Supporting Information

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SI Text

SI Materials and Methods

Our understanding of the early occupation of the Americas before fluted point technology became common is hindered by not dating discoveries correctly, the absence of regionally distinctive and diagnostic artifact types (analogous to the later Clovis fluted point) that can be easily recognized, and misinterpreting taphonomic relationships where animal and human intervention may overlap. At the Wally's Beach kill and butchering site, it is incorrect chronology that initially caused the site to be erroneously assigned to the Clovis complex, which resulted in masking the site's early age. The following information describes the methods used to directly date the fossil vertebrates at Wally's Beach and correct the original chronological misinterpretations and potential loss of information about these early sites.

AMS ^{14}C Dating. The Wally's Beach camel, horse and muskox fossil remains were originally buried 1.5–2 m beneath the modern ground surface in aeolian sand and a soil with a calcic horizon developed in these sediments (1, 2). Consequently, the fossil bones and teeth were subjected to pedogenesis, which continuously adds more recent carbon contaminants (e.g., humates) to the bone and to chemical processes, such as root etching that affected the bones' preservation (Fig. S1*A*) and dissolves bioapatite. These combined physical and chemical processes cause significant collagen loss and collagen contamination that hinder accurate radiocarbon dating.

Methods used in this report are continued improvements upon chemical purification methods used previously (3, 4) and were required because of the fossils' varying degrees of preservation and contamination, and the importance of distinguishing Clovis from pre-Clovis archaeological evidence. Multiple chemical fractions were dated from each bone to establish age trends as purification progressed and to contrast these dates with previous ^{14}C measurements.

Data Collection. Taxonomic, anatomical, and stratigraphic information for each specimen and museum and laboratory numbers are compiled in Table S1. Specimens were photographed as originally presented in the museum, during sampling, and at all stages of the chemical processing. Root etching, collagen distribution along cut surfaces (Fig. S1), and physical appearances of collagen and gelatin during progressive purification were photographed to record overall physical and chemical diagenesis (Figs. S2–S4). Mass yields were calculated after decalcification, KOH extraction, and gelatinization (Table S2). Fractions dated included KOH-extracted collagen, gelatin, and the XAD-purified gelatin hydrolyzate (Table 2). All δ^{13}C, δ^{15}N, %N, %C, and C/N values were determined on gelatin.

Laboratory Apparatus. Borosilicate glassware was combusted at 550 °C for 30 min; soda-lime glass pipettes were heated to 520 °C for 30 min. Quartz combustion tubes and CuO wire were combusted at 850 °C for 60 min. HCl solutions were made by diluting constant boiling, 6.0 M HCl made by distillation at 108 °C.

Chemical Purification of Collagen. After removing ≤500 μm of surficial bone, 0.5–1.9 g of dense cortical bone were used for chemical purification. One date on each sample could have been made using 250–400 mg of bone; however, multiple dates required additional mass. Clean, cortical bone was broken into 5- to 10-mm fragments and decalcified with 0.2 M HCl at 4 °C over 2–4 d, with three-to-four changes of HCl daily, and final washes in deionized (DI) water to neutrality.

Decalcified collagen was extracted with 0.1 M KOH at 4 °C over 2–3 d. The KOH-extracted collagen was washed voluminously with DI water to neutrality and finally acidified with 0.06 M HCl before freeze-drying.

Approximately 10–50 mg of dry, KOH-extracted decalcified collagen were gelatinized by heating the sample in 0.06 M HCl at 90 °C until the collagen dissolved, a step that required from 5 to 60 min. The gelatinized collagen (supernatant) was passed through a 0.45-μm Millipore Durapore membrane and the filtrate freeze-dried.

Approximately 10–20 mg of gelatin were hydrolyzed in Teflon-capped 10-mL tubes containing 1 mL of 6 M HCl. Sealed tubes were heated at 110 °C for 24 h, cooled, and the solution passed through a column of styrene-divinyl benzene resin that is identical to trademarked XAD-2 resin. The stock resin was Restek Ultra-Clean Resin (Cat. no. 24230, 20–60 mesh). Before use, the resin was wetted with acetone and fine particles decanted. The acetone-wetted resin was washed voluminously with DI water to remove the acetone and repeatedly in 1 M HCl before final storage in 1 M HCl in glass containers. A resin-HCl slurry containing ∼1 mL of resin was pipetted into a 3-mL, empty, solid-phase extraction (SPE) Resprep (Cat. no. 2601) column containing a 20-μm frit at the bottom. The resin-HCl slurry was added, the column filled with 1 M HCl and the top frit pressed into the SPE cartridge to compress the resin into a ∼1-cm-high resin bed. A 0.45-μm Durapore filter was placed on the Luer end of the SPE column and the resin washed with 20 mL of 1 M HCl, followed by equilibration with 10 mL of 6 M HCl. The gelatin hydrolyzate passed through the resin column and was collected in a 20-mL glass tube for drying by vortex evaporation. Sufficient resin was used to ensure that bead discoloration was less than one-third of the bed height.

Combustion, Graphitization, and AMS ^{14}C Measurement. Approximately 5–8 mg of amino acids as an XAD-purified syrup were transferred to a 6-mm OD × 4-mm ID quartz combustion tube and dried first by lyophilization and finally on a vacuum line pumped through a liquid nitrogen trap. After drying, ∼30–40 mg CuO and 5 mg of Alfa Aesar, 60–80 mesh, 99.99% silver powder were added, the tube evacuated to <10 mtorr through a liquid nitrogen trap, and sealed using a H_2/O_2 torch. Combustion was 1 h at 850 °C, with cooling to 250 °C at 60 °C per hour.

CO_2 from combustion tubes was separated at the University of California, Irvine, purified cryogenically, and converted to graphite (www.ess.uci.edu/researchgrp/ams/files/organic_graphitization_protocol.pdf) and ^{14}C measured at the University of California, Irvine W. M. Keck Carbon Cycle Accelerator Mass Spectrometer Facility (www.ess.uci.edu/researchgrp/ams/files/uci_kccams_ams_settings_to_14c_measurements.pdf).

SI Discussion

Four of six previous dates on Wally's Beach, fossil vertebrates were considered evidence for Clovis-aged human presence, and two other taxa (horse, caribou) predated Clovis (Table 1). Data presented in this report are evidence that all taxa predate the Clovis period and that the camel and horse skeletons have an average age of 11,445 ± 10 ^{14}C y B.P. (13,270–13,310 cal y B.P.), an age 300 calendar y older than established chronology for Clovis (3) (Table S3).

We interpret the difference between previous dates and this report's results as the progressive removal of humates, tannins, and possibly other compounds as chemical purification procedures

have improved over the decades. Slow decalcification of 5- to 10-mm fragments rather than powdered bone increases collagen yields, enables collagen preservation to be quantified, and rootlet or preservative presence to be detected earlier. Extraction with dilute KOH over more than 1 d is more efficient at removing humates than a few minutes of alkali extraction, the result being significantly more humate removal (Figs. S2 *A* and *B* and S3 *A* and *B*) and less humate needing to be removed in the final XAD step. Using cold, very dilute (0.1 M) KOH rather than 0.5 M to 1 M KOH significantly diminishes collagen loss that is common in alkali solutions.

Gelatinization only until dissolution occurs minimizes further degradation of the collagen and separates diagenetically cross-linked collagen from collagen that is soluble in hot, acidic water. Filtering the soluble collagen (gelatin) through 0.45-μm membranes removes inorganic and organic detritus that is too small for visual or physical removal (5) and yields particle-free solutions. Without gelatin extraction and filtration, rootlets, clays, silt, and other detritus remain in alkali-extracted collagen. An example of contaminant-retention by alkali-extracted collagen is Horse A, which was coated with Butvar. The specimen's KOH-collagen age was 13,540 ± 40 ^{14}C y B.P. (UCIAMS-127363), whereas its XAD-purified fraction was 11,440 ± 30 ^{14}C y B.P. (UCIAMS-127351). Petroleum-derived, ^{14}C-free Butvar remained in the alkali-collagen fraction and possibly to a small amount in the gelatin fraction, and caused the ages to be older than the geological age of the horse. The Butvar was finally removed by hydrolysis and subsequent XAD purification of the collagen hydrolyzate.

Gelatinization of KOH-extracted collagen yields gelatin with varying degrees of purity (Fig. S4). Visually, the freeze-dried Wally's Beach gelatins ranged from white (Fig. S4*A*) to light brown (Fig. S4*C*) and with lusters varying from subvitreous and glassine to matte. Except Horse A, which was treated with Butvar, ages on gelatin versus XAD-purified hydrolyzates overlapped at 1 SD for six specimens and at 2 SDs for the remainder (Table 2).

Only dates from XAD-purified collagen hydrolyzates are considered accurate ^{14}C ages because: (*i*) the theoretical purity of XAD treatment is higher than gelatin because hydrolysis separates humates (~fulvic acids) from the collagen, thereby enabling the humates to be adsorbed by the XAD resin; (*ii*) hydrolysis in 110 °C, 6M HCl causes the polymerization, precipitation, and removal-by-filtration of nonprotein constituents (e.g., Butvar); and (*iii*) the higher chemical purity of XAD fractions is shown by gelatin dates varying over 240 radiocarbon y versus 60 radiocarbon y for XAD-based ages.

The quantitative removal of humates from fossil collagen by using strong acid hydrolysis and XAD resins was first demonstrated by Stafford, et al. (6), who discovered that 6 M HCl hydrolysis of collagen at 110 °C for 24-h separated amino acids from humates bound to collagen by Maillard reactions. Strong acid hydrolysis reverses the Maillard reaction and yields free amino acids and the nonamino acid reaction product (7). Once collagen is hydrolyzed into its individual amino acids and humates are converted to nonpolar compounds, the humates are removed by adsorbing them onto hydrophobic resins, such as XAD-2. Subsequently, the need to separate humates from collagen by acid hydrolysis rather than by using alkali extractions and size filtration has been rediscovered (8). The only two methods using acid hydrolysis to separate collagen from covalently bound humates are the XAD (6) and SAA (single amino acid) (9, 10) techniques.

In the XAD technique, the hydrophobic resin adsorbs the fulvic acids and removes them from the free amino acids. The SAA protocol uses cation-exchange resins to separate fulvic acids and individual amino acids based on their retention times during chromatography (9, 10). One advantage of the XAD method over SAA dating is that XAD fractions enable dating of all collagen, whereas during SAA dating, the target amino acid, hydroxyproline, comprises only 9 M percent of collagen (9), thereby decreasing by 90% the amount of carbon dateable from a fossil. Alternative methods, such as ultrafiltration (UF) (11), use large molecular weight fractions (e.g., >30 kDa) that are believed to be more collagen-like than smaller fractions; however, all of these molecular weight fractions are derived from total gelatin and all are subject to humate contamination because the gelatin fractions have not been acid hydrolyzed. Experimental data comparing SAA and UF dating techniques demonstrate that UF fractions are often 1,000–5,000 radiocarbon y (10) and up to 15,000 y younger (12) than dates on hydroxyproline. Consequently, only dates using XAD or SAA techniques are suitable for paleoenvironmental, paleontological, and archaeological studies where accuracy and precision at ±20–30 radiocarbon y are required.

Data from this and previous studies (3–5, 9) are strong evidence that it is very difficult to assess site chronologies based on literature surveys because of high variability in sample chemistry. Dates based on the XAD and SAA techniques are presently considered the most accurate because only those techniques quantitatively remove humates by acid hydrolysis. Dates on gelatin or its derived molecular weight fractions are difficult to assess because unless gelatinized collagen is hydrolyzed, fulvic acids are theoretically still present. Ages from gelatin and XAD/SAA techniques are sometimes statistically similar; however, this could be caused by: (*i*) the collagen containing no humates; (*ii*) the ^{14}C/^{12}C value of the humates and collagen being similar, thereby making humate detection impossible; or (*iii*) the mass of humates was low enough that, regardless of the humates' ^{14}C/^{12}C value, this exogenous carbon was not detectable at ±50 y and larger dating precisions. Ages on alkali-extracted collagen should be rejected entirely because this chemical fraction contains significant amounts of particulate carbon and humates. The occurrence of alkali-treated collagen and gelatin ages that are occasionally similar to XAD or SAA ones should be considered fortuitous and not justification for using these fractions for final interpretations.

SI Conclusions

The difference in ages between previous dates and those cited in this report is predominantly a result of the retention of humates and possibly other compounds in the collagen. Humates can be removed effectively only if alkali extractions are done for one or more days instead of minutes or hours, if gelatin solutions are filtered instead of being centrifuged, and only when HCL hydrolysis is used to quantitatively separate humates bound to collagen by Maillard reactions and remove these small molecular weight humates (fulvic acids) by using XAD resins. Although 200–400 ^{14}C year differences in ^{14}C results may have been acceptable in previous decades, our results demonstrate that significant scientific discoveries can go unrecognized unless AMS ^{14}C dating accuracy is significantly improved.

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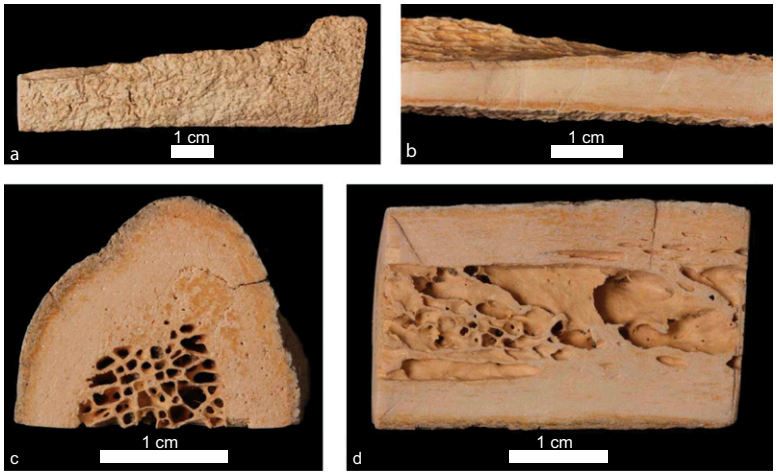

Fig. S1. Bone diagenesis and collagen distribution. (*A*) Exterior surface of *Equus* tibia Cat. no. 2988.1 with extensive root etching. (*B*) Longitudinal section along *Equus* tibia Cat. no. 2988.1 showing collagen better preserved at bone-sediment boundaries (exterior surface and medullary zone) of bone compared with the interior. (*C*) Transverse section of *Bootherium* rib Cat. no. 3293.1 showing heterogeneous collagen distribution, with higher amounts along the exterior surface. (*D*) Longitudinal section of *Bootherium* Cat. no. 3293.1 showing different zones of collagen preservation.

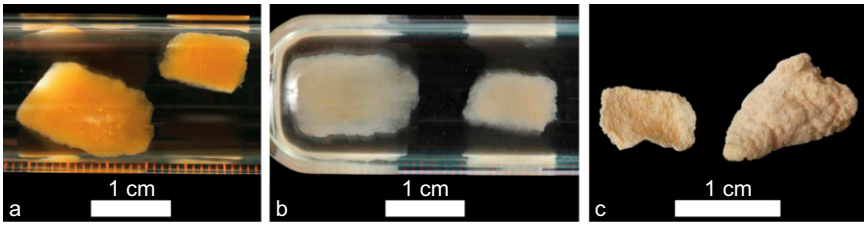

Fig. S2. Decalcification and KOH treatment of *Camelops hesternus* rib, Cat no. 3610.1. (*A*) Decalcified collagen from *Camelops* rib; brown color is humate contamination remaining after dilute HCl decalcification. (*B*) Decalcified collagen from same specimen after extraction in dilute KOH that has removed humates and yielded white collagen. (*C*) Freeze-dried, KOH-extracted, decalcified collagen from *B*.

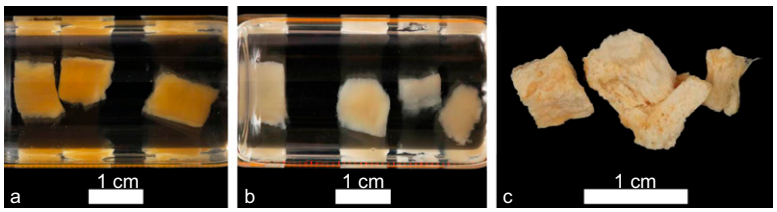

Fig. S3. Decalcification and KOH treatment of *Bootherium bombifrons* rib, Cat. no. 3293.1. (*A*) Decalcified collagen from *Bootherium* rib; brown color is humate contamination remaining after dilute HCl decalcification. (*B*) Decalcified collagen from sample specimen after extraction in dilute KOH that has removed humates and yielded white collagen. (*C*) Freeze-dried, KOH-extracted, decalcified collagen from *B*.

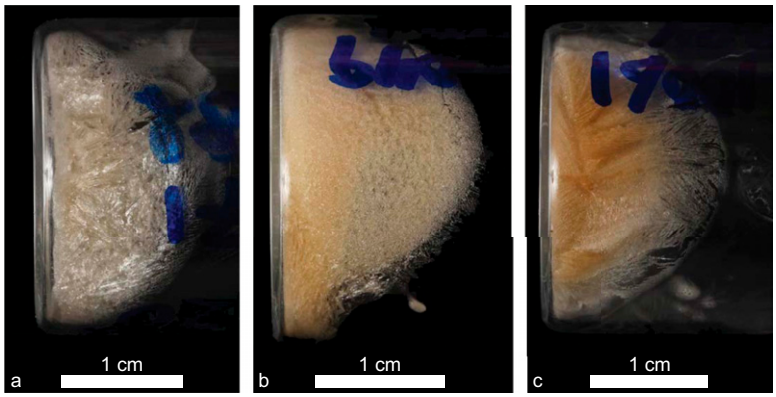

Fig. S4. Variations in gelatin preservation and purity. (*A*) *Equus* radius Cat. no. 944 gelatin with white, glassine, opalescent luster, and radiating blades. (*B*) *Equus* rib Cat. no. 315 gelatin with very light yellowish brown, fine granular. (*C*) *Equus* humerus Cat. no. 77.1 gelatin with yellowish brown, radiating blades.

Table S1. List of taxonomic, museum, archaeological, and stratigraphic data for specimens ^{14}C dated by AMS

SR no.	Field no.	Lab no.	Excavation unit	Stratum	Element	Taxon	Comments
SR-8171	Camel	Cat. no. 3610.1	87.71N 83.41E	Aeolian sand	Rib, partial proximal	*Camelops hesternus*	Previous date: 11,070 ± 80 ^{14}C y B.P. (TO-13513)
SR-8218	Horse 1	Cat. no. 3594		Aeolian sand	Rib, left	*Equus conversidens*	
SR-8219	Horse 2	Cat. no. 2988.1	5N, 106 E	Aeolian sand	Tibia, left	*Equus conversidens*	
SR-8220	Horse 3	Cat. no. 944	42.75N, 118.62E	Aeolian sand	Radius, left	*Equus conversidens*	
SR-8221	Horse A	Cat. no. 315	271N, 29 E	Aeolian sand	Rib, right	*Equus conversidens*	Coated with Butvar 98 (two coats) dissolved in ethanol. KOH fraction contaminated by Butvar
SR-8222	Horse B	Cat. no. 164		Aeolian sand	Rib, right	*Equus conversidens*	Rib No 15
SR-8225	Horse B	Cat. no. 159	7 cm N, 22 cm W	Aeolian sand	Ulna left	*Equus conversidens*	
SR-8223	Horse C	Cat. no. 683	115N, 34W; 78 cm N, 73 cm W	Aeolian sand	Femur, left	*Equus conversidens*	
SR-8224	Horse D	Cat. no. 77.1	509N, 49W	Aeolian sand	Humerus, left	*Equus conversidens*	
SR-8226	Muskox	Cat. no. 3293.1	0N, 0W	Aeolian sand	Rib, left	*Bootherium bombifrons*	Previously dated as 10,980 ± 80 ^{14}C y B.P. (TO-8972)

Table S2. Collagen yields for dated specimens

Number	Specimen	Catalog no.	KOH-collagen yield (%)
SR-8171	Camel	Cat. no. 3610.1	4.8
SR-8218	Horse 1	Cat. no. 3594	4.6
SR-8219	Horse 2	Cat. no. 2988.1	3.7
SR-8220	Horse 3	Cat. no. 944	1.5
SR-8221	Horse A	Cat. no. 315	5.5
SR-8222	Horse B	Cat. no. 164	3.1
SR-8225	Horse B	Cat. no. 159	5.2
SR-8223	Horse C	Cat. no. 683	3.4
SR-8224	Horse D	Cat. no. 77.1	7.2
SR-8226	Muskox	Cat. no. 3293.1	6.5

The yields are the mass of freeze-dried, alkali-extracted decalcified collagen in respect to the initial mass of bone decalcified.

Table S3. Radiocarbon and calibrated age averages for Wally's Beach

Animal	^{14}C y B.P. (1 σ)	Cal y B.P. (1 σ)
Camelops (n = 2)	11,440 ± 25	13,255–13,315
Equus (n = 8)	11,450 ± 10	13,270–13,310
Equus and *Camelops* (n = 10)	11,445 ± 10	13,270–13,310
Bootherium (n = 1)	11,320 ± 30	13,120–13,200