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1 **Increasing accuracy for the radiocarbon dating of sites occupied by the first Americans**

2

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15

16

17 **Abstract**

18 Genetic analysis of Paleoamerican human remains suggests that people first entered the Americas
19 sometime between ~14,000 and ~16,000 years ago. Evaluation of these data requires unequivocal
20 archaeological evidence in a solid geological context that is well dated. Accurately determining the
21 age of late Pleistocene sites is thus crucial in explaining when and how humans colonized the
22 Americas. There are, however, significant challenges to dating reliability, especially when vertebrate
23 fossils (i.e. bones, teeth and ivory) are often the only datable materials preserved at sites.

24 We re-dated vertebrate fossils associated with the North American butchering sites of Wally's Beach
25 (Canada), La Prele [also known as Fetterman (Wyoming)], Lindsay (Montana), and Dent (Colorado).

26 Our work illustrates the crucial importance of sample chemical preparation in completely removing
27 contaminants derived from sediments or museum curation. Specifically, our work demonstrates that

28 chromatographic methods, e.g. preparative High Performance Liquid Chromatography and column
29 chromatography using XAD resins, are currently the only efficient methods for removing
30 environmental and museum-derived contaminants. These advanced techniques yield demonstrably
31 more accurate AMS ¹⁴C measurements that refine the ages of these four sites and thereby
32 contribute to advancing our understanding of human dispersals across North America during the late
33 Pleistocene.

34

35

36 **Keywords**

37 AMS Radiocarbon dating, preparative HPLC, hydroxyproline, XAD resin, Clovis complex, Pre-Clovis

38

39

40 **1. Introduction**

41 The arrival time of the first humans into North America is an extremely debated topic within the
42 scientific community (Goebel et al., 2008; Meltzer, 2009, 2015). For most of the 20th century, it was
43 widely believed that near the end of the last Ice Age, when sea levels were lower, prehistoric hunters
44 from eastern Siberia followed prey animals across the Beringia Land Bridge into modern-day Alaska.
45 When the ice sheets receded and exposed a path southward, the colonizers moved across the vast
46 unpopulated continent, established a permanent human presence and, while doing so, possibly
47 caused the extinction of 30+ genera of large mammals (Grayson and Meltzer, 2003; Haynes, 2013;
48 Martin, 1958; Martin, 1973). These presumed earliest settlers were termed “Clovis”, a name derived
49 from the town of Clovis, New Mexico, where their distinctive tools, dating to ca. 13,000 Cal BP, were
50 first recognized at the site of Blackwater Draw (Waters and Stafford, 2007). The ensuing discovery of
51 Clovis stone and osseous tools across North America reinforced the idea that Clovis people were the
52 first Americans (Meltzer, 2009; Waters and Stafford, 2007). The discovery of new archaeological
53 sites and the re-evaluation of old collections, however, suggest that humans reached the Americas
54 several millennia before 13,000 Cal BP—the earliest time range for the Clovis complex (Amick,

55 2017; Bourgeon et al., 2017; Halligan et al., 2016; Waters and Stafford, 2007, 2014). Radiocarbon
56 dates on organic matter from the archaeological site of Monte Verde, Chile, for example, point to a
57 human occupation aged at around 12,300 BP or 14,200 Cal BP (MV-II).

58 To build robust chronologies for the peopling of the Americas, accurate radiocarbon dating is
59 required. For radiocarbon results to be accurate, however, samples must be free of contamination. In
60 this paper, we focus on the dating of collagen from vertebrate fossils that are commonly
61 contaminated with humates accumulated during burial and/or preservatives added by museum
62 curation processes. Humates were identified as primary contaminants in the early 1950s (Münnich,
63 1957), while post-excavation conservation was addressed somewhat later (Bronk Ramsey, 2008).

64 Inaccurate radiocarbon dates are rarely caused by problems associated with the measurement, but
65 are predominately the result of inadequate sample pretreatment. Over the last 35 years, numerous
66 methods have been used to chemically purify bone, teeth, and ivory for ^{14}C dating by accelerator
67 mass spectrometry (AMS). At the Oxford Radiocarbon Accelerator Unit (ORAU), the most commonly
68 used pre-treatment for bone samples is demineralization in HCl, followed by an alkali wash, and
69 gelatinization followed by ultrafiltration (Brock et al., 2010a; Higham et al., 2006). In some cases, this
70 method is unable to completely isolate uncontaminated collagen because of cross-linking and
71 degradation of the collagen molecule. This is particularly true when samples are heavily
72 contaminated with humic substances, conservation materials, or both. To resolve this problem, a few
73 laboratories have used an entirely different approach—chromatography—to isolate the compound of
74 interest. Following the work of Abelson and Hoering, who used ion exchange chromatography to
75 isolate individual amino acids for stable isotope analysis (Abelson and Hoering, 1961), Ho *et al.* used
76 cation exchange chromatography to purify petroleum-contaminated bones (Ho et al., 1969). They
77 were followed by groups isolating a specific amino acid, hydroxyproline (HYP), for direct radiocarbon
78 dating (Benders, 2010; Gillespie et al., 1984; Marom et al., 2013; Marom et al., 2012; Stafford et al.,
79 1982; Stafford et al., 1991). Reverse phase chromatography is another chromatographic technique
80 that separates humates from collagen hydrolyzates by using XAD resins (Stafford et al., 1988;
81 Stafford et al., 1987). There are different types of XAD resins that are commercially available and are

82 described in (Stafford et al., 1988). They can be used to isolate weakly or non-ionized aliphatic and
83 aromatic molecules from aqueous solutions. They are used, for example, to extract dilute organic
84 chemicals from environmental and physiological fluids, to concentrate humates from fresh and
85 marine waters, and in liquid chromatography, to separate weak polar compounds from aqueous
86 solutions (Stafford et al., 1988). The first application of this approach for ^{14}C dating archaeological
87 bones was by Stafford *et al.* in 1982 and 1988 (Stafford et al., 1988; Stafford et al., 1982). They
88 developed this procedure to remove humates from gelatin hydrolyzates for either subsequent
89 isolation of hydroxyproline and proline or in the dating the XAD-purified hydrolyzate directly (Stafford
90 et al., 1988; Stafford et al., 1982). A flow chart showing the different fractions that can be isolated
91 using this method is reported in Figure 1. Subsequently, the method has been applied to numerous
92 archaeological and paleontological dating projects, including samples from North and South America
93 (Waters and Stafford, 2007; Waters et al., 2015).

94 At the ORAU, attention has focused on dating the amino acid hydroxyproline, which is obtained after
95 the hydrolysis of collagen and separation by preparative High Performance Liquid Chromatography
96 (prep-HPLC) (Devièse et al., 2018; Gillespie et al., 1986; Gillespie et al., 1984; Marom et al., 2012;
97 Nalawade-Chavan et al., 2014). A flow chart showing the different steps of the procedure is reported
98 in Figure 2. The efficiency of the method in removing contaminants from heavily contaminated
99 samples and its ability to provide very accurate ^{14}C measurements has been demonstrated in
100 several recent notable cases involving Paleolithic sites in France (Bourrillon et al., 2017), Croatia
101 (Devièse et al., 2017), Russia (Marom et al., 2012; Nalawade-Chavan et al., 2014; Reynolds et al.,
102 2017; Sikora et al., 2017) and the Americas (Becerra-Valdivia et al., 2018).

103 Accurate AMS ^{14}C dates on fossil bone are crucial to testing archaeological and paleontological
104 hypotheses. In this paper, we evaluate the accuracy of two important chemical purification methods:
105 isolating HYP by prep-HPLC, and using XAD resins to purify collagen hydrolysates. To do this, we
106 chose four North American archaeological sites that had already been dated by XAD resin methods
107 and we re-dated the same bone specimens by extracting hydroxyproline. We also compare the
108 results against other ^{14}C determinations obtained using different pretreatment methods.

109

110

111 **2. Materials**

112 There are multiple Clovis-aged and older archaeological sites in North America where human
113 presence is established by lithic assemblages, taphonomy, cut marks or combinations of these. For
114 our experiment, seven vertebrate fossils associated with the North American butchering sites of
115 Wally's Beach (Canada), La Prele [also known as Fetterman (Wyoming)], Lindsay (Montana) and
116 Dent (Colorado) were selected (Table 1).

117 Three bone samples included in this study are from Wally's Beach (Table 1). This site is located at
118 St. Mary's Reservoir, Alberta (Canada), and represents the only known late Pleistocene kill and
119 butchery site at the southern margin of the ice-free corridor (Kooyman et al., 2006; Kooyman et al.,
120 2012; Waters et al., 2015). The animal assemblage includes extinct megafauna (camel and horse),
121 extinct muskox (*Bootherium bombifrons*), caribou and bison. The animal remains are being exposed
122 by eolian deflation, but were originally buried by 1.5 to 2.0 m of eolian silt and sand that overlies
123 Wisconsinan glacio-fluvial sediments. At the site, seven horses and one camel were killed and
124 butchered by humans based on cut marks on bones and the partial scattering and dismemberment
125 of the carcasses. Each of the carcasses was horizontally separated from one another by 25-100 m
126 over a distance of 500 m and were found with non-diagnostic lithic artifacts (Waters et al., 2015).

127 Two samples are from Dent, Colorado (USA). This site was originally discovered in 1932 when flood
128 runoff uncovered a mammoth bone near Milliken, Colorado (Brunswig, 2007). The initial excavation
129 in 1932 revealed fluted Clovis projectile points among the bones (Brunswig, 2007). Subsequent
130 excavations revealed the presence of 15 individual mammoths (Saunders, 1999) within a bone
131 stratum 1.5 m thick. Brunswig considers the site to represent humans killing a mammoth herd based
132 on the number and position of projectile points recovered among the bones. Both samples are from
133 mammoth (*Mammuthus columbi*) elements and contain humic acid contamination. One of the two
134 samples was also preserved with an unknown adhesive, possibly Gelva (Table 1).

135 Another mammoth sample selected for this study is from the La Prele site, Wyoming, USA (formerly
136 called the Fetterman site). Excavations at the site in 1986 produced a single sub-adult mammoth
137 (*Mammuthus columbi*), the fragmental remains of a bison (*Bison* sp.) and assorted lithic artifacts
138 (Byers, 2002). The mammoth was found 27 cm below the surface of soil 4, which was colluvium and
139 described as a “massive clayey sand” (Byers, 2002). Excavations during the last few years have also
140 produced artifacts of the Clovis complex (Byers, 2002; Mackie et al., 2017). The bones were deemed
141 physically unstable and were stabilized and reconstructed using adhesives, including Glyptol and
142 Paraloid B-72. A neural spine unquestionably from the mammoth was selected for dating (Table 1).
143 The final specimen was from a mammoth (*Mammuthus columbi*) that was excavated in 1967 from
144 late Pleistocene loess near Lindsay, Montana, USA (Davis and Wilson, 1985). At this site, loess
145 began to accumulate at the end of the Pleistocene and was derived from a nearby glacial lake bed
146 that was a few kilometers from the Wisconsin maximum ice margin (Davis and Wilson, 1985). The
147 Lindsay mammoth was interpreted as a cultural site based on the taphonomic patterns of
148 disarticulation and spiral fracturing (Davis and Wilson, 1985; Hill and Davis, 1998; Hill and Davis,
149 2014; Waters and Stafford, 2014). The absence of lithic artifacts was used to question the site’s
150 human presence (Grayson and Meltzer, 2015). However, based on taphonomic analyses by
151 Krasinski, the bones showed evidence of butchering with stone tools. Cut marks throughout the
152 skeleton suggests disarticulation and meat stripping (Krasinski, 2010). Eight sandstone blocks
153 (boulders) found underneath several bones further support the hypothesis of a butchery site (Davis
154 and Wilson, 1985; Krasinski, 2010; Waters and Stafford, 2014). The sandstone blocks are believed
155 to be manuports used to crack open the bones for marrow extraction.
156 The final fossil included in this study is a rib from a gray whale (*Eschrichtius robustus*), a chemically
157 and physically well-preserved bone used as a background reference sample (Table 1). It was
158 excavated from a site 20 km south of the Beaufort Sea coast in Alaska (USA). It had been preserved
159 in permafrost, dates >70,000 BP and is therefore significantly beyond the 55 ka limit of the
160 radiocarbon dating method (Stafford et al., 1987).

161

162 **Table 1:** Description of North American fossil bones dated using the single amino acid method at the
 163 ORAU. P, ORAU accession number; SR, Stafford Research lab number. Sample SR-8221 had to be
 164 resampled for additional radiocarbon measurements and was given a different P number.

ORAU P Number	SR Number	Site	Taxon	Contaminant
P39336	SR-5156	Beaufort Sea, Alaska, USA	<i>Eschrichtius robustus</i>	None identified
P39331	SR-8171	Wally's Beach, Alberta, Canada	<i>Camelops hesternus</i>	Humic acids
P39332	SR-8226	Wally's Beach, Alberta, Canada	<i>Bootherium bombifrons</i>	Humic acids
P39333 P45701	SR-8221	Wally's Beach, Alberta, Canada	<i>Equus conversidens</i>	Butvar B-98
P39334	SR-7616	Dent, Colorado, USA	<i>Mammuthus columbi</i>	Humic acids
P39335	SR-6606	Dent, Colorado, USA	<i>Mammuthus columbi</i>	Unknown adhesive
P39337	SR-7356	La Prele, Wyoming, USA	<i>Mammuthus columbi</i>	Paraloid B-72 & Glyptol
P39338	SR-8253	Lindsay, Montana, USA	<i>Mammuthus columbi</i>	Unknown adhesive

165

166

167 **3. Methods**

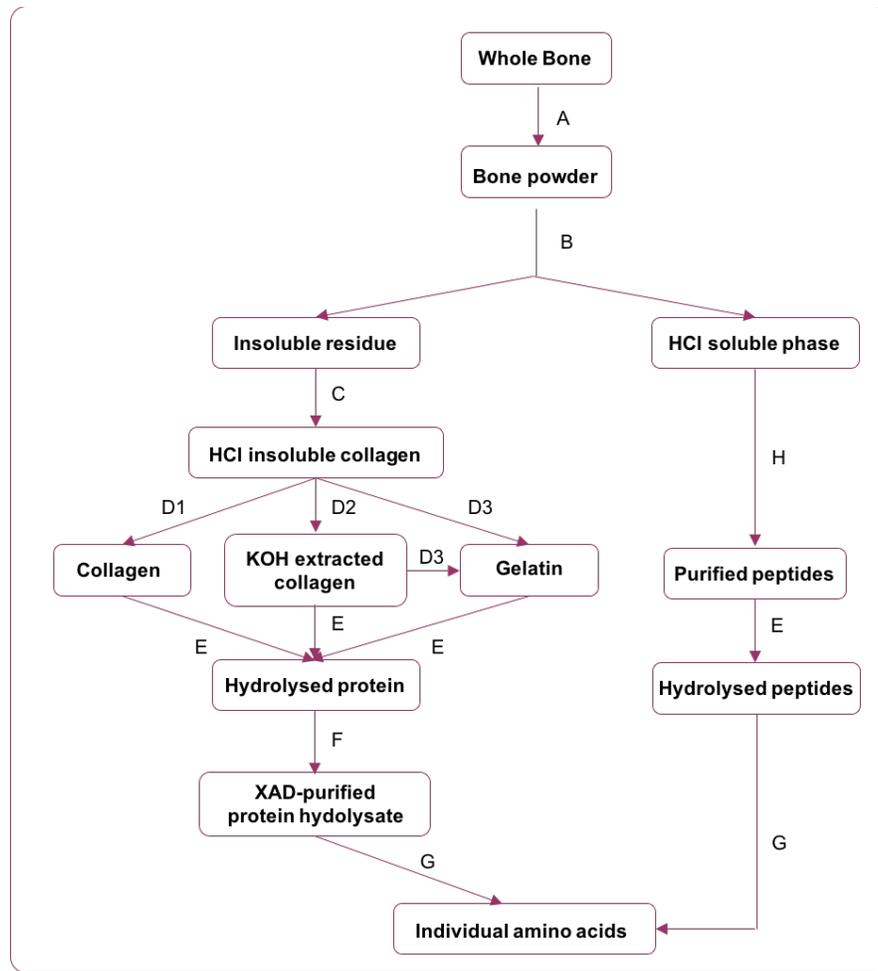
168 **3.1 Percent Nitrogen (%N)**

169 Before any chemical treatment was started, we tested collagen content by removing 3 to 5 mg of
 170 bone powder with an electrical drill with a tungsten carbide drill bit and measured the %C, %N and
 171 atomic C/N ratio of the bone powder. The method used an automated carbon and nitrogen elemental
 172 analyzer (Carlo Erba EA1108) coupled with a continuous-flow isotope monitoring mass spectrometer
 173 (Europa Geo 20/20).

174

175 **3.2 XAD procedure for radiocarbon dating**

176 All samples dated following XAD purification have been prepared using the procedure described in
 177 (Waters et al., 2015). Figure 1 shows a flow chart with the different steps used by Tom Stafford, and
 178 the different fractions that can be separated for AMS dating.



179

180 **Figure 1:** Flow diagram showing pretreatment methods used by Tom Stafford and discussed in this
 181 article. This diagram is based on (Stafford et al., 1987; Waters et al., 2015). **A:** Physical cleaning and
 182 grinding ; **B:** Decalcification with 0.6 N HCl; **C:** Water washes; **D1:** Lyophilisation; **D2:** KOH
 183 extraction; **D3:** Gelatinisation; **E:** Hydrolysis (6 N HCl at 110°C for 24 hr.); **F:** Purification on XAD
 184 resin; **G:** HPLC separation; **H:** Filtration and Reverse Phase Chromatography.

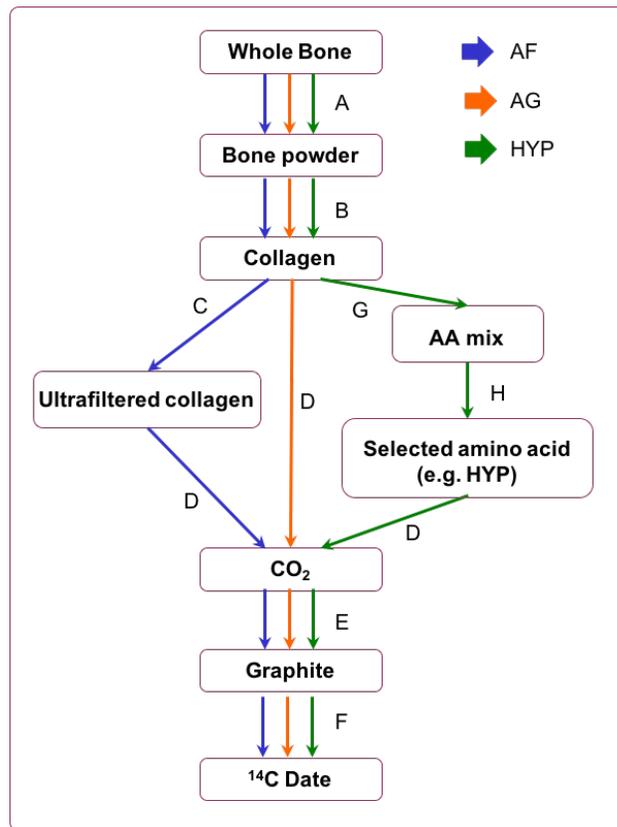
185

186 3.3 Radiocarbon dating of bones at the ORAU

187 Samples were first pre-treated following the routine procedure at the ORAU comprising
 188 decalcification in acid, a base wash, re-acidification, gelatinisation and ultrafiltration (coded 'AF '), as
 189 described in (Brock et al., 2010a) and illustrated on Fig. 2. Samples that had been preserved with
 190 glues or those contaminated by humics were washed with organic solvents (acetone, methanol and

191 chloroform) prior to AF treatment (coded 'AF*'). Samples were also re-dated using the single amino
 192 acid radiocarbon dating method optimised at ORAU (coded 'HYP'). Freeze-dried collagen samples
 193 (40-50 mg) were hydrolysed using 6M hydrochloric acid and hydroxyproline separated on a Varian
 194 ProStar HPLC system following the procedure detailed in (Devièse et al., 2018) and illustrated on
 195 Fig. 2.. Collagen or hydroxyproline samples were then combusted, graphitised and dated by AMS
 196 following the procedure as described in (Brock et al., 2010a).

197



198

199 **Figure 2:** Flow diagram showing pretreatment methods used at the ORAU and discussed in this
 200 article. **A:** Physical cleaning and grinding; **B:** Acid – Base – Acid treatment (ABA); **C:** Ultrafiltration;
 201 **D:** Combustion; **E:** Graphitization; **F:** AMS measurement; **G:** hydrolysis; **H:** Prep-HPLC. Technical
 202 details for each method can be found in (Brock et al., 2010a) and (Devièse et al., 2018)

203

204

205 **4. Results**

206 **4.1 Percent Nitrogen**

207 Percent total nitrogen (%N) of whole bone, used as an indicator of collagen preservation, was
208 measured for each fossil bone. All values were > 0.75% (Table 2), the threshold for accepting bones
209 for AMS ¹⁴C dating at ORAU (Brock et al., 2010b). Importantly, all samples had significantly different
210 %N values. This can be attributed to variable collagen preservation in the bone, the presence of
211 nitrogen containing contaminants, or both. Sample P39336, selected as a background reference
212 sample in this study, was already known to have good collagen preservation. Unsurprisingly, its %N
213 is higher than all the other samples and is in the range of values expected for modern bone (4.0 <
214 %N < 4.5).

215

216 **Table 2:** Total %N data for the 8 whole bone samples. To be acceptable, bone samples must have
217 %N values >0.75% at the ORAU. For this study, the 8 samples passed the %N test.

Oxford P Number	Sample Number	Site	Taxon	%N
P39336	SR-5156	Beaufort Sea, Alaska, USA	<i>Eschrichtius robustus</i>	4.08
P39331	SR-8171	Wally's Beach, Alberta, Canada	<i>Camelops hesternus</i>	0.94
P39332	SR-8226	Wally's Beach, Alberta, Canada	<i>Bootherium bombifrons</i>	2.09
P39333	SR-8221	Wally's Beach, Alberta, Canada	<i>Equus conversidens</i>	1.17
P39334	SR-7616	Dent, Colorado, USA	<i>Mammuthus columbi</i>	1.57
P39335	SR-6606	Dent, Colorado, USA	<i>Mammuthus columbi</i>	0.76
P39337	SR-7356	La Prele, Wyoming, USA	<i>Mammuthus columbi</i>	0.87
P39338	SR-8253	Lindsay, Montana, USA	<i>Mammuthus columbi</i>	0.92

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223 **4.2 Comparison of dates obtained on collagen (AF/AF*) and on Hydroxyproline (HYP)**

224 Collagen was extracted from the 8 bones included in this study in order to obtain a radiocarbon date
225 on bulk collagen after ultrafiltration and another one on hydroxyproline isolated by preparative HPLC.
226 The new chronometric data are reported reported in Table 3. The Beaufort Sea bone, used as a
227 background standard in this study, produced dates beyond the radiocarbon limit using both
228 protocols, as expected. For the other seven samples, Chi-squared tests were run on paired AF/AF*
229 and HYP dates using their modern carbon fractions (Table 4). In three of the cases, the dates
230 obtained on ultrafiltered collagen and hydroxyproline are not statistically distinguishable, indicating
231 either that all the contaminant had been removed by the AF/AF* treatment, or that there is no
232 significant contamination present in the bones. In the other four cases, the paired dates failed the
233 Chi-squared test. For sample SR-8221, the date obtained on collagen is slightly older than the date
234 obtained on hydroxyproline. It is important to note here that this specimen had to be resampled for
235 the AF treatment while, for the other 7 specimens, the two treatments were performed on the exact
236 same sample. For the 3 other pairs that failed the Chi-squared test, the dates obtained on
237 hydroxyproline are older than those obtained on collagen. For these samples, we suspect that some
238 contaminant had remained in the collagen (possibly crosslinked to it) but was removed by
239 hydrolysing the collagen and isolating hydroxyproline. Ages obtained on hydroxyproline are therefore
240 retained over those obtained from bulk collagen for the following sections of the paper.

241 **Table 3:** Radiocarbon determinations and analytical data for the eight late Pleistocene to early Holocene bones from North America used in this
242 study and dated at the Oxford Radiocarbon Accelerator Unit (ORAU). PCode refers to pretreatment code; 'AF' is ultrafiltered collagen; 'HYP'
243 denotes the extraction of hydroxyproline from hydrolysed bone collagen. Samples that had been preserved with glues were also washed with
244 solvents (acetone, methanol and chloroform) prior to AF treatment (coded 'AF*'). CRA is conventional radiocarbon age, expressed in years BP
245 with 1 σ standard deviation (Stuiver and Polach, 1977). Collagen yield is based on the mass of gelatin versus mass of bone powder
246 demineralized. Mass of Hyp is estimated by the peak area on the chromatogram. Stable isotope ratios are expressed in per mil (‰) relative to
247 VPDB with a mass spectrometric precision of $\pm 0.2\text{‰}$ (Coplen Tyler, 1994). C/N is the atomic ratio of carbon to nitrogen and is acceptable if it
248 ranges between 2.9-3.5 in the case of collagen, or ~ 5.0 in the case of hydroxyproline (Brock et al., 2010a; Devières et al., 2018).
249

SITE	P Number	P code	CRA ($\pm 1 \sigma$ SD)	AMS LAB No. OxA-X	Bone (mg)	Collagen (mg)	Collagen Yield %	Mass collagen hydrolysed (mg)	HYP (mg)	$\delta^{13}\text{C}$ (‰) (VPDB)	$\delta^{15}\text{N}$ (‰) (AIR)	C/N (Atomic %)
Beaufort (Whale)	P39336.0	AF	> 49,900	OxA-36957	1010	26.06	2.6	/	/	-14.3	14.0	3.2
	P39336.0	HYP	> 50,000	OxA-X-2736-13	1010	126.36	12.5	47.3	3.2	-19.4	25.8	5.1
Wally's Beach (Camelops)	P39331.1	AF*	11,530 \pm 55	OxA-36953	2000	33.25	1.7	/	/	-19.4	2.7	3.2
	P39331.1	HYP	11,530 \pm 50	OxA-X-2736-8	2000	94.17	4.7	48.5	3.6	-18.4	16.6	4.9
Wally's Beach (Bootherium)	P39332.0	AF*	11,295 \pm 50	OxA-36954	2030	19.52	1	/	/	-19.4	1.5	3.2
	P39332.1	HYP	11,255 \pm 50	OxA-X-2736-9	1140	70.63	6.2	48.8	4.5	-21.9	11.0	4.9
Wally's Beach	P45701.0	AF*	11,685 \pm 50	OxA-37337	867	28.24	3.3	/	/	-21.2	-0.6	3.4

250

<i>(Equus)</i>	P39333.2	HYP	11,445 ± 55	OxA-X-2736-10	880	39.62	4.5	39.6	3.5	-23.3	11.9	5.0
Dent <i>(Mammuthus)</i>	P39334.1	AF*	11,115 ± 50	OxA-36955	2100	28.61	1.4	/	/	-10.9	11.2	3.2
	P39334.1	HYP	11,055 ± 50	OxA-X-2736-11	2100	98.14	4.7	47.2	4.1	-13.6	18.4	5.0
Dent <i>(Mammuthus)</i>	P39335.2	AF*	10,870 ± 50	OxA-36956	796	20.14	2.5	/	/	-14.0	8.9	3.3
	P39335.1	HYP	11,155 ± 50	OxA-X-2736-12	1610	51.9	3.2	48.4	4.3	-18.0	14.7	5.0
La Prele <i>(Mammuthus)</i>	P39337.1	AF*	9,320 ± 45	OxA-36958	1380	38.73	2.8	/	/	-19.5	7.0	3.2
	P39337.1	HYP	11,035 ± 50	OxA-X-2736-14	1380	163.96	11.9	49.2	4.9	-22.7	13.0	4.9
Lindsay <i>(Mammuthus)</i>	P39338.2	AF*	11,720 ± 60	OxA-37113	1140	13.71	1.2	/	/	-20.7	3.8	3.3
	P39338.2	HYP	12,395 ± 55	OxA-X-2736-15	1140	66.13	5.8	48.6	4.4	-24.9	9.7	5.0

251 **Table 4:** Chi-squared test results on radiocarbon dates obtained using the UF/UF* or HYP protocols.
 252 The error weighted mean and the t values were calculated for each pair of samples using the AMS
 253 modern fraction values. If t is < 3.84, the error weighted mean is not significant and the 2 dates are
 254 therefore statistically identical.

Sample Numbers	AF/AF* dates	Hyp Dates	Error-weighted-means	Chi squared results
SR-5156	> 49,900 (OxA-36957)	> 50,000 (OxA-X-2736-13)	/	/
SR-8171	11,530 ± 55 (OxA-36953)	11,530 ± 50 (OxA-X-2736-8)	0.2381 ± 0.0110	Pass (t=0.00)
SR-8226	11,295 ± 50 (OxA-36954)	11,255 ± 50 (OxA-X-2736-9)	0.2458 ± 0.0011	Pass (t=0.33)
SR-8221	11,685 ± 50 (OxA-37337)	11,445 ± 55 (OxA-X-2736-10)	0.2368 ± 0.0011	Fail (t=10.43)
SR-7616	11,115 ± 50 (OxA-36955)	11,055 ± 50 (OxA-X-2736-11)	0.2515 ± 0.0011	Pass (t=0.75)
SR-6606	10,870 ± 50 (OxA-36956)	11,155 ± 50 (OxA-X-2736-12)	0.2539 ± 0.0011	Fail (t=16.46)
SR-7356	9,320 ± 45 (OxA-36958)	11,035 ± 50 (OxA-X-2736-14)	0.2823 ± 0.0012	Fail (t=658.89)
SR-8253	11,720 ± 60 (OxA-37113)	12,395 ± 55 (OxA-X-2736-15)	0.2220 ± 0.0011	Fail (t=70.04)

255
 256 **4.2.1 Beaufort Sea Coast Whale, Alaska, USA**
 257 The age of the sample from the Beaufort Sea, based on its stratigraphic position, is beyond the
 258 radiocarbon dating limit. Previous attempts to date the sample with different methods have provided
 259 greater than radiocarbon background ages but, with seemingly poor laboratory backgrounds, the
 260 minimum ages are very young (Table 5) (Stafford et al., 1987). More recently, Stafford obtained an
 261 age of 45,580 ± 270 BP after purification of the hydrolysed collagen using XAD resin (Stafford, 2014).
 262 We re-dated the same sample using the AF and HYP procedures. We obtained measurements of
 263 >49,900 years (OxA-36957) and >50,000 years (OxA-X-2736-13), respectively, which indicates they
 264 date to beyond the radiocarbon limit as expected for this sample.

265
 266

267 **Table 5:** Previously published dates for the sample from Beaufort Sea, Alaska, USA. Lab codes are
 268 AA for University of Arizona Accelerator Mass Spectrometry Laboratory; UCIAMS for University of
 269 California-Irvine W.M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory.

Sample Number	Sample Preparation	¹⁴ C y BP (1 σ)	References
AA-312A	HCl insoluble collagen (A+B+C, Fig. 1)	>26,700	(Stafford et al., 1987)
AA-312B	Gelatin (A+B+C+D3, Fig. 1)	>27,300	(Stafford et al., 1987)
AA-312C	Gelatin (A+B+C+D3, Fig. 1)	>38,000	(Stafford et al., 1987)
UCIAMS-116378	XAD purified hydrolysed protein (A+B+C+D3+ E +F, Fig. 1)	45,580 ± 270	(Stafford, 2014)

270

271 4.2.2 Wally's Beach, Alberta, Canada

272 The Wally's Beach site was initially dated by five radiocarbon ages ranging from 10,980 ± 80 BP (TO-
 273 7691) to 11,350 ± 80 BP (TO-8972) from bison, horse, muskox, caribou and camel bones from the
 274 eolian sediments (Kooyman et al., 2006; Kooyman et al., 2012; Waters et al., 2015). These ages were
 275 based on the unpurified gelatin fraction (Table 6). Twenty-seven radiocarbon ages were also obtained
 276 on XAD-purified collagen and other chemical fractions extracted from bones of all seven butchered
 277 horses, the butchered camel, and the un-butchered muskox (Waters et al., 2015). The eight XAD-
 278 purified collagen dates for the horses overlap at 1 σ and range from 11,410 ± 30 BP (UCIAMS-
 279 127349) to 11,470 ± 35 BP (UCIAMS-127348). The XAD-purified collagen from the camel bone
 280 yielded two ages [11,465 ± 40 BP (UCIAMS-116400) and 11,425 ± 30 BP (UCIAMS-127347)] that are
 281 statistically identical. The initial gelatin radiocarbon age of 10,980 ± 80 BP (TO-7691) for the muskox
 282 was revised to 11,320 ± 30 BP (UCIAMS-12737) using XAD collagen.

283 For this new study, three samples were selected for dating using the HYP method: the camel bone
 284 (*Camelops hesternus*), the muskox bone (*Bootherium bombifrons*) and one of the butchered horse
 285 bone (*Equus conversidens*; Horse A). Artifacts were found with the camel and the horse but no
 286 artifact was found with the unbutchered muskox. All samples were contaminated with humates; the
 287 horse bone was also coated with Butvar B-98 resin (Waters et al., 2015). Using the single amino acid
 288 dating approach, the *Camelops hesternus* (P39331) was dated at 11,530 ± 50 BP (OxA-X-2736-8),
 289 the *Bootherium bombifrons* (P39332) at 11,255 ± 50 BP (OxA-X-2736-9), and the *Equus*

290 *conversidens* (P39333) at $11,445 \pm 55$ BP (OxA-X-2736-10). We performed a Chi-squared test on
 291 these three new dates using the modern carbon fraction ($F^{14}C$) and its error. The error-weighted-
 292 mean in $F^{14}C$ was 0.2415 ± 0.0009 . The t value calculated for the Chi-squared test is 15.97. For a
 293 Chi-squared test with 3 values, if t is > 5.99 the error is significant. This test therefore shows that the
 294 results obtained on the three bones samples from Wally's Beach are not contemporaneous. The
 295 dates for the *Camelops hesternus* (P39331) and the *Equus conversidens* (P39333) are statistically
 296 the same but the date for the *Bootherium bombifrons* bone (P39332) is younger and therefore it
 297 appears that the unbutchered skeleton derives from a later, probably natural event. This is coherent
 298 with the ages obtained after XAD purification. From an archaeological perspective, the dates obtained
 299 on the *Camelops hesternus* (P39331) and the *Equus conversidens* (P39333) indicate that these
 300 animals were hunted some 300 years earlier than the earliest firmly dated Clovis site (Waters and
 301 Stafford, 2007).

302

303 **Table 6:** Previously published dates for the three samples from Wally's Beach, Alberta, Canada

304 included in this study. Lab code TO denotes the IsoTrace Laboratory.

Sample Number	Sample Preparation	^{14}C y BP (1 σ)	References
<i>Camelops hesternus</i>			
Cat No. 3610.1			
TO-13513	Gelatin (A+B+C+D3, Fig. 1)	$11,070 \pm 80$	(Waters et al., 2015)
UCIAMS-116390	KOH extracted collagen (A+B+C+D2, Fig. 1)	$11,425 \pm 35$	(Waters et al., 2015)
UCIAMS-116383	Gelatin (A+B+C+D3, Fig. 1)	$11,420 \pm 30$	(Waters et al., 2015)
UCIAMS-116400	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,465 \pm 40$	(Waters et al., 2015)
UCIAMS-127347	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,425 \pm 30$	(Waters et al., 2015)
<i>Bootherium bombifrons</i>			
Cat No. 3293.1			
TO-7691	Gelatin (A+B+C+D3, Fig. 1)	$10,980 \pm 80$	(Waters et al., 2015)
UCIAMS-127371	KOH extracted collagen (A+B+C+D2, Fig. 1)	$11,170 \pm 30$	(Waters et al., 2015)
UCIAMS-127372	Gelatin (A+B+C+D3, Fig. 1)	$11,255 \pm 30$	(Waters et al., 2015)
UCIAMS-127373	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	$11,320 \pm 30$	(Waters et al., 2015)
<i>Equus conversidens</i>			
Horse A, Cat No. 315			
UCIAMS-127363	KOH extracted collagen (A+B+C+D2, Fig. 1)	$13,540 \pm 40$	(Waters et al., 2015)

UCIAMS-127364	Gelatin (A+B+C+D2+D3, Fig. 1)	11,495 ± 30	(Waters et al., 2015)
UCIAMS-127351	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	11,440 ± 30	(Waters et al., 2015)

305

306 4.2.3 Dent, Colorado, USA

307 The Dent site was also believed to have been a kill and butchery site that occurred as a single event.

308 There have been several attempts at dating the bones from the site (Table 7). The first radiocarbon

309 date was 7,200 ± 200 BP (I-473) on whole bone. However, the age was considered too young due to

310 shellac contamination. After solvent extraction to remove the shellac, the same bone yielded a ¹⁴C

311 measurement of 11,200 ± 500 BP (I-622), which Haynes et al. argue to be the correct age (Haynes,

312 1966; Haynes et al., 1998; Trautman and Willis, 1966). The first attempts to date hydrolysed collagen

313 purified by XAD produced dates ranging from 10,590 ± 500 BP (AA-832) to 10,980 ± 90 BP (AA-

314 2941). Dates obtained at the same time on single amino acids produced similar ages (Table 7). More

315 recently, three additional dates were produced on collagen purified by XAD and they are older than

316 most of the dates previously obtained (Table 7). This may indicate that there was some contaminant

317 not being fully removed from the samples but, unfortunately, there is no material left to check this. The

318 two new hydroxyproline dates obtained at the ORAU are 11,055 ± 50 BP (OxA-X-2736-11) and

319 11,155 ± 50 BP (OxA-X-2736-12). Using the modern carbon fraction of these two HYP dates, we ran

320 a Chi-squared test. The error weighted mean in F¹⁴C was 0.2510 ± 0.0011 and *t* = 1.90. This *t* value

321 is <3.84. The two HYP dates are therefore statistically indistinguishable and provide a narrower time

322 window within which these mammoths were killed.

323

324 **Table 7:** Previously published dates for the samples from Dent, Colorado USA. Lab code I stands for

325 Teledyne Isotopes.

Sample Number	Sample Preparation	¹⁴ C y BP (1 σ)	References
I-473	HCl insoluble collagen (A+B+C, Fig. 1)	7,200 ± 200	(Trautman and Willis, 1966)
I-622	HCl insoluble collagen (A+B+C, Fig. 1)	11,200 ± 500	(Trautman and Willis, 1966)
AA-830	HCl insoluble collagen (A+B+C, Fig. 1)	8,250 ± 520	(Brunswig, 2007; Stafford et al., 1988)
AA-831	Gelatin (A+B+C+D3, Fig. 1)	9,240 ± 350	(Stafford et al., 1988; Stafford et al., 1987)

AA-832	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,590 ± 500	(Stafford et al., 1988; Stafford et al., 1991)
AA-833	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,950 ± 480	(Stafford et al., 1988; Stafford et al., 1987)
AA-2941	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,980 ± 90	(Stafford et al., 1988; Stafford et al., 1991)
AA-2942	Asp – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,750 ± 170	(Stafford et al., 1991)
AA-2943	Glu – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,890 ± 110	(Stafford et al., 1991)
AA-2944	Thr – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,380 ± 140	(Stafford et al., 1991)
AA-2945	Hyp – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,680 ± 90	(Stafford et al., 1988; Stafford et al., 1991)
AA-2946	Gly – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,780 ± 90	(Stafford et al., 1991)
AA-2947	Ala – Individual amino acid (A+B+C+D1+E+F+G, Fig. 1)	10,690 ± 120	(Stafford et al., 1991)
UCIAMS-11339	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	11,065 ± 35	(Waters and Stafford, 2007)
UCIAMS-11340	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	10,940 ± 30	(Waters and Stafford, 2007)
UCIAMS-116403	XAD purified hydrolysed protein (A+B+C+D3+E+F, Fig. 1)	10,960 ± 35	(Stafford, 2014)
UCIAMS-116394	KOH extracted collagen (A+B+C+D2, Fig. 1)	10,925 ± 35	(Stafford, 2014)
UCIAMS-116388	Gelatin (A+B+C+D2+D3, Fig. 1)	11,015 ± 30	(Stafford, 2014)

326

327 4.2.4 La Prele Mammoth (previously named Fetterman Mammoth), Wyoming, USA

328 The first ¹⁴C dating on the La Prele mammoth produced dates that were significantly younger than
329 Folsom or Clovis (Table 8). The fossil bone submitted as mammoth produced an early Holocene age
330 based on dating of two chemical fractions that overlapped at one standard deviation [9060 ± 50 BP
331 (CAMS-72350) and 8890 ± 60 BP (CAMS-74661) on KOH-collagen and gelatin, respectively] (Table
332 8). Three hypotheses were proposed to explain these Holocene ages; 1) the specimen represents a
333 relict (Holocene) mammoth population in that region; 2) the dated sample was contaminated with
334 modern carbon, possibly derived from a mixture of Glyptol and Paraloid B-72 (Byers, 2002) and; 3)
335 the first two AMS dates were on a nearby bison rather than the target mammoth. Stafford *et al.* re-
336 dated a different sample and used a neural spine that was unquestionably from the mammoth. Their
337 resulting date was 10,760 ± 30 BP (UCIAMS-40174) on gelatin and 10,965 ± 30 BP (UCIAMS-
338 206764) on XAD purified protein hydrolysate. This same neural spine was selected for dating by the
339 HYP method at the ORAU. After a solvent wash to remove the conservation material, we isolated
340 hydroxyproline and dated it. We obtained an age of 11,035 ± 50 BP (OxA-X-2736-14). These new
341 dates are significantly older than those published by (Byers, 2002) and allow us to confirm the Clovis
342 age attribution for this site.

343

344

345 **Table 8:** Previously dates for the samples from La Prele Mammoth, Wyoming, USA. Lab code CAMS
346 is for Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, California.
347 Although bone for CAMS-72350 and 74661 was submitted as *Mammuthus* sp., subsequent
348 examination of the collections indicates the specimen was probably *Bison* sp. and not *Mammuthus*. A
349 complete *Mammuthus* neural spine was located in the University of Wyoming collections and was
350 used for all ¹⁴C dates listed as *Mammuthus* sp. for the La Prele Mammoth.

Sample Number	Sample Preparation	¹⁴ C y BP (1 σ)	References
CAMS-72350	KOH extracted collagen (A+B+C+D2, Fig. 1)	9,060 ± 50	(Byers, 2002)
CAMS-74661	Gelatin (A+B+C+D3, Fig. 1)	8,890 ± 60	(Byers, 2002)
UCIAMS-40174	Gelatin (A+B+C+D2+D3, Fig. 1)	10,760 ± 30	This paper
UCIAMS-206764	XAD purified protein hydrolysate (A+B+C+D3+E+F, Fig. 1)	10,965 ± 30	This paper

351

352 4.2.5 Lindsay, Montana, USA

353 Five radiocarbon dates on bone were initially obtained from the Lindsay mammoth (Davis and Wilson,
354 1985; Hill and Davis, 1998; Hill, 2006; Huber and Hill, 2003). These dates are 9,490 ± 135 BP (I-
355 7028), 10,700 ± 290 BP (WSU-652), 10,980 ± 225 BP (I-9220), 11,500 ± 80 BP (Beta-102031), and
356 11,925 ± 350 BP (S-918). These ages were all derived from unpurified bone collagen and therefore
357 must represent minimum ages. Subsequently, three additional dates were obtained on a mammoth rib
358 from the site: KOH extracted collagen yielded an age of 12,105 ± 40 BP (CAMS-82416), gelatin
359 yielded an age of 12,175 ± 40 BP (CAMS-80541), and the XAD-purified collagen yielded an age of
360 12,330 ± 50 BP (CAMS-72348). To estimate the age of the Lindsay mammoth, Krasinski averaged all
361 eight radiocarbon measurements—from those on unpurified and purified collagen and spanning 9,490
362 to 12,330 BP—to derive an average age of 11,210 ± 190 BP or 12,920 to 13,300 Cal BP for the site.
363 Based on this averaging method, she suggested that this butchering likely represented the work of
364 Clovis hunters (Krasinski, 2010). Evaluation of the radiocarbon record indicates that the unpurified
365 bone collagen ages are minimum ages and cannot be used for valid age interpretations. Based on the
366 chemical fractions dated, the XAD-purified collagen that gave the age of 12,330 ± 40 BP or 14,140 to
367 14,400 Cal BP (CAMS-72348) provided the most accurate age from this second group of dates. Six
368 additional ages have been obtained on different chemical fractions from Lindsay mammoth bone;

369 three from the femur and three from the humerus (Waters and Stafford, 2014). Two of the dates were
 370 on XAD-purified collagen and are $12,300 \pm 35$ BP (UCIAMS-12308) for the femur and $12,270 \pm 35$ BP
 371 (UCIAMS-127316) for the humerus. The three XAD ages overlap at 1σ and average $12,300 \pm 25$ BP
 372 (Waters and Stafford, 2014). All of these radiocarbon measurements for the Lindsay Mammoth Site
 373 range from 9490 to 12,330 BP, with the youngest measurements being used to support the
 374 conclusion that mammoths survived into the Holocene and the older ages supporting evidence of pre-
 375 Clovis human presence (Krasinski, 2010; Waters and Stafford, 2014) (Table 9).

376 A bone from the same mammoth was selected for dating by the HYP method at the ORAU. Visual
 377 analysis of the sample revealed a coating of an unknown consolidant. Based on FTIR analyses we
 378 suspect that the contaminant was Butvar B-98. The collagen was therefore extracted after a solvent
 379 wash. The dating of hydroxyproline produced an age of $12,395 \pm 55$ BP (OxA-X-2736-15), which is
 380 congruent with all the XAD dates produced by Waters and Stafford (Huber and Hill, 2003; Waters and
 381 Stafford, 2014). It also overlaps at 2σ with the gelatin date of $12,290 \pm 35$. This new single-compound
 382 date reinforces the idea that this site predates the Clovis period by approximately 1,350 radiocarbon
 383 years. Despite the absence of classic lithic artifacts, taphonomic evidence from bone breakage and
 384 skeletal element distribution strongly implies human occurrence at the site.

385

386 **Table 9:** Previously published dates for the samples from Lindsay Wyoming, USA. Lab codes: S for
 387 Saskatchewan (Canada), Beta for Beta Analytic (USA) and WSU for Washington State University
 388 (USA).

Sample Number	Sample Preparation	^{14}C y BP (1σ)	References
I-7028	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$9,490 \pm 135$	(Davis and Wilson, 1985)
WSU-652	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$10,700 \pm 290$	(Davis and Wilson, 1985)
I-9220	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$10,980 \pm 225$	(Davis and Wilson, 1985)
Beta-102031	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$11,500 \pm 80$	(Davis and Wilson, 1985)
S-918	HCl insoluble collagen (A+B+C+D1, Fig. 1)	$11,925 \pm 350$	(Davis and Wilson, 1985)
CAMS-82416	KOH extracted collagen (A+B+C+D2, Fig. 1)	$12,105 \pm 40$	(Huber and Hill, 2003)
CAMS-80541	Gelatin (A+B+C+D3, Fig. 1)	$12,175 \pm 40$	(Huber and Hill, 2003)
CAMS-72348	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	$12,330 \pm 50$	(Huber and Hill, 2003)
UCIAMS-127306	KOH extracted collagen (A+B+C+D2, Fig. 1)	$12,230 \pm 35$	(Waters and Stafford, 2014)
UCIAMS-127307	Gelatin (A+B+C+D2+D3, Fig. 1)	$12,290 \pm 35$	(Waters and Stafford, 2014)

UCIAMS-127308	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	12,300 ± 35	(Waters and Stafford, 2014)
UCIAMS-127309	KOH extracted collagen (A+B+C+D2, Fig. 1)	12,220 ± 35	(Waters and Stafford, 2014)
UCIAMS-127310	Gelatin (A+B+C+D3, Fig. 1)	12,255 ± 35	(Waters and Stafford, 2014)
UCIAMS-127316	XAD purified hydrolysed protein (A+B+C+D1+E+F, Fig. 1)	12,270 ± 35	(Waters and Stafford, 2014)

389

390

391 **5. Discussion**

392 We obtained new ¹⁴C dates for seven vertebrate fossils associated with four North American
393 butchering sites: Wally's Beach (Canada), Dent (Colorado), La Prele, (Wyoming) and Lindsay
394 (Montana). In 4 cases, we observed a discrepancy between the dates obtained on ultrafiltered
395 collagen and the dates obtained on hydroxyproline. This shows that when samples are extremely
396 contaminated, it can be difficult to totally remove the contamination from the collagen as it can also be
397 chemically crosslinked. Similar observations have been made on a range of contaminated Palaeolithic
398 bone samples dated at the ORAU (Becerra-Valdivia et al., 2018; Bourrillon et al., 2017; Devière et al.,
399 2017; Reynolds et al., 2017). Ages obtained on hydroxyproline are therefore to be preferred over
400 those obtained from bulk collagen.

401 Radiocarbon ages obtained after the HYP and XAD procedures were compared using the Difference
402 function in the OxCal 4.3 platform (Bronk Ramsey, 2018). The new ages obtained on hydroxyproline
403 compare favorably with the previously reported XAD-derived ages for these sites with the exception of
404 the *Mammuthus columbi* sample (SR-6606) from Dent, Colorado (Table 10). We also observe here that
405 the ORAU dates tend to be slightly older. This is something currently being investigated, yet there are
406 2 likely explanations; (1) the dead carbon bleeding of the column is slightly underestimated for some
407 of the samples (we monitor it regularly but it cannot be measured for every individual sample) or (2)
408 the XAD is not removing a contaminant that is causing the collagen to date too young.

409 The new HYP ages from Wally's Beach (Canada) and Lindsay (Montana, USA) support the
410 conclusion that these sites pre-date the Clovis time period, considering its start at 11,050 BP (Waters
411 and Stafford, 2007). For the two Clovis sites, the HYP ages confirm the previous XAD purified protein
412 hydrolysates from the Dent site and provide the first accurate age for the La Prele site: 13,041 to
413 12,757 Cal BP. This age falls well within range of the other fourteen well-dated Clovis sites from
414 13,050 to 12,650 Cal BP (Waters and Stafford, 2007).

415 Because contaminants were not completely removed during earlier dating efforts, the majority of XAD
 416 and HYP ages are older than those previously obtained. However, a few of the dates derived on other
 417 chemical fractions do come close. Sample of *Bootherium bombifrons* (Cat No. 3293.1), for example,
 418 produced the same ages both on gelatin (UCIAMS-127372) and hydroxyproline (OxA-X-2736-9). This
 419 is likely the result of either the absence of contaminants in the bone or the removal of all contaminants
 420 during an earlier stage of pretreatment before purification using XAD and HYP methods (Fig. 1 and
 421 Fig.2). In addition, as can be observed with samples from Dent, accuracy and precision of the ages
 422 on chemical fractions from bone before XAD or HYP purification are inconsistent (Figure 3). It is
 423 impossible to predict analytically, to a high degree of confidence, when methods other than XAD or
 424 HYP are able to remove all of the contaminating carbon in dated bones. Sometimes they are reliable
 425 and sometimes not. Only the XAD and HYP methods will consistently remove contaminants and yield
 426 ages that are demonstrably contaminant free.

427

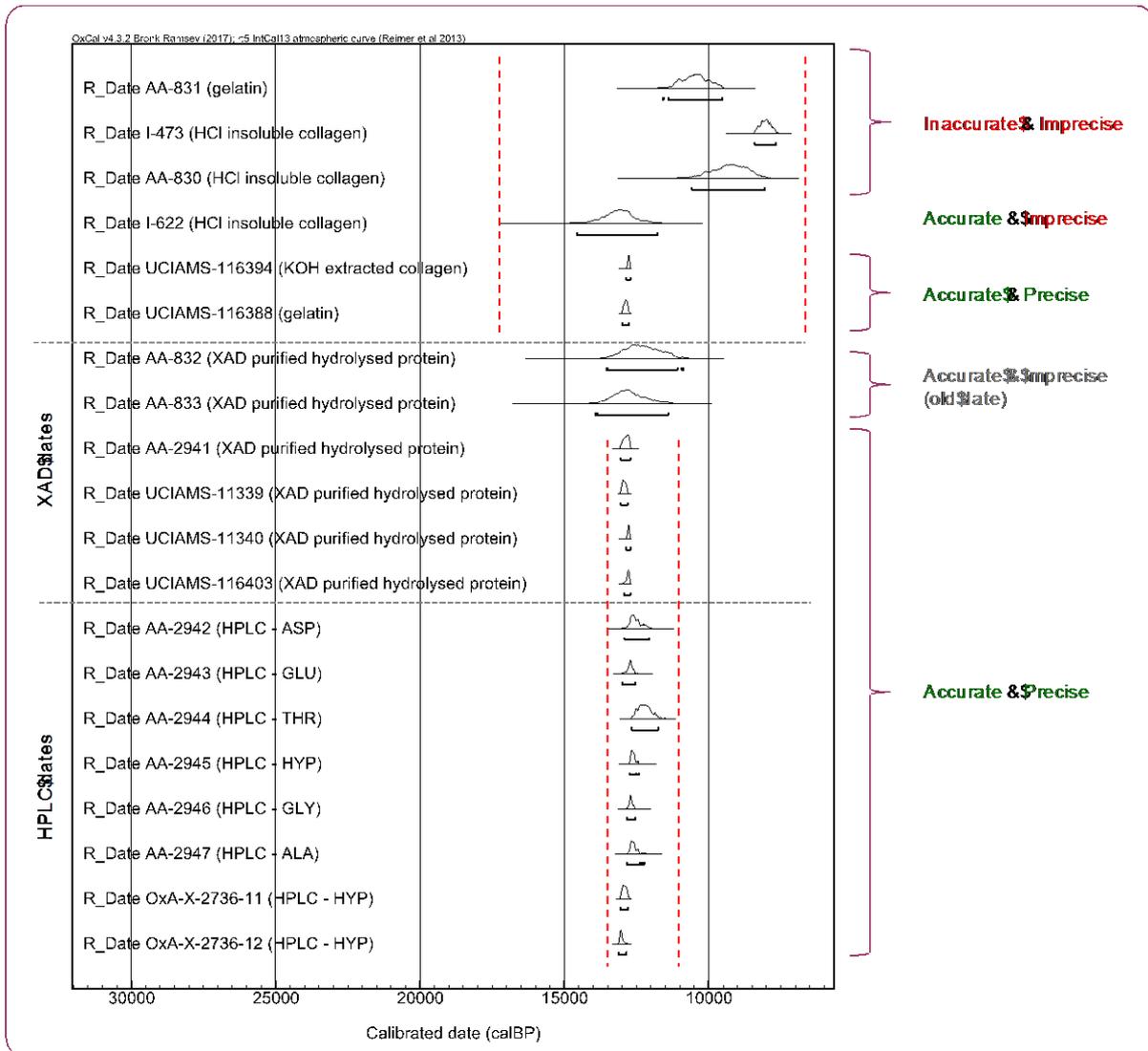
428 **Table 10:** Summary of HYP versus XAD ¹⁴C dates on fossil bones from North America.

SITE	Taxon	HYP Age ± 1σ RC yr.	AMS Lab No.	XAD Age ± 1σ RC yr.	AMS Lab No.	Age Difference, RC yr. (HYP - XAD)
Wally's beach, Canada	<i>Camelops hesternus</i>	11,530 ± 50	OxA-X-2736-8	11,465 ± 40	UCIAMS-116400	-194 (95.4 %) 92
	<i>Bootherium bombifrons</i>	11,255 ± 50	OxA-X-2736-9	11,425 ± 30	UCIAMS-127347	-237 (95.4 %) 21
	<i>Equus conversidens</i>	11,445 ± 55	OxA-X-2736-10	11,320 ± 30	UCIAMS-127373	-88 (95.4 %) 171
Dent, Colorado	<i>Mammuthus columbi</i>	11,055 ± 50	OxA-X-2736-11	10,960 ± 35	UCIAMS-116403	-290 (95.4 %) 70
	<i>Mammuthus columbi</i>	11,155 ± 50	OxA-X-2736-12			-355 (95.4 %) -36
La Prele, Wyoming	<i>Mammuthus columbi</i>	11,035 ± 50	OxA-X-2736-14	10,965 ± 30	UCIAMS-206764	-270 (95.4 %) 80
Lindsay,	<i>Mammuthus</i>	12,395 ± 55	OxA-X-2736-15	12,270 ± 35	UCIAMS-127316	-697 (95.4 %) 90

Montana	<i>columbi</i>					
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430



431

432 **Figure 3:** New calibrated hydroxyproline dates produced at ORAU for the samples from Dent,
 433 Colorado (USA), compared to previously-published measurements. This figure shows that most of the
 434 dates that have not been obtained using the XAD or HPLC approach, are too young and/or less
 435 precise.

436

437 Accurate dating of bones is necessary to properly reconstruct the story of the first Americans and Late
 438 Pleistocene megafaunal extinctions. Our study shows that XAD and HYP methods are more efficient
 439 in systematically removing contaminants from bone samples than other pretreatment methods and,

440 therefore, produce more reliable dates. We also demonstrated that when we examine determinations
441 obtained using less robust methods, we encounter a range of results, some of which are reliable and
442 some not. Ascertaining which samples are reliable is difficult as the analytical parameters used in
443 assessing the degree to which the extracted collagen is intact and not subject to contamination are
444 not always available. The C/N atomic ratio is, so far, the best quality indicator to identify if samples
445 are heavily contaminated. It is acceptable for collagen (or protein hydrolysate isolated by XAD) if it
446 ranges from 2.9-3.5. This indicator may however not be sensitive enough to detect contamination in
447 small quantity or with a C/N ratio similar to the one of collagen. Hydroxyproline acts as a nearly unique
448 biomarker for bone collagen and provides a chemically pure sample for accurate radiocarbon
449 measurement. Measuring the C/N ratio of the hydroxyproline isolated using prep-HPLC is therefore
450 the most sensitive and reliable indicator to check that the sample is totally free of contamination.

451

452

453 **6. Conclusions**

454 The debate concerning when and how humans first colonized the Americas is on-going and has been
455 further intensified by analyses of aDNA from human remains. The accurate dating of human fossils is
456 crucial to our understanding of this migration process. Due to the extreme rarity of Paleoindian human
457 skeletons in the Americas, it is also important to directly date animal bones from kill and butchering
458 sites. Our work has demonstrated how important sample purification chemistry is for obtaining
459 accurate AMS ¹⁴C measurements. More specifically, this dating effort on bones from Wally's Beach,
460 La Prele (Fetterman), Lindsay, and Dent, illustrates that chromatographic methods, such as
461 preparative High Performance Liquid Chromatography and column chromatography using XAD
462 resins, are the most efficient methods in the removal of contaminants from bone samples. To avoid
463 data misinterpretation, it is imperative that explanatory models in First Americans research only use
464 radiocarbon determinations previously shown to be demonstrably accurate. This increased degree of
465 accuracy is entirely dependent upon the most robust sample preparation chemistry that can be
466 applied.

467

468

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474

475

476 **References**

- 477 Abelson, P.H., Hoering, T.C., 1961. Carbon isotope fractionation in formation of amino acids
478 by photosynthetic organisms. *PNAS* 47, 623-632.
- 479 Amick, D.S., 2017. Evolving views on the Pleistocene colonization of North America.
480 *Quaternary International* 431, Part B, 125-151.
- 481 Becerra-Valdivia, L., Waters, M.R., Stafford, T.W., Anzick, S.L., Comeskey, D., Devière, T.,
482 Higham, T., 2018. Reassessing the chronology of the archaeological site of Anzick.
483 *Proceedings of the National Academy of Sciences*.
- 484 Benders, Q., 2010. *Agate Basin Archaeology in Alberta and Saskatchewan, Canada*,
485 Department of Anthropology. University of Alberta p. 178.
- 486 Bourgeon, L., Burke, A., Higham, T., 2017. Earliest Human Presence in North America Dated
487 to the Last Glacial Maximum: New Radiocarbon Dates from Bluefish Caves, Canada. *PLOS*
488 *ONE* 12, e0169486.
- 489 Bourrillon, R., White, R., Tartar, E., Chiotti, L., Mensan, R., Clark, A., Castel, J.C., Cretin, C.,
490 Higham, T., Morala, A., Ranlett, S., Sisk, M., Devière, T., Comeskey, D.J., 2017. A new
491 Aurignacian engraving from Abri Blanchard, France: Implications for understanding
492 Aurignacian graphic expression in Western and Central Europe. *Quaternary International*
493 10.1016/j.quaint.2016.09.063.
- 494 Brock, F., Higham, T., Ditchfield, P., Bronk Ramsey, C., 2010a. Current Pretreatment
495 Methods for AMS Radiocarbon Dating at the Oxford Radiocarbon Accelerator Unit (ORAU).
496 *Radiocarbon* 52, 103-112.
- 497 Brock, F., Higham, T., Ramsey, C.B., 2010b. Pre-screening techniques for identification of
498 samples suitable for radiocarbon dating of poorly preserved bones. *Journal of*
499 *Archaeological Science* 37, 855-865.
- 500 Bronk Ramsey, C., 2008. Radiocarbon Dating: Revolutions in Understanding. *Archaeometry*
501 50, 249-275.
- 502 Bronk Ramsey, C., 2018. *OxCal 4.3 Manual*. .
- 503 Brunswig, R.H., 2007. New Interpretations of the Dent Mammoth Site: A Synthesis of Recent
504 Multidisciplinary Evidence, in: Brunswig, R.H., Pitblado, B.L. (Eds.), *Frontiers in Colorado*
505 *Paleoindian Archaeology: From the Dent Site to the Rocky Mountains*. University Press of
506 Colorado, Boulder, pp. 87-121.
- 507 Byers, D.A., 2002. Taphonomic analysis, associational integrity, and depositional history of
508 the Fetterman Mammoth, eastern Wyoming, U.S.A. *Geoarchaeology* 17, 417-440.

509 Coplen Tyler, B., 1994. Reporting of stable hydrogen, carbon, and oxygen isotopic
510 abundances (Technical Report), Pure and Applied Chemistry, pp. 273-276.

511 Davis, L.B., Wilson, M.C., 1985. The late Pleistocene Lindsay mammoth (24DW501), eastern
512 Montana: Possible man-mammoth association. *Current Research in the Pleistocene* 2, 97-
513 98.

514 Devière, T., Comeskey, D., McCullagh, J., Bronk Ramsey, C., Higham, T., 2018. New protocol
515 for compound specific radiocarbon analysis of archaeological bones. *Rapid Communications*
516 *in Mass Spectrometry* 32, 373–379.

517 Devière, T., Karavanić, I., Comeskey, D., Kubiak, C., Korlević, P., Hajdinjak, M., Radović, S.,
518 Procopio, N., Buckley, M., Pääbo, S., Higham, T., 2017. Direct dating of Neanderthal remains
519 from the site of Vindija Cave and implications for the Middle to Upper Paleolithic transition.
520 *Proceedings of the National Academy of Sciences* 114, 10606-10611.

521 Gillespie, R., Hedges, R.E.M., Humm, M.J., 1986. Routine AMS Dating of Bone and Shell
522 Proteins. *Radiocarbon* 28, 451-456.

523 Gillespie, R., Hedges, R.E.M., Wand, J.O., 1984. Radiocarbon dating of bone by accelerator
524 mass spectrometry. *Journal of Archaeological Science* 11, 165-170.

525 Goebel, T., Waters, M.R., O'Rourke, D.H., 2008. The Late Pleistocene Dispersal of Modern
526 Humans in the Americas. *Science* 319, 1497-1502.

527 Grayson, D.K., Meltzer, D.J., 2003. A requiem for North American overkill. *Journal of*
528 *Archaeological Science* 30, 585-593.

529 Grayson, D.K., Meltzer, D.J., 2015. Revisiting Paleoindian exploitation of extinct North
530 American mammals. *Journal of Archaeological Science* 56, 177-193.

531 Halligan, J.J., Waters, M.R., Perrotti, A., Owens, I.J., Feinberg, J.M., Bourne, M.D., Fenerty,
532 B., Winsborough, B., Carlson, D., Fisher, D.C., Stafford, T.W., Jr., Dunbar, J.S., 2016. Pre-
533 Clovis occupation 14,550 years ago at the Page-Ladson site, Florida, and the peopling of the
534 Americas. *Science Advances* 2.

535 Haynes, C.V., 1966. Elephant-hunting in North America. *Scientific American*, 104-112.

536 Haynes, C.V., McFaul, M., Brunswig, R.H., Hopkins, K.D., 1998. Kersey–Kuner terrace
537 investigations at the Dent and Bernhardt sites, Colorado. *Geoarchaeology* 13, 201-218.

538 Haynes, G., 2013. Extinctions in North America's Late Glacial landscapes. *Quaternary*
539 *International* 285, 89-98.

540 Higham, T.G., Jacobi, R.M., Ramsey, C.B., 2006. AMS Radiocarbon Dating of Ancient Bone
541 using Ultrafiltration. *Radiocarbon* 48, 179-195.

542 Hill, C., Davis, L., 1998. Stratigraphy, AMS Radiocarbon Age, and Stable Isotope
543 Biogeochemistry of the Lindsay Mammoth, Eastern Montana. *Current Research in the*
544 *Pleistocene* 15, 110–112.

545 Hill, C.L., 2006. Stratigraphic and geochronologic contexts of mammoth (*Mammuthus*) and
546 other Pleistocene fauna, Upper Missouri Basin (northern Great Plains and Rocky
547 Mountains), U.S.A. *Quaternary International* 142, 87-106.

548 Hill, C.L., Davis, L.B., 2014. Multi-scalar Geological and Paleoenvironmental Analysis of the
549 Lindsay Mammoth, Yellowstone Basin, Montana, American Quaternary Association 23rd
550 Biennial Meeting, University of Washington, Seattle, pp. 64-65.

551 Ho, T.Y., Marcus, L.F., Berger, R., 1969. Radiocarbon Dating of Petroleum-Impregnated Bone
552 from Tar Pits at Rancho La Brea, California. *Science* 164, 1051-1052.

553 Huber, J.K., Hill, C.L., 2003. Paleoeological Inferences Based on Pollen and Stable Isotopes
554 for Mammoth Bearing Deposits of the Oahe Formation (Aggie Brown Member), Eastern
555 Montana. *Current Research in the Pleistocene*, 95–96.

556 Kooyman, B., Hills, L.V., McNeil, P., Tolman, S., 2006. Late Pleistocene Horse Hunting at the
557 Wally's Beach Site (DhPg-8), Canada. *American Antiquity* 71, 101-121.

558 Kooyman, B., Hills, L.V., Tolman, S., McNeil, P., 2012. Late Pleistocene western camel
559 (*Camelops Hesternus*) hunting in southwestern Canada. *American Antiquity* 77, 115-124.

560 Krasinski, K.E., 2010. Broken bones and cutmarks: Taphonomic analyses and implications for
561 the peopling of North America. University of Nevada, Reno, p. 556.

562 Mackie, M.M., Surovell, T.A., Kelly, R.L., O'Brien, M.J., 2017. New Excavations at the La Prele
563 Mammoth Site, Converse County, Wyoming, SAA 82nd Annual Meeting,, Vancouver, B.C.

564 Marom, A., McCullagh, J.S.O., Higham, T.F.G., Hedges, R.E.M., 2013. Hydroxyproline Dating:
565 Experiments on the ¹⁴C Analysis of Contaminated and Low-Collagen Bones. *Radiocarbon* 55,
566 698-708.

567 Marom, A., McCullagh, J.S.O., Higham, T.F.G., Sinitsyn, A.A., Hedges, R.E.M., 2012. Single
568 amino acid radiocarbon dating of Upper Paleolithic modern humans. *Proceedings of the*
569 *National Academy of Sciences* 109, 6878-6881.

570 Martin, P.S., 1958. "Pleistocene ecology and biogeography of North America" University of
571 Arizona. Program in Geochronology. *Contrib* 9, 375-420.

572 Martin, P.S., 1973. The Discovery of America. The first Americans may have swept the
573 Western Hemisphere and decimated its fauna within 1000 years 179, 969-974.

574 Meltzer, D.J., 2009. *First Peoples in a New World Colonizing Ice Age America*, First Edition
575 ed, University of California Press.

576 Meltzer, D.J., 2015. *The Great Paleolithic War: how science forged an understanding of*
577 *America's ice age past*. University of Chicago Press;

578 Münnich, K.O., 1957. Heidelberg Natural Radiocarbon Measurements I. *Science* 126, 194-
579 199.

580 Nalawade-Chavan, S., McCullagh, J.S.O., Hedges, R., 2014. New Hydroxyproline Radiocarbon
581 Dates from Sungir, Russia, Confirm Early Mid Upper Palaeolithic Burials in Eurasia. *PLoS ONE*
582 9, e76896.

583 Reynolds, N., Dinnis, R., Bessudnov, A., Devière, T., Higham, T., 2017. The Kostënki 18 child
584 burial and the cultural and funerary landscape of Mid Upper Palaeolithic European Russia.
585 *Antiquity* 91, 1435-1450.

586 Saunders, J.J., 1999. Morphometrical analyses of *Mammuthus columbi* from the Dent Site,
587 Weld County, Colorado. *Deinsea* 6, 55–78.

588 Sikora, M., Seguin-Orlando, A., Sousa, V.C., Albrechtsen, A., Korneliusen, T., Ko, A.,
589 Rasmussen, S., Dupanloup, I., Nigst, P.R., Bosch, M.D., Renaud, G., Allentoft, M.E.,
590 Margaryan, A., Vasilyev, S.V., Veselovskaya, E.V., Borutskaya, S.B., Devière, T., Comeskey, D.,
591 Higham, T., Manica, A., Foley, R., Meltzer, D.J., Nielsen, R., Excoffier, L., Lahr, M.M., Orlando,
592 L., Willerslev, E., 2017. Ancient genomes show social and reproductive behavior of early
593 Upper Paleolithic foragers. *Science* 358, 659-662.

594 Stafford, T.W., Jr., 2014. Chronology of the Kennewick Skeleton, Washington, in: Owsley ,
595 D.W., Jantz, R.L. (Eds.), *The Scientific Investigation of an Ancient American Skeleton*. Texas
596 A&M Press, College Station, pp. 58-89.

597 Stafford, T.W., Jr., Brendel, K., Duhamel, R.C., 1988. Radiocarbon, ¹³C and ¹⁵N analysis of
598 fossil bone: Removal of humates with XAD-2 resin. *Geochimica et Cosmochimica Acta* 52,
599 2257-2267.

600 Stafford, T.W., Jr., Duhamel, R.C., Vance Haynes, C., Brendel, K., 1982. Isolation of proline
601 and hydroxyproline from fossil bone. *Life Sciences* 31, 931-938.

602 Stafford, T.W., Jr., Hare, P.E., Currie, L., Jull, A.J.T., Donahue, D.J., 1991. Accelerator
603 radiocarbon dating at the molecular level. *Journal of Archaeological Science* 18, 35-72.

604 Stafford, T.W., Jr., Jull, A.J.T., Brendel, K., Duhamel, R.C., Donahue, D., 1987. Study of Bone
605 Radiocarbon Dating Accuracy at the University of Arizona NSF Accelerator Facility for
606 Radioisotope Analysis. *Radiocarbon* 29, 24-44.

607 Stuiver, M., Polach, H.A., 1977. Discussion: Reporting of C-14 data. *Radiocarbon* 19, 355-
608 363.

609 Trautman, M.A., Willis, E.H., 1966. Isotopes, Inc. radiocarbon measurements V. *Radiocarbon*
610 8, 161-203.

611 Waters, M.R., Stafford, T.W., Jr., 2007. Redefining the Age of Clovis: Implications for the
612 Peopling of the Americas. *Science* 315, 1122-1126.

613 Waters, M.R., Stafford, T.W., Jr., 2014. The First Americans : A Review of the Evidence for
614 the Late-Pleistocene Peopling of the Americas, in: Graf, K.E., Ketron, C.V., Waters, M.R.
615 (Eds.), *Paleoamerican Odyssey*. Texas A&M University Press, College Station, Texas, pp. 543-
616 562.

617 Waters, M.R., Stafford, T.W., Jr., Kooyman, B., Hills, L.V., 2015. Late Pleistocene horse and
618 camel hunting at the southern margin of the ice-free corridor: Reassessing the age of
619 Wally's Beach, Canada. *Proceedings of the National Academy of Sciences* 112, 4263-4267.
620