

Phylogeography of lions (*Panthera leo* ssp.) reveals three distinct taxa and a late Pleistocene reduction in genetic diversity

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Abstract

Lions were the most widespread carnivores in the late Pleistocene, ranging from southern Africa to the southern USA, but little is known about the evolutionary relationships among these Pleistocene populations or the dynamics that led to their extinction. Using ancient DNA techniques, we obtained mitochondrial sequences from 52 individuals sampled across the present and former range of lions. Phylogenetic analysis revealed three distinct clusters: (i) modern lions, *Panthera leo*; (ii) extinct Pleistocene cave lions, which formed a homogeneous population extending from Europe across Beringia (Siberia, Alaska and western Canada); and (iii) extinct American lions, which formed a separate population south of the Pleistocene ice sheets. The American lion appears to have become genetically isolated around 340 000 years ago, despite the apparent lack of significant barriers to gene flow with Beringian populations through much of the late Pleistocene. We found potential evidence of a severe population bottleneck in the cave lion during the previous interstadial, sometime after 48 000 years, adding to evidence from bison, mammoths, horses and brown bears that megafaunal populations underwent major genetic alterations throughout the last interstadial, potentially presaging the processes involved in the subsequent end-Pleistocene mass extinctions.

Keywords: American lion, ancient DNA, Beringia, cave lion, extinction, megafauna

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Introduction

Lions currently survive only in parts of Africa and India, yet as recently as the late Pleistocene (LP), large and morphologically diverse forms of lion were distributed across the Northern Hemisphere. Lions survived in Europe,

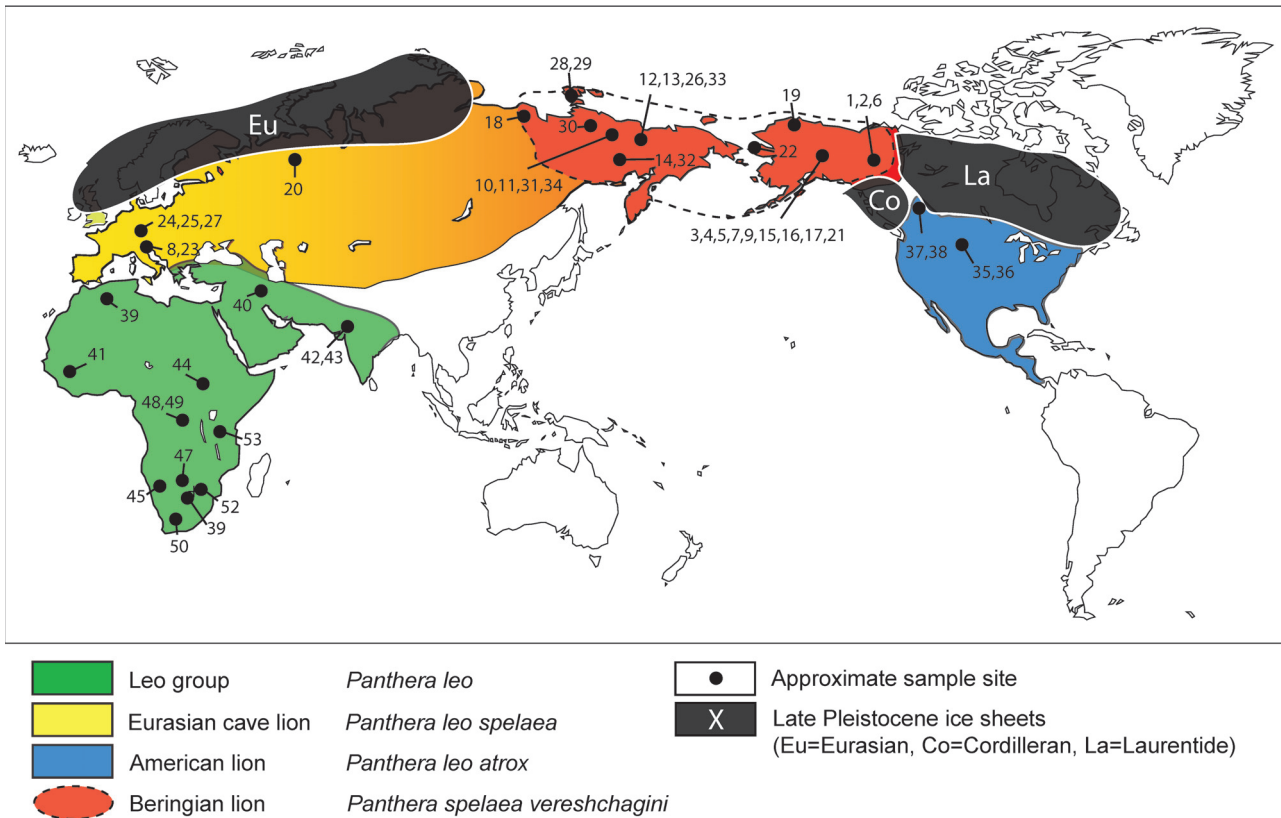


Fig. 1 Pleistocene lion distribution and sample origin. Colours correspond to commonly cited population subdivisions (e.g. Baryshnikov & Boeskorov 2001). Numbers refer to particular samples used in this analysis (Table 1).

northern Asia, and North America until around 11 000 BP (Fig. 1), when they disappeared as part of the end-Pleistocene megafaunal mass extinctions (Martin & Steadman 1999). Human hunting and/or environmental alteration, and climate changes associated with the last glacial maximum (LGM; 22 000–18 000 BP) and the end of the Pleistocene have variously been implicated (Barnosky *et al.* 2004).

In eastern Beringia, a Pleistocene landmass consisting of present-day Alaska and Canada’s Yukon Territory, genetic and palaeontological data from large mammal populations suggest that a key period of change occurred around 40 000–21 000 BP, in the period before the LGM (Barnes *et al.* 2002; Guthrie 2003; Shapiro *et al.* 2004). Due to their worldwide distribution, genetic analyses of Pleistocene lions potentially hold key insights into how megafaunal carnivore populations responded to environmental changes in different regions. Additionally, a better understanding of how and when lion populations went extinct is vital for reconstructing community-level dynamics during the LP, which in turn is fundamental for testing hypotheses about the cause of the end-Pleistocene extinctions.

All Pleistocene and modern lion specimens are assigned to the genus *Panthera*, but there is little consensus as to the

number of distinct species or the extent of overlap of their distributions (for a comprehensive overview of the taxonomy of pantherine cats, please refer to Burger *et al.* 2004). Morphological identification is complicated by the presence of shared primitive features (e.g. Sotnikova & Nikolskiy 2006), although characteristics such as body size or the presence/absence of a mane in males have been used to suggest taxonomic distinctiveness between geographical regions (e.g. Kurtén 1968; Yamaguchi *et al.* 2004; Sotnikova, Nikolskiy 2006). Pleistocene lions in Europe are usually referred to as *spelaea*, while North American lions are given the designation *atrox*. However, the status of these groups as species or subspecies, and their relationships to each other and to extant *Panthera* remains unclear (e.g. Simpson 1941; Kurtén 1985; Harington 1971; Vereshchagin 1971; Kurtén & Anderson 1980; Herrington 1986; Groiss 1996; Turner & Antón 1997; Baryshnikov & Boeskorov 2001; Burger *et al.* 2004; Sotnikova, Nikolskiy 2006).

In this study, the first to examine intraspecific diversity in an extinct Pleistocene felid, we investigate the relationships among the putative lion subspecies. This was carried out by obtaining mitochondrial DNA sequences from modern and ancient lions. The evolutionary and

demographic history of the sequences is inferred using phylogenetic analysis, and the results are interpreted in the context of environmental changes in the late Pleistocene.

Materials and methods

More than 200 cortical bone samples were obtained from museum specimens of modern, Pleistocene, and American lions (Table S1, Supporting information). Specimens ranged in age from modern to > 62 000 years. To prevent duplicate sampling within a site, either the same skeletal element or samples associated with widely separated radiocarbon dates were chosen. DNA extraction was performed as described in Barnett *et al.* (2006) in dedicated ancient DNA (aDNA) facilities at the University of Oxford. Four European lions were also extracted and amplified at Mainz University under similarly stringent conditions (Burger *et al.* 2004). One of the cave lions from Mainz (sample 8 in Table 1) was previously analysed for cytochrome *b* in an earlier study showing the phylogenetic position of the cave lion among the *Panthera* cats (Burger *et al.* 2004).

A ~215-bp fragment of the mitochondrial hypervariable region 1 (HVR1) and a 143-bp fragment of *ATP8* were polymerase chain reaction (PCR) amplified, and cloned, purified, and sequenced following Barnett *et al.* (2006). Strict aDNA protocols were followed, including the use of multiple PCRs and negative controls, overlapping fragments and independent replication. Nuclear mitochondrial inserts, which are known to be widespread among felids (e.g. Kim *et al.* 2006), were identified and excluded from the analysis. All specimens possessing unique haplotypes, along with a subset of those with shared haplotypes, were amplified several times and cloned to check for the presence of contamination (details are given in Supporting Information: supporting text, Tables S1–S3).

Thirty-three samples were submitted to the Oxford Radiocarbon Accelerator Unit (University of Oxford) for radiocarbon analysis. Analyses were performed using 0.2 g of bone taken from a site adjacent to the sample used for DNA extraction. An additional sample (sample 8 in Table 1) was radiocarbon dated at the Leibniz laboratory of the University of Kiel. For all samples, total bone collagen was extracted, graphitized, and dated by accelerator mass spectrometry. Dates are presented as uncalibrated radiocarbon values. Two of the oldest specimens (samples 31 and 34 in Table 1) were dated twice, to test the reproducibility of dates in the > 50 000-year time frame.

Phylogenetic analyses were performed on the HVR1 and *ATP8* data sets separately, due to the differing number of specimens that yielded sequences from the two regions (Supporting Information: supporting text, Table S3). Median-joining networks were produced for both data sets using the program Network version 4.1.0.3 (Bandelt *et al.* 1999).

Bayesian Markov chain Monte Carlo (MCMC) analyses were then performed using BEAST (Drummond & Rambaut 2007), first for HVR1 and *ATP8* data sets separately, and then for a smaller, combined analysis consisting of only those samples from which both sequence fragments could be amplified and which were associated with finite radiocarbon dates. For all three data sets, comparison of Akaike information criterion scores suggested the HKY85 model of nucleotide substitution. Each BEAST analysis assumed this substitution model as well as a constant population size and a strict molecular clock calibrated using the age of the split between the *spelaea* and *leo* groups (with a normal prior mean of 550 000 years, standard deviation 25 000 years), based on the first appearance of *Panthera leo fossilis* (Garcia Garcia 2001; Burger *et al.* 2004). Demographic and evolutionary model parameters were then estimated simultaneously along with the phylogeny, with samples drawn from the posterior every 5000 MCMC steps over a total of 5 000 000 steps. The first 500 000 steps were discarded as burn-in. Acceptable mixing and convergence to stationarity were checked using the program Tracer version 1.4 (Rambaut & Drummond 2007).

Results

DNA was successfully amplified from 38 LP and 14 modern lions (Fig. 1, Table S3, Supporting Information). The repeated extractions, amplifications, and cloning experiments yielded consistent results and negative controls at the extraction and PCR stages were uniformly clean. Collagen levels in the samples submitted for radiocarbon analysis were consistently high, indicating good biochemical preservation. The specimens older than 50 000 years that were dated twice gave results within one standard deviation (Supporting information). The sequences contained 48 parsimony-informative sites, distinguishing 24 distinct haplotypes. These are subdivided into three well-supported clusters in both Bayesian (Fig. 2) and median-joining network analyses (Fig. 3): modern lion (*Panthera leo*, from Africa and southwest Asia), cave lion (*spelaea*, from Europe, Siberia, Alaska, and the Yukon) and American lion (*atrox*, from south of the LP glacial ice sheets in North America). All late Pleistocene lion samples produced sequences that grouped strongly with modern lion data (Jae-Heup *et al.* 2001; Barnett *et al.* 2006), rejecting any postulated link between *atrox* and jaguar (*Panthera onca*) (Simpson 1941; Christiansen 2008) or *spelaea* and tiger (*Panthera tigris*) (Herrington 1986; Groiss 1996).

Previous work has shown nuclear translocations of mitochondrial DNA (numts) to be common in cats (Lopez *et al.* 1994; Lopez *et al.* 1996; Cracraft *et al.* 1998; Kim *et al.* 2006). A possible numt of the lion HVR1 was found in only one of the 15 modern lions (sample 50 in Table 1). This numt was similar to that previously reported for other

Table 1 List of samples used in this study along with museum accession numbers, sample provenance, haplotype assignment, radiocarbon dates and whether the extract was also used in an earlier study

Sample number	Control region haplotype	Extract	Element	Museum accession no.	Origin	Uncalibrated radiocarbon date	OxAU accession no.	Partial sequence also in Barnett <i>et al.</i> (2006)
1	A	IB133	L. Humerus	CMN 47294	Gold Run Ck, Yukon, Canada	12 640 ± 75	OxA-10083	YES
2	A	RB112	Canine Root	CMN 43789	Caribou Ck, Yukon, Canada, 1985	n/a	n/a	n/a
3	A	RB74	Phalanx	FAM69073	Fairbanks Ck, Alaska, USA, 1949	n/a	n/a	n/a
4	A	RB75	Tibia	FAM69167	Ester Ck, Alaska, USA, 1938	12 090 ± 80	OxA-13451	YES
5	B	IB134	Ramus	FAM 69016	Gold Hill, Alaska, USA, 1952	18 240 ± 90	OxA-10084	n/a
6	B	IB136	R. Femur	CMN 35609	Hunker Ck, Dawson, Canada, 1978	15 550 ± 90	OxA-10086	n/a
7	B	IB138	Humerus	FAM A-780-1435	Fairbanks Ck, Alaska, USA, 1955	11 925 ± 70	OxA-10080	n/a
8	B	MZ124	Pelvis	MKU	Tischhofer Höhle, Austria	31 890 ± 300	KIA 16510	n/a
9	B	RB18	Humerus	FAM 69105	Engineer Ck, Alaska, USA, 1941	n/a	n/a	n/a
10	B	RB36	Radius	PIN 3020-350	Alazeya River, Russia, 1972	13 770 ± 55	OxA-13835	n/a
11	B	RB39	Ulna	IEM 153-003	Arga-Yurekh River, Russia, 1982	12 525 ± 50	OxA-13833	n/a
12	B	RB41	Calcaneus	GIN 772-95/1341	Duvanny Yar, Loc.1341, Russia, 1964	46 200 ± 1500	OxA-13024	YES
13	B	RB45	Radius	GIN xDYa-84	Duvanny Yar, Russia, 1984	28 720 ± 160	OxA-12981	YES
14	B	RB47	Ulna	PIN 3020-73	Beryozovka River, Russia, 1970	27 950 ± 140	OxA-13831	n/a
15	B	RB72	Humerus	FAM69101	Fairbanks Ck, Alaska, USA, 1950	n/a	n/a	n/a
16	B	RB73	Humerus	FAM69104	Lower Gold Stream, Alaska, USA, 1939	n/a	n/a	n/a
17	B	RB77	Ulna	FAM69138	Fairbanks Ck, Alaska, USA, 1951	17 890 ± 100	OxA-13452	n/a
18	B	RB91	Femur	ZIN 29421(2)	Lena River, Russia	12 450 ± 60	OxA-12901	n/a
19	B	RB96	Bone	UAF IK-01-409	North Slope, Alaska, USA	12 630 ± 60	OxA-13473	n/a
20	B	RB157	Bone	UB J.S.951	Ural Mountains, Russia	~13 000	Date supplied by A. Hufthammer	n/a
21	C	RB17	Ulna	FAM 69139	Fairbanks Ck, Alaska, USA, 1954	16 005 ± 65	OxA-13834	n/a
22	C	RB24	Ramus	FAM 30757	Banner Ck, Alaska, USA, 1938	15 975 ± 65	OxA-13832	n/a
23	D	RB60	Tibia	VIE GS-27	Gamssulzen, Höhle, Austria	49 900 ± 1500	OxA-13110	YES
24	E	MZ477b	Femur	UEN	Gremsdorf, Germany	28 310 ± 50	OxA-14862	n/a
25	F	MZ475	Phalanx	UMB	Zoolithenhöhle, Germany	47 600 ± 900	OxA-14863	n/a
26	G	RB43	Mandible	IPF AR(DYA)2002	Duvanny Yar, Russia, 2002	> 53 200	OxA-13022	n/a
27	G	RB64	Calcaneus	STU 9995.2	Sibyllen Höhle, Germany	> 48 100	OxA-15354	YES
28	H	RB115	Tibia	IEM bl-0418-1	Bolshoi Liakhovsky Island, Russia, shore 1998	> 62 100	OxA-13837	n/a
29	H	RB116	Bone	IEM bl-0192-1	Bolshoi Liakhovsky Island, Russia, in situ 1998	> 60 700	OxA-13836	n/a
30	H	RB42	Femur	PIN 3915-121	Khromskaya Guba, Khaptashinskiy, Russia, 1978	> 50 600	OxA-13023	n/a
31	I	RB38	Calcaneus	PIN 2998-217	Chukochya, Loc. N 27, Kolyma, Russia, 1969	55 700 ± 3000	OxA-13025	n/a
						54 600 ± 1700	OxA-13475	
32	J	RB44	Radius	PIN 3916-162	Krestovka River, Loc.6, Russia, 1979	54 100 ± 1800	OxA-13830	n/a
33	K	RB46	Femur	PIN 3752-24A	Duvanny Yar, Russia, 1976	> 61 500	OxA-13829	n/a
34	L	RB48	Calcaneus	GIN KON.345	Alazeya River, Russia, 1950s	55 300 ± 2500	OxA-13021	n/a
						58 200 ± 3500	OxA-13474	

Table 1 Continued

Sample number	Control region haplotype	Extract	Element	Museum accession no.	Origin	Uncalibrated radiocarbon date	ORAU accession no.	Partial sequence also in Barnett <i>et al.</i> (2006)
35	M	IB174/RB153	Humerus	KU44409	Natural Trap Cave, Wyoming, USA	24 080 ± 170	OxA-10078	n/a
36	N	IB177/RB154	Humerus	KU44000	Natural Trap Cave, Wyoming, USA	n/a	n/a	n/a
37	O	RB113	R. Mandible	EDM P98.5.404	Consolidated pit 48, Edmonton, Canada	28 940 ± 240	OxA-13453	n/a
38	P	RB95	L. Metatarsal II	EDM P89.13.546	Consolidated pit 48, Edmonton, Canada	11 355 ± 55	OxA-12900	n/a
39	Q	RB206	Mandible	STK A58:5287	Barbary, 1831	n/a	n/a	YES
40	R	IB 4, RB243	Drilled Bone	PAR 1962-2854	Iran	n/a	n/a	YES
41	S	IB 27	Drilled Bone	PAR cA-1892	Senegal	n/a	n/a	n/a
42	T	IB43	Tissue	EDI	India	n/a	n/a	YES
43	T	PLP	Bone	MAN	India	n/a	n/a	n/a
44	U	IB 6	Drilled Bone	PAR 1995-164	Sudan	n/a	n/a	YES
45	V	RB209	Drilled Bone	STK A58:1971	Namibia	n/a	n/a	YES
46	V	PLE-181	Published Sequence	Jae-Heup <i>et al.</i>	Serengeti, Tanzania	n/a	n/a	n/a
47	V	RB215	Skull	BAW 5728	Zambia	n/a	n/a	YES
48	V	RB216	Skull	STK A59:5062	DRC (NE), 1921	n/a	n/a	YES
49	V	RB217	Mandible	STK A59:5066	DRC (NE), 1921	n/a	n/a	YES
50	W	RB210	Drilled Bone	AMA 15904	South Africa	n/a	n/a	YES
51	W	RB213	Skull	BAW 29119	Botswana	n/a	n/a	YES
52	W	RB214	Skull	BAW 63591	Zimbabwe, 1967	n/a	n/a	YES
53	X	RB208	Drilled Bone	AMA 107.1	Tanzania	n/a	n/a	YES
54	?	IB135	Ulna	FAM 69158	Cripple Ck Sump, Alaska, USA, 1950	53 900 ± 2300	OxA-10085	n/a
55	?	IB153	Bone	KRA 6857	Wierchowska, Poland	38 650 ± 600	OxA-10087	n/a
56	?	IB81	Humerus	FAM69094	Lower Gold Stream, Alaska USA, 1939	12 540 ± 75	OxA-10081	n/a
Leopard	<i>P. pardus</i>	IB123	Tissue	EDI	Saudi Arabia	n/a	n/a	n/a
Tiger	<i>P. tigris</i>	RB100	Tissue	EDI	Siberia, Russia	n/a	n/a	n/a
Jaguar	<i>P. onca</i>	RB52	Tissue	EDI	South America	n/a	n/a	n/a

Museum code: American Museum of Natural History (AMNH), Canadian Museum of Nature (CMN), University of Vienna (VIE), Mannheim Museum (MAN), University of Bergen (UB), State Museum of Natural History Stuttgart (STU), University of Alaska (UAF), Palaeontological Institute Moscow (PIN), Russian Academy of Sciences (IEM), Moscow State University (GIN), Zoological Institute St. Petersburg (ZIN), Polish Academy of Sciences (KRA), Royal Alberta Museum (EDM), University of Kansas (KU), Muséum national d'Histoire naturelle (PAR), Amathole Museum (AMA), Natural History Museum of Zimbabwe (BUW), Swedish Museum of Natural History (STK), University of Erlangen-Nuremberg (UEN), Urweltmuseum Bayreuth (UMB), Museum Kufstein (MKU), and the Royal Museum of Scotland (EDI). All sequences have been deposited in GenBank (DQ899900–DQ899945, DQ318552–DQ318562).

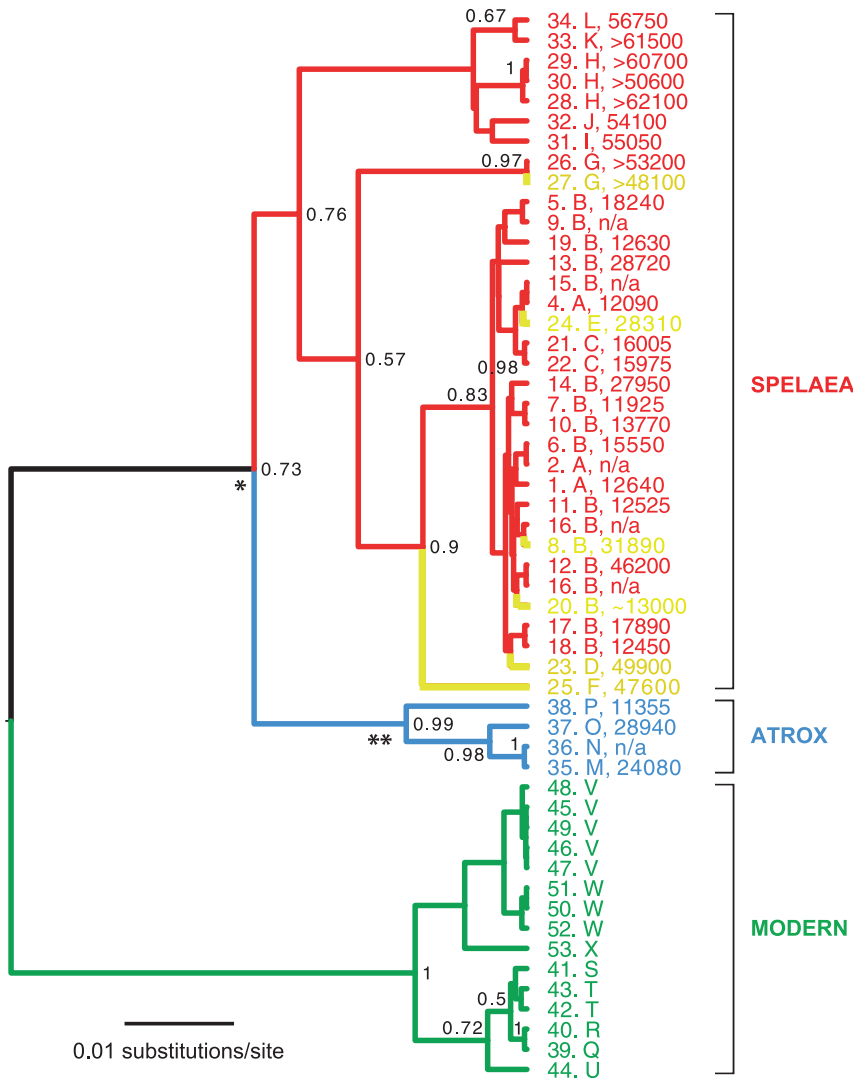


Fig. 2 Evolutionary relationships among lions estimated using Bayesian phylogenetic analysis of the mitochondrial HVR1. Colours and numbers correspond to those in Fig. 1. Nodes with posterior probabilities greater than 0.5 are labelled. Haplotypes and mean uncalibrated radiocarbon dates are given (where available). TMRCA estimated from a combined HVR1 and *ATP8* data set are indicated:
 *337 000 BP (95% HPD: 194 000–489 000 BP).
 **200 000 BP (95% HPD: 118 000–346 000 BP).

pantherines (Cracraft *et al.* 1998; Kim *et al.* 2006) and was highly divergent from the target mitochondrial locus. A nuclear copy of the *ATP8* gene was also detected in several lion specimens, as well as in leopard and tiger. The *ATP8* numt sequences clustered in a basal position in the phylogeny, and were also distinguished by an adenine insertion in the tiger sequence and a stop codon in the leopard sequence.

Under the assumption of a molecular clock, the substitution rate for the data set of combined HVR1 and *ATP8* sequences was estimated at 5.9×10^{-8} substitutions/site/year, with a 95% highest posterior density (HPD) of $3.7\text{--}8.3 \times 10^{-8}$ substitutions/site/year. Due to the relatively low information content of the sequences, it was not possible to employ a relaxed molecular clock to allow for changes in evolutionary rate over the time period examined (Ho *et al.* 2005). The combined HVR1/*ATP8* analysis resulted in an estimate for the divergence between *spelaea* and *atrox* at

337 000 BP (95% HPD: 194 000–489 000 BP). Estimates for the time to most recent common ancestor (TMRCA) for LP lions are given in Fig. 2. The large temporal range of these estimates reflects both the considerable uncertainty in the primary fossil calibration and the relatively low information content of the sequences.

Discussion

Three distinct types of lion survived during the LP: the American lion (*atrox*), cave lion (*spelaea*) and the ancestors of the modern lion (*Panthera leo*) (Fig. 1). The phylogenetic topology (Fig. 2) indicates that the mitochondrial lineages of *atrox* and *spelaea* form sister clades.

Network analyses (Fig. 3) produce a similar topology, but place *atrox* inside the basal diversity of *spelaea*, suggesting *atrox* was derived from a Beringian population that dispersed into North America and was subsequently isolated.

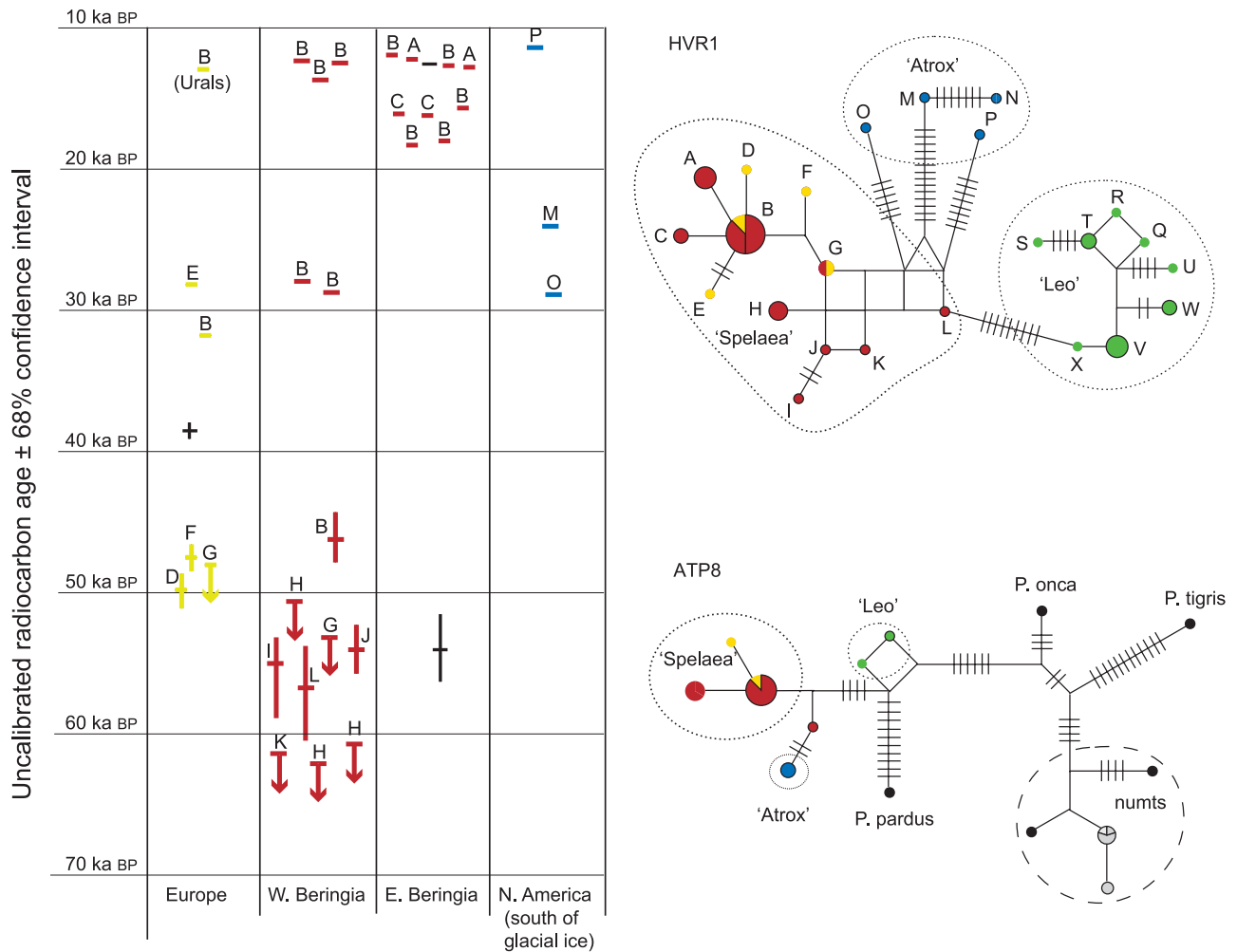


Fig. 3 Graph of uncalibrated radiocarbon date against haplotype sorted by geographical area. Median-joining network analysis of HVR1 and ATP8. Different letters represent different haplotypes, corresponding to those in Table 1. Colours represent area of origin as in Fig. 1. Area of circles are proportional to haplotype frequencies, and the lengths of connecting lines are proportional to the distance between haplotypes (defined as the number of substitutions estimated using Network version 4.1.0.3). Points at which lines intersect without associated circles indicate putative ancestral states.

Molecular date estimates place this initial divergence at 337 000 BP (95% HPD: 194 000–489 000 BP) with a most recent common ancestor of the *atrox* lineage c. 200 000 BP (95% HPD: 118 000–346 000 BP; Fig. 2). This is consistent with palaeontological evidence of lions in eastern Beringia during the Illinoian glacial (c. 300 000–130 000 BP, Harington 1969), and in central North America from the Sangamon interglacial (c. 130 000–120 000 BP). Lions persisted in North America, both north and south of the ice, until the end of the Pleistocene (Harington 1969; Kurtén & Anderson 1980).

The most recent directly radiocarbon dated *atrox* is that from Edmonton, at 11 355 ± 55 BP. (Table 1) This is younger than the lion metatarsal from Jaguar Cave, Idaho, which previously yielded a date of 11 900 ± 130 BP (Kurtén &

Anderson 1980; Stuart 1991). The date of 10 370 ± 150 BP, which has been used previously as the terminal date for *Panthera leo atrox* (Kurtén & Anderson 1980; Stuart 1991) is based indirectly on hearth charcoal from Jaguar Cave. The radiocarbon-dated cave lions from western Beringia reveal a reasonably continuous chronological distribution with a terminal date from the Lena River of 12 450 ± 60 BP (Table 1). The terminal date for eastern Beringia (11 925 ± 70 BP, Fairbanks Creek) is 1500 years older than the widely reported Lost Chicken Creek date of 10 370 ± 160 BP (Kurtén & Anderson 1980; Stuart 1991; Harington 1980), which comes not directly from a lion, but a bison found at the same site. In Eurasia, the youngest dated lion is that reported from Zigeuenerfels, Sigmaringen, Germany at 12 375 ± 50 BP (Stuart & Lister 2007). The widely reported

date from Lathum in the Netherlands ($10\,670 \pm 160$ BP) (Stuart 1991) has recently been shown to be much older, and therefore invalid (Stuart & Lister 2007).

Modern and LP European cave lions have been shown previously (Burger *et al.* 2004) to be distinct, but this is the first genetic evidence for a grouping of European *spelaea* with lions from Beringia as a single population. Similarly, the genetic separation between LP lions in Beringia and those south of the North American ice sheets is reported for the first time. The reasons for the lack of gene flow within North America remain unclear, as there is no evidence of a barrier between Beringia and central North America during interstadial periods for other taxa such as horse and bison (Burns 1996; Shapiro *et al.* 2004; Weinstock *et al.* 2005). *Atrox* is also present in Edmonton, Alberta, within the region postulated to be an ice-free corridor at various times in the late Pleistocene (Burns 1996; Wilson 1996) both before and after the LGM (Fig. 1, samples 37 and 38 in Table 1). While this indicates that the region was habitable by lions during interstadial periods, it is possible that currently unidentified ecological barriers prevented movement between the Yukon and southern Canada, such as the intermittent presence of forested regions (Wilson 1996) suggested to have been a barrier to the dispersal of bison following the LGM (Shapiro *et al.* 2004). Alternatively, some form of interspecific competition may have existed between *atrox* and *spelaea*, preventing interchange between the areas, as it seems unlikely that environmental or ecological barriers operated over the entire 200 000-year period of separation. Interestingly, there is also no evidence for genetic interchange between cave lions and modern lions, despite a long-term contact zone in the Near East (Fig. 1; Kurtén 1968; Burger *et al.* 2004). These observations are consistent with the suggestion that the three forms may represent separate species, rather than subspecies (Sotnikova, Nikolskiy 2006). However, pronounced mitochondrial phylogeographical structure has also been observed in Pleistocene eastern Beringian brown bear populations, albeit for much shorter time periods (Barnes *et al.* 2002). To fully resolve the species status of the three lion forms, it will be necessary to include both nuclear and morphological data.

The clear genetic distinction between the *atrox* and *spelaea* groups in North America is in sharp contrast to the paucity of genetic divisions within *spelaea* across Europe, Asia, and Alaska/Yukon, suggesting genetic interchange across an immense geographical range. This is particularly apparent after *c.* 46 000–48 000 BP when, despite the small sample size, we observe a dramatic and widespread decline in genetic diversity within *spelaea* across western Beringia and Europe (Figs 2 and 3). Before 46 000 BP, nine diverse mitochondrial haplotypes (B, D, F–L; Figs 2 and 3) are observed from 12 specimens. In contrast, all 18 *spelaea* specimens sampled across the entire range of *spelaea* after this point form part of a closely related star-shaped radiation, consistent with a

rapid population expansion, of four derived haplotypes (A, B, C, and E; Figs 2 and 3) centred around B, the only haplotype shared with the earlier time period. This marked decline in diversity may simply reflect genetic drift, combined with the relatively sparse temporal–geographical sampling. However, this seems somewhat unlikely given the pattern of loss, where all seven known haplotypes from outside the star cluster disappear, including apparently common and widespread haplotypes such as H and G. An alternative is that a geographically widespread genetic bottleneck occurred in *spelaea* populations sometime after 48 000 BP (the last occurrence of a haplotype external to the star radiation) which removed the earlier lineages. A subsequent re-invasion and/or range expansion of haplotype B is consistent with it being the most widespread and numerous mitochondrial sequence across the *spelaea* range (12 out of 17 specimens) after 48 000 BP. Members of the star radiation (haplotypes B and D) are first detected at 46 000 and 50 000 BP, suggesting that this group had started diversifying before the putative genetic bottleneck. The other derived haplotypes (A, C and E) may have also existed at this point, or evolved subsequently as populations containing haplotype B expanded in range and numbers. Such a pattern could be produced by the localized extinction of populations (e.g. across Eurasia), followed by the replacement with individuals from other parts of the range (e.g. eastern Beringia), as has previously been detected in Pleistocene bison and mammoth populations (Shapiro *et al.* 2004; Debruyne *et al.* 2008).

It is difficult to identify a cause for the putative bottleneck in *spelaea* populations without a precise date estimate for the event. However, it is notable that a major genetic alteration is also detectable in west Beringian bison populations between 48 000 and 50 000 BP (Cooper and Shapiro, unpublished data). This is especially significant because bison are thought to be a primary prey item for Beringian *spelaea* (Guthrie 1990; Matheus *et al.* 2003), suggesting that a population bottleneck might reflect major changes in prey availability or ecology. Alternatively, it is possible that a larger environmental change is occurring across the entire Beringian community around 48 000–50 000 BP, as this period has been identified as Heinrich event 5a (Hemming 2004), a pronounced cold stadial between Dansgaard-Oeschger (DO) interstadials 14 and 15 (GRIP 1993). Heinrich events occur during the coldest DO stadials, and are associated with catastrophic iceberg outbursts and rafting in the North Atlantic (Heinrich 1988). Heinrich events are associated with major environmental changes across the Holarctic, including neighbouring terrestrial and marine areas such as the Northwest Pacific (Gorbarenko *et al.* 2007).

With the current data, it is impossible to identify the precise nature of the genetic changes taking place in *spelaea* populations during the late Pleistocene. However, our data

add to the growing body of evidence that large-scale shifts in climate during MIS 3 were associated with major alterations in genetic structure in megafaunal populations (Barnes *et al.* 2002, 2007; Guthrie 2003; Shapiro *et al.* 2004; Hofreiter *et al.* 2007; Debruyne *et al.* 2008) well before the end-Pleistocene megafaunal extinctions in the northern hemisphere. As more data become available, integrated with detailed records of vegetative change, it should become possible to reconstruct the environment in Beringia throughout the LP to investigate the relationship between climate change and the response of megafaunal populations, providing key background information to understand their subsequent extinctions.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 List of all samples extracted during this study along with museum accession numbers and sample provenance. Calibrated radiocarbon dates were calculated using CalPal (Danzeglocke *et al.* 2008).

Table S2 Primer sequences, primer combinations, annealing temperatures (T_a), and number of thermal cycles used.

Table S3 Number of amplifications, cloning, and replication for lion samples.

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