

Megafaunal Extinctions and the Disappearance of a Specialized Wolf Ecomorph

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Summary

The gray wolf (*Canis lupus*) is one of the few large predators to survive the Late Pleistocene megafaunal extinctions [1]. Nevertheless, wolves disappeared from northern North America in the Late Pleistocene, suggesting they were affected by factors that eliminated other species. Using skeletal material collected from Pleistocene permafrost deposits of eastern Beringia, we present a comprehensive analysis of an extinct vertebrate by exploring genetic (mtDNA), morphologic, and isotopic ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) data to reveal the evolutionary relationships, as well as diet and feeding behavior, of ancient wolves. Remarkably, the Late Pleistocene wolves are genetically unique and morphologically distinct. None of the 16 mtDNA haplotypes recovered from a sample of 20 Pleistocene eastern-Beringian wolves was shared with any modern wolf, and instead they appear most closely related to Late Pleistocene wolves of Eurasia. Moreover, skull shape, tooth wear, and isotopic data suggest that eastern-Beringian wolves were specialized hunters and scavengers of extinct megafauna. Thus, a previously unrecognized, uniquely adapted, and genetically distinct wolf ecomorph suffered extinction in the Late Pleistocene, along with other megafauna. Consequently, the survival of the species in North America depended on the presence of more generalized forms elsewhere.

Results

Temporal Continuity

Radiocarbon dating of 56 ancient gray wolf specimens from permafrost deposits in Alaska shows a continuous

population from 12,500 radiocarbon years before present (BP) to beyond the capacity of radiocarbon dating (Table S1 in the Supplemental Data available online). A single wolf was dated to $7,674 \pm 66$ BP (Figure S1). The near absence of wolves dated as younger than 12,500 BP likely reflects population decline rather than taphonomic bias because other extinct and extant megafauna (e.g., bison, elk, and moose) have been recovered from eastern Beringia dating between 12,500 and 10,000 BP [2].

Genetic Relationships

To determine the relationship between ancient and modern Alaskan gray wolves, we successfully amplified the 5' end of the mitochondrial control region and sequenced it in three overlapping fragments [3] from 20 ancient individuals, yielding 16 haplotypes. These sequences were compared to those from 436 recent wolves from throughout the world. None of the haplotypes identified in the ancient material was found in modern conspecifics. A phylogeny constructed with parsimony, maximum-likelihood, and distance approaches showed that the ancient American wolf haplotypes are generally basal to extant wolves (excluding Indian wolves), and no recent North American haplotypes clustered within them (Figure 1). Thus modern North American wolves are not their direct lineal descendants. Rather, nearly all modern Holarctic wolves share a common ancestry, whereas ancient North American wolves are phylogenetically associated with a distinct group of modern European haplotypes (lu5, lu6, lu15 and lu16), suggesting evolutionary turnover among wolves within North America. Genetic diversity of ancient wolves is also higher than that of their modern counterparts, implying that the population in the Late Pleistocene was larger than it is at present (Table S2).

The distinctness of the Pleistocene American wolves from recent American wolves is further supported by a comparison of our sequences of ancient eastern-Beringian wolves to much shorter 57 bp sequences of Pleistocene Old World wolves [4]. None of these ancient Old World sequences were identical to any modern wolf, and they differed from recent North American and European haplotypes by 1–10 bp (2%–18%). However, three (dated 30,000 BP and 28,000 BP from Ukraine, and 33,000 BP from Altai) had the same sequence found in six eastern-Beringian wolves (haplotypes PW1–PW4). Another haplotype from a 44,000 BP wolf from the Czech Republic matched those of two ancient Beringian wolves (haplotypes PW5 and PW9). This sequence identity between ancient Old World wolves and eight of the ancient eastern-Beringian wolves reaffirms the validity of our sequences and supports the existence of a separate origin for ancient and extant North American wolves.

Craniodental Morphology

Skull measurements were taken from recent and Pleistocene gray wolves from Alaska and elsewhere in North

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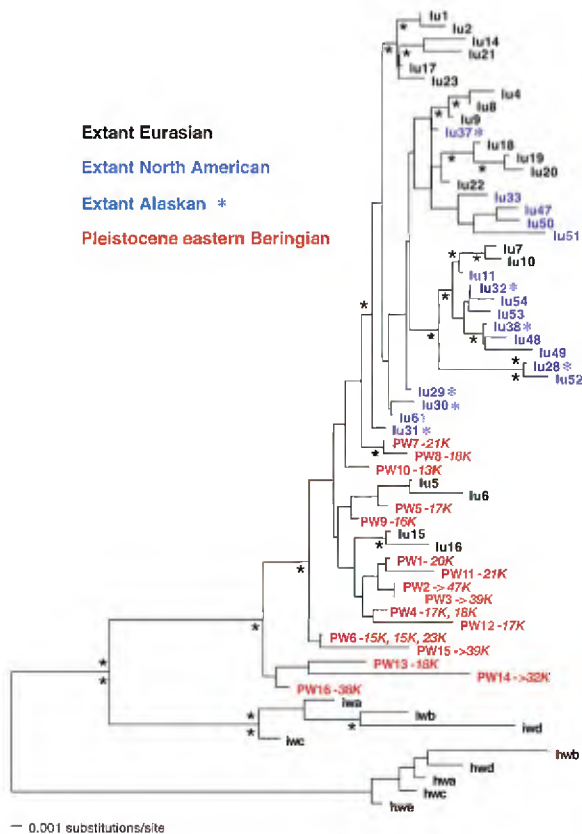


Figure 1. Neighbor-Joining Phylogeny of Pleistocene and Modern Wolves

Phylogeny based on 421–427 bp of mitochondrial control region I sequence (variation due to indels) rooted with Indian (iw) and Himalayan (hw) wolves [17]. Radiocarbon dates associated with each of the ancient haplotypes are listed in italics next to the relevant haplotype (K = thousand years BP). The bootstrap support for nodes in a neighbor-joining phylogeny (based on 1000 pseudoreplicates) and the percent of the 7200 most parsimonious trees when equal to or greater than 90% are indicated with an asterisk above and below nodes, respectively. Values for all support indices, including likelihood-reliability values, are available by request from the authors.

America (Tables S3 and S4). Both analysis of variance (ANOVA) of the raw morphological data and principal components analysis (PCA) of geometric-mean-transformed shape variables (see Experimental Procedures) revealed that eastern-Beringian wolves differed significantly in shape from both the sample of modern North American wolves and Rancho La Brea (California) Pleistocene wolves (Figures 2A and 2B; Table S5). The disparity is more apparent for cranial than dentary shape, but Beringian wolves tend to cluster in a different space than other gray wolves in both plots of the first two PCA factors (Figures 2A and 2B). Cranial dimensions show almost no overlap (Figure 2B), with most of the separation occurring on the second PCA axis. Modern Alaskan and Pleistocene La Brea specimens overlap the distribution of other extant North American wolves, and they are distinct from the Pleistocene eastern-Beringian group. The Beringian wolves tend to have short, broad palates with large carnassials, relative to their overall skull size (Figure 2C; Tables S5 and S6).

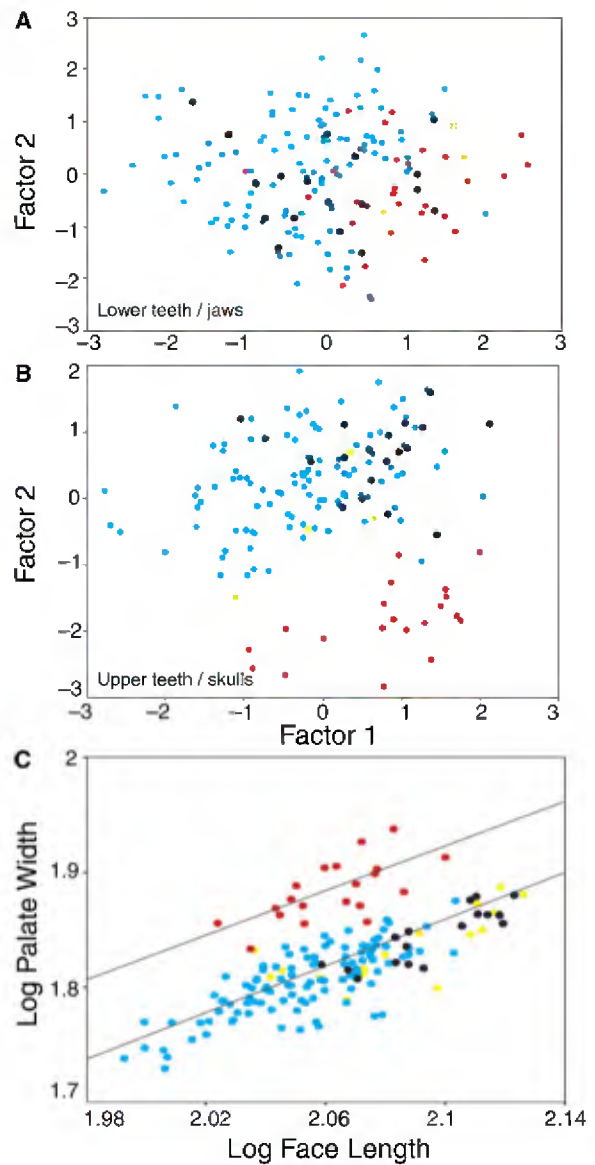


Figure 2. Plot of the First Two Factors of a PCA on Log₁₀-Transformed Shape Variables

(A and B) Dentary (A) and cranial (B) data. (C) Linear regression of log₁₀ palate width against log₁₀ face length. The equation for the eastern-Beringian sample is the following: $\log_{10} PW = 0.928 \cdot \log_{10} FL - 0.003$; $r^2 = 0.44$. The equation for modern North American sample is the following: $\log PW = 0.906 \cdot \log FW - 0.005$; $r^2 = 0.64$. The following symbols are used: Late Pleistocene eastern-Beringian wolves (red), La Brea wolves (yellow), modern Alaskan wolves (light blue), and non-Alaskan modern North American wolves (black).

Together, these features suggest a gray wolf adapted for producing relatively large bite forces. The short, broad rostrum increased the mechanical advantage of a bite made with the canine teeth and strengthened the skull against torsional stresses imposed by struggling prey [5, 6]. Relatively deep jaws are characteristic of habitual bone crackers, such as spotted hyenas (*Crocuta crocuta*) [7], as well as canids that take prey as large as or larger than themselves [8]. In all of these features, the eastern-Beringian wolves differed from

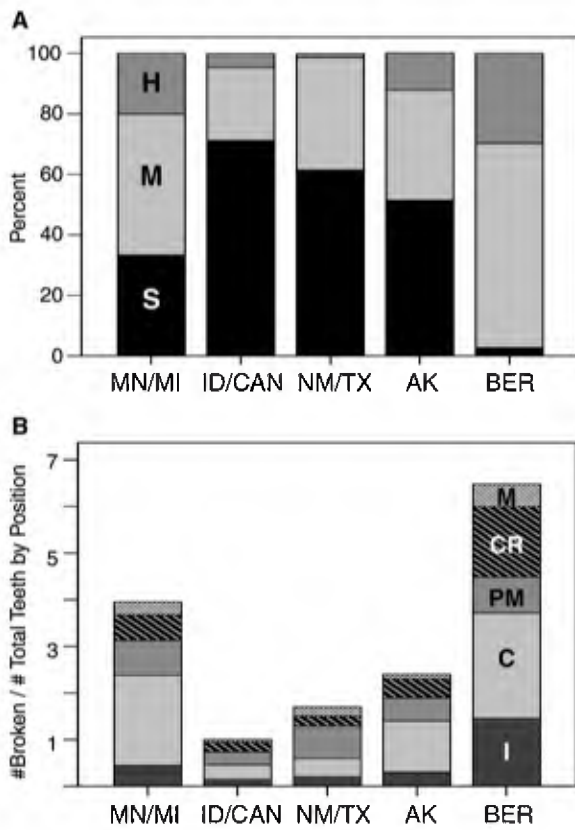


Figure 3. Tooth Wear Data from Five Gray Wolf Samples
(A) Percent of individuals in each wear-stage category. The following abbreviations are used: slight (S), moderate (M), and heavy (H), as indicated in the leftmost bar.
(B) Percentage of broken teeth for each tooth type. The following abbreviations are used: incisors (I), canines (C), premolars (PM), carnassials (CR), and molars (M), as indicated in the rightmost bar. The following sample labels are used: Minnesota and Michigan (MN/MI), Idaho and adjacent Canada (ID/CAN), New Mexico and Texas (NM/TX), Alaska (AK), and Late Pleistocene eastern Beringia (BER). For sample sizes, see Table S7.

their extant counterparts and Pleistocene Rancho La Brea gray wolves in a direction that suggests greater specialization for killing and consuming relatively large prey and/or habitual scavenging.

Tooth Wear and Fracture

All eastern-Beringian wolves used in the morphometric analysis and 313 modern, wild-caught, adult wolves from four North American subspecies (Table S7) were scored for overall tooth wear (slight, moderate, and heavy; see Experimental Procedures). In addition, the number and position of teeth broken in life were recorded for each individual. Relative to modern populations from a range of environments (Alaska to Arizona), the sample of Beringian wolves includes many more individuals with moderately and heavily worn teeth (97%; Figure 3A). In addition, eastern-Beringian gray wolves exhibit heavier wear and significantly greater numbers of broken teeth. Overall fracture frequencies ranged from a low of 2% in *C. lupus irremotus* (Canada, Idaho) to a high of 11% in the Pleistocene eastern-Beringian wolves (Table S7). The distribution of fractures

across the tooth row differs as well, with eastern-Beringian wolves having much higher fracture frequencies of incisors, carnassials, and molars (Figure 3B). A similar pattern was observed for spotted hyenas, relative to those of canids (including gray wolves) and felids [9], suggesting that increased incisor and carnassial fracture reflects habitual bone consumption because bones are gnawed with incisors and subsequently cracked with cheek teeth.

Stable Isotopes

Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) data were collected from bone collagen of Late Pleistocene wolves ($n = 40$) and their potential prey, including horses (*Equus lambei*), caribou (*Rangifer tarandus*), bison (*Bison bison*), yak (*Bos grunniens*), and woodland muskox (*Symbos cavifrons*) (Table S8). We also included published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data collected from undated Pleistocene mammoths (*Mammuthus primigenius*) from multiple sites in Alaska [10]. To eliminate potential isotopic variability among individuals due to spatial differences, we obtained all samples (except mammoth) from the Fairbanks area. The $\delta^{13}\text{C}$ values of Beringian wolves range from -18.3‰ to -20.5‰ , and $\delta^{15}\text{N}$ values range from 10.3‰ to 5.4‰ (excluding one outlying full-glacial individual with a value of 12.7‰) (Table S8). All preglacial and postglacial wolf values (corrected for trophic-level isotopic enrichments) fall within a dietary isospace defined by megafaunal prey $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Figure 4). Two full-glacial wolves have high $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values that seem difficult to explain with existing prey values, but we do not have a large sample of full-glacial caribou, and this might better explain these deviant values. The variances of wolf $\delta^{13}\text{C}$ values are similar among time periods (Levene's test; $F = 0.4$, $p = 0.65$), whereas those of $\delta^{15}\text{N}$ values differ significantly (Levene's test; $F = 4.9$, $p = 0.013$), with postglacial wolves being less variable than preglacial and full-glacial wolves. If the outlier with a very high $\delta^{15}\text{N}$ value (12.7‰) is removed, the difference between full-glacial and postglacial samples is no longer significant, but the preglacial sample remains significantly more variable than do the younger samples (Levene's test; $F = 8.8$, $p = 0.0008$). In each of the three time periods, isotope values from wolves are intermediate among prey types, suggesting that they had diets comprised of a mix of species. Horse and bison appear to have been prey for wolves during all three time periods, whereas woodland muskox show up in the wolves' diets only in preglacial times, and mammoth seems to have been consumed only after this time. Thus, eastern-Beringian wolves as a group preyed on a wide diversity of species, including megafauna that are now extinct, supporting the conclusion of the morphologic analysis that they were capable of killing and dismembering large prey.

Discussion

Our morphological and isotopic data demonstrate the existence of a previously undetected ecomorph of the gray wolf in the Late Pleistocene of eastern Beringia and suggest an evolutionary plasticity of craniodental form within *C. lupus* greater than previously observed in modern North American gray wolves. This wolf was

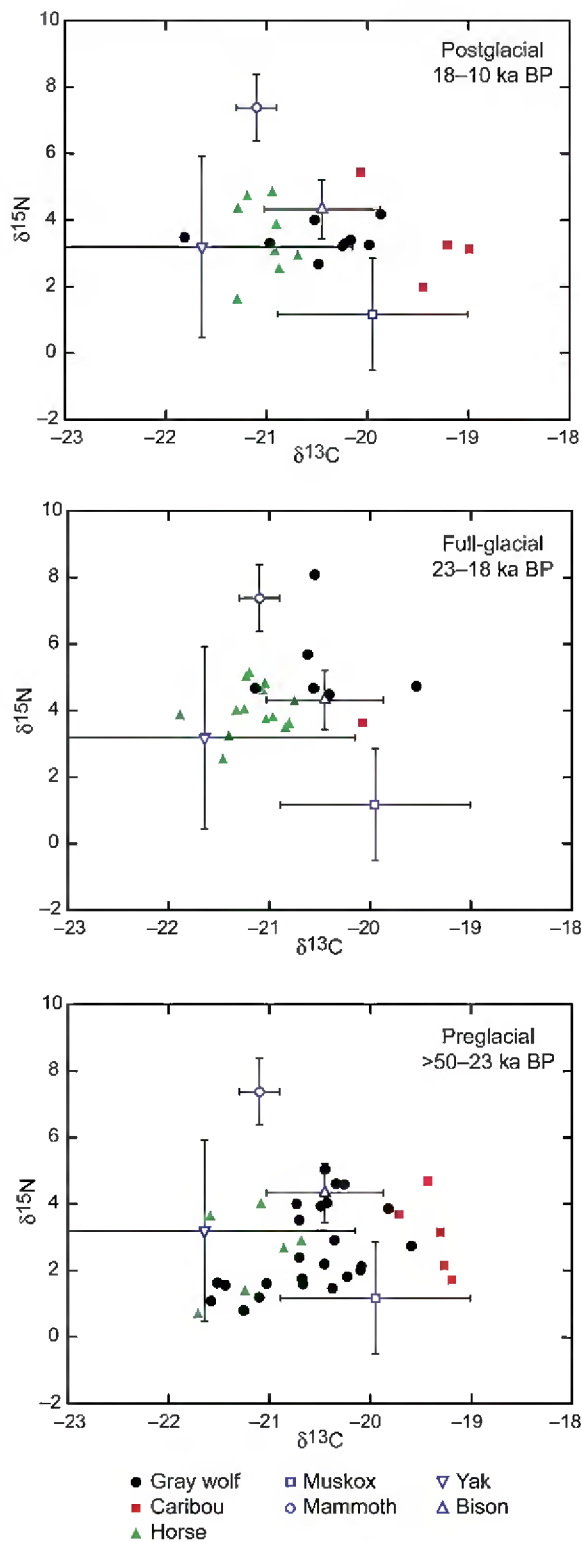


Figure 4. Dietary Reconstructions for Late Pleistocene Gray Wolves from the Fairbanks Region

In each reconstruction, the wolf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values have been corrected for trophic-level isotopic fractionation (-1.3% for $\delta^{13}\text{C}$, and -4.6% for $\delta^{15}\text{N}$) and are compared to isotope values of mega-faunal prey species from the Fairbanks area (except for mammoth, which are from sites throughout Alaska). Horse and caribou individuals were ^{14}C dated, and discrete isotopic values are included for individuals from each time period. Bison, yak, muskox, and

similar in size to coeval Rancho La Brea wolves and modern Alaskan wolves, but it had stronger jaws and teeth. Moreover, tooth fracture frequency was high (11%) and comparable to that observed among four large Pleistocene predators from south of the ice sheets [11]—from 7% in dire wolves (*Canis dirus*), coyotes (*C. latrans*), and saber-toothed cats (*Smilodon fatalis*), to 17% in American lions (*Panthera atrox*). The greater tooth fracture in Pleistocene carnivores suggests heavier carcass utilization in the past, probably as a result of higher carnivore densities and increased competition. This same explanation could apply to the eastern-Beringian gray wolves because they coexisted with several formidable predators, including the American lion, short-faced bear (*Arctodus simus*), brown bear (*Ursus arctos*), scimitar-tooth sabercat (*Homotherium serum*), and dhole (*Cuon alpinus*).

Compared with extant gray wolves and Pleistocene gray wolves from Rancho La Brea, the eastern-Beringian ecomorph was hypercarnivorous with a craniodental morphology more capable of capturing, dismembering, and consuming the bones of very large mega-herbivores, such as bison. When their prey disappeared, this wolf ecomorph did as well, resulting in a significant loss of phenotypic and genetic diversity within the species. Conceivably, the robust ecomorph also was present in western Beringia in the Late Pleistocene, but specimens were not available for this study.

A plausible scenario for the presence of two distinct Pleistocene gray wolves in North America relies on an early arrival of the more gracile wolf from the Old World and migration to areas below the Wisconsin ice sheet. This gray wolf insinuated itself into a carnivore guild that already contained forms both larger (dire wolf) and smaller (coyote) than itself. The presence of these two relatively common species (especially the dire wolf) seems to have prevented gray wolves from reaching high densities until after the demise of the dire wolf, approximately 10 ka BP [12]. The appearance of a more robust form of the gray wolf in eastern Beringia in the Late Pleistocene might represent evolution in situ or a secondary invasion from the Old World. Its success was favored by the absence of dire wolves north of the ice sheet [12]. Otherwise, the eastern-Beringian gray wolf would probably not have evolved in the same direction as the dire wolf, acquiring a more robust skull and dentition, because that would have increased ecological overlap between the two species. Instead, the eastern-Beringian gray wolf was well positioned as the dominant large, pack-hunting canid within a predator guild that included large felids, ursids, and two smaller canids, the dhole and coyote [1].

Greater extinction vulnerability of relatively specialized forms has been shown for other Pleistocene carnivores [13]. Species that did not survive were usually the largest and most carnivorous within their family. Among the felids, the large lion and saber tooth disappeared, whereas the relatively eurytopic puma and bobcat (*Lynx rufus*) survived. Among the bears, the species with the greatest dental specialization for carnivory, the

mammoth were not ^{14}C dated, and a range of isotopic values (means \pm standard deviations) are included for each time period. Mammoth isotopic data are from reference [10].

short-faced bear, vanished, but the more omnivorous and smaller black (*Ursus americanus*) and brown bears remain [13]. Similarly, the dire wolf was lost, whereas the smaller gray wolf and coyote survived. However, the gray wolf did not survive unscathed—we show that at least one ecologically distinct form was lost and replaced by a smaller and more generalized form. Past studies of Pleistocene survivors [14–16] also demonstrated a loss of genotypic diversity but did not explore changes in morphologic diversity. Thus, there may be other extinctions of unique Pleistocene ecomorphs yet to be discovered.

Experimental Procedures

Details of the experimental procedures are given in the [Supplemental Data](#). Our eastern-Beringian gray wolf sample was restricted to specimens positively identified as *Canis lupus* and a few specimens of uncertain identity (labeled “*Canis sp.*”) that were radiocarbon dated as greater than 7 ka BP (to eliminate the possibility of recent wolves and dogs) (Table S1). Specimens are from the collections of the American Museum of Natural History (AMNH) (New York) and the Canadian Museum of Nature (CMN) (Ottawa, Canada). We also examined the morphology of a sample ($n = 15$) of gray wolves from the Pleistocene Rancho La Brea tar seeps of California that are in the collections of the Natural History Museum of Los Angeles County (LACM). All specimens used in the analyses are listed in Table S1, along with radiocarbon dates when available.

Supplemental Data

Experimental Procedures, one figure, and eight tables are available at <http://www.current-biology.com/cgi/content/full/17/13/1146/DC1/>.

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