

UC Santa Cruz

UC Santa Cruz Previously Published Works

Title

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

Permalink

<https://escholarship.org/uc/item/4ss3r1z3>

Journal

Proceedings of the National Academy of Sciences of the United States of America, 114(13)

ISSN

0027-8424

Authors

Froese, Duane
Stiller, Mathias
Heintzman, Peter D
et al.

Publication Date

2017-03-28

DOI

10.1073/pnas.1620754114

Peer reviewed

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

Duane Froese^{a,1}, Mathias Stiller^{b,c}, Peter D. Heintzman^b, Alberto V. Reyes^a, Grant D. Zazula^d, André E. R. Soares^b, Matthias Meyer^e, Elizabeth Hall^d, Britta J. L. Jensen^{a,f}, Lee J. Arnold^g, Ross D. E. MacPhee^h, and Beth Shapiro^{b,i,1}

^aDepartment of Earth and Atmospheric Sciences, University of Alberta, Edmonton, AB, Canada T6G 2E3; ^bDepartment of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA 95064; ^cGerman Cancer Consortium, German Cancer Research Center, Institute for Translational Skin Cancer Research, D-45141 Essen, Germany; ^dYukon Palaeontology Program, Department of Tourism & Culture, Government of Yukon, Whitehorse, YT, Canada Y1A 2C6; ^eDepartment of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany; ^fRoyal Alberta Museum, Edmonton, AB, Canada T5N 0M6; ^gSchool of Physical Sciences, Environment Institute, and Institute for Photonics and Advanced Sensing, University of Adelaide, Adelaide, SA 5005, Australia; ^hDivision of Vertebrate Zoology, American Museum of Natural History, New York, NY 10024; and ⁱUniversity of California, Santa Cruz Genomics Institute, University of California, Santa Cruz, CA 95064

Edited by Donald K. Grayson, University of Washington, Seattle, WA, and approved February 3, 2017 (received for review December 20, 2016)

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.

Author contributions: D.F. and B.S. designed research; D.F., M.S., P.D.H., A.V.R., G.D.Z., A.E.R.S., M.M., E.H., B.J.L.J., L.J.A., R.D.E.M., and B.S. performed research; G.D.Z. and L.J.A. contributed new reagents/analytic tools; P.D.H., A.V.R., A.E.R.S., and L.J.A. analyzed data; and D.F., P.D.H., A.V.R., G.D.Z., M.M., L.J.A., R.D.E.M., and B.S. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. [KX269109](#), and [KX269112–KX269145](#)).

¹To whom correspondence may be addressed. Email: duane.froese@ualberta.ca or beth.shapiro@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1620754114/-DCSupplemental.

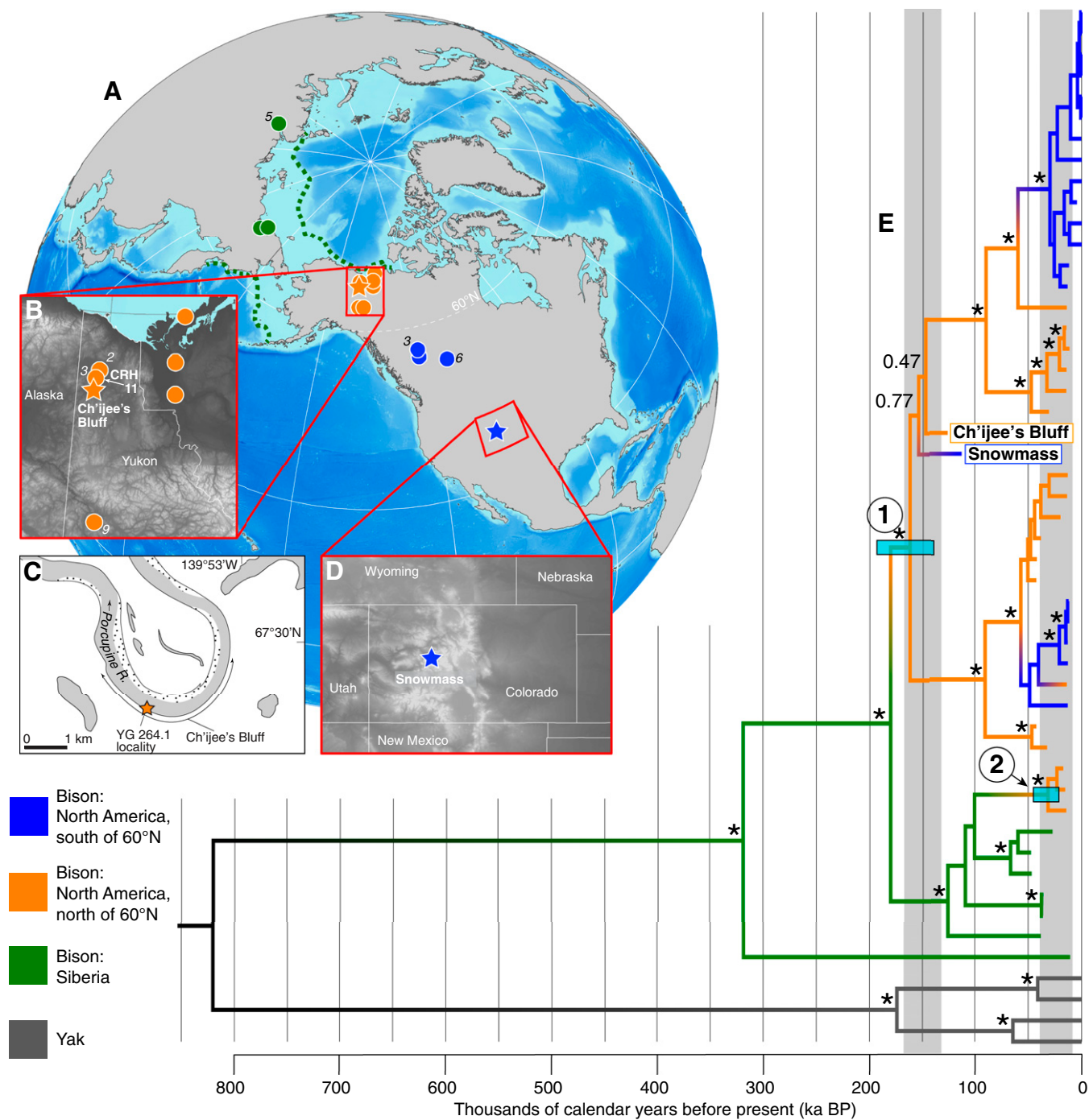


Fig. 1. (A) Localities of the 36 fossil bison. Siberia: green; North America north of 60° N: orange; North America south of 60° N: blue. The number of samples is given in italics if >one sample was recovered. The green dashed lines outline the last glacial maximum Bering Land Bridge extent. Insets showing 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

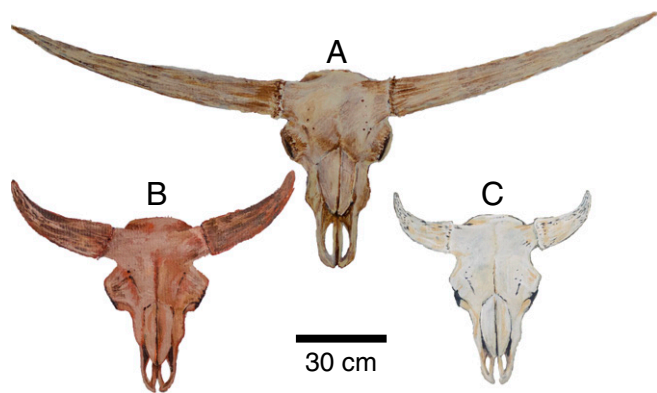


Fig. 2. Reconstructions of bison skulls based on fossils attributed to (A) a giant long-horned bison, *B. latifrons*; (B) a Late Pleistocene steppe bison, *B. priscus*; and (C) a present-day *B. bison*. Giant long-horned bison were significantly larger than present-day bison; adult males may have weighed 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*),

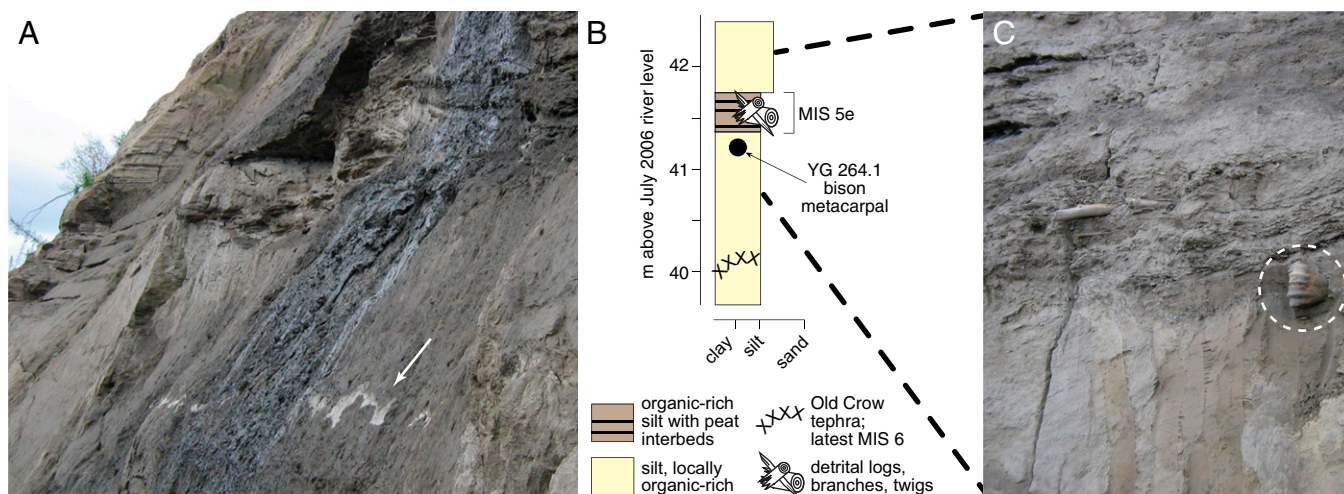


Fig. 3. Features of the Ch'ijee's Bluff locality. (A) the Old Crow tephra (124 ± 10 kyBP; UA1206) highlighted by the white arrow, (B) the stratigraphic setting of the Old Crow tephra, bison metacarpal YG 264.1, and the MIS 5e forest bed, and (C) the in situ metacarpal was found several centimeters beneath 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.

ACKNOWLEDGMENTS. We thank Andrew Fields, Brandon Letts, Dan Chang, Joshua Kapp, and Amanda Hart for technical assistance; Ben J. Novak for the bison skull paintings in Fig. 2; Ian Miller, Joe Sertich, and the Denver Museum of Nature and Science for access to the Snowmass sample; and Harold Frost, Jr. and the late Stephen Charlie for assistance in the field. Access and support to work at the Old Crow sites was facilitated by the Heritage Department of the Vuntut Gwitchin Government. This work used the Vincent J. Coates Genomics Sequencing Laboratory at University of California, Berkeley, supported by NIH S10 Instrumentation Grants S10RR029668 and S10RR027303. Support was provided from Yukon Heritage Branch; Natural Resources Canada Polar Continental Shelf Program (D.F.), Canada Research Chairs program, and Natural Sciences and Engineering Research Council (D.F.); National Science Foundation ARC-09090456 and ARC-1417046, and Gordon and Betty Moore Foundation GBMF3804 (to B.S.); and Australian Research Council DP0878604 and FT130100195 (to L.J.A.).

- Knapp AK, et al. (1999) The keystone role of bison in North American tallgrass prairie. *Bioscience* 49:39–50.
- Guthrie RD (1970) Bison evolution and zoogeography in North America during the Pleistocene. *Q Rev Biol* 45(1):1–15.
- Shapiro B, et al. (2004) Rise and fall of the Beringian steppe bison. *Science* 306(5701):1561–1565.
- Frisen GC (1978) *Prehistoric Hunters of the High Plains* (Academic, New York).
- Freeze CH, et al. (2007) Second chance for the plains bison. *Biol Conserv* 136:175–184.
- MacFadden BJ (1988) Horses, the fossil record, and evolution. *Evolutionary Biology*, eds Hecht MK, Wallace B, Prance GT (Springer, New York), Vol 22, pp 131–158.
- Lister AM, Sher AV (2015) Evolution and dispersal of mammoths across the Northern Hemisphere. *Science* 350(6262):805–809.
- Bell CJ, et al. (2004) The North American Pliocene and Pleistocene sequences. *Cenozoic Mammals of North America*, ed Woodburne MO (Univ of California Press, Berkeley).
- Savage DE (1951) Late Cenozoic vertebrates of the San Francisco Bay region, California. *Publ Depart Geol Sci* 28:1–30.
- Barnosky AD, et al. (2014) Prelude to the Anthropocene: Two new North American land mammal ages (NALMAs). *Anthropol Rev* 1:225–242.
- McDonald JN, Morgan GS (1999) The appearance of bison in North America. *Curr Res Pleist* 16:127–129.
- Péwé TL (1975) *Quaternary Geology of Alaska*, USGS Professional Paper 835 (US Government Print Office, Washington, DC), Vol 835.
- Hamilton TD, Craig JL, Sellman PV (1988) The Fox permafrost tunnel: A Late Quaternary geologic record in central Alaska. *GSA Bull* 100:948–969.
- Preece SJ, Westgate JA, Stemper BA, Pewe TL (1999) Tephrochronology of late Cenozoic loess at Fairbanks, central Alaska. *GSA Bull* 111:71–90.
- Sanders AE, Weems RE, Albright LB, III (2009) Formalization of the Middle Pleistocene 'Ten Mile Hill Beds' in South Carolina with evidence for placement of the Irvingtonian-Rancholabrean boundary. *Papers on Geology, Vertebrate Paleontology, and Biostratigraphy in Honor of Michael O. Woodburne*, ed Albright, III LB (Museum of Northern Arizona, Flagstaff, AZ), Vol 65, pp 363–370.
- Szabo BJ (1985) Uranium-series dating of fossil corals from marine sediments of southeastern United States Atlantic Coastal Plain. *GSA Bull* 96:398–406.
- Hollin JT, Hearty PJ (1990) South Carolina interglacial sites and stage 5 sea levels. *Quat Res* 33:1–17.
- Bell CJ, Repenning CA (1999) Observations on dental variation in *Microtus* from the Cudahy Ash Pit Fauna, Meade County, Kansas and implications for Irvingtonian microtine rodent biochronology. *J Vert Paleontol* 19:757–766.
- Schultz CB, Martin LD (1977) Biostratigraphy of the Neogene-Quaternary boundary in North America. Proceedings of the 2nd Symposium of the Neogene-Quaternary Boundary, Bologna. *G Geol* XLI:285–295.
- Forman SL, Pierson J (2002) Late Pleistocene luminescence chronology of loess deposition in the Missouri and Mississippi river valleys, United States. *Paleo3* 186:25–46.
- Orlando L, et al. (2013) Recalibrating *Equus* evolution using the genome sequence of an early Middle Pleistocene horse. *Nature* 499(7456):74–78.
- Storer JE (2004) A Middle Pleistocene (late Irvingtonian) Mammalian fauna from Thistle Creek, Klondike Goldfields Region of Yukon Territory, Canada. *Paludicola* 4:115–155.
- Westgate JA, et al. (2008) Changing ideas on the identity and stratigraphic significance of the Sheep Creek tephra beds in Alaska and the Yukon Territory, northwestern North America. *Quat Int* 178:183–209.
- Miller IM, et al. (2014) Summary of the Snowmastodon Project Special Volume: A high-elevation, multi-proxy biotic and environmental record of MIS 6–4 from the Ziegler Reservoir fossil site, Snowmass Village, Colorado, USA. *Quat Res* 82:618–634.
- Pinsof JD (1998) The American Falls local fauna: Late Pleistocene (Sangamonian) Vertebrates from Southeastern Idaho. *And Whereas...: Papers on the Vertebrate Paleontology of Idaho Honoring John A. White, Volume 1., Occasional Paper 36*, eds Akersten WA, McDonald HG, Meldrum DJ, Flint MEJ (Idaho Museum of Natural History, Pocatello, ID.) pp 121–145.
- Drummond A, Rodrigo AG (2000) Reconstructing genealogies of serial samples under the assumption of a molecular clock using serial-sample UPGMA. *Mol Biol Evol* 17(12):1807–1815.
- Jopling AV, Irving WN, Beebe BF (1981) Stratigraphic, sedimentological and faunal evidence for the occurrence of pre-Sangamonian artefacts in northern Yukon. *Arctic* 34:3–33.
- Kuzmina S, Froese DG, Jensen BJL, Hall E, Zazula GD (2014) Middle Pleistocene (MIS 7) to Holocene fossil insect assemblages from the Old Crow basin, northern Yukon, Canada. *Quat Int* 341:216–242.
- Preece S, et al. (2011) Old Crow tephra across eastern Beringia: A single cataclysmic eruption at the close of Marine Isotope Stage 6. *Quat Sci Rev* 30:2069–2090.
- Reyes AV, Froese DG, Jensen BJL (2010) Permafrost response to last interglacial warming: Field evidence from non-glaciated Yukon and Alaska. *Quat Sci Rev* 29:3256–3274.
- Matthews JV, Jr, Schweger CE, Janssens JA (1990) The last (Koy-Yukon) interglaciation in the northern Yukon: Evidence from Unit 4 at Ch'j'ee's Bluff, Bluefish Basin. *Geogr Phys Quat* 44:341–362.
- Shapiro B, Hofreiter M (2014) A paleogenomic perspective on evolution and gene function: New insights from ancient DNA. *Science* 343(6169):1236573.
- Douglas KC, et al. (2011) Complete mitochondrial DNA sequence analysis of Bison bison and bison-cattle hybrids: Function and phylogeny. *Mitochondrion* 11(1):166–175.

34. Achilli A, et al. (2008) Mitochondrial genomes of extinct aurochs survive in domestic cattle. *Curr Biol* 18(4):R157–R158.
35. Kirillova IV, et al. (2015) An ancient bison from the mouth of the Rauchua River (Chukotka, Russia). *Quat Res* 84:232–245.
36. Hu A, et al. (2010) Influence of Bering Strait flow and North Atlantic circulation on glacial sea-level changes. *Nat Geosci* 3:118–121.
37. Vaelbroeck C, et al. (2002) Sea-level and deep water temperature changes derived from benthic foraminifera isotopic records. *Quat Sci Rev* 21:295–305.
38. Reyes AV, et al. (2010) A late-Middle Pleistocene (Marine Isotope Stage 6) vegetated surface buried by Old Crow tephra at the Palisades, interior Alaska. *Quat Sci Rev* 29: 801–811.
39. Aitken MJ (1998) *An Introduction to Optical Dating: The Dating of Quaternary Sediments by the Use of Photon-Stimulated Luminescence* (Oxford Univ Press, Oxford).
40. Arnold LJ, Demuro M, Navazo Ruiz M, Benito-Calvo A, Pérez-González A (2013) OSL dating of the Middle Palaeolithic Hotel California site, Sierra de Atapuerca, north-central Spain. *Boreas* 42:285–305.
41. Arnold LJ, Demuro M, Navazo Ruiz M (2012) Empirical insights into multi-grain averaging effects from 'pseudo' single-grain OSL measurements. *Radiat Meas* 47:652–658.
42. Arnold LJ, Roberts RG (2009) Stochastic calculation of multi-grain equivalent dose (De) distributions: Implications for OSL dating of sediment mixtures. *Quat Geochronol* 4:204–230.
43. Galbraith RF, Roberts RG, Laslett GM, Yoshida H, Olley JM (1999) Optical dating of single and multiple grains of quartz from Jinmium rock shelter, northern Australia: Part I, experimental design and statistical models. *Archaeometry* 41:339–364.
44. Dabney J, et al. (2013) Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc Natl Acad Sci USA* 110(39):15758–15763.
45. Rohland N, Siedel H, Hofreiter M (2010) A rapid column-based ancient DNA extraction method for increased sample throughput. *Mol Ecol Resour* 10(4):677–683.
46. Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protoc* 2010(6):pbp.5448.
47. Gansauge MT, Meyer M (2013) Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nat Protoc* 8(4):737–748.
48. Slon V, et al. (2016) Mammalian mitochondrial capture, a tool for rapid screening of DNA preservation in faunal and undiagnostic remains, and its application to Middle Pleistocene specimens from Qesem Cave (Israel). *Quat Int* 398:210–218.
49. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754–1760.
50. Briggs AW, et al. (2009) Targeted retrieval and analysis of five Neandertal mtDNA genomes. *Science* 325(5938):318–321.
51. Meyer M, et al. (2014) A mitochondrial genome sequence of a hominin from Sima de los Huesos. *Nature* 505(7483):403–406.
52. Jónsson H, Ginolhac A, Schubert M, Johnson PL, Orlando L (2013) mapDamage2.0: Fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* 29(13):1682–1684.
53. Lanfear R, Calcott B, Ho SYW, Guindon S (2012) Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol* 29(6):1695–1701.
54. Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol* 29(8):1969–1973.
55. Gill MS, et al. (2013) Improving Bayesian population dynamics inference: A coalescent-based model for multiple loci. *Mol Biol Evol* 30(3):713–724.
56. Shapiro B, et al. (2011) A Bayesian phylogenetic method to estimate unknown sequence ages. *Mol Biol Evol* 28(2):879–887.
57. Guthrie RD (1990) *Frozen Fauna of the Mammoth Steppe* (Univ of Chicago Press, Chicago, IL).
58. Skinner MF, Kaisen OC (1947) The fossil bison of Alaska and preliminary revision of the genus. *Bull Am Mus Nat Hist* 89:123–256.
59. Berger GW (2003) Luminescence chronology of late Pleistocene loess-paleosol and tephra sequences near Fairbanks, Alaska. *Quat Res* 60:70–83.
60. Audclair M, Lamothe M, Lagroix F, Banerjee SK (2007) Luminescence investigation of loess and tephra from Halfway House section, Central Alaska. *Quat Geochronol* 2:34–38.
61. Péwé TL, Berger GW, Westgate JA, Brown PM, Leavitt SW (1997) Eva Interglaciation Forest Bed, unglaciated east-central Alaska: Global warming 125,000 years ago. *GSA Special Publication*.
62. Hamilton TD, Brigham-Grette J (1991) The last interglaciation in Alaska: Stratigraphy and paleoecology of potential sites. *Quat Int* 10-12(C):49–71.
63. Mathews P, Begét J, Mason O, Gelvin-Reymiller C (2003) Late Pliocene to late Pleistocene environments preserved at the Palisades Site, central Yukon River, Alaska. *Quat Res* 60:33–43.
64. McDowell PF, Edwards ME (2001) Evidence of Quaternary climatic variations in a sequence of loess and related deposits at Birch Creek, Alaska: Implications for the stage 5 climatic chronology. *Quat Sci Rev* 20:63–76.
65. Hughes OL, et al. (1981) Upper Pleistocene stratigraphy, paleoecology and archeology of northern Yukon interior, eastern Beringia 1. Bonnet Plume Basin. *Arctic* 34:329–365.
66. Harington CR (2011) Pleistocene vertebrates of the Yukon Territory. *Quat Sci Rev* 30: 2341–2354.
67. Schweger CE (1989) The Old Crow and Bluefish basin, Northern Yukon: development of the Quaternary history. *Late Cenozoic History of the Interior Basins of Alaska and the Yukon*, eds Carter DL, Hamilton TD, Galloway, JP (US Geological Survey, Washington, DC) pp 30–33.
68. Kennedy KE, Froese DG, Zazula GD, Lauriol B (2010) Last Glacial Maximum age for the northwest Laurentide maximum from the Eagle River spillway and delta complex, northern Yukon. *Quat Sci Rev* 29:1288–1300.
69. Zazula GD, Duk-Rodkin A, Schweger CE, Morlan RE (2004) Late Pleistocene chronology of glacial Lake Old Crow and the north-west margin of the Laurentide Ice Sheet. *Quaternary Glaciations - Extent and Chronology, Part II*. (Elsevier, Amsterdam) pp 347–362.
70. Murray AS, Wintle AG (2000) Luminescence dating of quartz using an improved single-aliquot regenerative-dose procedure. *Radiat Meas* 32:57–73.
71. Arnold LJ, et al. (2011) Paper II—Dirt, dates and DNA: OSL and radiocarbon chronologies of perennally frozen sediments in Siberia, and their implications for sedimentary ancient DNA studies. *Boreas* 40:417–445.
72. Jacobs Z, Duller GAT, Wintle AG (2006) Interpretation of single-grain De distributions and calculation of De. *Radiat Meas* 41:264–277.
73. Yoshida H, Roberts RG, Olley JM, Laslett GM, Galbraith RF (2000) Extending the age range of optical dating using single 'supergrains' of quartz. *Radiat Meas* 32:439–446.
74. Wintle AG, Murray AS (2006) A review of quartz optically stimulated luminescence characteristics and their relevance in single-aliquot regeneration dating protocols. *Radiat Meas* 41:369–391.
75. Arnold LJ, Roberts RG (2011) Paper I—Optically stimulated luminescence (OSL) dating of perennally frozen deposits in north-central Siberia: OSL characteristics of quartz grains and methodological considerations regarding their suitability for dating. *Boreas* 40:389–416.
76. Arnold LJ, Roberts RG, Galbraith RF, DeLong SB (2009) A revised burial dose estimation procedure for optical dating of young and modern-age sediments. *Quat Geochronol* 4:306–325.
77. Galbraith RF, Green PF (1990) Estimating the component ages in a finite mixture. *Nucl Tracks Radiat Meas* 17:197–206.
78. Lisiecki LE, Raymo ME (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records. *Paleoceanography* 20:PA1003.
79. Morlan RE (1989) Paleoeological implications of Late Pleistocene and Holocene microtine rodents from the Bluefish Caves, northern Yukon Territory. *Can J Earth Sci* 26:149–156.
80. Harington CR (2003) *Annotated Bibliography of Quaternary Vertebrates of Northern North America—With Radiocarbon Dates* (Univ of Toronto Press, Toronto), p 360.
81. Weinstock J, et al. (2005) Evolution, systematics, and phylogeography of pleistocene horses in the new world: A molecular perspective. *PLoS Biol* 3(8):e241.
82. McDonald HG, Harington CR, de Iulius G (2000) The ground sloth *Megalonyx* from Pleistocene deposits of the Old Crow Basin, Yukon, Canada. *Arctic* 53:213–220.
83. Hoganson J, McDonald HG (2007) First report of Jefferson's ground sloth (*Megalonyx jeffersonii*) in North Dakota: Paleobiogeographical and paleoecological significance. *J Mammal* 88:73–80.
84. Harington CR (1989) Soergelia: An indicator of Holarctic Middle Pleistocene deposits. Second Annual Muskox Symposium (NRC Canada, Ottawa), pp A1–A9.
85. Hughes OL (1972) *Surficial geology of northern Yukon Territory and northwestern District of Mackenzie, Northwest Territories*, Geological Survey of Canada, Paper, (Geological Survey of Canada, Ottawa) Vol 69-36, pp 1–11.
86. Reyes AV, Zazula GD, Kuzmina S, Ager TA, Froese DG (2011) Identification of last interglacial deposits in eastern Beringia: A cautionary note from the Palisades, interior Alaska. *J Quat Sci* 26:345–352.
87. Fulton TL (2012) Setting up an ancient DNA laboratory. *Methods Mol Biol* 840:1–11.
88. Heintzman PD, et al. (2015) Genomic data from extinct North American *Camelops* revise camel evolutionary history. *Mol Biol Evol* 32(9):2433–2440.
89. Pigati JS, et al. (2014) Geologic setting and stratigraphy of the Ziegler Reservoir fossil site, Snowmass Village, Colorado. *Quat Res* 82:477–489.
90. Mahan SA, et al. (2014) A geochronologic framework for the Ziegler Reservoir fossil site, Snowmass Village, Colorado. *Quat Res* 82:490–503.
91. Kircher M, Sawyer S, Meyer M (2012) Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res* 40(1):e3.
92. Maricic T, Whitten M, Pääbo S (2010) Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PLoS One* 5(11):e14004.
93. Renaud G, Stenzel U, Kelso J (2014) IeeHom: Adaptor trimming and merging for Illumina sequencing reads. *Nucleic Acids Res* 42(18):e141.
94. Meyer M, et al. (2012) A high-coverage genome sequence from an archaic Denisovan individual. *Science* 338(6104):222–226.
95. Dabney J, Meyer M, Pääbo S (2013) Ancient DNA damage. *Cold Spring Harb Perspect Biol* 5(7):1–7.
96. Briggs AW, et al. (2007) Patterns of damage in genomic DNA sequences from a Neandertal. *Proc Natl Acad Sci USA* 104(37):14616–14621.
97. Verkaar ELC, Nijman IJ, Beeke M, Hanekamp E, Lenstra JA (2004) Maternal and paternal lineages in cross-breeding bovine species. Has wisent a hybrid origin? *Mol Biol Evol* 21(7):1165–1170.
98. Heintzman PD, et al. (2016) Bison phylogeography constrains dispersal and viability of the Ice Free Corridor in western Canada. *Proc Natl Acad Sci USA* 113(29):8057–8063.
99. Duller GAT (2003) Distinguishing quartz and feldspar in single grain luminescence measurements. *Radiat Meas* 37:161–165.
100. Aitken MJ (1985) *Thermoluminescence Dating* (Academic, London).
101. Adamiec G, Aitken M (1998) Dose-rate conversion factors: update. *Anc TL* 16:37–50.
102. Stokes S, et al. (2003) Alternative chronologies for Late Quaternary (Last Interglacial–Holocene) deep sea sediments via optical dating of silt-sized quartz. *Quat Sci Rev* 22:925–941.
103. Brennan BJ (2003) Beta doses to spherical grains. *Radiat Meas* 37:299–303.
104. Prescott JR, Hutton JT (1994) Cosmic ray contributions to dose rates for luminescence and ESR dating: Large depths and long-term time variations. *Radiat Meas* 23:497–500.
105. Pawley SM, et al. (2008) Age limits on Middle Pleistocene glacial sediments from OSL dating, north Norfolk, UK. *Quat Sci Rev* 27:1363–1377.
106. Duller GAT (2007) Assessing the error on equivalent dose estimates derived from single aliquot regenerative dose measurements. *Anc TL* 25:15–24.