# Supporting Information

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## SI Materials and Methods

### Radiocarbon ($^{14}$C) Methods.

***Arizona Accelerator Mass Spectrometry Laboratory.*** For radiocarbon analyses at the Arizona Accelerator Mass Spectrometry Laboratory (AA), collagen was extracted using an automated flow cell apparatus, following a modified Longin method (1): Fossil subsamples were mechanically cleaned of surface contaminants, ground in a mortar and pestle, demineralized in 0.5M HCl, rinsed with $H_2O$, treated with 0.1 M NaOH to remove humics and base-soluble contaminants, rinsed with 0.1 M HCl and water, solubilized in $10^{-3}$M HCl at 75 °C for 20 h, and freeze dried. The resulting collagen was combusted with CuO at >800 °C, reduced to graphite, and Accelerator Mass Spectrometry (AMS) radiocarbon dated (2).

***Center for Accelerator Mass Spectrometry at the Lawrence Livermore Laboratory.*** Preparation of bone collagen for specimens analyzed at Center for Accelerator Mass Spectrometry (CAMS) was conducted at the University of Alaska, Fairbanks, using a modified Longin (1) method. The surface of samples was cleaned with water and sonicated in a bath for about 1 min and soaked for about 8 h each in ethanol, methanol, and acetone, with a final rinse in water. The bone was soaked in weak HCl at low temperature to separate the collagen fraction until demineralization was complete, then rinsed in water to the neutralize acid. Bone was soaked in 5% KOH solution for ∼8 h and then rinsed with water to neutral. Bone collagen was gelatinized with HCl at pH 3–4, heated at 100 °C for 1–4 h, centrifuged, lyophilized, and then freeze dried. Collagen products were submitted to CAMS, where they were combusted, reduced to graphite, and radiocarbon dated.

***Keck Carbon Cycle AMS facility at the University of California, Irvine.*** For radiocarbon analyses at the Keck Carbon Cycle AMS facility at the University of California, Irvine (UCIAMS), collagen from the fossil subsamples was extracted using another modified version of the Longin method (1) with ultrafiltration (3). Briefly, specimens were manually cleaned with a handheld grinding tool, and samples of ∼150 mg of crushed bone were decalcified for 24–36 h at room temperature in 0.5 M HCl, rinsed with Milli-Q water, and hydrolyzed overnight at 60 °C with 0.01 M HCl. For all but two specimens, the high molecular mass fraction (>30 kDa) of the resulting gelatin solution was freeze dried, and aliquots were used for AMS radiocarbon dating (4) and for stable isotope and elemental (C and N) analyses. Samples YG 43.2 (UCIAMS 75320) and CMN 42552 (UCIAMS 78703) gave very low >30-kDa yields, and the 10- to 30-kDa molecular mass fraction was used instead (see Table S2). For samples where the presence of consolidants was known or suspected, decalcification was preceded by sonication for 30 min each in acetone, methanol, and water at ∼45 °C, and repeated if the initial treatment produced obvious flocculent material or solvent coloration.

***Oxford Radiocarbon Accelerator Unit.*** Surfaces of the samples were carefully cleaned by air abrasion using aluminum oxide powder, before drilling 600–700 mg of bone powder from each using a tungsten carbide drill bit at low speed. The samples were then subjected to sequential solvent washes with acetone (45 °C), methanol (45 °C), and chloroform (room temperature) for a minimum of 1 h for each procedure to remove any remaining consolidant. Sample F:AM:103277 underwent two sequential acetone washes initially due to the presence of contaminants. The samples were then left to air dry thoroughly before undergoing routine collagen extraction procedures involving sequential washes with HCl (0.5M, three to four rinses over ∼18 h), NaOH (0.1 M, 30 min), and HCl (0.5 M, 15 min) at room temperature, with thorough rinses with ultrapure MilliQ water after each stage (5). The crude collagen was then gelatinized in pH 3 solution at 75 °C for 20 h and the resultant solution filtered with a cleaned Ezee-filter (Elkay). The filtrate was transferred to a precleaned Vivaspin 15 30-kDa MWCO ultrafilter (Sartorius) and centrifuged until 0.5–1.0 mL of the >30-kDa fraction remained. This fraction was then removed from the ultrafilter with ultrapure water before being freeze dried. The final collagen products were converted to $CO_2$ and graphitized before AMS dating (6). During the conversion to $CO_2$, the C:N atomic mass ratio and carbon and nitrogen stable isotopic values of the collagen were measured.

A second approach at Oxford Radiocarbon Accelerator Unit (ORAU) was to separate the single amino acid (SAA) hydroxyproline from hydrolyzed bone collagen using mixed-mode preparative chromatography (7, 8). The hydroxyproline (Hyp) fractions collected were evaporated to dryness using a vacuum evaporator and reconstituted in pH 3 water so as to facilitate their addition onto Chromosorb in tin capsules on the day of combustion. ORAU's routine procedures were followed for combustion, graphitization, and AMS dating of the hydroxyproline (9). Hydroxyproline fractions, using the same procedures, were separated from a laboratory background age standard, an Alaskan bison bone considered to be 70,000–60,000 y B.P. (10).

**Contamination by Natural Exogenous Carbon.** As demonstrated in Table 1 and Table S2, some samples were radiocarbon dated several times, with different collagen preparation methods (i.e., collagen ultrafiltration or SAAs). We obtained duplicate $^{14}$C dates, using more stringent pretreatments, when initial $^{14}$C analysis yielded outlying finite dates (Fig. 2), especially those obtained with standard collagen preparation (1). Other specimens suspected to have yielded erroneous dates as a result of exogenous carbon contamination (either natural or from museum conservation) were also reanalyzed, this time using ultrafiltered collagen or SAAs taken from the same specimen.

The effects of natural exogenous carbon contamination can be appreciated by examining $^{14}$C dates on mastodon fossils from the North Slope of Alaska (Ikpikpuk and Kigalik Rivers) (Table S2). Out of the 12 Alaskan North Slope specimens listed in Fig. 2, Table 1, and Table S2—UAMES 12060, UAMES 9705, and UAMES 34125—originally yielded radiocarbon dates younger than 40,000 $^{14}$C years B.P. The initial $^{14}$C dates on the first two specimens, respectively 36,370 ± 790 $^{14}$C years B.P. (AA 48275) and 38,800 ± 1100 $^{14}$C years B.P. (CAMS 53904), were obtained by use of standard collagen pretreatment methods (1) with no ultrafiltration of bone protein components (i.e., "collagen," which makes up 80–90% of all bone proteins). These dates approach the limit of reliable $^{14}$C dating but might well be regarded as acceptable. However, our radiocarbon analyses of ultrafiltered collagen extracted from the same specimens (3, 4) produced $^{14}$C age estimates that are best interpreted as nonfinite and therefore consistent with the rest of our the mastodon dataset. UAMES 12060 yielded an age estimate of 49,800 ± 3300 $^{14}$C years B.P. (UCIAMS 117236) while UAMES 9705 was found to be >51,700 $^{14}$C years B.P. (UCIAMS 117239). In both cases, the $^{14}$C ages on ultrafiltered collagen were >10,000 y older than those obtained on collagen prepared using standard methods. Thus, even in the absence of contamination from museum treatments/consolidants, careful $^{14}$C pretreatment protocols are required to remove carbon contamination from specimens recovered in certain depositional settings.

Radiocarbon dating of the remaining "young" North Slope specimen, UAMES 34125 (Fig. S1 and Table S2), illustrates the value of careful screening of collagen extracts. We initially obtained a seemingly plausible finite $^{14}$C date of 31,780 ± 360 $^{14}$C years B.P. (UCIAMS 117238) for this specimen using ultrafiltered collagen (3, 4). However, the gelatin solution produced during sample preparation was a golden color and the >30-kDa collagen was tan rather than white, suggesting that some exogenous carbon may have remained in the final collagen extract that was analyzed for radiocarbon. A subsequent reanalysis of the specimen (5) with special attention paid to remove the suspected contaminant by the addition of a sequential solvent extraction of the bone powder before collagen ultrafiltration and extraction yielded a date of >50,100 $^{14}$C years B.P. (OxA-29838).

***Contamination by museum preparation and conservation techniques.*** For $^{14}$C analysis of museum specimens, strict attention must be paid to the identification and removal of consolidants, varnishes, and glues because, in our experience, treated specimens yield inconsistent and erroneous radiocarbon dates (9) (Table 1, Table S2, and Figs. S2 and S3). Since studies of megafaunal chronology and extinction have historically relied heavily on museum collections, problematic dates will continue to engender problematic interpretations if precautions are not taken. Not all radiocarbon ages reported in the literature should be accepted at face value, and careful consideration should always be given to the possible influence of contaminants, known or unknown. Caveat investigator!

Our results demonstrate that consolidants and glues routinely used in museum preparation and conservation, even in apparently small quantities, have demonstrable effects on $^{14}$C age determinations (9) (Table 1, Table S2, Fig. 2, and Figs. S2 and S3). This is best revealed in the case of our efforts to date a mastodon cranium (UAMES 7663; Fig. S3) from Livengood, Alaska. Visual inspection of the cranium revealed that surfaces were heavily consolidated with a varnish or glue of unknown origin. Samples from this specimen, independently analyzed at the Keck and Oxford laboratories, produced notably disparate age estimates of 33,090 ± 470 $^{14}$C years B.P. (UCIAMS 88768) and 20,440 ± 130 $^{14}$C years B.P. (OxA-25401), respectively. Even though both laboratories tried to physically remove the consolidant, and used solvent treatment as well as collagen ultrafiltration procedures, their age estimates differed by >10,000 $^{14}$C years and are considerably younger than the majority of specimens in the dataset.

Subsequent analyses of UAMES 7663 using SAA (hydroxyproline) dating (9) yielded estimates of 43,000 ± 2400 $^{14}$C years B.P. (OxA-X-2457-7) and 48,200 ± 2600 $^{14}$C years B.P. (OxA-X-2492-15), which are very close to the effective radiocarbon limit, thus best interpreted as nonfinite. Although certainty is once again not possible, in view of this enormous range of age estimates it is abundantly clear that this specimen is exceedingly problematic from a $^{14}$C dating perspective and for that reason alone should be rejected.


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**Fig. S1.** American mastodon molar, specimen UAMES 34125 from the North Slope of Alaska.


**Fig. S2.** American mastodon molar, specimen YG 26.1 from Gold Run Creek, Yukon. Note the heavy presence of consolidants to repair missing parts of the molar.

**Fig. S3.** American mastodon cranium UAMES 7663 from Livengood, Alaska. Note the shiny coating of unknown varnish or consolidant on the surface of the bone and molars.

## Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)