

Fossil and genomic evidence constrains the timing of bison arrival in North America

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The arrival of bison in North America marks one of the most successful large-mammal dispersals from Asia within the last million years, yet the timing and nature of this event remain poorly determined. Here, we used a combined paleontological and paleogenomic approach to provide a robust timeline for the entry and subsequent evolution of bison within North America. We characterized two fossil-rich localities in Canada's Yukon and identified the oldest well-constrained bison fossil in North America, a 130,000-y-old steppe bison, *Bison cf. priscus*. We extracted and sequenced mitochondrial genomes from both this bison and from the remains of a recently discovered, ~120,000-y-old giant long-horned bison, *Bison latifrons*, from Snowmass, Colorado. We analyzed these and 44 other bison mitogenomes with ages that span the Late Pleistocene, and identified two waves of bison dispersal into North America from Asia, the earliest of which occurred ~195–135 thousand y ago and preceded the morphological diversification of North American bison, and the second of which occurred during the Late Pleistocene, ~45–21 thousand y ago. This chronological arc establishes that bison first entered North America during the sea level lowstand accompanying marine isotope stage 6, rejecting earlier records of bison in North America. After their invasion, bison rapidly colonized North America during the last interglaciation, spreading from Alaska through continental North America; they have been continuously resident since then.

Beringia | *Bison latifrons* | *Bison priscus* | paleogenomics | Rancholabrean | steppe bison

The invasion of bison (*Bison*) from Asia across the Bering Isthmus profoundly affected the North American faunal community. Bison, or American buffalo, are large-bodied, aggressive, and highly fecund. Following their establishment in North America, bison rapidly became the most important competitor for forage within the established large mammal community (1). Early North American bison were morphologically and ecologically diverse (2, 3). In addition to extant *Bison bison*, taxa traditionally recognized in systematic treatments include the steppe bison (*Bison priscus*), which first colonized northwestern North America from Asia, and the giant long-horned bison (*Bison latifrons*) of the central and southern continent (Figs. 1*A* and 2). The latter is the largest bison known. It inhabited woodlands and forest openings through much of the continental United States and southern Canada; however, their fossils have not been found in northern Canada or Alaska. Bison eventually became an important hunting resource for Indigenous North Americans (4) and remain an icon of the American plains (5).

At the time of bison arrival, the North American megaherbivore grazing community was dominated by mammoths (*Mammuthus*) and caballine equids (*Equus*). Equids have a deep evolutionary history in North America that is closely associated with the rise of grasslands during the Early Miocene (~18 Ma)

(6). Mammoths dispersed from Asia to North America during the Early Pleistocene (~1.35 Ma), becoming the continent's largest-bodied obligatory grazer (7). The subsequent arrival of *Bison* markedly affected a faunal community dominated by *Equus* and *Mammuthus*, but when that process actually began has been difficult to determine.

Paleontologically, *Bison* is the index taxon for the Rancholabrean, the final North American Land Mammal Age (8–10). Land Mammal ages are important because, in the absence of other chronological data, they provide a means to infer the age of a locality based on taxonomic assemblages. This assumes, however, that the first appearance datum of the index taxon can be reliably tied to a specific interval, which is surprisingly not the case for bison (8). Here, we used a combined paleontological and paleogenomic approach to establish the timing of bison entry into North America.

Models of the timing of *Bison* entry into North America range, based on fossil occurrences, from arrival during the Late Pliocene or Early Pleistocene, approximately 2–3 Ma, to the Late Pleistocene (8). The oldest of these dates are based on fossil sites in Florida (11) and Alaska (12) that have now been shown to be of poor stratigraphic and chronologic integrity (8, 13, 14). A bison astragalus dating to 290–230 thousand y before present (kyBP), or marine isotope stage (MIS) 7, was recovered from South Carolina (15), but the reliability of this age and its association with the fossils have also been questioned (16, 17). Bison fossils are absent in well-dated Middle Pleistocene (780–130 kyBP) localities from both central and northern North America (8). In

Significance

The appearance of bison in North America is both ecologically and paleontologically significant. We analyzed mitochondrial DNA from the oldest known North American bison fossils to reveal that bison were present in northern North America by 195–135 thousand y ago, having entered from Asia via the Bering Land Bridge. After their arrival, bison quickly colonized much of the rest of the continent, where they rapidly diversified phenotypically, producing, for example, the giant long-horned morphotype *Bison latifrons* during the last interglaciation.

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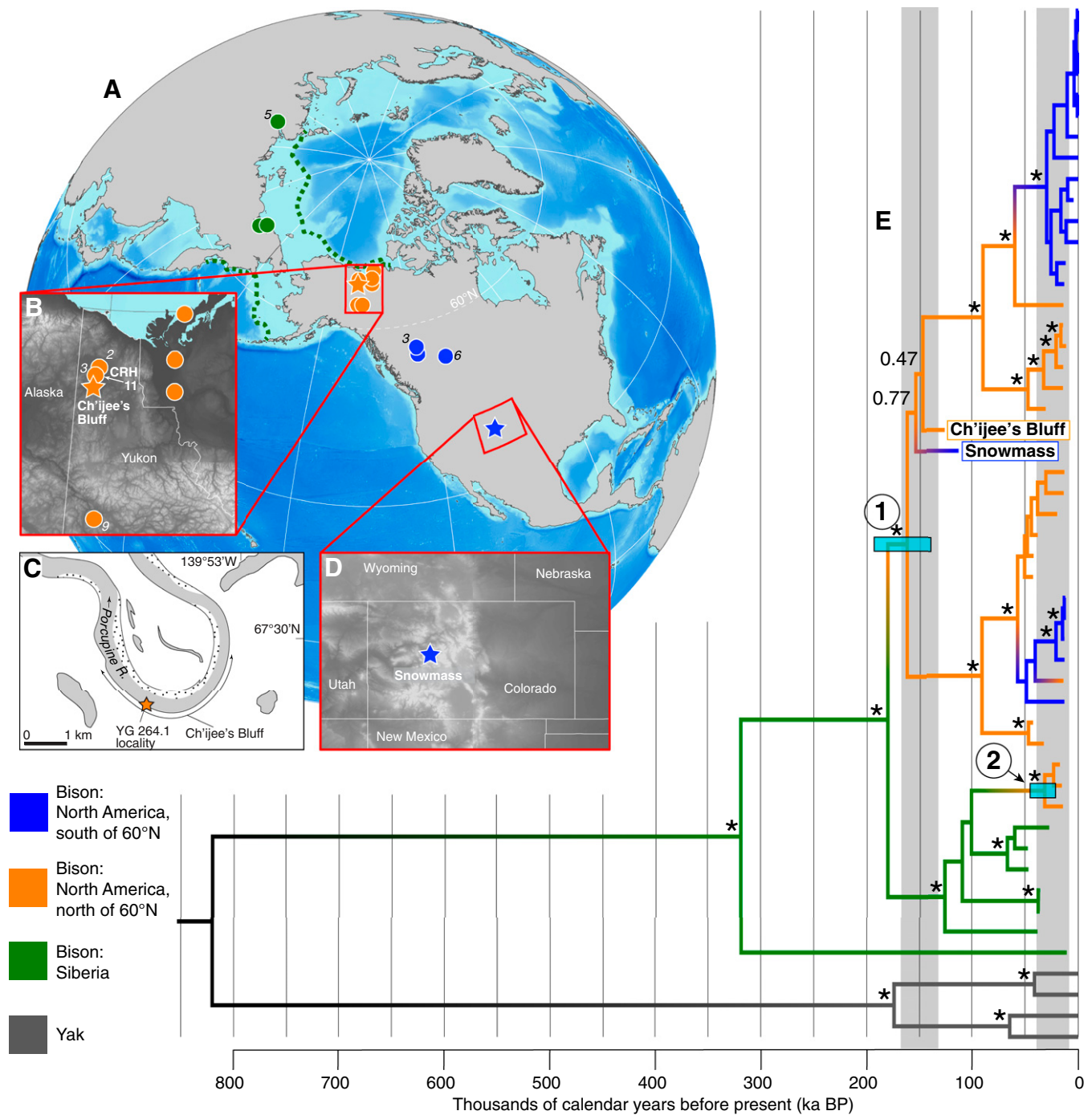


Fig. 1. (A) Localities of the 36 fossil bison. Siberia: green; North America north of 60° N: orange; North America south of 60° N: blue. The number of samples is given in italics if >one sample was recovered. The green dashed lines outline the last glacial maximum Bering Land Bridge extent. Insets showing locations of (B) CRH 11 (labeled orange circle) and Ch'ijee's Bluff (orange star) in northern Yukon, with (C) a zoom-in on Ch'ijee's Bluff, and (D) Snowmass, Colorado (blue star). (E) Bayesian phylogeny resulting from analysis of the reduced mitochondrial alignment, calibrated using the ages of the bison fossils from which data were generated. Nodes with posterior support of >0.99 are indicated with a black asterisk and other values are provided for deep nodes. The positions of the Ch'ijee's Bluff and Snowmass bison are highlighted. We identify two waves of dispersal from Asia into North America via the Bering Land Bridge (nodes 1 and 2), with date ranges indicated as light blue bars. Areas of gray shading indicate intervals of lowered sea level sufficient to expose the Bering Land Bridge (36).

Kansas, bison remains are all younger than the Lava Creek B Ash, which has a maximum constraining age of 640 kyBP (18). Bison fossils are also absent from the rich Sheridanian fauna “*Equus* beds” of Nebraska (19), which are overlain by the Loveland loess, dated regionally using optically stimulated luminescence to 180–130 kyBP, or MIS 6 (20). In Yukon, a fossil assemblage in stratigraphic association with the Middle Pleistocene

Gold Run tephra (735 ± 88 kyBP) includes horse (*Equus*), proboscideans (*Mammuthus*), sheep (*Ovis*), and biostratigraphically diagnostic microtine rodent species, but bison fossils are absent (21–23). The oldest site in the midcontinental United States possessing both bison and a firm chronology is near Snowmass, Colorado, where bison fossils were recovered within sediments associated with the last interglaciation (MIS 5d), thus dating to

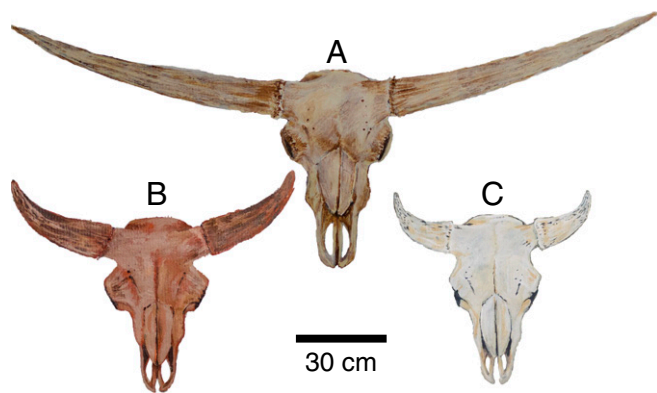


Fig. 2. Reconstructions of bison skulls based on fossils attributed to (A) a giant long-horned bison, *B. latifrons*; (B) a Late Pleistocene steppe bison, *B. priscus*; and (C) a present-day *B. bison*. Giant long-horned bison were significantly larger than present-day bison; adult males may have weighed in excess of 2,000 kg, which is twice as large as present-day bison, and had horns that spanned as much as 2.2 m (57, 58).

~120 kyBP (24). Bison fossils also occur at American Falls, Idaho, associated with a lava-dammed lake that dates to ~72 kyBP (8, 25). Bison from both of these sites represent the giant long-horned bison, *B. latifrons* (24, 25), generally considered to be the earliest form of bison developed in the continental United States.

To establish a reliable first appearance datum for bison in North America, we first characterized the in situ fossil assemblage and chronology of two, well-dated fossil-rich localities in the Old Crow area of northern Yukon, Canada: CRH 11 and Ch'ijee's Bluff (Figs. 1 B and C and 3). Next, we isolated and sequenced mitochondrial genomes from two of the oldest *Bison* fossils yet identified: a partial metacarpal found at Ch'ijee's Bluff that dates to ~130 kyBP (Fig. 3C), and a humerus from the site near Snowmass recovered within a layer dated to ~120 kyBP (Fig. 1D). These two bison were identified, based on their size and geographic location, as a steppe bison and a giant long-horned bison (24), respectively. We then used a coalescent-based approach to infer a mitochondrial genealogy for these and 44 other Late Pleistocene and Holocene bison, taking advantage of an approach to calibrate a molecular clock within a coalescent framework using the ages of each sampled bison (26).

This approach allows an estimate of the age of relevant nodes in the bison mitochondrial genealogy, making it possible to test hypotheses not only about the timing of bison entry into North America but also about the relationship between these morphologically distinct bison forms.

Results and Discussion

Characterizing Two Fossil Assemblages in Yukon, Canada. To assess the chronology of bison presence in high-latitude northwest North America, we first characterized the in situ fossil assemblage and chronology at CRH 11 (27, 28) and Ch'ijee's Bluff (Fig. 3). The chronologies of both of these sites rely heavily on identification of volcanic ash layers, or tephra, in sediment exposures. Individual tephra are readily characterized geochemically and, when combined with other correlative indicators—such as stratigraphy or paleoecology—can provide isochronous stratigraphic markers across a region (29). The Old Crow tephra has an isothermal plateau glass fission-track age of 124 ± 10 kyBP at the 1σ confidence level (29). This age determination spans the transition from the late MIS 6 glacial to the MIS 5e interglaciation, but paleoecological evidence for cool climate conditions during tephra deposition indicates a late MIS 6 age (29, 30). The Old Crow tephra thus provides a useful marker horizon for this study: interglacial deposits below the tephra must date to MIS 7 or older, whereas sediment above the tephra but below the prominent interglacial deposits represents a narrow time interval between latest MIS 6 and the beginning of MIS 5e (~135 to ~125 kyBP).

CRH 11 is one of the classic localities for Quaternary paleontology in North American Beringia. This bluff, on the left bank of the Old Crow River ($67^\circ 49' N$, $139^\circ 51' W$) comprises ~30 m of silt and sand that is locally organic-rich (28). We recovered 294 vertebrate fossils from the lowest bone-bearing unit at CRH 11, including specimens of woolly mammoth (*Mammuthus primigenius*), horse (*Equus* sp.), caribou (*Rangifer tarandus*), giant beaver (*Castoroides ohioensis*), beaver (*Castor canadensis*), wolverine (*Gulo gulo*), and Jefferson's ground sloth (*Megalonyx jeffersonii*) (for a complete list, see *SI Text*). *Bison* fossils are absent, consistent with earlier in situ assemblages recovered from the site (27). The Old Crow tephra is present 14 m above the in situ fossils (Fig. S1), establishing that the fossiliferous sediments must be older than the last interglaciation (pre-MIS 5e) and, based on paleoecology, date from the penultimate interglaciation, MIS 7 (28). To confirm this age, we performed direct single-grain optically stimulated luminescence dating of these sediments (*SI Text*),

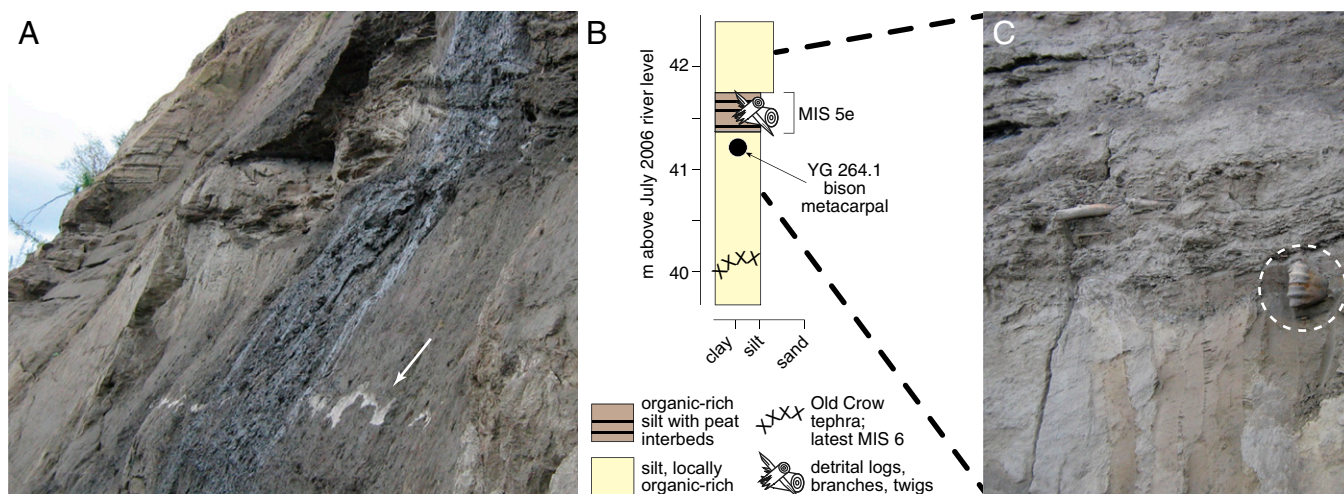


Fig. 3. Features of the Ch'ijee's Bluff locality. (A) the Old Crow tephra (124 ± 10 kyBP; UA1206) highlighted by the white arrow, (B) the stratigraphic setting of the Old Crow tephra, bison metacarpal YG 264.1, and the MIS 5e forest bed, and (C) the in situ metacarpal was found several centimeters beneath the prominent MIS 5e forest bed and ~125 cm above Old Crow tephra (the latter is not shown). The stratigraphy indicates a latest MIS 6 age for YG 264.1.

which gave a weighted mean age of 208 ± 6 kyBP (Figs. S2–S4), consistent with a MIS 7 age. Bison fossils were absent not only from this stratigraphic level at CRH 11, but also from nearby sites of comparable age (27), suggesting that bison were not present in Yukon before MIS 6.

Bison fossils are present, however, at nearby Ch'ijee's Bluff 40 km southwest ($67^{\circ} 29' N$, $139^{\circ} 56' W$), where 50 m of unconsolidated Late Cenozoic sediment are exposed along a prominent 4-km-long cut bank on the Porcupine River (Figs. 1C and 3). The Old Crow tephra (29) is present across much of Ch'ijee's Bluff (Fig. 3A), where it underlies a prominent bed of dark-brown macrofossil-rich organic silt. Paleocological indicators within this organic layer represent a closed boreal forest and a warmer than present climate, indicating a MIS 5e age (30, 31). In support of this age and the stability of the organic layer, logs and organic detritus from the organic bed all have nonfinite ^{14}C ages (30, 31). Although most bison fossils at the site are detrital, we recovered a single in situ bison metacarpal (YG 264.1) 125 cm above the Old Crow tephra and directly below the prominent MIS 5e woody peat bed (Fig. 3B and C). The tephra- and bone-bearing silt containing the fossil is sharply overlain by the organic-rich bed, an unconformity related to the thawing of permafrost during the last interglaciation that is common across northwestern Canada and Alaska (30). Given this stratigraphic context, we conclude that the bison fossil dates to ~ 130 kyBP: it is younger than late MIS 6 (the age of Old Crow tephra), and older than the onset of the MIS 5e interglaciation. This is the oldest reliably dated fossil evidence of bison in North America (cf. ref. 8).

The Oldest Fossil Bison in North America. Bison fossils are common throughout Yukon, Alaska, and Siberia. All are medium-horned bison, most commonly referred to as steppe bison, *B. priscus* (2), and published genetic data are consistent with this interpretation (3). The discovery of giant long-horned bison at a last interglacial site (MIS 5 *sensu lato*) near Snowmass, Colorado, establishes the presence of a morphologically distinct bison in continental North America (Fig. 2A). These giant long-horned forms have never been recovered from northern locales between Yukon and Siberia (2). The age of the Snowmass site places the long-horned bison fossil slightly younger than the bison from Ch'ijee's Bluff.

Because of their antiquity and the poor preservation conditions of continental compared with northern (permafrost) localities, giant long-horned bison fossils have thus far failed to yield usable DNA. However, recent advances in paleogenomics have expanded the range of fossils from which DNA can be recovered (32). Capitalizing on these, we used a hybridization capture approach to enrich for bison mitochondrial DNA from both the Ch'ijee's Bluff bison and from a giant long-horned bison from Snowmass (DMNH EPV.67609) that dates to the last interglaciation, ~ 120 kyBP (24). We recovered a complete mitochondrial genome (159 \times coverage) from the Ch'ijee's Bluff bison and a near-complete mitochondrial genome (6.6 \times mean coverage) from the Snowmass bison (*Materials and Methods*). We assembled complete mitochondrial genomes from an additional 6 Siberian and 26 North American bison ranging in age from ~ 0.4 –45 kyBP (*Dataset S1*). We then estimated the evolutionary relationship between these and 10 present-day American (33, 34) and an ancient Siberian bison (35), using stratigraphic ages and radiocarbon dates to inform the molecular clock (*Materials and Methods* and *SI Text*).

Both the Ch'ijee's Bluff bison and the Snowmass bison mitochondrial lineages fall near the root of sampled bison mitochondrial diversity, indicating that both bison were early descendants of the first bison dispersing into North America (Fig. 1E). North American bison share a common maternal ancestor 195–135 kyBP (Fig. 1E, node 1, *SI Text*, and Fig. S5), consistent with the MIS 6 glaciation (Fig. 1E), and precluding models for a significantly older bison presence in North America. This timing is coincident with an interval of reduced eustatic sea level that would have enabled interchanges across the Bering Isthmus (36, 37). We also identify a second, later dispersal of bison from Asia into North America during the Late Pleistocene,

~ 45 –21 kyBP (Fig. 1E, node 2, *SI Text*, and Fig. S5), within a period of lowered sea level during the last glaciation (36).

The Rancholabrean is the most recent of the North American Land Mammal Ages, and has long been defined by the presence of bison in continental records (9). However, Bell et al. (8) argued that this North American Land Mammal Age should only apply to localities or faunules recovered from south of 55° North. Their reasoning was that, given its proximity to the Bering Land Bridge and eastern Eurasia, the northern part of the continent required a distinct chronology because of the potential for faunal mixing. Our results, however, show temporal and genetic affinity between the arrival of bison in northwestern Canada and their dispersal further south. The close genetic relationship between maternal lineages found in the earliest northern bison and the earliest continental bison argues for a rapid expansion of bison across the continent in a period of approximately 20,000 y between late MIS 6 and MIS 5d. These records also demonstrate the rapid phenotypic change from northern forms of bison (e.g., *B. priscus*, *B. alaskensis*) found in Siberia through Yukon, to *B. latifrons* in the continental United States.

The integration of independent geochronological data with faunal collections and a molecular dating approach constrains the history and dynamics of bison dispersal into North America. These complementary approaches provide a remarkably consistent picture of this grazer as it entered the continent during the sea level lowstand accompanying MIS 6 and spread from Alaska through the continental United States. The rapid dispersal and success of bison in North America make a strong case for bison as an index taxon for the Rancholabrean at a continental scale. Although full nuclear genomic resources for bison are not yet available, these well preserved specimens will be important to future work to better understand the genetic basis for the remarkable phenotypic variability in early North American bison. Given their relatively shallow history and success in North American ecosystems, the entry of bison stands with human arrival as one of the most successful mammalian dispersals into North America during the last million years.

Materials and Methods

This section provides an overview of the methods of this study; full details can be found in *SI Text*.

Geochronology. Chronology at Ch'ijee's Bluff and CRH 11 relies on identification of tephra in sediment exposures and optically stimulated luminescence (OSL) dating. Old Crow tephra was identified based on stratigraphic position, glass shard morphology, and grain-discrete glass major element geochemistry. Glass geochemical analyses were by wavelength dispersive spectrometry on a JEOL 8900 electron microprobe at University of Alberta following Reyes et al. (38), with correlations confirmed by concurrent analyses of an Old Crow tephra reference sample (Fig. S1).

We obtained four samples for single-grain OSL dating from immediately above and below the lower fossil-bearing horizon at CRH 11. Samples were processed under safe (dim red) light conditions using standard procedures (39) to isolate refined quartz fractions. We performed equivalent dose (D_e) measurements on 1,800–2,400 individual quartz grains per sample using the experimental apparatus described by Arnold et al. (40) and the single-aliquot regenerative-dose procedure shown in Table S1. We considered 3–6% of the measured grain populations suitable for D_e determination after applying the SAR quality assurance criteria (41). D_e estimation over high dose ranges (>300 Gy) was well-supported by the single-grain dose saturation characteristics and dose-recovery test results (Figs. S2 and S3). The natural D_e datasets exhibited low overdispersion values (11–14%) and are dominated by experimental rather than field-related D_e scatter (42) (Fig. S4). We therefore calculated the final burial doses using the central age model (43). Dose rates were calculated using a combination of field γ -ray spectrometry (FGS), high-resolution γ -ray spectrometry (HRGS), and inductively coupled plasma-mass spectrometry (ICP-MS) (Table S2). To calculate the final OSL ages, we assumed that the measured radionuclide activities and present-day field water/organic contents prevailed throughout the burial period of these perennially frozen deposits. An uncertainty of 10% was assigned to long-term water and organic content estimates to accommodate minor variations during the burial periods.

DNA Extraction, Sequencing, and Mitochondrial Genome Assembly. We assembled mitochondrial genomes for 35 ancient bison, including the Ch'j'ee's Bluff steppe bison and a giant long-horned bison from a site near Snowmass, Colorado (24). Of these, 21 were not associated with any stratigraphic or age information, and were sent to accelerator mass spectrometry (AMS) radiocarbon dating facilities for dating using ultrafiltered collagen (Dataset S1). We extracted DNA from 23 ancient bison using silica-based methods optimized for recovery of ancient DNA (44, 45), and included in our dataset 12 previously extracted bison (Dataset S1, and references therein). We converted extracted DNA to either double-stranded (46) or single-stranded (47) Illumina-compatible libraries. Mitochondrial DNA molecules were enriched using biotinylated RNA baits based on either the bison mitochondrial genome (GenBank: NC_012346) or a 242 mammal mitochondrial genome reference panel (48). We sequenced enriched libraries on the Illumina MiSeq or HiSeq platforms using paired-end chemistry. Sequencing read pairs were merged and adapter trimmed in SeqPrep. Merged and remaining unmerged reads were mapped to the bison mitochondrial genome using either Burrows-Wheeler Aligner (BWA) (49) or the iterative short-read assembler, MIA (50). We collapsed PCR duplicates using either bam-rmdup or MIA. For consensus sequence calling, we required each position to have a minimum of 3 \times coverage and a base agreement greater than 67% (51). To evaluate DNA preservation in these oldest bison, we used mapDamage (52) to assess patterns of DNA fragmentation and cytosine deamination (Fig. S6). The resulting ancient mitochondrial genomes ranged in coverage from 6.6 \times to 898.4 \times (Dataset S1). The Ch'j'ee's Bluff steppe bison had an average fragment length of 54 bp and was sequenced to 159 \times coverage, with all bases called following consensus calling, as above. The Snowmass bison, which was much more poorly preserved (Fig. S6 D–F), had an average fragment length of 35 bp, with 5,596 missing bases following consensus calling.

Phylogenetic Analysis. We aligned mitochondrial genomes from the 35 ancient bison described above with previously published mitochondrial genomes from 10 present-day American bison (33, 34), an ancient Siberian bison (35), and 4 yaks (*Bos grunniens*). We then created two datasets for phylogenetic analysis: one comprising the complete mitochondrial genome (full dataset), and another limited to only those sites in the mitochondrial genome in which we were able to call a consensus base for the Snowmass bison (reduced dataset). We partitioned both alignments and selected appropriate models of molecular evolution using PartitionFinder (53), and

inferred the evolutionary relationships among the sampled mitochondrial lineages using BEAST v1.8.3 (54). Following model testing (SI Text), our final analyses assumed a strict molecular clock and the skygrid coalescent prior (55). We calibrated the molecular clock using median calibrated radiocarbon ages for each sampled mitochondrial genome, and sampled the ages of Ch'j'ee's Bluff and Snowmass bison using a mean and SD of 125 \pm 4.5 kyBP and 124 \pm 8.5 kyBP, respectively (56). For each analysis, we ran two Markov chain-Monte Carlo chains for 60 million iterations each, sampling priors and trees every 3,000 iterations, and discarding the first 10% as burn-in, and combining the remainder. We visually inspected log files for run convergence using Tracer and summarized the sampled trees using TreeAnnotator. Phylogenies presented in the text and SI Text are maximum clade credibility trees (Fig. 1E and Fig. S5).

Data Accessibility. Dataset S1 includes repository and radiocarbon accession details for all fossil specimens analyzed. The Ch'j'ee's Bluff steppe bison specimen, YG 264.1, is archived in the fossils collections of the Vuntut Gwitchin First Nation Government in Old Crow, Yukon. The giant long-horned bison specimen from the site near Snowmass, DMNH EPV.67609, is archived in the vertebrate paleontology collections of the Denver Museum of Natural History, Colorado. Mitochondrial genome sequences have been deposited in GenBank, with accession numbers KX269109, and KX269112–KX269145. Input BEAST files are available as Dataset S2.

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