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8   **Lions and brown bears colonised North America in multiple synchronous waves of**

9   **dispersal across the Bering Land Bridge**

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48

49   **Abstract:**

50   The Bering Land Bridge connecting North America and Eurasia was periodically exposed and

51   inundated by oscillating sea levels during the Pleistocene glacial cycles. This land connection

52   allowed the intermittent dispersal of animals, including humans, between Western Beringia

53   (far north-east Asia) and Eastern Beringia (north-west North America), changing the faunal

54   community composition of both continents. The Pleistocene glacial cycles also had profound

55   impacts on temperature, precipitation, and vegetation, impacting faunal community structure

56   and demography. While these palaeoenvironmental impacts have been studied in many large

57   herbivores from Beringia (e.g., bison, mammoths, horses), the Pleistocene population

58   dynamics of the diverse guild of carnivorans present in the region are less well understood,

59   due to their lower abundances. In this study, we analyse mitochondrial genome data from

60   ancient brown bears (*Ursus arctos;* n = 103) and lions (*Panthera* spp.; n = 39), two

61   megafaunal carnivorans that dispersed into North America during the Pleistocene. Our results

62   reveal striking synchronicity in the population dynamics of Beringian lions and brown bears,

63   with multiple waves of dispersal across the Bering Land Bridge coinciding with glacial

64   periods of low sea levels, as well as synchronous local extinctions in Eastern Beringia during

65   Marine Isotope Stage 3. The evolutionary histories of these two taxa underscore the crucial

66   biogeographic role of the Bering Land Bridge in the distribution, turnover, and maintenance

67   of megafaunal populations in North America.

68

69   **Keywords**

70   Ancient DNA, brown bears, lions, phylogeography, Beringia

71

72   **Introduction:**

73   During the Pleistocene (2.58 million to 11,700 years ago), Eastern Beringia — the area

74   comprising Alaska and parts of Yukon Territory — was inhabited by numerous species of

75   megafauna (Harington, Naughton, Dalby, Rose, & Dawson, 2003). Many of these taxa

76   belonged to endemic New World lineages, such as the giant short-faced bear (*Arctodus*

77   *simus*), Jefferson's ground sloth (*Megalonyx jeffersonii*), and the stilt-legged horse

78   (*Haringtonhippus francisci*) (Harington et al., 2003; Kurtén & Anderson, 1980). However,

79   Eastern Beringian megafaunal diversity also included non-endemic species that dispersed

80   from Western Beringia — the area of Russia east of the Lena River — during the Pleistocene

81   (Elias & Crocker, 2008; Elias, Short, Nelson, & Birks, 1996; Harington et al., 2003). Some of

82   these immigrant taxa, including moose (*Alces alces*) and elk/wapiti (*Cervus canadensis*),

83   appear to have arrived during the Last Glacial Maximum (LGM) when the Bering Land

84   Bridge connecting Western and Eastern Beringia was most recently exposed (Guthrie, 2006;

85   Hundertmark et al., 2002; Meiri et al., 2014). Other taxa apparently invaded much earlier in

86   the Pleistocene, including bison (*Bison* spp.) (Froese et al., 2017; Shapiro et al., 2004), and

87   mammoth (*Mammuthus* spp.) (Enk et al., 2016; Lister & Sher, 2015). However, the exact

88   timeline and processes underlying early Pleistocene dispersals are currently poorly

89   characterised, and it remains uncertain whether the arrivals of individual species represented

90   independent chance events or were temporally concentrated in waves of dispersal following

91   changes in climate and environment.

92

93   Sea level records from the Northern Pacific indicate that the Bering Land Bridge opened and

94   closed multiple times during the Pleistocene (Hopkins, 1973; Hu et al., 2010) during glacial

95   and interglacial periods, respectively. During glacial Marine Isotope Stage 6 (MIS 6) ~185

96   thousand years ago (kya) to 135 kya, sea levels were low enough to allow the Bering Land

97   Bridge to be uncovered (Colleoni, Wekerle, Näslund, Brandefelt, & Masina, 2016; Hopkins,

98   1973). In the subsequent MIS 5, interglacial sea levels increased to higher than present-day,

99   flooding the Bering Land Bridge from approximately 135 to 70 kya before it re-emerged

100   again ~70 to 60 kya during glacial MIS 4 (Hu et al., 2010). Intermittent connections may have

101   occurred again during MIS 3, before the final emergence during MIS 2/LGM starting ~34 kya

102   and finishing 11 kya (Hu et al., 2010; Jakobsson et al., 2017).

103

104   Repeated glacial cycles had profound effects on vegetation, which could also influence animal

105   dispersal. For example, increased temperature during interstadials is likely to have resulted in

106   the landscape becoming wetter, in turn facilitating the accumulation of organic matter

107   ("paludification") and the expansion of peatlands (Mann et al., 2015; Treat et al., 2019).

108   Paludification is thought to have lowered nutrient availability and favoured less palatable

109   plant species, negatively impacting megafaunal herbivore populations. Indeed, Mann et al.

110   (2015) observed that during interstadials in Alaska there was an initial increase in megafaunal

111   herbivore abundance followed by a decrease coincident with peatland expansion. In addition,

112   bone nitrogen isotopes demonstrate that the diet of horses in Alaska changed radically

113   coincident with an increase in peatlands during Greenland Interstadial 1 (14.7-12.9 kya)

114   (Mann et al., 2015). Changes in herbivore communities are likely to have impacted

115   populations of megafaunal carnivores and omnivores, potentially affecting their ability to

116   colonise or persist in Eastern Beringia through multiple glacial cycles. However, our
117   understanding of fine-scale carnivore responses to environmental change in Eastern Beringia
118   has been limited by their relative rarity in the fossil record. Although several studies have
119   used ancient DNA to examine megafaunal carnivoran population dynamics (e.g., Barnes,
120   Matheus, Shapiro, Jensen, & Cooper, 2002; Barnett et al., 2009), sample sizes have generally
121   been small and resolution limited.

122

123   During the Late Pleistocene, a number of megafaunal carnivorans roamed Eastern Beringia,
124   including the giant short-faced bear (*Arctodus simus*), grey wolves (*Canis lupus*), and
125   scimitar-toothed cats (*Homotherium serum*) (Harington et al., 2003; Kurtén & Anderson,
126   1980). Lions (*Panthera* spp.) and brown bears (*Ursus arctos*) appear to have dispersed into
127   northern North America from Eurasia via the Bering Land Bridge during the Pleistocene
128   (Kurtén & Anderson, 1980), but genetic data from North American lion and brown bear
129   subfossils (preserved non-mineralised animal remains) have revealed a complicated
130   evolutionary history (Barnes et al., 2002; Barnett et al., 2009; Davison et al., 2011; Ersmark et
131   al., 2015; Leonard, Wayne, & Cooper, 2000). North American brown bears appear to
132   represent distinct mitochondrial lineages with diverse origins. Likewise, Pleistocene lions
133   from North America have been grouped into two distinct lineages based on both fossil
134   evidence and genetic data, potentially representing two species (or alternatively two
135   subspecies of the extant lion, *Panthera leo*) with different origins.

136

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

146 Beringian lions (Barnett et al., 2009; Christiansen & Harris, 2009; Stuart & Lister, 2011); the
147 genetic divergence between the American lion and its relatives is estimated to have occurred
148 ~340 kya (Barnett et al., 2009), suggesting that the ancestors of the American lion entered
149 North America prior to MIS 6, consistent with fossil records in Eastern Beringia where lion
150 remains are found in Illinoian deposits onwards (Kurtén & Anderson, 1980; Yamaguchi,
151 Cooper, Werdelin, & Macdonald, 2004). In contrast, molecular data suggest that brown bears
152 first colonised North America ~70 kya (around the MIS 5/MIS 4 transition), and subsequently
153 appear to have become locally extinct in Eastern Beringia between ~35 kya and 21 kya
154 (Barnes et al., 2002; Davison et al., 2011; Kurtén, 1985).

155

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

166 **Materials and Methods:**

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

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

175

176 Sample preparation, DNA extraction and library construction were conducted in purpose-built

177 ancient DNA (aDNA) clean-room facilities at the University of Adelaide's Australian Centre

178 for Ancient DNA (ACAD) or the Henry Wellcome Ancient Biomolecules Centre at the

179 University of Oxford, and a number of precautions were taken to minimise contamination of

180 samples with exogenous DNA (Cooper & Poinar, 2000).

181

182 DNA extraction was performed on bone or tooth powder using either an in-house silica-based

183 extraction protocol adapted from Dabney et al. (2013) or a phenol-chloroform-based

184 extraction protocol from Bray et al. (2013). Double-stranded Illumina libraries were

185 constructed following the protocol of Meyer et al. (2012) with truncated Illumina adapters

186 with unique dual 7-mer internal barcodes added to allow identification and exclusion of any

187 downstream contamination. Further, partial uracil-DNA glycosylase (UDG) treatment

188 (Rohland, Harney, Mallick, Nordenfelt, & Reich, 2015) was included to restrict cytosine

189 deamination to terminal nucleotides.

190

191 Brown bear libraries were enriched with home-made RNA baits following Richards et al.

192 (2019) produced from long-range PCR fragments amplified from modern brown bear DNA

193 using primers from Hwang et al. (2008). For lion libraries, commercially synthesised

194 biotinylated 80-mer RNA baits (Arbor Biosciences, MI, USA) were used to enrich for

195 mammalian mitochondrial DNA (Mitchell et al., 2016). DNA-RNA hybridisation enrichment

196 was performed according to manufacturer's recommendations (MYbaits protocol v3).

197 Libraries were pooled and sequenced on an Illumina NextSeq using 2 x 75 bp PE (150 cycle)

198 High Output chemistry. A more detailed description of the laboratory methods is available in

199 the Supplementary Material.

200

201 ***Data processing***

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

223

224 *Phylogenetic analyses*

225 Brown bear consensus sequences were aligned using MUSCLE v3.8.425 (Edgar, 2004) in
226 Geneious Prime v2019.0.4 with an additional 46 brown bear and polar bear mitogenomes
227 downloaded from GenBank (Supplementary Table S5). Lion sequences were aligned
228 separately also using MUSCLE v3.8.425. PartitionFinder v2.1.1 (Lanfear, Frandsen, Wright,
229 Senfeld, & Calcott, 2016) was used to find the best-fitting partitioning scheme using the
230 Bayesian information criterion, separating the data into five partitions for each alignment
231 (Supplementary Table S6). Bayesian tip-dating analyses were then performed on each taxon
232 using BEAST v2.6.1 (Bouckaert et al., 2019). The temporal signal in our dataset was

233   evaluated using leave-one-out cross-validation (e.g., Stiller et al., 2014), using only the finite-
234   dated specimens (Supplementary Fig. S1). The ages of undated specimens were then
235   estimated one at a time using the dated specimens as calibration for the molecular clock
236   (Supplementary Fig. S2). Once all samples were assigned an age (either based on radiocarbon
237   dating or Bayesian date estimation), we conducted a date-randomisation test (Ramsden,
238   Holmes, & Charleston, 2009; Stiller et al., 2014), to test for sufficient temporal signal within
239   the datasets (Supplementary Fig. S3). Runs described above were performed with a strict
240   clock with a uniform prior on rate (0–10^{-5} mutations per site per year), constant population
241   coalescent tree prior with a 1/x distribution on population size, a uniform prior (0–500,000)
242   on the age of the sequence being estimated (if required), and run for 30 million steps with
243   sampling every 3,000 steps. Convergence was checked in Tracer v1.7.1 (Rambaut,
244   Drummond, Xie, Baele, & Suchard, 2018). Final BEAST analyses were conducted using a
245   strict clock with a uniform prior on rate (0–10^{-5} mutations per site per year), and a Bayesian
246   skyline coalescent tree prior. We ran three independent MCMC chains, each run for 50
247   million steps, sampling every 5,000 steps. Results from individual runs were combined using
248   LogCombiner after discarding the first 10% of steps as burn-in. Maximum clade credibility
249   trees were generated in TreeAnnotator using the median node age.

250

251   To test for the association of migrations between Eurasia and North America with glacial
252   periods, phylogeographic model testing was performed in BEAST (Suchard et al., 2018). The
253   same substitution model settings were used as described above, but the alignments were
254   combined in a single analysis, with a separate tree estimated simultaneously for each taxon.
255   Clade 2 brown bears were excluded from the analysis due to lack of sampling, and the
256   introgressed nature with polar bears resulting in a complicated evolutionary history of the
257   clade (Cahill et al., 2013; Cahill et al., 2018; Cahill et al., 2015; Edwards et al., 2011; Hailer,
258   2015; Hailer & Welch, 2016; Miller et al., 2012). Each tip was assigned a binary
259   phylogeographic character (Eurasia vs North America), and the rate of evolution of this
260   character was estimated directly from the data. Two models for the evolution of this character
261   were tested: a strict clock, where rates of evolution were constant through time, and a two-
262   epoch clock that had two separate rates (one for interglacial periods and one for glacial
263   periods). Note, in this method, tree topology and dispersal times for the two clades are

264 essentially estimated separately (unlinked trees), but dispersal rates for the two epochs

265 (combined glacial and combined interglacial) are estimated based on the pooled data from

266 both trees, and have identical priors. Bayes factors were estimated and compared using

267 Akaike's Information Criterion for MCMC samples in Tracer v1.7.1 (Rambaut et al., 2018).

268 Four independent MCMC chains were run for 20 million steps each, sampling every 2,000

269 steps. We checked for convergence and sufficient sampling of parameters in Tracer. A more

270 detailed description of the phylogenetic analysis methods is available in the Supplementary

271 Material.

272

273 **Results:**

274 ***Brown bears***

275 We produced 103 new near-complete (i.e., >70% coverage) mitogenomes from

276 Pleistocene/Holocene subfossil *Ursus arctos* specimens from North America (n=53) and

277 Eurasia (n=50), which we analysed along with previously published data from 47 brown bears

278 and polar bears (Hirata et al., 2013; Lindqvist et al., 2010; Liu et al., 2014; Miller et al., 2012;

279 Rey-Iglesia et al., 2019), spanning 107 unique mitochondrial haplotypes. We used BEAST2

280 (Bouckaert et al., 2019) to create a time-calibrated phylogenetic tree (Fig. 1), which was

281 largely concordant with previous studies in grouping Beringian brown bear mitochondrial

282 diversity into four major spatio-temporally restricted clades: clade 2 (including clade 2a, 2b,

283 and 2c, and also encompassing extant polar bears), clade 3 (including 3a, 3b, and 3c), clade 4,

284 and clade 5 (Barnes et al., 2002; Davison et al., 2011; Hirata et al., 2013; Leonard et al., 2000;

285 Talbot & Shields, 1996; Waits, Talbot, Ward, & Shields, 1998). The temporal and geographic

286 distributions of the different clades appear to result from dispersals into Eastern Beringia at

287 widely different points in time.

288

289 Within Eurasia we identified three ancient specimens (A155, A156, and A1945) with

290 haplotypes closely related to North American clade 3b bears, and five deeply divergent

291 Eurasian clade 3b bears (A138, A1944, A1946, A5889, and MH255807), including a

292 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

323 belonged to either clade 3b or 3a. Clade 3b is the dominant group through MIS 2, comprising

324 13 samples, and appears to have arrived during the LGM with a TMRCA ~25 kya (95% HPD:

325 22.9–28.1 kya) (Fig. 1). The upper limit of this dispersal is constrained by a 39 kya estimate

326 for the TMRCA with the closely related Eurasian clade 3b brown bears (95% HPD: 31.9–46.4

327 kya). In contrast, clade 3a is represented by only a single Holocene specimen and two

328 previously published modern bears, presumably constituting a terminal-Pleistocene dispersal

329 into North America, as clade 3a bears arrive in Japan at a similar time (Hirata et al., 2013).

330

331 Lastly, we recovered mitochondrial data from ten ancient clade 2a bears from Haida Gwaii

332 and Prince of Wales Island (Alexander Archipelago). Clade 2a is closely related to the polar

333 bear mitochondrial clade 2b, and a divergent clade 2a specimen (A308) was also recovered

334 from Engineer Creek Mine near Fairbanks, Alaska dating to 23.3 kya, the first record of clade

335 2a in interior Alaska. This specimen was previously reported as belonging to clade 2b based

336 on control region sequences (Barnes et al., 2002; Davison et al., 2011), although doubts about

337 species assignment (polar bear versus brown bear) and provenance have been raised (Barnes

338 et al., 2002; Edwards et al., 2011). In any case, the TMRCA of all Haida Gwaii and

339 Alexander Archipelago specimens dates to ~20 kya (95% HPD: 17–24 kya), while the

340 TMRCA between the Engineer Creek sample and all other clade 2a bears is 41 kya (95%

341 HPD: 32.7–28.7 kya).

342

343 ***Lions***

344 We produced 39 new near-complete mitogenomes from lion subfossil material from North

345 America (n=24) and Eurasia (n=15), and analysed these along with two mitogenomes

346 reconstructed from previously published data (Barnett et al., 2016), represented by 35 unique

347 haplotypes. The topological results of our phylogenetic analyses were in broad agreement

348 with past studies, supporting the existence of two geographically restricted clades (Fig. 1B)

349 corresponding to *Panthera* (*leo*) *spelaea* (Eastern Beringia and Eurasia) and *Panthera* (*leo*)

350 *atrox* (all other North American specimens from Edmonton southwards). We observed one

351 important exception to this pattern: a specimen from Sixtymile River in Yukon Territory

352 (~64°N), A181, possessing an *atrox* (American lion) mitochondrial haplotype (Fig. 1;

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

360

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

370

371 Within Beringian lion diversity we were able to identify a genetically distinct pre-LGM
372 mitochondrial clade of Eastern Beringian *Panthera* (*leo*) *spelaea* specimens with a TMRCA
373 of 63 kya (95% HPD: 58.9–67.6 kya). These pre-LGM samples are genetically distinct from
374 the two clades that include all younger Eastern Beringian lion specimens, which have
375 TMRCAs of 23 kya (95% HPD: 22.1–24.5 kya) and 22 kya (95% HPD: 18.9–25.5 kya), and a
376 combined TMRCA of 33 kya (95% HPD: 29.2–37.0 kya). This suggests that in addition to the
377 original dispersal of the ancestors of *Panthera* (*leo*) *atrox*, lions appear to have dispersed into
378 North America on at least two other occasions during the Late Pleistocene. It is notable that
379 the hiatus in the fossil record between the pre- and post-LGM lion clades falls between 33 and
380 22 kya, closely mirroring the pattern of local extinction observed in brown bears (Fig. 2).

381

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

412   concentrated in glacials, yet the combined glacial epochs occupy less time and shorter tree

413   length (compared to the combined interglacials).

414

415   **Discussion:**

416   Our new data regarding the age and genetic identity of brown bear and lion specimens from

417   North America revealed previously unrecognised patterns of synchronous dispersal and

418   extirpation in Eastern Beringia. For the first time we have detected a member of the *atrox*

419   mitochondrial clade in Pleistocene lions from Eastern Beringia, and further revealed that

420   Eastern Beringian lions from the *spelaea* clade comprise two temporally and genetically

421   distinct sub-clades. This suggests that lions dispersed from Eurasia into Eastern Beringia in

422   multiple waves that appear to have replaced earlier populations. Our results show that the

423   timing of these dispersal and extinction events are mirrored by patterns observed in brown

424   bears, and that Pleistocene glacial cycles were an important driver of population dynamics in

425   both species. In particular, dispersal between Western and Eastern Beringia was heavily

426   influenced by presence of the Bering Land Bridge, with inferred dispersal rates across both

427   species being over an order of magnitude higher during colder periods (Fig. 3; Supplementary

428   Fig. S6).

429

430   The respective origins of the American lion (*atrox*) mitochondrial lineage (~165 kya) and

431   North American clade 4 brown bear lineage (~177 kya) — the earliest representatives of both

432   species observed in North America (Fig. 2) — occurred during MIS 6, the Illinoian glaciation

433   (Fig. 1), when the Bering Land Bridge was likely exposed (Fig. 4A). This is consistent with

434   the first lion fossil records from Eastern Beringia in Illinoian deposits (MIS 6) (Kurtén &

435   Anderson, 1980; Yamaguchi et al., 2004) followed by the first recorded lions occurring in

436   Sangamonian (MIS 5) deposits in Kansas and Texas (Dalquest, 1962; Harington, 1969;

437   Hibbard & Taylor, 1960). Notably, this also aligns with evidence that steppe bison (*Bison*

438   *priscus*) and red foxes (*Vulpes vulpes*) arrived in North America during MIS 6 (Froese et al.,

439   2017) or immediately prior (Kutschera et al., 2013; Statham et al., 2014), respectively.

440   Further, a dispersal of caballine horses (*Equus* spp.) into North America from Eurasia likely

441 occurred during MIS 6, after caballine horses initially expanded out of North America across

442 the Bering Land Bridge into Eurasia earlier in the Pleistocene (Vershinina et al., 2021).

443

444 While our results suggest that clade 4 bears and *atrox* lions likely arrived in Eastern Beringia

445 ~170 kya during MIS 6, they must have dispersed southwards soon afterwards, as individuals

446 belonging to these lineages are never observed farther north than Edmonton (~53°N)

447 following the end of MIS 3. The TMRCAs of the North American clade 4 brown bear clade at

448 92 kya and *atrox* lion clade (including all North American samples) at 81 kya both occurred

449 during MIS 5, suggesting that both species dispersed southwards during this warmer period

450 when ice sheets retreated and opened an ice-free north-south corridor (Fig. 4B). This

451 movement coincides with the first southward dispersal of bison through the ice-free corridor

452 between late MIS 6 and early MIS 5 (Froese et al., 2017; Heintzman et al., 2016; Shapiro et

453 al., 2004). The dispersal and subsequent isolation of lions south of the ice was previously

454 thought to have initiated the divergence between the American lion (*Panthera atrox*) and cave

455 lion (*P. spelaea*) (Barnett et al., 2009). However, our discovery of a ~66.7 thousand-year-old

456 *P. atrox* specimen north of the ice sheets in the Yukon Territory instead suggests that the

457 formation of the endemic American lion lineage was more likely the result of their isolation in

458 North America after the flooding of the Bering Land Bridge during MIS 5. Alternatively, this

459 Yukon *atrox* sample could plausibly represent a migrant from south of the ice sheets, but we

460 favour the former hypothesis as the timing of the split between *atrox* and *spelaea* coincides

461 with the emergence of the Bering Land Bridge and there are no putative later examples of

462 lions dispersing northwards.

463

464 Following MIS 6, the second wave of lion and brown bear dispersal into North America

465 appears to have occurred during MIS 4 when lowered sea levels next exposed the Bering

466 Land Bridge (Fig. 4C), corresponding with the respective TMRCAs of the North American

467 endemic clade 3c bears and the clade comprising the four pre-LGM Eastern Beringian *spelaea*

468 lions. However, during the interglacial period MIS 3, as the Bering Land Bridge was again

469 submerged (Hu et al., 2010) (Fig. 4D), all lions (*atrox* and *spelaea*) and brown bears (clades

470 2c, 3c, and 4) appear to have become locally extinct in Eastern Beringia (Fig. 2), with *atrox*

471 lions and clade 4 brown bears — descendants of the first wave of dispersal — surviving only

472 in the contiguous USA and southern Canada. The absence of both brown bears and lions from

473 the Eastern Beringian fossil record between 35 and 25 kya does not appear to be due to a

474 taphonomic bias, as remains of the giant short-faced bear (*Arctodus simus*) are abundant

475 during the same period (Fig. 2). Indeed, the reappearance of both lion and bear populations

476 appears to be closely linked in time to the extinction of short-faced bears in the area,

477 suggesting some form of competition (Barnes et al., 2002; Barnett et al., 2009; Davison et al.,

478 2011; Ersmark et al., 2015; Leonard et al., 2000). Importantly, the timing of these carnivoran

479 extinctions in Eastern Beringia coincides with evidence for widespread vegetation change in

480 the region, namely expansion of peatlands caused by significant paludification (Mann et al.,

481 2015; Reuther et al., 2020; Treat et al., 2019).

482

483 Populations of a number of megafaunal herbivores appear to have decreased during MIS 3,

484 possibly related to the expansion of peatlands and restrictions on foraging and nutrition

485 (Mann et al., 2015), which may have had reciprocal impacts on the megafaunal carnivores and

486 omnivores that preyed upon them, plausibly causing the local extinction of both lions and

487 brown bears. For example, musk-ox populations experienced a dramatic decrease in diversity

488 and effective population size during MIS 3 (Campos et al., 2010), mammoth populations were

489 steadily declining (Debruyne et al., 2008), and bison began to experience dramatic declines

490 towards the end of MIS 3 into MIS 2 (Drummond, Rambaut, Shapiro, & Pybus, 2005;

491 Lorenzen et al., 2011; Shapiro et al., 2004). In addition, it appears that non-caballine horses

492 (i.e., *Haringtonhippus*) underwent a bottleneck during MIS 3 with only a single fossil

493 specimen found in Eastern Beringia after ~31 kya (Guthrie, 2003; Heintzman et al., 2017)

494 around the time that the brown bear and lion populations went extinct.

495

496 In contrast to lions and brown bears, the giant short-faced bear appears to have persisted in

497 Eastern Beringia throughout MIS 3. It is possible that the mobility, large home range, and

498 solitary behaviour that has been proposed for the giant short-faced bear (Matheus, 1995;

499 Schubert & Wallace, 2009) may have allowed them to exploit food resources that were less

500 available to lions or brown bears. Grey wolves also appear to be present in eastern Beringia

501 throughout MIS 3, with no evidence of genetic turnovers (Leonard et al., 2007; Loog et al.,

502   2020). Isotope analyses have suggested Beringian wolves had similar diets to that of
503   Pleistocene lions and brown bears, consisting largely of large herbivores (Fox-Dobbs,
504   Leonard, & Koch, 2008; Leonard et al., 2007; Pilot et al., 2010). However, dietary analysis of
505   a mummified MIS 3 Beringian wolf indicated a diet with a significant proportion of aquatic
506   resources (Meachen et al., 2020), possibly indicating wolves may have exploited resources
507   that were less available to lions or brown bears in Beringia during MIS 3.

508

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

521

522   The reappearance of lions and brown bears in Eastern Beringia during MIS 2 occurred at
523   around the same time as the local extinction of *Arctodus*, which may relate to previously
524   proposed competition between brown bears and *Arctodus* (Barnes et al., 2002; Steffen &
525   Fulton, 2018). The apparent timing of the extinction of *Arctodus* in Eastern Beringia ~23 kya
526   could be linked to the sharp climatic cooling associated with Heinrich Event 2 (24.3–23.3 ka
527   BP), a period characterised by drastic climatic changes caused by large discharges of ice into
528   the North Atlantic following the collapse of the Northern Hemisphere ice sheets (Heinrich,
529   1988; Hemming, 2004). In any case, the fact that Eastern Beringia was not instead
530   recolonised by *atrox* lions and clade 4 bears from the contiguous USA may either reflect that
531   conditions had not improved sufficiently to support lion and brown bear populations in

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

539

540 All modern and ancient clade 2a brown bears from the Alexander and Haida Gwaii
541 archipelagos coalesce at ~20 kya (95% HPD: 17.0–24.0 kya), comparable to the TMRCAs for
542 Beringian clade 3b bears and *spelaea* lions. This supports the model proposed by Cahill et al.
543 (2013) for the origin of clade 2a bears, under which the mitochondrial lineage was captured
544 by brown bears following male-biased gene-flow into a population of polar bears stranded in
545 the Alexander Archipelago after the retraction of ice sheets post-LGM. Assuming all our
546 ancient Alexander and Haida Gwaii archipelago samples represent brown bears (or at least
547 brown-polar hybrids), and that mtDNA diversity in the stranded polar bear population was
548 low, the coalescence of our samples can be considered a proxy for the minimum age of
549 hybridisation between polar and brown bears, and hence a minimum age for the arrival of
550 brown bears in the Alexander and Haida Gwaii archipelagos post-LGM. If this is the case,
551 then brown bears arrived in the islands no later than 17 kya (the lower bound of the 95%
552 HPD). That timing is coincident with the first records of brown bears on the Haida Gwaii
553 archipelago ~17.5 kya (Ramsey, Griffiths, Fedje, Wigen, & Mackie, 2004) and the existence
554 of unglaciated western Alaskan coastline, which represents an alternative southward dispersal
555 pathway into the continent that may also have been exploited by humans (Lesnek, Briner,
556 Lindqvist, Baichtal, & Heaton, 2018; Shaw, Barrie, Conway, Lintern, & Kung, 2020).

557

558 Overall, our results highlight the key role of Pleistocene glacial cycles in driving the
559 distribution and diversity of North American carnivorans. Glacial cycles may also have driven
560 parallel waves of dispersal in other regions, such as across the Sakhalin land bridge that
561 connected Japan with mainland Asia. Such a dispersal event may explain the origin and

562   distribution of Japanese wolves (Matsumura, Inoshima, & Ishiguro, 2014; Niemann et al.,
563   2021). Further, genetic evidence from modern Japanese brown bears suggests multiple waves
564   of Pleistocene dispersal in a similar temporally staggered sequence, with present day Japanese
565   mitochondrial diversity closely mirroring that observed in modern Eastern Beringia (i.e.,
566   clades 3a, 3b, and 4) and also exhibiting a marked phylogeographic structure (Hirata et al.,
567   2013). Analysis of ancient Japanese brown bear specimens might allow determination of
568   whether extinct Eastern Beringian clades such as 3c were also present in Japan during the
569   Late Pleistocene.

570

571   **Conclusion:**

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

582

583   A combined biogeographical approach may also be useful for exploring the timing of faunal
584   dispersals through the ice-free corridor between the North American ice sheets, which
585   available data suggest are biased southwards, with few observed northward dispersals.
586   However, this apparent bias may be due to many ancient DNA studies focusing on recently
587   immigrated taxa (e.g., brown bears, bison, wapiti, humans) for which Eastern Beringia acts as
588   a source, with the contiguous USA likely a sink. Endemic North American species may
589   exhibit different patterns of phylogeography and dispersal, and large ancient DNA datasets
590   from species like the giant short-faced bear or the western camel (*Camelops hesternus*) would
591   be valuable in evaluating this possibility. In any case, our densely-sampled study of two

592 carnivorans contributes to the growing body of research suggesting remarkably concerted
593 responses to Pleistocene geographical and environmental changes across many megafaunal
594 taxa (e.g., Cooper et al., 2015).

595

621

622   **References:**

623   Ardelean, C. F., Becerra-Valdivia, L., Pedersen, M. W., Schwenninger, J. L., Oviatt, C. G.,
624   Macias-Quintero, J. I., . . . Willerslev, E. (2020). Evidence of human occupation in
625   Mexico around the Last Glacial Maximum. *Nature, 584*, 87–92. doi:10.1038/s41586-
626   020-2509-0

627   Barnes, I., Matheus, P., Shapiro, B., Jensen, D., & Cooper, A. (2002). Dynamics of
628   Pleistocene population extinctions in Beringian brown bears. *Science, 295*(5563),
629   2267-2270. doi:10.1126/science.1067814

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

634   Barnett, R., Shapiro, B., Barnes, I., Ho, S. Y. W., Burger, J., Yamaguchi, N., . . . Cooper, A.
635   (2009). Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a
636   late Pleistocene reduction in genetic diversity. *Molecular Ecology, 18*(8), 1668-1677.
637   doi:10.1111/j.1365-294X.2009.04134.x

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

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

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

647   Bray, S. C. E., Austin, J. J., Metcalf, J. L., Østbye, K., Østbye, E., Lauritzen, S.-E., . . .
648   Cooper, A. (2013). Ancient DNA identifies post-glacial recolonisation, not recent
649   bottlenecks, as the primary driver of contemporary mtDNA phylogeography and

650   diversity in Scandinavian brown bears. *Diversity and Distributions, 19*(3), 245-256.
651   doi:10.1111/j.1472-4642.2012.00923.x

652   Cahill, J. A., Green, R. E., Fulton, T. L., Stiller, M., Jay, F., Ovsyanikov, N., . . . Shapiro, B.
653   (2013). Genomic evidence for island population conversion resolves conflicting
654   theories of polar bear evolution. *PLoS Genetics, 9*(3), e1003345.
655   doi:10.1371/journal.pgen.1003345

656   Cahill, J. A., Heintzman, P. D., Harris, K., Teasdale, M. D., Kapp, J., Soares, A. E. R., . . .
657   Shapiro, B. (2018). Genomic evidence of widespread admixture from polar bears into
658   brown bears during the last ice age. *Molecular Biology and Evolution, 35*(5), 1120-
659   1129. doi:10.1093/molbev/msy018

660   Cahill, J. A., Stirling, I., Kistler, L., Salamzade, R., Ersmark, E., Fulton, T. L., . . . Shapiro, B.
661   (2015). Genomic evidence of geographically widespread effect of gene flow from
662   polar bears into brown bears. *Molecular Ecology, 24*(6), 1205-1217.
663   doi:10.1111/mec.13038

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

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

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

676   Cooper, A., & Poinar, H. N. (2000). Ancient DNA: Do it right or not at all. *Science,*
677   *289*(5482), 1139. doi:10.1126/science.289.5482.1139b

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

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

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

687   Davison, J., Ho, S. Y. W., Bray, S. C., Korsten, M., Tammeleht, E., Hindrikson, M., . . .
688   Saarma, U. (2011). Late-Quaternary biogeographic scenarios for the brown bear
689   (*Ursus arctos*), a wild mammal model species. *Quaternary Science Reviews, 30*(3-4),
690   418-430. doi:10.1016/j.quascirev.2010.11.023

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

732 Harington, C. R. (1969). Pleistocene Remains of the Lion-Like Cat (*Panthera atrox*) from
733   Yukon Territory and Northern Alaska. *Canadian Journal of Earth Sciences, 6*(5),
734   1277-1288. doi:10.1139/e69-127

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

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

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

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

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

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

752 Hibbard, C. W., & Taylor, D. W. (1960). Two late Pleistocene faunas from southwestern
753   Kansas. *Contributions from the Museum of Paleontology, University of Michigan,*
754   *16*(1), 1-223.

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

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

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

764 Hundertmark, K. J., Shields, G. F., Udina, I. G., Bowyer, R. T., Danilkin, A. A., & Schwartz,
765   C. C. (2002). Mitochondrial phylogeography of moose (*Alces alces*): Late Pleistocene
766   divergence and population expansion. *Molecular Phylogenetics and Evolution, 22*(3),
767   375-387. doi:10.1006/mpev.2001.1058

768 Hwang, D. S., Ki, J. S., Jeong, D. H., Kim, B. H., Lee, B. K., Han, S. H., & Lee, J. S. (2008).
769   A comprehensive analysis of three Asiatic black bear mitochondrial genomes
770   (subspecies *ussuricus*, *formosanus* and *mupinensis*), with emphasis on the complete
771   mtDNA sequence of *Ursus thibetanus ussuricus* (Ursidae). *DNA Sequence, 19*(4),
772   418-429. doi:10.1080/19401730802389525

773 Jakobsson, M., Pearce, C., Cronin, T. M., Backman, J., Anderson, L. G., Barrientos, N., . . .
774   O'Regan, M. (2017). Post-glacial flooding of the Bering Land Bridge dated to
775   11 cal ka BP based on new geophysical and sediment records. *Climate of the Past,*
776   *13*(8), 991-1005. doi:10.5194/cp-13-991-2017

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

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

783 Kurtén, B. (1985). The Pleistocene Lion of Beringia. *Annales Zoologici Fennici, 22*(1), 117-
784   121.

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

787 Kutschera, V. E., Lecomte, N., Janke, A., Selva, N., Sokolov, A. A., Haun, T., . . . Hailer, F.
788   (2013). A range-wide synthesis and timeline for phylogeographic events in the red fox
789   (*Vulpes vulpes*). *BMC Evolutionary Biology, 13*, 114. doi:10.1186/1471-2148-13-114

790 Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2016). PartitionFinder
791   2: New Methods for Selecting Partitioned Models of Evolution for Molecular and
792   Morphological Phylogenetic Analyses. *Molecular Biology and Evolution, 34*(3), 772-
793   773. doi:10.1093/molbev/msw260

794 Leonard, J. A., Vila, C., Fox-Dobbs, K., Koch, P. L., Wayne, R. K., & Van Valkenburgh, B.
795   (2007). Megafaunal extinctions and the disappearance of a specialized wolf ecomorph.
796   *Current Biology, 17*(13), 1146-1150. doi:10.1016/j.cub.2007.05.072

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

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

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

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

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

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

814 Liu, S. P., Lorenzen, E. D., Fumagalli, M., Li, B., Harris, K., Xiong, Z. J., . . . Wang, J.
815   (2014). Population genomics reveal recent speciation and rapid evolutionary
816   adaptation in polar bears. *Cell, 157*(4), 785-794. doi:10.1016/j.cell.2014.03.054

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

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

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

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

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

834 Meachen, J., Wooller, M. J., Barst, B. D., Funck, J., Crann, C., Heath, J., . . . Zazula, G.
835   (2020). A mummified Pleistocene gray wolf pup. *Current Biology, 30*(24), R1467-
836   R1468. doi:10.1016/j.cub.2020.11.011

837 Meiri, M., Lister, A. M., Collins, M. J., Tuross, N., Goebel, T., Blockley, S., . . . Barnes, I.
838   (2014). Faunal record identifies Bering isthmus conditions as constraint to end-
839   Pleistocene migration to the New World. *Proceedings of the Royal Society B:*
840   *Biological Sciences, 281*(1776). doi:10.1098/rspb.2013.2167

841   Meyer, M., Kircher, M., Gansauge, M. T., Li, H., Racimo, F., Mallick, S., . . . Paabo, S.
842   (2012). A high-coverage genome sequence from an archaic Denisovan individual.
843   *Science, 338*(6104), 222-226. doi:10.1126/science.1224344

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

849   Mitchell, K. J., Bray, S. C., Bover, P., Soibelzon, L., Schubert, B. W., Prevosti, F., . . .
850   Cooper, A. (2016). Ancient mitochondrial DNA reveals convergent evolution of giant
851   short-faced bears (Tremarctinae) in North and South America. *Biology Letters, 12*(4),
852   20160062. doi:10.1098/rsbl.2016.0062

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

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

860   Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior
861   summarization in bayesian phylogenetics using Tracer 1.7. *Systematic Biology, 67*(5),
862   901-904. doi:10.1093/sysbio/syy032

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

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

868   Ramsey, C. L., Griffiths, P. A., Fedje, D. W., Wigen, R. J., & Mackie, Q. (2004). Preliminary

869   investigation of a late Wisconsinan fauna from K1 cave, Queen Charlotte Islands

870   (Haida Gwaii), Canada. *Quaternary Research, 62*(1), 105-109.

871   doi:10.1016/j.yqres.2004.05.003

872   Reimer, P. J., Bard, E., Bayliss, A., Beck, J. W., Blackwell, P. G., Ramsey, C. B., . . . van der

873   Plicht, J. (2013). Intcal13 and Marine13 radiocarbon age calibration curves 0-50,000

874   years cal BP. *Radiocarbon, 55*(4), 1869-1887. doi:10.2458/azu_js_rc.55.16947

875   Reuther, J. D., Rogers, J., Druckenmiller, P., Bundtzen, T. K., Wallace, K., Bowman, R., . . .

876   Cherkinsky, A. (2020). Late Quaternary (≥MIS 3 to MIS 1) stratigraphic transitions in

877   a highland Beringian landscape along the Kuskokwim River, Alaska. *Quaternary*

878   *Research, 93*, 139-154. doi:10.1017/qua.2019.51

879   Rey-Iglesia, A., Garcia-Vazquez, A., Treadaway, E. C., van der Plicht, J., Baryshnikov, G. F.,

880   Szpak, P., . . . Lorenzen, E. D. (2019). Evolutionary history and palaeoecology of

881   brown bear in North-East Siberia re-examined using ancient DNA and stable isotopes

882   from skeletal remains. *Scientific Reports, 9*(1), 4462. doi:10.1038/s41598-019-40168-

883   7

884   Richards, S. M., Hovhannisyan, N., Gilliham, M., Ingram, J., Skadhauge, B., Heiniger, H., . . .

885   Cooper, A. (2019). Low-cost cross-taxon enrichment of mitochondrial DNA using in-

886   house synthesised RNA probes. *PLoS ONE, 14*(2), e0209499.

887   doi:10.1371/journal.pone.0209499

888   Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., & Reich, D. (2015). Partial uracil-DNA-

889   glycosylase treatment for screening of ancient DNA. *Philosophical Transactions of*

890   *the Royal Society of London B Biological Sciences, 370*(1660), 20130624.

891   doi:10.1098/rstb.2013.0624

892   Sanmartin, I., Van der Mark, P., & Ronquist, F. (2008). Inferring dispersal: a Bayesian

893   approach to phylogeny-based island biogeography, with special reference to the

894   Canary Islands. *Journal of Biogeography, 35*(3), 428-449. doi:10.1111/j.1365-

895   2699.2008.01885.x

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

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

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

906   Shapiro, B., Drummond, A. J., Rambaut, A., Wilson, M. C., Matheus, P. E., Sher, A. V., . . .
907   Cooper, A. (2004). Rise and fall of the Beringian steppe bison. *Science, 306*(5701),
908   1561-1565. doi:10.1126/science.1101074

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

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

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

919   Steffen, M. L., & Fulton, T. L. (2018). On the association of giant short-faced bear (*Arctodus*
920   *simus*) and brown bear (*Ursus arctos*) in late Pleistocene North America. *Geobios,*
921   *51*(1), 61-74. doi:10.1016/j.geobios.2017.12.001

922   Stiller, M., Molak, M., Prost, S., Rabeder, G., Baryshnikov, G., Rosendahl, W., . . . Knapp,
923   M. (2014). Mitochondrial DNA diversity and evolution of the Pleistocene cave bear

924   complex. *Quaternary International, 339-340*, 224-231.

925   doi:10.1016/j.quaint.2013.09.023

926   Stuart, A. J., & Lister, A. M. (2011). Extinction chronology of the cave lion *Panthera*

927   *spelaea*. *Quaternary Science Reviews, 30*(17-18), 2329-2340.

928   doi:10.1016/j.quascirev.2010.04.023

929   Subramanian, S., & Lambert, D. M. (2011). Time dependency of molecular evolutionary

930   rates? Yes and no. *Genome Biology and Evolution, 3*, 1324-1328.

931   doi:10.1093/gbe/evr108

932   Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018).

933   Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus*

934   *Evolution, 4*(1), vey016. doi:10.1093/ve/vey016

935   Talbot, S. L., & Shields, G. F. (1996). Phylogeography of brown bears (*Ursus arctos*) of

936   Alaska and paraphyly within the Ursidae. *Molecular Phylogenetics and Evolution,*

937   *5*(3), 477-494. doi:DOI 10.1006/mpev.1996.0044

938   Treat, C. C., Kleinen, T., Broothaerts, N., Dalton, A. S., Dommain, R., Douglas, T. A., . . .

939   Brovkin, V. (2019). Widespread global peatland establishment and persistence over

940   the last 130,000 y. *Proceedings of the National Academy of Sciences of the United*

941   *States of America, 116*(11), 4822-4827. doi:10.1073/pnas.1813305116

942   Vershinina, A. O., Heintzman, P. D., Froese, D. G., Zazula, G., Cassatt-Johnstone, M., Dalen,

943   L., . . . Shapiro, B. (2021). Ancient horse genomes reveal the timing and extent of

944   dispersals across the Bering Land Bridge. *Molecular Ecology*. doi:10.1111/mec.15977

945   Waits, L. P., Talbot, S. L., Ward, R. H., & Shields, G. F. (1998). Mitochondrial DNA

946   phylogeography of the North American brown bear and implications for conservation.

947   *Conservation Biology, 12*(2), 408-417. doi:10.1046/j.1523-1739.1998.96351.x

948   Whitmore, F. C., & Foster, H. L. (1967). *Panthera atrox* (Mammalia: Felidae) from Central

949   Alaska. *Journal of Paleontology, 41*(1), 247-251.

950 Yamaguchi, N., Cooper, A., Werdelin, L., & Macdonald, D. W. (2004). Evolution of the
951   mane and group-living in the lion (Panthera leo): a review. *Journal of Zoology, 263*,
952   329-342. doi:10.1017/S0952836904005242

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Data Availability Statement

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

959

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Author Contributions

962 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
963 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.,
964 N.Y., and J.A.M. contributed new reagents/analytic tools; A.T.S., M.S.Y.L., and K.J.M.
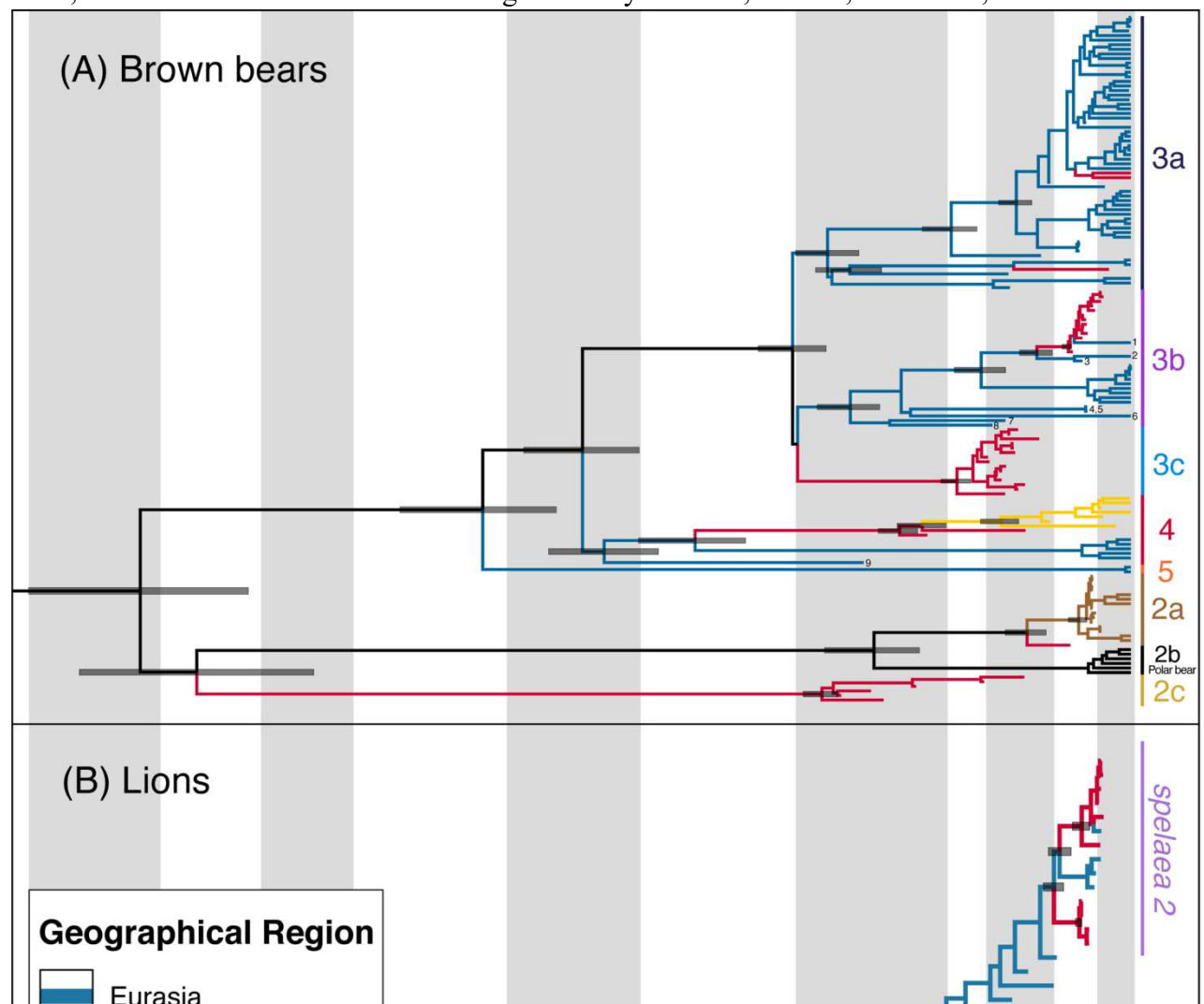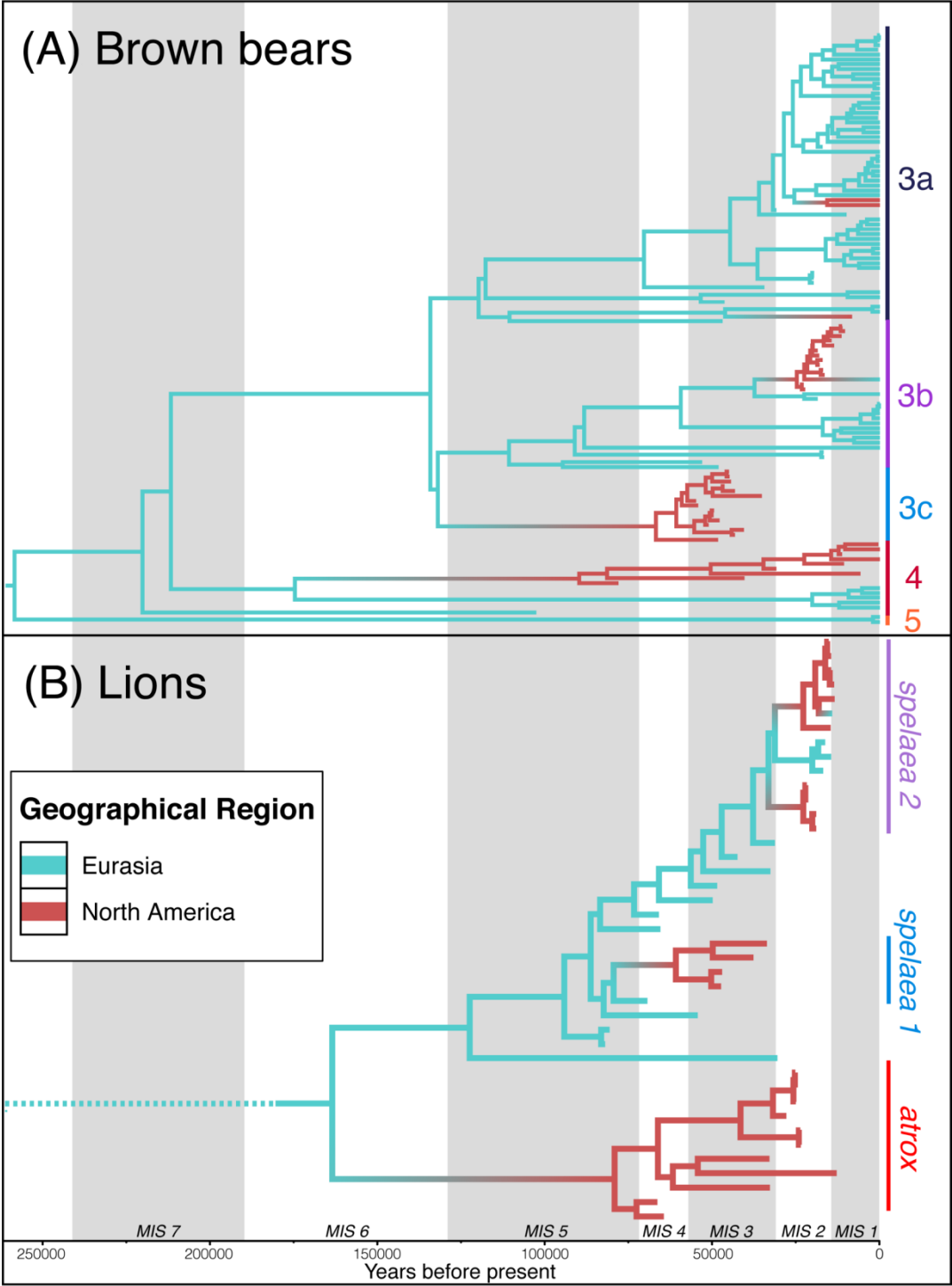
990 **Fig. 1.** Bayesian phylogenetic trees inferred from (A) brown bear and (B) lion mitogenomes.
991   The grey vertical columns represent odd-numbered MIS stages (interglacials) and white
992   columns even-numbered MIS stages (glacials). Bars on nodes represent 95% Highest
993   Posterior Densities for node age estimates indicated for modes leading to major clades
994   and those reported in main text. Numbers on tips in (A) refer to selected specimens
995   mentioned in text: 1 = A155, 2 = A156, 3 = A1945, 4 = A1944, 5 = A1946, 6 = A138, 7
996   = A5889, 8 = MH255807, 9 = A5883. For detailed trees with tip labels, and posterior
997   support values see Supplementary Figs S4 and S5.

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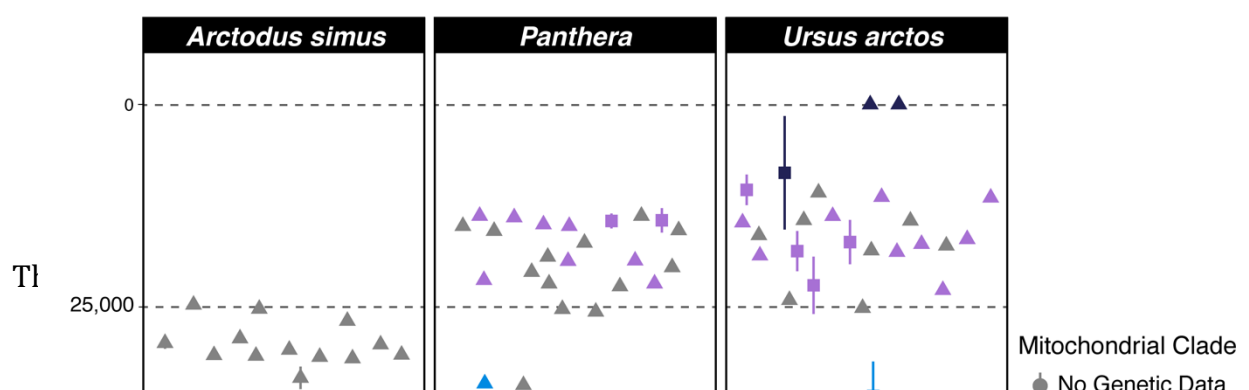
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1018 **Fig. 2.** Temporal distribution of Eastern Beringian giant short-faced bear (*Arctodus simus*),

1019 lion (*Panthera* spp.), and brown bear (*Ursus arctos*) specimens based on radiocarbon

1020 and molecular age estimates. Ages are shown with one standard error and are coloured

1021 by genetic clade. For additional radiocarbon dates used to produce this plot see

1022 Supplementary Table S3.

1023

(A) Brown bears

3a

3b

3c

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(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

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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.

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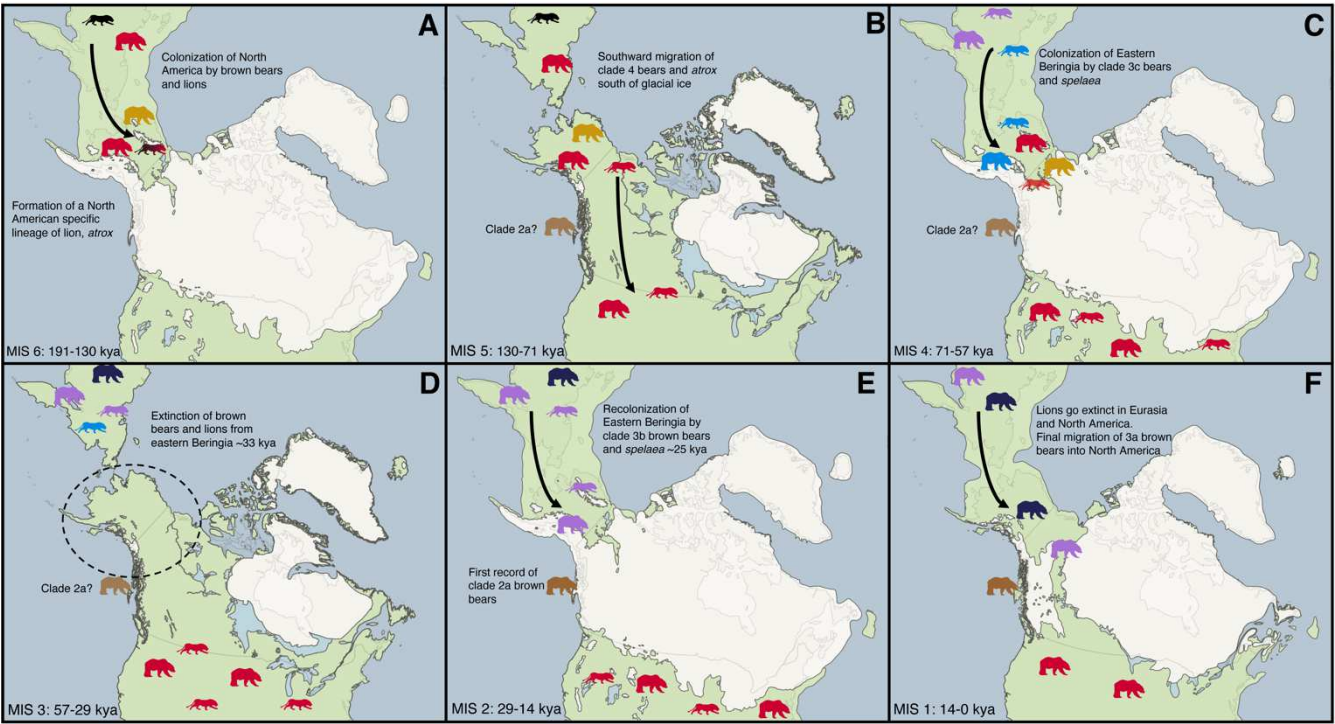
1083 **Fig. 4.** Map of Late Quaternary phylogeography of North American brown bears and lions

1084 during six time periods. A) MIS 6, 191–130 kya, brown bears and lions first colonise

1085 North America via the Bering Land Bridge; B) MIS 5, 130–71 kya, Bering Land Bridge

1086 is flooded, dispersal of brown bears and lions south of continental ice sheets; C) MIS 4,

1087 71–57 kya, dispersal of clade 3c bears and *spelaea* lions (*spelaea* 1) across the Bering

1088 Land Bridge; D) MIS 3, 57–29 kya, flooding of Bering Land Bridge and extinction of

1089 brown bears and lions in Eastern Beringia; E) MIS 2, Last Glacial Maximum, 29–14

1090 kya, dispersal of clade 3b bears and second wave of *spelaea* lions (*spelaea* 2); and F)

1091 MIS 1, Holocene, 14 kya to present, lions go extinct in North America and Eurasia,

1092 additionally clade 3a bears disperse into Eastern Beringia before the Bering Land

1093 Bridge is flooded for the last time. Different coloured silhouettes of brown bears and

1094 lions represent different genetic clades, corresponding to clade colouring in Figs 1 and

1095 2. White area represents the approximate extent of glacial ice along with rough

1096 estimates of Bering Land Bridge extent during the different time periods using spatial

1097 data from Dyke, Moore, and Robertson (2003).