

Megafaunal Extinctions and the Disappearance of a Specialized Wolf Ecomorph

Jennifer A. Leonard, Carles Vilà, Kena Fox-Dobbs,
Paul L. Koch, Robert K. Wayne,
and Blaire Van Valkenburgh

Supplemental Experimental Procedures

Genetic Analysis

DNA was extracted from tooth and bone samples by phenol-chloroform extraction as in Leonard et al. [S1]. Two blank (no bone powder) extractions were processed concurrently throughout all steps of the protocol to serve as negative controls. Approximately 425 bp of mitochondrial control region I was amplified in three overlapping regions with the following primers: Thr-L 5'-GAATTCCTCCGGTCTTGTAACC-3' and dogd15 5'-CATTAATGCACGACGTACATAGG-3' (yielded polymerase chain reaction [PCR] products of 207–227 bp in length); dogd11g 5'-GTGCTATGTCAGTATCTCCA GG-3' and dogd12 5'-GCAAGGGTTGATGGTTTCTCG-3' (235 bp); and dogd14 5'-GCATATCACTTAGTCCAATAAGGG-3' and DLH-can 5'-CCTGAGGTAAGAACCAGATG-3' (150 bp) as in Leonard et al. [S1]. The setup for PCR amplification of DNA was performed in a separate lab exclusively devoted to ancient-DNA extraction, and each reaction consisted of 3–6 μ l of extract and 25 ng bovine serum albumin (BSA), 1X buffer, 0.1 mM deoxyribonucleotide triphosphate (dNTP), 2.5 U AmpliTaq Gold (Perkin-Elmer), 2.5 mM Mg^{2+} , and 2.5 μ M each primer in a total volume of 25 μ l. The product of each successful reaction was either directly sequenced or used as a source for reamplification, and this product was then sequenced.

PCR products were purified with the UltraClean kit (MoBio, Carlsbad, California). Products were cycle-sequenced in a Primus 96 plus (MWG-Biotech, High Point, North Carolina) PCR instrument with BigDye terminator (Perkin-Elmer, Boston) chemistry. Sequences were then separated on an Applied Biosystems automated sequencer 377 according to the manufacturer's protocols. The same primers were used for both PCR and sequencing. Fragments were sequenced in both directions.

Precautions for analysis of low-copy DNA include nucleic-acid isolation in a pre-PCR ancient-DNA facility separated from the PCR-amplification laboratory by five floors and two corridors, as well as replications as in Leonard et al. [S1]. The ancient-DNA lab utilized ultraviolet lights to inactivate DNA, had an independent air-handling system with hepa filters on all vents, and was under positive air displacement. Two PCR blanks, as well as both extraction blanks, were run with each PCR reaction to monitor for contamination. We used three different sets of PCR primers to produce overlapping sequences that could be compared to detect contamination. Further, 80% of the sequences were confirmed independently by amplification and sequencing multiple times. No canid contamination was detected [S2].

The eastern-Beringian wolf sequences were compared with those of 32 modern wolves from Alaska and the Yukon, as well as with previously reported sequences from 401 recent wolves throughout the world [S3–S5]. Nucleotide and haplotype diversity, as well as their standard deviations, were calculated with DnaSP 4.10.9 [S6]. Three different criteria were used to construct phylogenies (maximum parsimony, maximum likelihood, and distance-based neighbor joining) in the program PAUP* 4.0b10 [S7]. Himalayan and Indian wolves were used as outgroups in all analyses [S5]. For the parsimony analysis, a heuristic search including indels as fifth state was run, and a consensus tree of all of the most parsimonious trees was constructed. The model of sequence evolution which best fit the data was estimated in ModelTest [S8] and used in the likelihood and distance analyses. The best-fit model was the HKY85 with a γ parameter ($G = 0.9082$) and a portion of invariant sites ($I = 0.5213$). For the likelihood analysis, the puzzle quartet algorithm was used and reliability values were estimated. Lastly, for the distance analysis, a neighbor-joining phylogeny was constructed, and bootstrap support for branches was estimated with 1000 pseudoreplications.

Morphological Analysis

Twenty-seven craniodental measurements were taken by J.A.L. and C.V. with digital calipers on 116 modern-wolf skulls from North America, ranging from Ellesmere Island, Canada to northern Mexico (Table S3). All were adult wild-caught individuals in the mammal collections of the American Museum of Natural History (AMNH) or the National Museum of Natural History (USNM). Specimen numbers are available from the authors on request. In addition, data were collected from 35 crania and 37 dentaries of Pleistocene wolves (Table S3). Most of the Pleistocene specimens are incomplete, and dentaries are not usually associated with crania, so the two skull parts were analyzed separately. Moreover, because of their fragmentary nature, only a subset of measurements was possible. To maximize the sample of Pleistocene wolves used in statistical analyses, the number of measurements was reduced to 11 for the skull and upper teeth and seven for the lower jaw and teeth (Table S4). With the software SPSS 11 for Mac OS X, measurements were subjected to bivariate and multivariate analyses, including analysis of variance (ANOVA), principal components analysis (PCA), and linear regression. The ANOVA tested for significant differences in single measures between the ancient and modern populations of wolves. For better identification of shape differences among wolves, PCA was performed on size-transformed data, with Mosimann shape variables [S9, S10]. Each of the raw measures was divided by the geometric mean (GM), which is defined as the n^{th} root of the product of n raw measurements. For the minimization of the effect of outliers, ratios were $\log_{10}$ transformed prior to entry into the PCA. For testing for remaining correlations between shape variables and size, correlations between principal component scores on the first two axes and size were determined with the $\log_{10}$ of the GM as an overall estimate of body size.

Tooth-Wear Analysis

All eastern-Beringian wolves used in the morphometric analysis and 313 modern, wild-caught, adult wolves from four subspecies were examined for tooth wear and fracture by B.V.V. (Tables S1 and S7). The modern-wolf skulls are part of the USNM mammal collection.

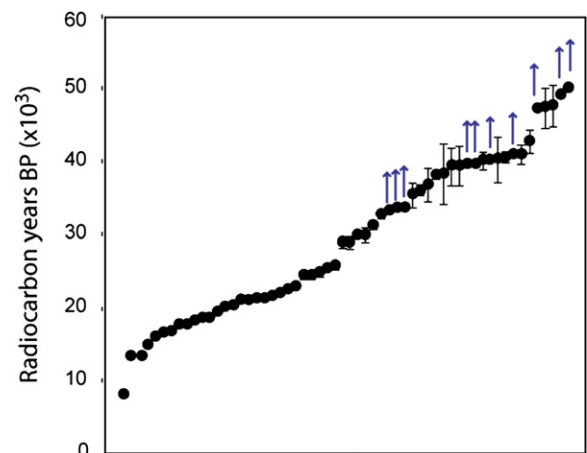


Figure S1. Radiocarbon Dates for 58 *Canis lupus* from East-Beringian Permafrost Deposits

Arrows indicate specimens with infinite dates. Specimens are from the AMNH and NMC collections of Pleistocene mammals (Table S1).

Table S1. Fossil Wolf Specimens Included in the Study, Mitochondrial DNA Haplotypes Identified, and Their Uncalibrated Radiocarbon Dates, if Available

Specimen Number	Species	Element	DNA	DATE	Lab #
AMNH FM 68008-6#	<i>Canis lupus</i>	ulna	PW6	23,380 ± 470	AA35222
AMNH FM 30447#	" <i>C. lupus</i> "	femur		15,680 ± 190	AA35223
AMNH FM 67224*#	" <i>C. lupus</i> "	ramus	PW4	17,640 ± 240	AA35226
AMNH FM 58009-A#	" <i>C. lupus</i> "	femur	PW12	16,800 ± 210	AA35227
AMNH FM 67240#	" <i>C. lupus</i> "	ramus	PW13	28,610 ± 860	AA35228
AMNH FM 67199*#	" <i>C. lupus</i> "	ramus	PW8	18,380 ± 390	AA35230
AMNH FM 67227*#	" <i>C. lupus</i> "	ramus	PW9	15,870 ± 190	AA35231
AMNH FM 70958#	" <i>C. lupus</i> "	ramus		37,700 ± 2600	AA37615
AMNH FM 67212#	<i>Canis sp.</i>	ramus			
AMNH FM 67208*	" <i>C. lupus</i> "	ramus		41,040 ± 1530	CAMS 115759
AMNH FM 67204*	" <i>C. lupus</i> "	ramus			
AMNH FM 67179*	" <i>C. lupus</i> "	ramus		36,700 ± 4000	AA37617
AMNH FM 67197*	" <i>C. lupus</i> "	ramus		39,300 ± 1230	CAMS 115760
AMNH FM 67216#	" <i>C. lupus</i> "	ramus	PW5	17,330 ± 290	AA42301
AMNH FM 30480*	" <i>C. lupus</i> "	ramus			
AMNH FM 67228#	<i>C. lupus</i>	ramus	PW14	>32,100	AA42302
AMNH FM 67203*#	" <i>C. lupus</i> "	ramus	PW15	>39,200	AA42303
AMNH FM 67207*	<i>C. lupus</i>	ramus			
AMNH FM 67202#	" <i>C. lupus</i> "	ramus		38,000 ± 2,700	AA35224
AMNH FM 67230*	" <i>C. lupus</i> "	ramus			
AMNH FM 30438*#	" <i>C. lupus</i> "	cranium		45,500 ± 2700	CAMS 115778
AMNH FM 67235*#	" <i>C. lupus</i> "	ramus		>38,000	AA42310
AMNH FM 70945*#	" <i>C. lupus</i> "	ramus	PW16	37,733 ± 2633	AA38448
AMNH FM 70944*#	" <i>C. lupus</i> "	ramus	PW6	15,268 ± 169	AA38449
AMNH FM 142411	" <i>C. lupus</i> "	humerus		24,130 ± 190	CAMS 115761
AMNH FM 142410	" <i>C. lupus</i> "	humerus		>45,400	CAMS 115762
AMNH FM 67184	" <i>C. lupus</i> "	ramus		34,600 ± 700	CAMS 115763
AMNH FM 30476#	" <i>C. lupus</i> "	ramus		20,050 ± 430	AA42312
AMNH FM 70946*#	" <i>C. lupus</i> "	ramus	PW3	>38,570	AA42313
AMNH FM 30440*#	" <i>C. lupus</i> "	ramus		>38,000	AA42314
AMNH FM 67256*	" <i>C. lupus</i> "	ramus			
AMNH FM 67250*	" <i>C. lupus</i> "	ramus			
AMNH FM 30477*	" <i>C. lupus</i> "	ramus			
AMNH FM 67210*	" <i>C. lupus</i> "	ramus			
AMNH FM 68006-I#	<i>Canis sp.</i>	humerus			
AMNH FM 30445#	<i>Canis sp.</i>	humerus			
NMC 42388#	<i>C. lupus</i>			33,900 ± 1700	AA35221
AMNH FM 97079*	<i>C. lupus</i>	cranium		23,660 ± 490	AA48690
AMNH FM 30430*	<i>C. lupus</i>	cranium		19,280 ± 290	AA48691
AMNH FM 67160*	<i>C. lupus</i>	cranium		24,630 ± 520	AA48692
AMNH FM 67163*	<i>C. lupus</i>	cranium		>31,800	AA48693
AMNH FM 67170*	<i>C. lupus</i>	cranium		27,620 ± 580	AA48694
AMNH FM 67169*#	<i>C. lupus</i>	ramus		20,305 ± 385	
AMNH FM 97079#	<i>C. lupus</i>	ramus		>32,100	
AMNH FM 30450*#	<i>C. lupus</i>	cranium		7,674 ± 66	AA48695
AMNH FM 30433*	<i>C. lupus</i>	cranium		12,690 ± 130	AA48697
AMNH FM 30457*	<i>C. lupus</i>	cranium		38,600 ± 3000	AA48698
AMNH FM 67162*	<i>C. lupus</i>	cranium		23,230 ± 440	AA48700
AMNH FM 67168* ^a	<i>C. lupus</i>	cranium		27,690 ± 790	AA48701
AMNH FM 67168* ^a	<i>C. lupus</i>	cranium		31,200 ± 450	CAMS 115767
AMNH FM 30453*	<i>C. lupus</i>	cranium		19,210 ± 260	AA48702
AMNH FM 30458*	<i>C. lupus</i>	cranium			
AMNH FM 67158*	<i>C. lupus</i>	cranium			
AMNH FM 67159*	<i>C. lupus</i>	cranium		35,200 ± 2300	AA48703
AMNH FM 70935*	<i>C. lupus</i>	cranium			
AMNH FM 30432*	<i>C. lupus</i>	cranium		17,670 ± 230	AA48704
AMNH FM 67167*	<i>C. lupus</i>	cranium		45,800 ± 2800	CAMS 115768
AMNH FM 67166*	<i>C. lupus</i>	cranium			
AMNH FM 30431*	<i>C. lupus</i>	cranium		28,500 ± 300	CAMS 115776
AMNH FM 30452*	<i>C. lupus</i>	cranium		20,550 ± 120	CAMS 115769
AMNH FM 67164*	<i>C. lupus</i>	cranium			
AMNH FM 67211*	" <i>C. lupus</i> "	ramus			
AMNH FM 67201*	<i>C. lupus</i>	ramus			
AMNH FM 67194*	<i>C. lupus</i>	ramus			
AMNH FM 67209*	<i>C. lupus</i>	ramus			
AMNH FM 67243*	<i>C. lupus</i>	ramus		38,500 ± 1,100	CAMS 115772
AMNH FM 67182*	<i>C. lupus</i>	ramus			
AMNH FM 30478*	<i>C. lupus</i>	ramus			
AMNH FM 67248*	<i>C. lupus</i>	ramus		29,800 ± 400	CAMS 115773

Table S1. Continued

Specimen Number	Species	Element	DNA	DATE	Lab #
AMNH FM 67231*	<i>C. lupus</i>	ramus		21,900 ± 140	CAMS 115774
AMNH FM 67239*	" <i>C. lupus</i> "	ramus			
AMNH FM 70951*	" <i>C. lupus</i> "	ramus			
AMNH FM 70943*	" <i>C. lupus</i> "	ramus			
AMNH FM 70942*	<i>C. lupus</i>	ramus		20,150 ± 110	CAMS 115775
AMNH FM 70950*	" <i>C. lupus</i> "	ramus			
AMNH FM 67169#	" <i>C. lupus</i> "	cranium	PW1	20,305 ± 385	AA35216A
AMNH FM 67165*#	<i>C. lupus</i>	cranium	PW10	12,600 ± 150	AA42317
AMNH FM 67157*#	<i>C. lupus</i>	cranium	PW6	14,030 ± 50	UCR 3760
AMNH FM 30451*#	<i>C. lupus</i>	cranium	PW4	16,800 ± 90	UCR 3761
NMC 17311#	<i>C. lupus</i>	cranium	PW17	38,780 ± 540	UCR 3762
NMC 45574#	<i>C. lupus</i>	cranium	PW11	21,490 ± 110	UCR 3763
NMC 9929#	<i>C. lupus</i>	cranium	PW7	20,920 ± 70	UCR 3764
NMC CR-95100#	<i>C. lupus</i>	cranium	PW2	>47,170	Beta 89988
NMC 44726#	<i>C. lupus</i>	humerus		36,490 ± 410	UCR 3765
LACM 1414*	<i>C. lupus</i>	cranium			
LACM 314*	<i>C. lupus</i>	cranium			
LACM h6606*	<i>C. lupus</i>	cranium			
LACM 615*	<i>C. lupus</i>	cranium			
LACM 2300-384*	<i>C. lupus</i>	cranium			
LACM 2600*	<i>C. lupus</i>	cranium			
LACM HC607*	<i>C. lupus</i>	cranium			
LACM2300-353*	<i>C. lupus</i>	cranium			
LACM 2600-2*	<i>C. lupus</i>	cranium			
LACM 604*	<i>C. lupus</i>	cranium			
LACM 609*	<i>C. lupus</i>	cranium			
LACM 11505*	<i>C. lupus</i>	cranium			
LACM 608*	<i>C. lupus</i>	cranium			
LACM 6909*	<i>C. lupus</i>	cranium			
LACM 11505*	<i>C. lupus</i>	cranium			

Specimens used in the morphological and/or tooth fracture analyses are indicated by an asterisk. Specimens used in the genetic analyses are indicated by the pound sign, and those which yielded complete sequences have the haplotype indicated in the "DNA" column. Specimens listed as "*C. lupus*" were labeled as *Canis sp.* in the collection but considered to be *C. lupus* by R.H. Tedford on the basis of craniodental morphology. The following museum abbreviations are used: Frick Collection of the American Museum of Natural History, New York (AMNH FM); Museum of Natural History of Los Angeles County collections of the George C. Page Museum, Los Angeles (LACM); and Nature Museum of Canada, Ottawa (NMC). "Lab #" indicates the catalogue number at the radiocarbon-dating laboratory. Radiocarbon dating was done at Arizona Accelerator Mass Spectrometry Laboratory (AA), University of California, Riverside Radiocarbon Laboratory (UCR), Beta Analytic (Beta), and Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory (CAMS).

^a These two specimens are partial skulls with the same catalog number but different field numbers.

As in previous studies of tooth-fracture frequency [S11, S12], each individual was scored for overall wear as one of three stages: (1) *slight*, little or no apparent wear observed as shear facets or blunting of cusps; (2) *moderate*, shear facets apparent on carnassial teeth, and cusps blunted on most teeth; or (3) *heavy*, carnassial teeth with strong shear facets or blunted cusps, and premolars and molars with well-rounded cusps. The number and identity of teeth

broken in life were recorded for each individual. A tooth was counted as broken in life only if it exhibited wear that must have occurred after fracture. Total fracture frequency was calculated as a weighted average of tooth fracture frequencies for each tooth type (incisors, canines, premolars, carnassial, and molars) [S11]. The weighted average was used because the relative representation of tooth types (e.g., incisors) often differs between fossil and modern samples

Table S2. Nucleotide and Haplotype Diversity of North American Gray Wolf Populations

Population	n	Haplotype Diversity	Nucleotide Diversity
Pleistocene	19	0.977 ± 0.027	0.0125 ± 0.0021
Pleistocene, 12–18K bp only	10	0.956 ± 0.059	0.0103 ± 0.0026
Recent Alaska	29	0.791 ± 0.038	0.0093 ± 0.0008
Recent Inuvik	40	0.595 ± 0.085	0.0044 ± 0.0008
Recent <i>C. l. nubilus</i> ^a	22	0.831 ± 0.054	0.0135 ± 0.0012

Pleistocene populations are from eastern Beringia, and the 12–18 ka BP population is a subset of the entire Pleistocene population. The number of samples used to calculate the haplotype and nucleotide diversities is indicated in the column labeled "n." Each haplotype- and nucleotide-diversity value is followed by its standard deviation. The genetic diversity of Pleistocene wolves is higher than that of their modern counterparts. We find 16 haplotypes in 20 ancient Beringian wolves, compared with only seven haplotypes in a sample of 32 modern Alaska and Yukon wolves. The sample of ancient wolves represents a time-averaged sample that might increase the apparent genetic diversity. However, even in a narrow time range of 12 to 18 thousand years before present we find eight haplotypes in 10 wolves from a single locality, a higher diversity than that in any modern North American population. Assuming that the populations were at demographic equilibrium, such differences in genetic diversity would imply that the ancient-wolf population was larger than that of the current day, and might have been so for some time.

^a Historic wolves from the contiguous United States.

Table S3. Sample of Pleistocene *Canis lupus* and Subspecies of Extant *C. lupus* Measured

Population	n (crania)	n (dentaries)	Provenance
<i>C. lupus</i> (Pleistocene)	20	34	Alaska
<i>C. lupus</i> (Pleistocene)	15	3	California (Rancho La Brea)
<i>C. l. baileyi</i>	9	10	Mexico, New Mexico
<i>C. l. lycaon</i>	22	24	Eastern Canada, Minnesota, Michigan
<i>C. l. mongollensis</i>	12	11	Arizona, New Mexico
<i>C. l. monstabilis</i>	10	9	Mexico, New Mexico, Texas
<i>C. l. nubilus</i>	26	26	Texas, Nebraska, Colorado, Kansas. Oklahoma, South Dakota
<i>C. l. youngi</i>	12	12	Colorado, New Mexico, Utah, Wyoming
<i>C. l. pambasileus</i>	17	15	Alaska
<i>C. l. arctos</i>	5	5	Northwest Territories, Ellesmere Island
<i>C. l. tundarum</i>	3	4	Alaska

because of biases in preservation. The weighted average is the sum across all tooth types of the product of the observed fracture frequency per tooth type and the proportion of the tooth row represented by that tooth type in a complete dentition. Differences in frequency of fracture were assessed with the Pearson chi-square

Table S4. Measurements Used in the Morphometric Analyses and Their Definitions

Measurement	Definition
FL	Face length: distance from just behind the incisors to the distal margin of the hard palate.
PL	Palatine length: distance from just behind the incisors to the distal margin of the palatines (at the palatine-maxilla suture)
TPRL	Total premolar row length: distance from the mesial border of the upper first premolar to the distal border of the upper fourth premolar.
PW	Palate width: hard palate breadth anterior to the upper fourth premolar.
P4L	Upper carnassial length: maximum mesiodistal length.
P4W	Upper carnassial width: maximum buccolingual breadth of the blade (behind the protocone).
M1L	Upper first molar length: maximum mesiodistal length along the buccal margin.
M1W	Upper first molar width: maximum buccolingual breadth.
M2L	Upper second molar length: maximum mesiodistal length along the buccal margin.
M2W	Upper second molar width: maximum buccolingual breadth.
SD	Snout depth: maximum rostrum height just distal to the upper-canine-tooth alveolus.
dd	Dentary depth: maximum dorsoventral height just posterior to the lower-first-molar alveolus.
p4L	Lower fourth premolar length: maximum mesiodistal length.
m1L	Lower carnassial length: maximum mesiodistal length.
m1TRL	Lower carnassial blade length: maximum mesiodistal length of the trigonid of the lower first molar.
m1W	Lower carnassial width: maximum buccolingual breadth of the trigonid of the lower first molar.
m2L	Lower second molar length: maximum mesiodistal length.
m2W	Lower second molar width: maximum buccolingual breadth.

statistic (with a continuity correction in the case of 2×2 comparisons), and 95% confidence intervals were calculated based on the binomial distribution [S13].

Stable-Isotope Analyses

As noted in the results, we collected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data from fossil bone collagen for wolves and their potential prey, including 32 horses (*Equus lambei*), ten caribou (*Rangifer tarandus*), six bison (*Bison bison*), five yak (*Bos grunniens*), and ten woodland muskox (*Symbos cavifrons*) from the Fairbanks area (Table S8). Also included are published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data collected from undated Pleistocene mammoths (*M. primigenius*) from multiple sites in Alaska [S14]. Thirty-two horses, ten caribou and 58 wolves were radiocarbon dated (Tables S1 and S8).

Bone samples (~150 mg) were crushed into coarse powder in a mortar and pestle, and collagen was extracted according to the methods of Brown et al. [S15]. Collagen preservation was assessed with atomic carbon/nitrogen (C/N) ratios, and all samples presented here were between 2.2:1 and 2.8:1, well within the range for viable collagen [S16]. For stable-isotope analyses, collagen samples (1.0 mg) were weighed into tin capsules. Carbon and nitrogen stable-isotope ratios were measured with an elemental analyzer coupled with a mass spectrometer (Europa Hydra 20/20) at the University of California Davis Stable Isotope Facility. Stable-isotope compositions are reported with standard δ notation and are referenced to Vienna PeeDee Belemnite and air for carbon and nitrogen, respectively. The standard deviation for replicates of a gelatin standard was <0.2‰ for carbon and nitrogen. Pleistocene collagen samples analyzed for radiocarbon were prepared with the same method outlined above. Because there is no agreed-upon calibration curve for ^{14}C ages >26 ka BP [S17], we present all dates as uncalibrated ^{14}C ages.

Because of climate driven change in isotope values, the ^{14}C dated wolves, horses, and caribou were grouped into three climatically relevant time periods: postglacial (18 to 10 ka BP), full-glacial (23 to 18 ka BP), and preglacial (>50 to 23 ka BP). The preglacial time period encompasses mild conditions that persisted until approximately 25 ka BP, followed by the onset of last glacial maximum (LGM) cooling. The full-glacial group captures the 5000 year period when LGM conditions were the coldest and driest in eastern Beringia, and the post-glacial time period is characterized by a rapid transition toward the warmer, mesic conditions of the Holocene.

Supplemental References

- Leonard, J., Wayne, R., and Cooper, A. (2000). Population genetics of ice age brown bears. *Proc. Natl. Acad. Sci. USA* 97, 1651–1654.
- Leonard, J., Shanks, O., Hofreiter, M., Kreuz, E., Hodges, L., Ream, W., Wayne, R., and Fleischer, R. (2006). Animal DNA in PCR reagents plagues ancient DNA research. *Journal of Archaeological Science*. Published online December 5, 2006. 10.1016/j.jas.2006.10.023.
- Leonard, J.A., Vila, C., and Wayne, R.K. (2005). Legacy lost: Genetic variability and population size of extirpated US grey wolves (*Canis lupus*). *Mol. Ecol.* 14, 9–17.

Table S5. Mean and Standard Deviations for Raw Measurements Used in the Bivariate and Multivariate Analyses of the Extant and Two Late Pleistocene Wolf Samples

Measurement	Mean (SD) for Extant Wolves	Mean (SD) for Rancho La Brea Wolves	Mean (SD) for Beringian Wolves
Face length (FL)	114.5 (7.36)	115.8 (7.05)	115.6 (4.97)
Palatine length (PL)	73.4 (4.64)	75.2 (5.16)	73.9 (3.62)
Premolar row length (TPRL)*	63.4 (4.32)	63.6 (4.56)	69.3 (3.69)
Palate width (PW)*	64.9 (4.68)	67.6 (2.06)	76.6 (4.66)
P4 length (P4L)*	25.1 (1.61)	26.3 (2.01)	26.7 (1.59)
P4 width (P4W) *	10.1 (0.88)	10.6 (0.65)	11.35 (0.71)
M1 length (M1L)	16.4 (1.07)	16.5 (1.69)	16.6 (0.84)
M1 width (M1W)	22.2 (1.43)	22.1 (1.51)	22.1 (1.06)
M2 length (M2L)*	8.7 (0.67)	8.92 (0.54)	9.2 (0.48)
M2 width (M2W)	13.5 (1.01)	13.8 (0.65)	13.6 (0.84)
Snout depth (SD)	42.6 (3.98)	40.9 (2.86)	43.5 (2.63)
dentary depth (dd)*	30.4 (2.88)	31.6 (0.53)	32.7 (2.41)
p4 length (p4l)*	15.4 (1.03)	16.6 (1.64)	16.5 (0.75)
m1 length (m1L)*	28.2 (1.86)	28.9 (2.26)	29.6 (1.91)
m1 trigonid length (m1trl)*	19.6 (1.49)	21.9 (1.28)	20.9 (1.43)
m1 width (m1w)*	10.7 (0.85)	11.3 (0.08)	11.1 (0.86)
m2 length (m2L)	11.7 (0.81)	11.4 (1.12)	11.6 (0.75)
m2 width (m2w)	8.95 (0.62)	8.39 (0.19)	8.8 (0.63)

For measurement definitions, see Table S4. “***” denotes eastern-Beringian samples that differ significantly ($p < 0.05$) from the other two (for cranial measurements) or from modern wolves (for dentary measurements).

- S4. Vilà, C., Amorim, I.R., Leonard, J.A., Posada, D., Castroviejo, J., Petrucci-Fonseca, F., Crandall, K.E., Ellegren, H., and Wayne, R.K. (1999). Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Mol. Ecol.* 8, 2089–2103.
- S5. Sharma, D.K., Maldonado, J.E., Jhala, Y.V., and Fleischer, R.C. (2003). Ancient wolf lineages in India. *Proc. R. Soc. Lond. B. Biol. Sci.* 271, S1–S4.
- S6. Rozas, J., Sanchez-DelBarrio, J.C., Messeguer, X., and Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19, 2496–2497.

- S7. Swofford, D.L. (2002). PAUP*. Phylogenetic Analysis Using Patrimony (*And Other Methods), Version 4.0b10 (Sunderland, Massachusetts: Sinauer Associates).
- S8. Posada, D., and Crandall, K.A. (1998). MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- S9. Mosimann, J.E., and James, F.C. (1979). New statistical methods for allometry with application to Florida red-winged blackbirds. *Evolution Int. J. Org. Evolution* 33, 444–459.
- S10. Jungers, W.L., Falsetti, A.B., and Wall, C.E. (1995). Shape, relative size, and size adjustments in morphometrics. *Yearb. Phys. Anthropol.* 38, 137–162.
- S11. Van Valkenburgh, B., and Hertel, F. (1993). Tough times at La Brea: Tooth breakage in large carnivores of the late Pleistocene. *Science* 261, 456–459.
- S12. Van Valkenburgh, B. (1988). Incidence of tooth breakage among large, predatory mammals. *Am. Nat.* 131, 291–302.
- S13. Wonnacott, R., and Wonnacott, T. (1985). *Introductory Statistics*, Fourth Edition (New York: Wiley).
- S14. Bocherens, H., Fizet, M., Mariotti, A., Gangloff, R.A., and Burns, J.A. (1994). Contribution of isotopic biogeochemistry (^{13}C , ^{15}N , ^{18}O) to the paleoecology of mammoths (*Mammuthus primigenius*). *Historical Biology* 7, 187–202.
- S15. Brown, T.A., Nelson, D.E., Vogel, J.S., and Southon, J.R. (1988). Improved collagen extraction by modified Longin method. *Radiocarbon* 30, 171–177.
- S16. Ambrose, S.H. (1990). Preparation and characterization of bone and tooth collagen for isotopic analysis. *J. Archaeol. Sci.* 17, 431–451.
- S17. Reimer, P.J., Baillie, M.G.L., Bard, E., Beck, J.W., Blackwell, P.G., Buck, C.E., Burr, G.S., Edwards, R.L., Guilderson, M.F.T.P., Hogg, A.G., et al. (2006). Comment on “Radiocarbon calibration curve spanning 0 to 50,000 years BP based on paired $^{230}\text{Th}/^{234}\text{U}/^{238}\text{U}$ and ^{14}C dates on pristine corals” by R.G. Fairbanks et al. (*Quaternary Science Reviews* 24 (2005) 1781–1796) and “Extending the radiocarbon calibration beyond 26,000 years before present using fossil corals” by T.-C. Chiu et al. (*Quaternary Science Reviews* 24 (2005) 1797–1808). *Quaternary Science Reviews* 25, 855–862.

Table S6. Results of Principal Components Analysis

Shape Variable	Factor 1	Factor 2
<i>Crania</i>		
M2W	−0.764	0.141
TPRL	0.755	−0.217
M2L	−0.624	−0.004
PW	0.617	−0.529
M1L	−0.588	0.104
M1W	−0.58	0.361
SD	0.408	0.354
FL	0.474	0.769
P4W	−0.004	−0.707
PL	0.473	0.672
P4L	−0.109	−0.39
% variance	29.4	21
<i>Dentaries</i>		
m2W	−0.891	0.116
m2L	−0.853	0.007
m1TR	0.718	0.319
m1L	0.568	0.516
dh	0.488	−0.716
m1W	0.222	0.519
p4L	0.351	−0.357
% variance	39.6	18.5

Variable loadings and percent variance explained on the first two axes of a principal-components analysis of the $\log_{10}$ -transformed shape variables for the cranial and dentary samples, respectively. For variable abbreviations, see Table S5.

Table S7. Observed Number of Total and Broken Teeth in Four Historical and One Pleistocene Eastern-Beringian Wolf Populations

Population	Provenance	n (skulls)	Total Teeth (No.)	Broken Teeth (No.)	Fracture Frequency	
					With Incisors	Without Incisors
<i>C. l. lycaon</i>	Minnesota, Michigan	90	2955	193	0.07 (0.009)	0.07 (0.009)
<i>C. l. irremotus</i>	Canada, Idaho	66	2459	48	0.02 (0.007)	0.01 (0.009)
<i>C. l. nubilus</i>	New Mexico, Texas	83	3250	123	0.04 (0.009)	0.05 (0.009)
<i>C. l. pambasileus</i>	Alaska	74	2910	125	0.04 (0.009)	0.05 (0.01)
<i>C. lupus</i>	Eastern Beringia	37 ^a	373	36	0.11 (0.04)	0.1 (0.04)

Fracture frequency is equal to the weighted average of tooth-breakage frequencies for each tooth type (incisors, canines, premolars, carnassials, and molars). See Supplemental Experimental Procedures for further explanation. Shown in parentheses are $\pm$ 95% confidence intervals for the fracture frequencies.

^aFor the Pleistocene sample, n is the total number of specimens (lower jaws and skulls).

Table S8. List of Fossil Specimens Used in the Stable Isotope Analyses and Their Radiocarbon Dates

Species	Specimen ID	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	^{14}C Age	Lab ID
<i>Clu</i>	AMNH FM 30450	-19.4	5.6		AA48695
<i>Clu</i>	AMNH FM 70944	-19.2	7.3		AA38449
<i>Clu</i>	AMNH FM 30447	-19.7	7.9		AA35223
<i>Clu</i>	AMNH FM 67227	-18.9	7.9		AA35231
<i>Clu</i>	AMNH FM 68009-A	-18.9	8.0		AA35227
<i>Clu</i>	AMNH FM 67224	-19.0	7.8		AA35226
<i>Clu</i>	AMNH FM 30432	-20.5	8.1		AA48704
<i>Clu</i>	AMNH FM 30453	-18.3	9.3		AA48702
<i>Clu</i>	AMNH FM 70942	-19.8	9.3		CAMS115775
<i>Clu</i>	AMNH FM 30452	-19.1	9.1		CAMS115769
<i>Clu</i>	AMNH FM 67231	-19.3	9.3		CAMS115774
<i>Clu</i>	AMNH FM 68008-G	-19.2	8.5		AA35222
<i>Clu</i>	AMNH FM 67170	-18.3	7.3		AA48694
<i>Clu</i>	AMNH FM 30431	-19.1	8.6		CAMS115776
<i>Clu</i>	AMNH FM 67248	-19.7	6.2		CAMS115773
<i>Clu</i>	AMNH FM 67168	-19.0	9.2		CAMS115767
<i>Clu</i>	AMNH FM 67163	-18.8	6.6		AA48693
<i>Clu</i>	AMNH FM 67228	-19.4	7.0		AA42302
<i>Clu</i>	NMC 42388	-18.5	8.5		AA35221
<i>Clu</i>	AMNH FM 67184	-20.2	6.2		CAMS115763
<i>Clu</i>	AMNH FM 67159	-19.4	8.1		AA48703
<i>Clu</i>	AMNH FM 70958	-19.1	7.5		AA37615
<i>Clu</i>	AMNH FM 70945	-18.8	6.7		AA38448
<i>Clu</i>	AMNH FM 67202	-19.2	6.8		AA35224
<i>Clu</i>	AMNH FM 67243	-20.1	6.2		CAMS115772
<i>Clu</i>	AMNH FM 67197	-20.3	5.7		CAMS115760
<i>Clu</i>	AMNH FM 67208	-19.2	9.7		CAMS115759
<i>Clu</i>	AMNH FM 30438	-19.4	6.4		CAMS115778
<i>Clu</i>	AMNH FM 67167	-19.0	9.2		CAMS115768
<i>Clu</i>	AMNH FM 67235	-19.8	5.8		AA42310
<i>Clu</i>	AMNH FM 70946	-20.0	5.4		AA42313
<i>Clu</i>	AMNH FM 30440	-18.9	6.4		AA42314
<i>Clu</i>	AMNH FM 142410	-19.4	6.2		CAMS115762
<i>Clu</i>	AMNH FM 142409	-19.4	8.6		CAMS115777
<i>Clu</i>	AMNH FM 67165	-19.2	8.6		AA42317
<i>Clu</i>	AMNH FM 67157	-18.7	7.9		AA42315
<i>Clu</i>	AMNH FM 30451	-18.6	8.8		UCR 3761
<i>Clu</i>	AMNH FM 67169	-19.3	10.3		AA35216A
<i>Clu</i>	NMC 9929	-19.3	12.7		UCR 3764
<i>Clu</i>	NMC 17311	-19.1	6.1		UCR 3762
<i>Eq</i>	AMNH FM 60003	1.41	-21.25	24260 $\pm$ 200	CAMS120077
<i>Eq</i>	AMNH FM 60004	3.08	-20.93	16370 $\pm$ 80	CAM119968
<i>Eq</i>	AMNH FM 60005	1.64	-21.29	14630 $\pm$ 60	CAMS119969
<i>Eq</i>	AMNH FM 60044	3.47	-20.85	18630 $\pm$ 100	CAMS119970
<i>Eq</i>	AMNH FM 142419	4.52	-21.07	20520 $\pm$ 120	CAMS119971
<i>Eq</i>	AMNH FM 60017	3.58	-21.58	41000 $\pm$ 1500	CAMS119972
<i>Eq</i>	AMNH FM 142420	4.96	-21.23	19870 $\pm$ 110	CAMS119973
<i>Eq</i>	AMNH FM 142421	4.79	-20.95	14860 $\pm$ 60	CAMS119974
<i>Eq</i>	AMNH FM 142422	2.98	-21.50	>48500	CAMS119975

Table S8. *Continued*

Species	Specimen ID	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	^{14}C Age	Lab ID
<i>Eq</i>	AMNH FM 142423	3.85	-20.90	12560 $\pm$ 50	CAMS119976
<i>Eq</i>	AMNH FM 142424	2.94	-20.70	15460 $\pm$ 70	CAMS119977
<i>Eq</i>	AMNH FM 142425	4.23	-20.76	20440 $\pm$ 120	CAMS119978
<i>Eq</i>	AMNH FM 142426	5.07	-21.20	20300 $\pm$ 120	CAMS119979
<i>Eq</i>	AMNH FM 142427	4.73	-21.05	19960 $\pm$ 110	CAMS119980
<i>Eq</i>	AMNH FM 142428	3.74	-21.03	21280 $\pm$ 130	CAMS119981
<i>Eq</i>	AMNH FM 142429	4.29	-21.28	12310 $\pm$ 45	CAMS119982
<i>Eq</i>	AMNH FM 142430	3.23	-21.40	19950 $\pm$ 110	CAMS119983
<i>Eq</i>	AMNH FM 60019	1.65	-21.50	>48500	CAMS119984
<i>Eq</i>	AMNH FM 142431	3.94	-21.09	25710 $\pm$ 230	CAMS119985
<i>Eq</i>	AMNH FM 142432	2.47	-21.48	48500	CAMS119986
<i>Eq</i>	AMNH FM 142433	2.85	-20.70	25960 $\pm$ 240	CAMS119987
<i>Eq</i>	AMNH FM 142434	3.76	-20.97	22610 $\pm$ 150	CAMS119988
<i>Eq</i>	AMNH FM 142435	3.82	-21.87	21840 $\pm$ 140	CAMS119989
<i>Eq</i>	AMNH FM 60023	2.53	-21.45	19000 $\pm$ 100	CAMS120058
<i>Eq</i>	AMNH FM 60027	3.60	-20.81	19590 $\pm$ 110	CAMS120059
<i>Eq</i>	AMNH FM 60026	3.96	-21.32	21310 $\pm$ 140	CAMS120060
<i>Eq</i>	AMNH FM 60025	4.65	-21.19	13710 $\pm$ 60	CAMS120061
<i>Eq</i>	AMNH FM 60020	3.99	-21.25	19950 $\pm$ 120	CAMS120062
<i>Eq</i>	AMNH FM 60028	4.43	-20.63	>48400	CAMS120064
<i>Eq</i>	AMNH FM 60221	0.74	-21.70	43700 $\pm$ 2000	CAMS120067
<i>Eq</i>	AMNH FM 60032	2.54	-20.89	15850 $\pm$ 70	CAMS120068
<i>Eq</i>	AMNH FM 60033	2.64	-20.87	39910 $\pm$ 1330	CAMS120069
<i>Rta</i>	AMNH FM 142438	3.63	-20.08	21142 $\pm$ 361	AA48680
<i>Rta</i>	AMNH FM 142439	1.97	-19.45	16721 $\pm$ 207	AA48682
<i>Rta</i>	AMNH FM 142440	5.43	-20.07	17287 $\pm$ 222	AA48683
<i>Rta</i>	AMNH FM 142441	3.26	-19.21	16070 $\pm$ 190	AA48686
<i>Rta</i>	AMNH FM 142442	3.13	-18.99	16420 $\pm$ 202	AA48687
<i>Rta</i>	AMNH FM 142443	2.13	-19.27	>40707	AA48681
<i>Rta</i>	AMNH FM 142444	4.67	-19.43	>40673	AA48685
<i>Rta</i>	AMNH FM 142445	3.65	-19.72	>41100	AA48688
<i>Rta</i>	AMNH FM 142446	3.13	-19.31	29640 $\pm$ 370	CAMS120070
<i>Rta</i>	AMNH FM 142447	1.69	-19.19	>45200	CAMS120071
<i>Bbi</i>	AMNH FM 5092	4.38	-20.08		
<i>Bbi</i>	AMNH FM 46555	5.21	-20.10		
<i>Bbi</i>	AMNH FM 46858	3.73	-20.88		
<i>Bbi</i>	AMNH FM 46856	2.93	-19.77		
<i>Bbi</i>	AMNH FM 3135	5.22	-20.51		
<i>Bbi</i>	AMNH FM 1636	4.38	-21.34		
<i>Bos</i>	AMNH FM 142448	2.65	-20.73		
<i>Bos</i>	AMNH FM 142449	0.47	-24.16		
<i>Bos</i>	AMNH FM 142450	0.98	-20.38		
<i>Bos</i>	AMNH FM 142451	4.77	-21.68		

The following species abbreviations are used: *Canis lupus* (*Clu*), *Equus lambei* (*Eq*), *Rangifer tarandus* (*Rta*), *Bison bison* (*Bbi*), *Bos grunniens* (*Bos*), and *Symbolus cavifrons* (*Sca*). Museum and radiocarbon dating laboratory abbreviations are as in Table S1. Radiocarbon dates for the wolf specimens are in Table S1. Methods: Collagen samples (1.0 mg) were weighed into tin capsules. Carbon and nitrogen stable-isotope ratios were measured with an elemental analyzer coupled with a mass spectrometer (Europa Hydra 20/20) at the University of California Davis Stable Isotope Facility. Stable-isotope compositions are reported with standard δ notation and are referenced to Vienna Pee Dee Belemnite and air for carbon and nitrogen, respectively. The standard deviation for replicates of a gelatin standard was <0.2‰ for carbon and nitrogen.