagos post-LGM. If this is the case, then brown bears arrived in the islands no later than 17 kya (the lower bound of the 95% HPD). That timing is coincident with the first records of brown bears on the Haida Gwaii archipelago ~17.5 kya (Ramsey, Griffiths, Fedje, Wigen, & Mackie, 2004) and the existence of unglaciated western Alaskan coastline, which represents an alternative southward dispersal pathway into the continent that may also have been exploited by humans (Lesnek, Briner, Lindqvist, Baichtal, & Heaton, 2018; Shaw, Barrie, Conway, Lintern, & Kung, 2020).

Overall, our results highlight the key role of Pleistocene glacial cycles in driving the distribution and diversity of North American carnivorans. Glacial cycles may also have driven parallel waves of dispersal in other regions, such as across the Sakhalin land bridge that connected Japan with mainland Asia. Such a dispersal event may explain the origin and distribution of Japanese wolves (Matsumura, Inoshima, & Ishiguro, 2014; Niemann et al., 2021). Further, genetic evidence from modern Japanese brown bears suggests multiple waves of Pleistocene dispersal in a similar temporally staggered sequence, with present day Japanese mitochondrial diversity closely mirroring that observed in modern Eastern Beringia (i.e., clades 3a, 3b, and 4) and also exhibiting a marked phylogeographic structure (Hirata et al., 2013). Analysis of ancient Japanese brown bear specimens might allow determination of whether extinct Eastern Beringian clades such as 3c were also present in Japan during the Late Pleistocene.

Conclusion:

Lions and brown bears appear to display remarkably synchronous responses to Pleistocene glacial cycles. By combining phylogenetic data from these two Pleistocene carnivoran species in a shared common biogeographic model, we demonstrate a 13-fold increase in dispersal rate between Eastern and Western Beringia during glacials, when the Bering Land Bridge was present. This suggests that the Bering Land Bridge was the dominant route of dispersal for these taxa, as opposed to — for example — rafting, traversal of winter sea ice, or dispersal via island hopping (e.g., through the Aleutian Islands). By combining additional ancient DNA datasets from other species with trans-Beringian Pleistocene distributions (e.g., foxes), future studies may further refine the timing and magnitude of waves of faunal dispersal across the Bering Land Bridge.

A combined biogeographical approach may also be useful for exploring the timing of faunal dispersals through the ice-free corridor between the North American ice sheets, which available data suggest are biased southwards, with few observed northward dispersals. However, this apparent bias may be due to many ancient DNA studies focusing on recently immigrated taxa (e.g., brown bears, bison, wapiti, humans) for which Eastern Beringia acts as a source, with the contiguous USA likely a sink. Endemic North American species may exhibit different patterns of phylogeography and dispersal, and large ancient DNA datasets from species like the giant short-faced bear or the western camel (*Camelops hesternus*) would be valuable in evaluating this possibility. In any case, our densely-sampled study of two carnivorans contributes to the growing body of research suggesting remarkably concerted responses to Pleistocene geographical and environmental changes across many megafaunal taxa (e.g., Cooper et al., 2015).

Acknowledgements:

This article is dedicated to the memory of Dick Harington, who made many foundational contributions to the study of North American Quaternary palaeontology and palaeoecology, and who passed away during the final revisions of the manuscript. We would like to thank the following institutions for allowing access to specimens in their collections: University of Alaska Fairbanks Museum, University of Kansas Natural History Museum, University of Wyoming Geological Museum, Yukon Government, American Museum of Natural History, Cincinnati Museum, Bureau of Land Management, St. Petersburg Institute of Zoology, Krakow Institute of Zoology, the Russian Academy of Sciences, Palaeontological Institute Moscow, Zoological Museum of Moscow University, the Museum of the Institute of Plant and Animal Ecology of the Ural Branch of the Russian Academy of Sciences, Natural History Museum Stuttgart, University of Vienna, Museum of Natural History Vienna, Idaho Museum of Natural History, Royal Alberta Museum, Parks Canada, the Canadian Museum of Nature, Gwaii Haanas National Park Reserve and the Haida Nation. In addition, we are grateful to the following individuals who helped to collect and identify specimens and/or provided laboratory support during the early stages of the project: L. Orlando, T. Heaton, K. Chen, I. Barnes, A. Derevianko, E. Pankeyeva, I. Chernikov, M. Shunkov, M. Thompson, B. Breithaupt, G. Hurley, A. Sher, N. Ovodov, C. Beard, D. Miao, D. Burnham, L. Vietti, M. Clementz, G. Zazula, P. Matheus, P. Wrinn, D. McLaren, and J. Austin. Specimens held by the University of Wyoming were collected from Natural Trap Cave under permit PA13-WY-207 awarded to JAM. Gaadu Din Haida Gwaii fieldwork was funded by Social Science and Humanities Research Council of Canada Standard Grant awarded to DF (410-2005-0778). This research was funded by an Australian Research Council Laureate Fellowship awarded to AC (FL140100260) and U.S. National Science Foundation grant (EAR/SGP# 1425059) awarded to JAM and AC.

References:

Ardelean, C. F., Becerra-Valdivia, L., Pedersen, M. W., Schwenninger, J. L., Oviatt, C. G., Macias-Quintero, J. I., . . . Willerslev, E. (2020). Evidence of human occupation in Mexico around the Last Glacial Maximum. *Nature, 584*, 87–92. doi:10.1038/s41586-020-2509-0

Barnes, I., Matheus, P., Shapiro, B., Jensen, D., & Cooper, A. (2002). Dynamics of Pleistocene population extinctions in Beringian brown bears. *Science, 295*(5563), 2267-2270. doi:10.1126/science.1067814

Barnett, R., Lisandra, M., Zepeda Mendoza, M. L., Soares, A., Soares, R., Ho, S., . . . Gilbert, P. (2016). Mitogenomics of the extinct cave lion, *Panthera spelaea* (Goldfuss, 1810), resolve its position within the *Panthera* cats. *Open Quaternary, 2*(4), 1-11. doi:10.5334/oq.24

Barnett, R., Shapiro, B., Barnes, I., Ho, S. Y. W., Burger, J., Yamaguchi, N., . . . Cooper, A. (2009). Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity. *Molecular Ecology, 18*(8), 1668-1677. doi:10.1111/j.1365-294X.2009.04134.x

Baryshnikov, G., & Boeskorov, G. (2001). The Pleistocene cave lion, *Panthera spelaea* (Carnivora, Felidae) from Yakutia, Russia. *Cranium, 18*, 7-23.

Bielejec, F., Lemey, P., Baele, G., Rambaut, A., & Suchard, M. A. (2014). Inferring heterogeneous evolutionary processes through time: from sequence substitution to phylogeography. *Systematic Biology, 63*(4), 493-504. doi:10.1093/sysbio/syu015

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchene, S., Fourment, M., Gavryushkina, A., . . . Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology, 15*(4), e1006650. doi:10.1371/journal.pcbi.1006650

Bray, S. C. E., Austin, J. J., Metcalf, J. L., Østbye, K., Østbye, E., Lauritzen, S.-E., . . . Cooper, A. (2013). Ancient DNA identifies post-glacial recolonisation, not recent bottlenecks, as the primary driver of contemporary mtDNA phylogeography and diversity in Scandinavian brown bears. *Diversity and Distributions, 19*(3), 245-256. doi:10.1111/j.1472-4642.2012.00923.x

Cahill, J. A., Green, R. E., Fulton, T. L., Stiller, M., Jay, F., Ovsyanikov, N., . . . Shapiro, B. (2013). Genomic evidence for island population conversion resolves conflicting theories of polar bear evolution. *PLoS Genetics, 9*(3), e1003345. doi:10.1371/journal.pgen.1003345

Cahill, J. A., Heintzman, P. D., Harris, K., Teasdale, M. D., Kapp, J., Soares, A. E. R., . . . Shapiro, B. (2018). Genomic evidence of widespread admixture from polar bears into brown bears during the last ice age. *Molecular Biology and Evolution, 35*(5), 1120-1129. doi:10.1093/molbev/msy018

Cahill, J. A., Stirling, I., Kistler, L., Salamzade, R., Ersmark, E., Fulton, T. L., . . . Shapiro, B. (2015). Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. *Molecular Ecology, 24*(6), 1205-1217. doi:10.1111/mec.13038

Campos, P. F., Willerslev, E., Sher, A., Orlando, L., Axelsson, E., Tikhonov, A., . . . Gilbert, M. T. P. (2010). Ancient DNA analyses exclude humans as the driving force behind late Pleistocene musk ox (*Ovibos moschatus*) population dynamics. *Proceedings of the National Academy of Sciences of the United States of America, 107*(12), 5675-5680. doi:10.1073/pnas.0907189107

Christiansen, P., & Harris, J. M. (2009). Craniomandibular Morphology and Phylogenetic Affinities of *Panthera Atrox*: Implications for the Evolution and Paleobiology of the Lion Lineage. *Journal of Vertebrate Paleontology, 29*(3), 934-945. doi:10.1671/039.029.0314

Colleoni, F., Wekerle, C., Näslund, J.-O., Brandefelt, J., & Masina, S. (2016). Constraint on the penultimate glacial maximum Northern Hemisphere ice topography (≈140 kyrs BP). *Quaternary Science Reviews, 137*, 97-112. doi:10.1016/j.quascirev.2016.01.024

Cooper, A., & Poinar, H. N. (2000). Ancient DNA: Do it right or not at all. *Science, 289*(5482), 1139. doi:10.1126/science.289.5482.1139b

Cooper, A., Turney, C., Hughen, K. A., Brook, B. W., McDonald, H. G., & Bradshaw, C. J. A. (2015). Abrupt warming events drove Late Pleistocene Holarctic megafaunal turnover. *Science, 349*(6248), 602-606. doi:10.1126/science.aac4315

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., . . . Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences of the United States of America, 110*(39), 15758-15763.

Dalquest, W. W. (1962). The Good Creek Formation, Pleistocene of Texas, and Its Fauna. *Journal of Paleontology, 36*(3), 568-582.

Davison, J., Ho, S. Y. W., Bray, S. C., Korsten, M., Tammeleht, E., Hindrikson, M., . . . Saarma, U. (2011). Late-Quaternary biogeographic scenarios for the brown bear (*Ursus arctos*), a wild mammal model species. *Quaternary Science Reviews, 30*(3-4), 418-430. doi:10.1016/j.quascirev.2010.11.023

Debruyne, R., Chu, G., King, C. E., Bos, K., Kuch, M., Schwarz, C., . . . Poinar, H. N. (2008). Out of America: Ancient DNA evidence for a New World origin of Late Quaternary woolly mammoths. *Current Biology, 18*(17), 1320-1326. doi:10.1016/j.cub.2008.07.061

Drummond, A. J., Rambaut, A., Shapiro, B., & Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution, 22*(5), 1185-1192. doi:10.1093/molbev/msi103

Dyke, A., Moore, A., & Robertson, L. (2003). *Deglaciation of North America*. Ottawa, ON: Natural Resources Canada.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research, 32*(5), 1792-1797. doi:10.1093/nar/gkh340

Edwards, C. J., Suchard, M. A., Lemey, P., Welch, J. J., Barnes, I., Fulton, T. L., . . . Shapiro, B. (2011). Ancient hybridization and an Irish origin for the modern polar bear matriline. *Current Biology, 21*(15), 1251-1258. doi:10.1016/j.cub.2011.05.058

Elias, S. A., & Crocker, B. (2008). The Bering Land Bridge: a moisture barrier to the dispersal of steppe–tundra biota? *Quaternary Science Reviews, 27*(27), 2473-2483. doi:10.1016/j.quascirev.2008.09.011

Elias, S. A., Short, S. K., Nelson, C. H., & Birks, H. H. (1996). Life and times of the Bering land bridge. *Nature, 382*(6586), 60-63. doi:10.1038/382060a0

Enk, J., Devault, A., Widga, C., Saunders, J., Szpak, P., Southon, J., . . . Poinar, H. (2016). *Mammuthus* population dynamics in Late Pleistocene North America: Divergence, phylogeography, and introgression. *Frontiers in Ecology and Evolution, 4*(42), 42. doi:10.3389/fevo.2016.00042

Ersmark, E., Orlando, L., Sandoval-Castellanos, E., Barnes, I., Barnett, R., Stuart, A., . . . Dalén, L. (2015). Population demography and genetic diversity in the Pleistocene cave lion. *Open Quaternary, 1*(4), 1-14. doi:10.5334/oq.aa

Fox-Dobbs, K., Leonard, J. A., & Koch, P. L. (2008). Pleistocene megafauna from eastern Beringia: Paleoecological and paleoenvironmental interpretations of stable carbon and nitrogen isotope and radiocarbon records. *Palaeogeography, Palaeoclimatology, Palaeoecology, 261*(1-2), 30-46. doi:10.1016/j.palaeo.2007.12.011

Froese, D., Stiller, M., Heintzman, P. D., Reyes, A. V., Zazula, G. D., Soares, A. E., . . . Shapiro, B. (2017). Fossil and genomic evidence constrains the timing of bison arrival in North America. *Proceedings of the National Academy of Sciences of the United States of America, 114*(13), 3457-3462. doi:10.1073/pnas.1620754114

Guthrie, R. D. (2003). Rapid body size decline in Alaskan Pleistocene horses before extinction. *Nature, 426*(6963), 169-171. doi:10.1038/nature02098

Guthrie, R. D. (2006). New carbon dates link climatic change with human colonization and Pleistocene extinctions. *Nature, 441*(7090), 207-209. doi:10.1038/nature04604

Hailer, F. (2015). Introgressive hybridization: brown bears as vectors for polar bear alleles. *Molecular Ecology, 24*(6), 1161-1163. doi:10.1111/mec.13101

Hailer, F., & Welch, A. J. (2016). Evolutionary history of polar and brown bears. *eLS*, 1-8.

Harington, C. R. (1969). Pleistocene Remains of the Lion-Like Cat (*Panthera atrox*) from Yukon Territory and Northern Alaska. *Canadian Journal of Earth Sciences, 6*(5), 1277-1288. doi:10.1139/e69-127

Harington, C. R. (1996). American Lion. *Beringian Research Notes, 5*, 1-4.

Harington, C. R., Naughton, D., Dalby, A., Rose, M., & Dawson, J. (2003). *Annotated Bibliography of Quaternary Vertebrates of Northern North America*. Toronto: University of Toronto Press.

Heinrich, H. (1988). Origin and Consequences of Cyclic Ice Rafting in the Northeast Atlantic-Ocean during the Past 130,000 Years. *Quaternary Research, 29*(2), 142-152. doi:10.1016/0033-5894(88)90057-9

Heintzman, P. D., Froese, D., Ives, J. W., Soares, A. E., Zazula, G. D., Letts, B., . . . Shapiro, B. (2016). Bison phylogeography constrains dispersal and viability of the Ice Free Corridor in western Canada. *Proceedings of the National Academy of Sciences of the United States of America, 113*(29), 8057-8063. doi:10.1073/pnas.1601077113

Heintzman, P. D., Zazula, G. D., Macphee, R. D. E., Scott, E., Cahill, J. A., McHorse, B. K., . . . Shapiro, B. (2017). A new genus of horse from Pleistocene North America. *Elife, 6*, e29944. doi:10.7554/eLife.29944

Hemming, S. R. (2004). Heinrich events: Massive late pleistocene detritus layers of the North Atlantic and their global climate imprint. *Reviews of Geophysics, 42*(1), RG1005. doi:10.1029/2003rg000128

Hibbard, C. W., & Taylor, D. W. (1960). Two late Pleistocene faunas from southwestern Kansas. *Contributions from the Museum of Paleontology, University of Michigan, 16*(1), 1-223.

Hirata, D., Mano, T., Abramov, A. V., Baryshnikov, G. F., Kosintsev, P. A., Vorobiev, A. A., . . . Masuda, R. (2013). Molecular phylogeography of the brown bear (*Ursus arctos*) in Northeastern Asia based on analyses of complete mitochondrial DNA sequences. *Molecular Biology and Evolution, 30*(7), 1644-1652. doi:10.1093/molbev/mst077

Hopkins, D. M. (1973). Sea level history in Beringia during the past 250,000 years. *Quaternary Research, 3*(4), 520-540. doi:10.1016/0033-5894(73)90029-X

Hu, A. X., Meehl, G. A., Otto-Bliesner, B. L., Waelbroeck, C., Han, W. Q., Loutre, M. F., . . . Rosenbloom, N. (2010). Influence of Bering Strait flow and North Atlantic circulation on glacial sea-level changes. *Nature Geoscience, 3*(2), 118-121. doi:10.1038/Ngeo729

Hundertmark, K. J., Shields, G. F., Udina, I. G., Bowyer, R. T., Danilkin, A. A., & Schwartz, C. C. (2002). Mitochondrial phylogeography of moose (*Alces alces*): Late Pleistocene divergence and population expansion. *Molecular Phylogenetics and Evolution, 22*(3), 375-387. doi:10.1006/mpev.2001.1058

Hwang, D. S., Ki, J. S., Jeong, D. H., Kim, B. H., Lee, B. K., Han, S. H., & Lee, J. S. (2008). A comprehensive analysis of three Asiatic black bear mitochondrial genomes (subspecies *ussuricus*, *formosanus* and *mupinensis*), with emphasis on the complete mtDNA sequence of *Ursus thibetanus ussuricus* (Ursidae). *DNA Sequence, 19*(4), 418-429. doi:10.1080/19401730802389525

Jakobsson, M., Pearce, C., Cronin, T. M., Backman, J., Anderson, L. G., Barrientos, N., . . . O'Regan, M. (2017). Post-glacial flooding of the Bering Land Bridge dated to 11 cal ka BP based on new geophysical and sediment records. *Climate of the Past, 13*(8), 991-1005. doi:10.5194/cp-13-991-2017

Kass, R. E., & Raftery, A. E. (1995). Bayes Factors. *Journal of the American Statistical Association, 90*(430), 773-795. doi:10.1080/01621459.1995.10476572

Kim, J. H., Antunes, A., Luo, S. J., Menninger, J., Nash, W. G., O'Brien, S. J., & Johnson, W. E. (2006). Evolutionary analysis of a large mtDNA translocation (numt) into the nuclear genome of the *Panthera* genus species. *Gene, 366*(2), 292-302. doi:10.1016/j.gene.2005.08.023

Kurtén, B. (1985). The Pleistocene Lion of Beringia. *Annales Zoologici Fennici, 22*(1), 117-121.

Kurtén, B., & Anderson, E. (1980). *Pleistocene Mammals of North America*. New York: Columbia University Press.

Kutschera, V. E., Lecomte, N., Janke, A., Selva, N., Sokolov, A. A., Haun, T., . . . Hailer, F. (2013). A range-wide synthesis and timeline for phylogeographic events in the red fox (*Vulpes vulpes*). *BMC Evolutionary Biology, 13*, 114. doi:10.1186/1471-2148-13-114

Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. *Molecular Biology and Evolution, 34*(3), 772-773. doi:10.1093/molbev/msw260

Leonard, J. A., Vila, C., Fox-Dobbs, K., Koch, P. L., Wayne, R. K., & Van Valkenburgh, B. (2007). Megafaunal extinctions and the disappearance of a specialized wolf ecomorph. *Current Biology, 17*(13), 1146-1150. doi:10.1016/j.cub.2007.05.072

Leonard, J. A., Wayne, R. K., & Cooper, A. (2000). Population genetics of Ice age brown bears. *Proceedings of the National Academy of Sciences of the United States of America, 97*(4), 1651-1654. doi:10.1073/pnas.040453097

Lesnek, A. J., Briner, J. P., Lindqvist, C., Baichtal, J. F., & Heaton, T. H. (2018). Deglaciation of the Pacific coastal corridor directly preceded the human colonization of the Americas. *Science Advances, 4*(5), eaar5040. doi:10.1126/sciadv.aar5040

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics, 25*(14), 1754-1760. doi:10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., . . . Genome Project Data Processing, S. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics, 25*(16), 2078-2079. doi:10.1093/bioinformatics/btp352

Lindqvist, C., Schuster, S. C., Sun, Y. Z., Talbot, S. L., Qi, J., Ratan, A., . . . Wiig, O. (2010). Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. *Proceedings of the National Academy of Sciences of the United States of America, 107*(11), 5053-5057. doi:10.1073/pnas.0914266107

Lister, A. M., & Sher, A. V. (2015). Evolution and dispersal of mammoths across the Northern Hemisphere. *Science, 350*(6262), 805. doi:10.1126/science.aac5660

Liu, S. P., Lorenzen, E. D., Fumagalli, M., Li, B., Harris, K., Xiong, Z. J., . . . Wang, J. (2014). Population genomics reveal recent speciation and rapid evolutionary adaptation in polar bears. *Cell, 157*(4), 785-794. doi:10.1016/j.cell.2014.03.054

Loog, L., Thalmann, O., Sinding, M. H. S., Schuenemann, V. J., Perri, A., Germonpr?, M., . . . Manica, A. (2020). Ancient DNA suggests modern wolves trace their origin to a Late Pleistocene expansion from Beringia. *Molecular Ecology, 29*(9), 1596-1610. doi:10.1111/mec.15329

Lorenzen, E. D., Nogues-Bravo, D., Orlando, L., Weinstock, J., Binladen, J., Marske, K. A., . . . Willerslev, E. (2011). Species-specific responses of Late Quaternary megafauna to climate and humans. *Nature, 479*(7373), 359-364. doi:10.1038/nature10574

Mann, D. H., Groves, P., Reanier, R. E., Gaglioti, B. V., Kunz, M. L., & Shapiro, B. (2015). Life and extinction of megafauna in the ice-age Arctic. *Proceedings of the National Academy of Sciences of the United States of America, 112*(46), 14301-14306. doi:10.1073/pnas.1516573112

Matheus, P. E. (1995). Diet and co-ecology of Pleistocene short-faced bears and brown bears in eastern Beringia. *Quaternary Research, 44*(3), 447-453. doi:10.1006/qres.1995.1090

Matsumura, S., Inoshima, Y., & Ishiguro, N. (2014). Reconstructing the colonization history of lost wolf lineages by the analysis of the mitochondrial genome. *Molecular Phylogenetics and Evolution, 80*, 105-112. doi:10.1016/j.ympev.2014.08.004

Meachen, J., Wooller, M. J., Barst, B. D., Funck, J., Crann, C., Heath, J., . . . Zazula, G. (2020). A mummified Pleistocene gray wolf pup. *Current Biology, 30*(24), R1467-R1468. doi:10.1016/j.cub.2020.11.011

Meiri, M., Lister, A. M., Collins, M. J., Tuross, N., Goebel, T., Blockley, S., . . . Barnes, I. (2014). Faunal record identifies Bering isthmus conditions as constraint to end-Pleistocene migration to the New World. *Proceedings of the Royal Society B: Biological Sciences, 281*(1776). doi:10.1098/rspb.2013.2167

Meyer, M., Kircher, M., Gansauge, M. T., Li, H., Racimo, F., Mallick, S., . . . Paabo, S. (2012). A high-coverage genome sequence from an archaic Denisovan individual. *Science, 338*(6104), 222-226. doi:10.1126/science.1224344

Miller, W., Schuster, S. C., Welch, A. J., Ratan, A., Bedoya-Reina, O. C., Zhao, F. Q., . . . Lindqvist, C. (2012). Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. *Proceedings of the National Academy of Sciences of the United States of America, 109*(36), E2382-E2390. doi:10.1073/pnas.1210506109

Mitchell, K. J., Bray, S. C., Bover, P., Soibelzon, L., Schubert, B. W., Prevosti, F., . . . Cooper, A. (2016). Ancient mitochondrial DNA reveals convergent evolution of giant short-faced bears (Tremarctinae) in North and South America. *Biology Letters, 12*(4), 20160062. doi:10.1098/rsbl.2016.0062

Niemann, J., Gopalakrishnan, S., Yamaguchi, N., Ramos-Madrigal, J., Wales, N., Gilbert, M. T. P., & Sinding, M. S. (2021). Extended survival of Pleistocene Siberian wolves into the early 20th century on the island of Honshu. *iScience, 24*(1), 101904. doi:10.1016/j.isci.2020.101904

Pilot, M., Branicki, W., Jedrzejewski, W., Goszczynski, J., Jedrzejewska, B., Dykyy, I., . . . Tsingarska, E. (2010). Phylogeographic history of grey wolves in Europe. *BMC Evolutionary Biology, 10:104*. doi:10.1186/1471-2148-10-104

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in bayesian phylogenetics using Tracer 1.7. *Systematic Biology, 67*(5), 901-904. doi:10.1093/sysbio/syy032

Ramsden, C., Holmes, E. C., & Charleston, M. A. (2009). Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for codivergence. *Molecular Biology and Evolution, 26*(1), 143-153. doi:10.1093/molbev/msn234

Ramsey, C. B. (2009). Bayesian analysis of radiocarbon dates. *Radiocarbon, 51*(1), 337-360. doi:10.1017/S0033822200033865

Ramsey, C. L., Griffiths, P. A., Fedje, D. W., Wigen, R. J., & Mackie, Q. (2004). Preliminary investigation of a late Wisconsinan fauna from K1 cave, Queen Charlotte Islands (Haida Gwaii), Canada. *Quaternary Research, 62*(1), 105-109. doi:10.1016/j.yqres.2004.05.003

Reimer, P. J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Ramsey, C. B., . . . van der Plicht, J. (2013). Intcal13 and Marine13 radiocarbon age calibration curves 0-50,000 years cal BP. *Radiocarbon, 55*(4), 1869-1887. doi:10.2458/azu_js_rc.55.16947

Reuther, J. D., Rogers, J., Druckenmiller, P., Bundtzen, T. K., Wallace, K., Bowman, R., . . . Cherkinsky, A. (2020). Late Quaternary (≥MIS 3 to MIS 1) stratigraphic transitions in a highland Beringian landscape along the Kuskokwim River, Alaska. *Quaternary Research, 93*, 139-154. doi:10.1017/qua.2019.51

Rey-Iglesia, A., Garcia-Vazquez, A., Treadaway, E. C., van der Plicht, J., Baryshnikov, G. F., Szpak, P., . . . Lorenzen, E. D. (2019). Evolutionary history and palaeoecology of brown bear in North-East Siberia re-examined using ancient DNA and stable isotopes from skeletal remains. *Scientific Reports, 9*(1), 4462. doi:10.1038/s41598-019-40168-7

Richards, S. M., Hovhannisyan, N., Gilliham, M., Ingram, J., Skadhauge, B., Heiniger, H., . . . Cooper, A. (2019). Low-cost cross-taxon enrichment of mitochondrial DNA using in-house synthesised RNA probes. *PLoS ONE, 14*(2), e0209499. doi:10.1371/journal.pone.0209499

Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., & Reich, D. (2015). Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philosophical Transactions of the Royal Society of London B Biological Sciences, 370*(1660), 20130624. doi:10.1098/rstb.2013.0624

Sanmartin, I., Van der Mark, P., & Ronquist, F. (2008). Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography, 35*(3), 428-449. doi:10.1111/j.1365-2699.2008.01885.x

Schubert, B. W., & Wallace, S. C. (2009). Late Pleistocene giant short-faced bears, mammoths, and large carcass scavenging in the Saltville Valley of Virginia, USA. *Boreas, 38*(3), 482-492. doi:10.1111/j.1502-3885.2009.00090.x

Schubert, M., Ermini, L., Sarkissian, C. D., Jonsson, H., Ginolhac, A., Schaefer, R., . . . Orlando, L. (2014). Characterization of ancient and modern genomes by SNP detection and phylogenomic and metagenomic analysis using PALEOMIX. *Nature Protocols, 9*(5), 1056-1082. doi:10.1038/nprot.2014.063

Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes, 9*, 88. doi:10.1186/s13104-016-1900-2

Shapiro, B., Drummond, A. J., Rambaut, A., Wilson, M. C., Matheus, P. E., Sher, A. V., . . . Cooper, A. (2004). Rise and fall of the Beringian steppe bison. *Science, 306*(5701), 1561-1565. doi:10.1126/science.1101074

Shaw, J., Barrie, J. V., Conway, K. W., Lintern, D. G., & Kung, R. (2020). Glaciation of the northern British Columbia continental shelf: the geomorphic evidence derived from multibeam bathymetric data. *Boreas, 49*(1), 17-37. doi:10.1111/bor.12411

Sotnikova, M., & Nikolskiy, P. (2006). Systematic position of the cave lion *Panthera spelaea* (Goldfuss) based on cranial and dental characters. *Quaternary International, 142*, 218-228. doi:10.1016/j.quaint.2005.03.019

Statham, M. J., Murdoch, J., Janecka, J., Aubry, K. B., Edwards, C. J., Soulsbury, C. D., . . . Sacks, B. N. (2014). Range-wide multilocus phylogeography of the red fox reveals ancient continental divergence, minimal genomic exchange and distinct demographic histories. *Molecular Ecology, 23*(19), 4813-4830. doi:10.1111/mec.12898

Steffen, M. L., & Fulton, T. L. (2018). On the association of giant short-faced bear (*Arctodus simus*) and brown bear (*Ursus arctos*) in late Pleistocene North America. *Geobios, 51*(1), 61-74. doi:10.1016/j.geobios.2017.12.001

Stiller, M., Molak, M., Prost, S., Rabeder, G., Baryshnikov, G., Rosendahl, W., . . . Knapp, M. (2014). Mitochondrial DNA diversity and evolution of the Pleistocene cave bear complex. *Quaternary International, 339-340*, 224-231. doi:10.1016/j.quaint.2013.09.023

Stuart, A. J., & Lister, A. M. (2011). Extinction chronology of the cave lion *Panthera spelaea*. *Quaternary Science Reviews, 30*(17-18), 2329-2340. doi:10.1016/j.quascirev.2010.04.023

Subramanian, S., & Lambert, D. M. (2011). Time dependency of molecular evolutionary rates? Yes and no. *Genome Biology and Evolution, 3*, 1324-1328. doi:10.1093/gbe/evr108

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution, 4*(1), vey016. doi:10.1093/ve/vey016

Talbot, S. L., & Shields, G. F. (1996). Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution, 5*(3), 477-494. doi:DOI 10.1006/mpev.1996.0044

Treat, C. C., Kleinen, T., Broothaerts, N., Dalton, A. S., Dommain, R., Douglas, T. A., . . . Brovkin, V. (2019). Widespread global peatland establishment and persistence over the last 130,000 y. *Proceedings of the National Academy of Sciences of the United States of America, 116*(11), 4822-4827. doi:10.1073/pnas.1813305116

Vershinina, A. O., Heintzman, P. D., Froese, D. G., Zazula, G., Cassatt-Johnstone, M., Dalen, L., . . . Shapiro, B. (2021). Ancient horse genomes reveal the timing and extent of dispersals across the Bering Land Bridge. *Molecular Ecology*. doi:10.1111/mec.15977

Waits, L. P., Talbot, S. L., Ward, R. H., & Shields, G. F. (1998). Mitochondrial DNA phylogeography of the North American brown bear and implications for conservation. *Conservation Biology, 12*(2), 408-417. doi:10.1046/j.1523-1739.1998.96351.x

Whitmore, F. C., & Foster, H. L. (1967). *Panthera atrox* (Mammalia: Felidae) from Central Alaska. *Journal of Paleontology, 41*(1), 247-251.

Yamaguchi, N., Cooper, A., Werdelin, L., & Macdonald, D. W. (2004). Evolution of the mane and group-living in the lion (Panthera leo): a review. *Journal of Zoology, 263*, 329-342. doi:10.1017/S0952836904005242

Data Availability Statement

Consensus mitochondrial sequences have been uploaded to GenBank (accessions OK512879-OK513020). Demultiplexed and filtered sequencing data has been uploaded to the European Nucleotide Archive (study accession PRJEB48139).

Author Contributions

A.T.S., A.C., J.A.M., and K.J.M. designed research; A.T.S., S.C.E.B., H.H., R.B., J.W., and K.J.M. performed research; J.A.B., V.D., D.F., L.G., C.R.H., B.H., P.K., X.L., Q.M., S.V., N.Y., and J.A.M. contributed new reagents/analytic tools; A.T.S., M.S.Y.L., and K.J.M.

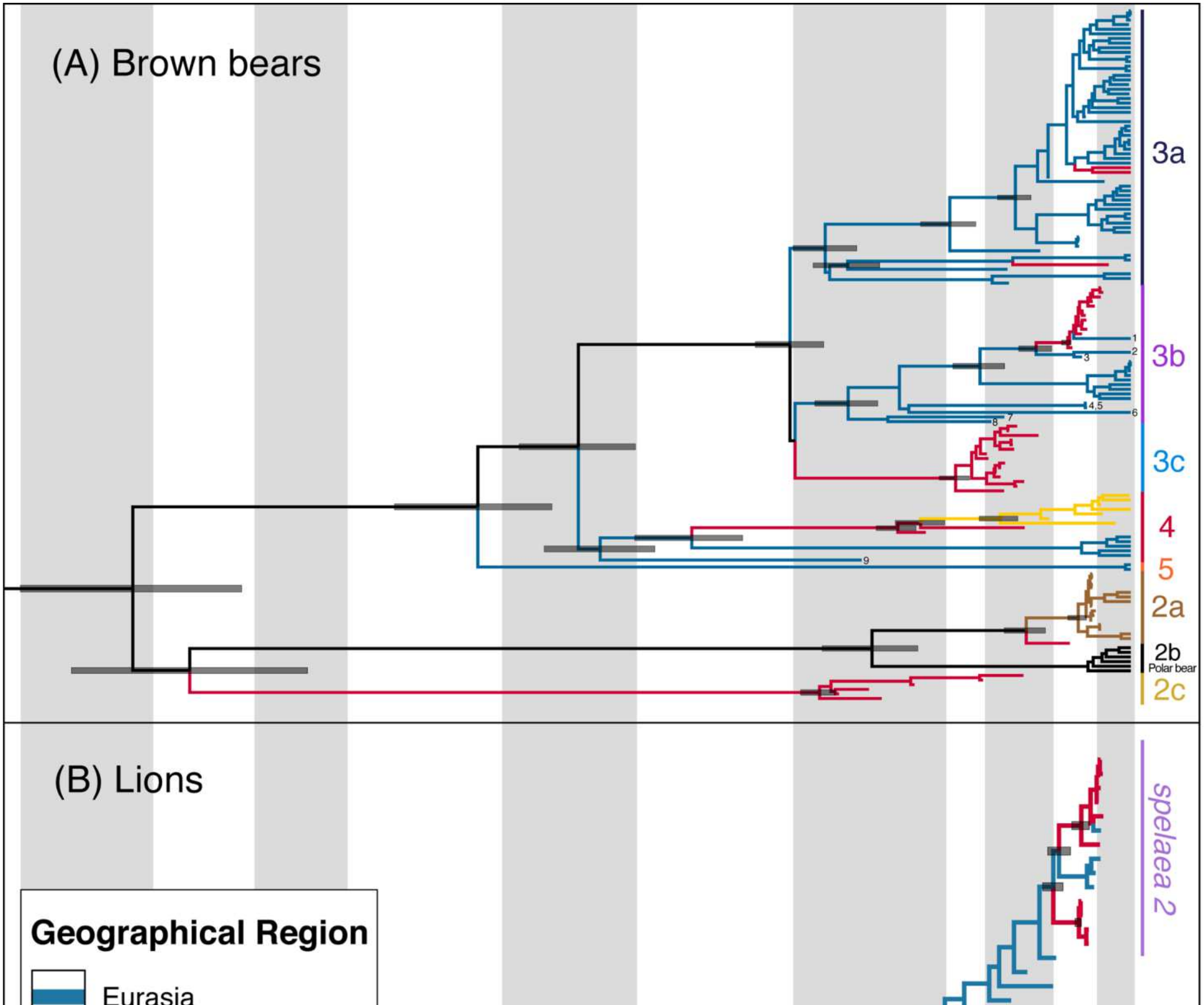

Fig. 1. Bayesian phylogenetic trees inferred from (A) brown bear and (B) lion mitogenomes. The grey vertical columns represent odd-numbered MIS stages (interglacials) and white columns even-numbered MIS stages (glacials). Bars on nodes represent 95% Highest Posterior Densities for node age estimates indicated for modes leading to major clades and those reported in main text. Numbers on tips in (A) refer to selected specimens mentioned in text: 1 = A155, 2 = A156, 3 = A1945, 4 = A1944, 5 = A1946, 6 = A138, 7 = A5889, 8 = MH255807, 9 = A5883. For detailed trees with tip labels, and posterior support values see Supplementary Figs S4 and S5.

Tl

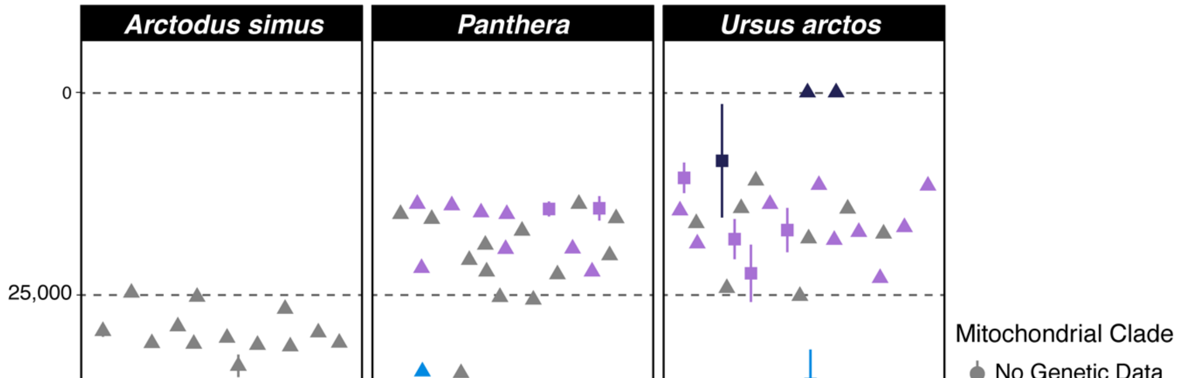

Fig. 2. Temporal distribution of Eastern Beringian giant short-faced bear (*Arctodus simus*), lion (*Panthera* spp.), and brown bear (*Ursus arctos*) specimens based on radiocarbon and molecular age estimates. Ages are shown with one standard error and are coloured by genetic clade. For additional radiocarbon dates used to produce this plot see Supplementary Table S3.

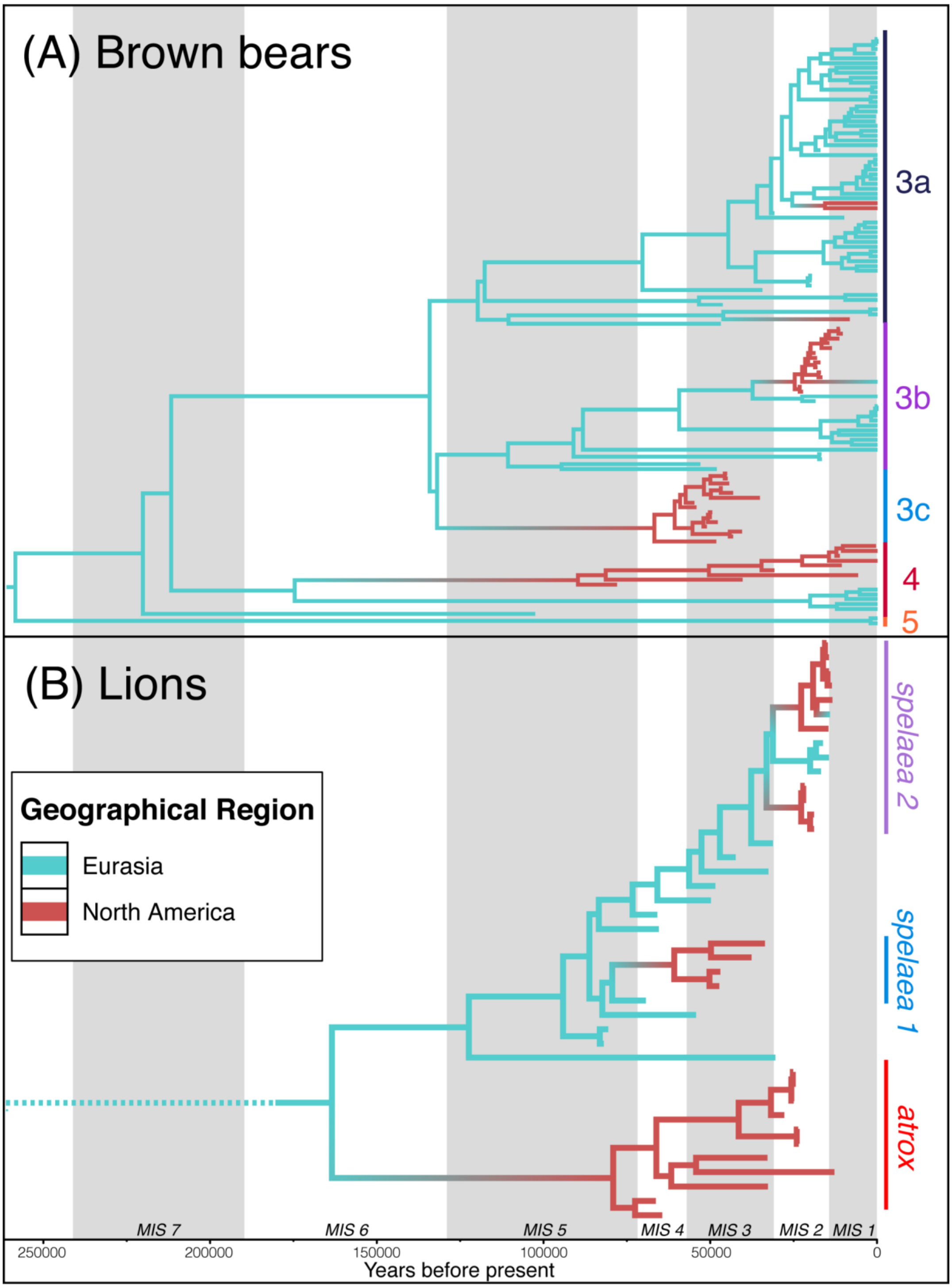
(A) Brown bears
3a
3b
3c
4
5
(B) Lions
Geographical Region
🟦 Eurasia
🟥 North America
spelaea 2
spelaea 1
atrox
MIS 7
MIS 6
MIS 5
MIS 4
MIS 3
MIS 2
MIS 1
250000
200000
150000
100000
50000
0
Years before present

Fig. 3. Bayesian phylogenetic trees for (A) brown bear and (B) lion resulting from our combined phylogeographic analysis performed using BEAST. The grey vertical columns represent odd-numbered MIS stages (interglacials) and white columns even-numbered MIS stages (glacials), modelled as different epochs in our analysis. Colours of branches correspond to phylogeographic character state; blue is Eurasia and red is North America. Shifts from blue to red and vice versa denote branches along which we inferred migration across the Bering Land Bridge. The combined glacials (white) occupy less time overall, and subtend less tree length, than combined interglacials (grey), yet branches with inferred migration events tend to span glacials: of 11 such branches, two are entirely restricted to glacials and two are largely restricted to glacials, whereas none are restricted to interglacials and only one is largely restricted to interglacials (the other six broadly span both glacial and interglacial time slices). For detailed trees with tip labels, see Supplementary Fig S6.

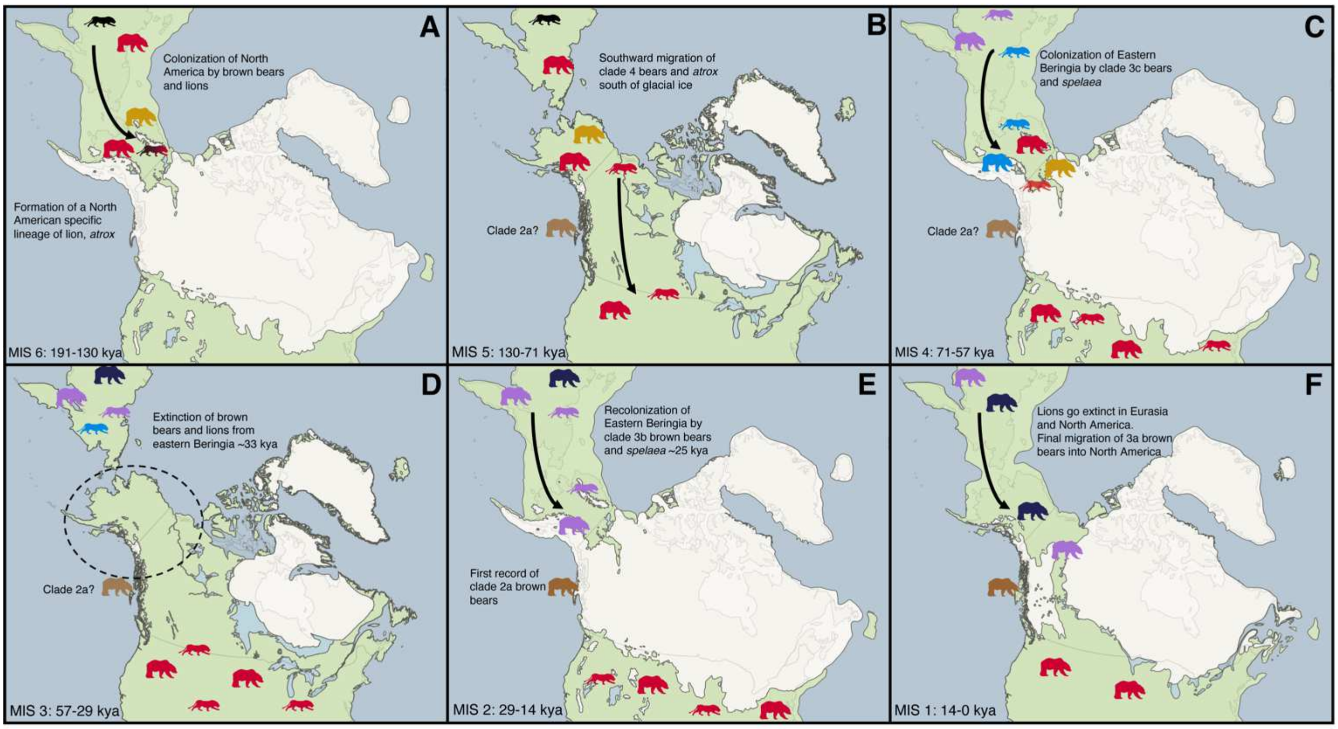

Fig. 4. Map of Late Quaternary phylogeography of North American brown bears and lions during six time periods. A) MIS 6, 191–130 kya, brown bears and lions first colonise North America via the Bering Land Bridge; B) MIS 5, 130–71 kya, Bering Land Bridge is flooded, dispersal of brown bears and lions south of continental ice sheets; C) MIS 4, 71–57 kya, dispersal of clade 3c bears and *spelaea* lions (*spelaea* 1) across the Bering Land Bridge; D) MIS 3, 57–29 kya, flooding of Bering Land Bridge and extinction of brown bears and lions in Eastern Beringia; E) MIS 2, Last Glacial Maximum, 29–14 kya, dispersal of clade 3b bears and second wave of *spelaea* lions (*spelaea* 2); and F) MIS 1, Holocene, 14 kya to present, lions go extinct in North America and Eurasia, additionally clade 3a bears disperse into Eastern Beringia before the Bering Land Bridge is flooded for the last time. Different coloured silhouettes of brown bears and lions represent different genetic clades, corresponding to clade colouring in Figs 1 and 2. White area represents the approximate extent of glacial ice along with rough estimates of Bering Land Bridge extent during the different time periods using spatial data from Dyke, Moore, and Robertson (2003).