Reassessing the chronology of the archaeological site of Anzick

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Found in 1968, the archaeological site of Anzick, Montana, contains the only known Clovis burial. Here, the partial remains of a male infant, Anzick-1, were found in association with a Clovis assemblage of over 100 lithic and osseous artifacts—all red-stained with ochre. The incomplete, unstained cranium of an unassociated, geologically younger individual, Anzick-2, was also recovered. Previous chronometric work has shown an age difference between Anzick-1 and the Clovis assemblage (represented by dates from two antler rod samples). This discrepancy has led to much speculation, with some discounting Anzick-1 as Clovis. To resolve this issue, we present the results of a comprehensive radiocarbon dating program that utilized different pretreatment methods on osseous material from the site. Through this comparative approach, we obtained a robust chronometric dataset that suggests that Anzick-1 is temporally coeval with the dated antler rods. This implies that the individual is indeed temporally associated with the Clovis assemblage.

AMS radiocarbon dating | First Americans | Clovis | Anzick | hydroxyproline

The timing and process of the initial peopling of the Americas have been, and continue to be, highly debated (1). Archaeological evidence suggests that humans first reached the American continent during the late Pleistocene through, or bordering, ancient Beringia (2, 3). In North America, evidence of early, widespread human settlement is found with the Clovis archaeological complex, recognized by eponymous and distinctive fluted bifacial points (4) and dated to 11,050–10,800 radiocarbon years B.P. [ref. 5; see Waters et al. (6) on the exclusion of earlier dates from the sites of El Fin del Mundo and Aubrey]. Although Clovis artifacts are widespread in the North American record (7), human remains associated with the Clovis complex are rare. The only known Clovis burial was found in the archaeological site of Anzick.

Anzick (24PA506) was accidentally discovered in 1968 near Wilsall, Montana, by construction workers. The partial remains of a male infant, Anzick-1, were found in association with an assemblage of over 100 Clovis lithic and osseous artifacts—all red-stained with ochre (8–11). The incomplete, unstained cranium of an unassociated, geologically younger individual (12, 13), Anzick-2, was also recovered. Paleogenomic data obtained for Anzick-1 suggest that this individual is (*i*) genetically closer to modern Native Americans than any other group, (*ii*) shares genetic information with the Upper Paleolithic Siberian Mal'ta population, and (*iii*) shows a closer affinity to Central and South American indigenous groups than northern counterparts—likely indicating a divergence in Native American populations that predates Anzick-1 (11). Considering the geographic location and antiquity of the burial, these findings hold important spatiotemporal implications that further add to the complexity of the peopling process and render Anzick one of the most important archaeological sites in First Americans research. However, the chronology of the site has a ^{14}C age discrepancy that puts its status as the only Clovis burial into question.

For the last two-and-a-half decades, the site of Anzick has been the subject of multiple chronometric investigations. These have produced radiocarbon dates obtained from different chemical fractions, for example, bulk collagen and "compound-specific" single amino acids, from Anzick-1, Anzick-2, and Clovis artifacts (two antler rods) found within the same archaeological context as Anzick-1 (5, 11–14). Although dates for Anzick-2 are consistent (*SI Appendix*, Table S1), Anzick-1 results show significant variation among different chemical fractions, with ages ranging from 8,690 ± 310 B.P. (10,575–9,005 cal B.P. at 95.4% confidence) on decalcified collagen (AA-313A), to 11,550 ± 60 B.P. (13,490–13,265 cal B.P. at 95.4% confidence) on 0.45-μm–filtered gelatin (CAMS-35912; Fig. 1). With the exception of AA-2979 (glutamic acid; 10,820 ± 100 B.P. or 12,960–12,565 cal B.P. at 95.4% confidence) and AA-2981 (glycine; 10,940 ± 90 B.P. or 13,020–12,700 at 95.4% confidence), most of the measurements for Anzick-1 fall outside the 95.4% confidence range of the antler rods, dated to 13,000–12,795 cal B.P. with good agreement (Fig. 1 and *SI Appendix*, Table S2). The temporal discrepancy between the younger Anzick-1 ages and the artifacts has caused much speculation, with some archaeologists questioning the confidence ascribed to their association and discounting Anzick-1 as a Clovis individual (15). The compound-specific dates that agree with the antler rods should not be dismissed, however. Produced from a more pure fraction than bulk collagen, these likely point to the presence of a modern-carbon contaminant that might have eluded collagen pretreatment and, in this

Significance

The site of Anzick contains the only known Clovis burial. As such, it presents a significant opportunity to explore biocultural processes attributed to a key prehistoric complex within First Americans research. Considering the site's uniqueness and the existing ^{14}C age discrepancy between the human remains (Anzick-1) and the associated Clovis assemblage, obtaining robust chronometric data for this site is crucial. Through the use of different pretreatment methods, this investigation has yielded a comprehensive chronometric dataset that shows, most relevantly, that Anzick-1 is temporally coeval with the Clovis artifacts found at the site.

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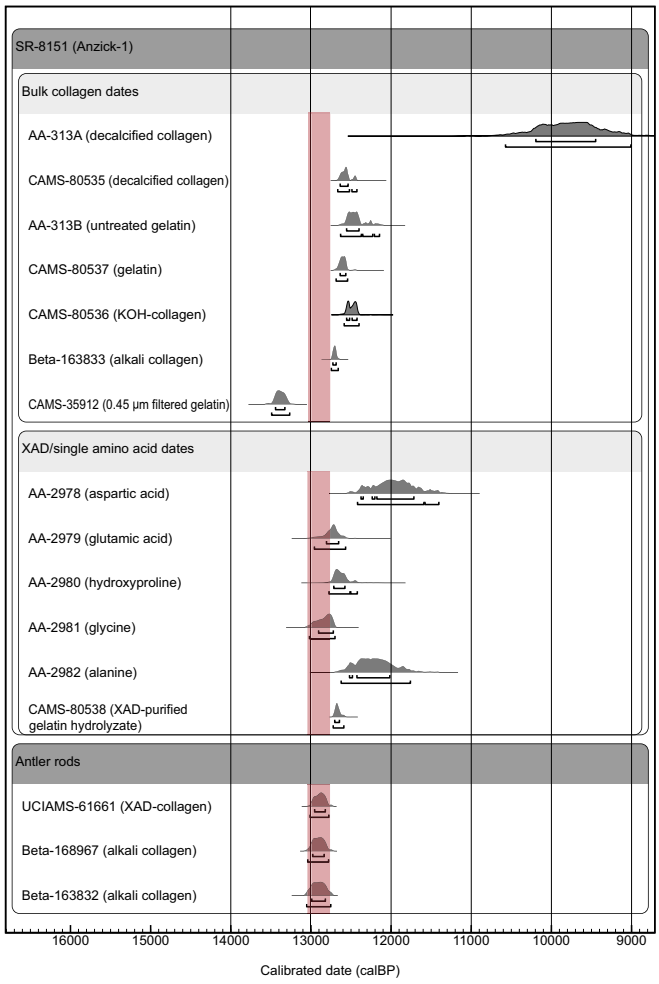

Fig. 1. Previously published radiocarbon dates (calibrated) for sample SR-8151 (Anzick-1) and two antler rods (SR-7599 and rod #118/11) found within the same burial context. Data were compiled from multiple publications (refs. 5 and 11–14; more detail in *SI Appendix*, Table S1). The red band illustrates how a majority of the radiocarbon results for Anzick-1, with the exception of AA-2981 and AA-2979, fall outside the 95.4% confidence range of the dates obtained for the Clovis antler rods. This might suggest that Anzick-1 and the material assemblage represented by the antler rod samples are not temporally coeval and, in turn, unassociated.

case, coeluted with other amino acids, for example, aspartic acid and alanine.

In radiocarbon dating, contamination can be a major source of error (16). Methodological improvements, however, have seen a significant effect in dating accuracy and reliability (17–27). Given this, and the variability shown by the Anzick-1 dataset, we sought to better define the chronology at the site by obtaining new radiocarbon measurements for Anzick-1, Anzick-2, and two Clovis bone artifacts using four different pretreatment procedures. This approach would allow for cross-examination and a more comprehensive assessment of the chronometric data. While three of the protocols center on the decontamination of bulk collagen and are routinely employed in radiocarbon laboratories, the fourth was optimized at the Oxford Radiocarbon Accelerator Unit (ORAU) and entails the extraction of a single amino acid, hydroxyproline (HYP), from bone collagen, using preparative high-performance liquid chromatography (prep-HPLC) (28). This protocol ensures sample purity through the complete removal of exogenous carbon, producing more accurate, robust results than alternative methods, particularly in the case of dating heavily contaminated bones (20, 25, 28–32).

Materials

Four osseous samples from the Anzick site were selected for accelerator mass spectrometry (AMS) radiocarbon dating. These include Anzick-1 (SR-8151), Anzick-2 (SR-8147), and two Clovis artifacts (antler rods; SR-7599 and SR-7602). Three of these (SR-8151, SR-8147, and SR-7599) were previously dated using different pretreatment methods (Fig. 1 and *SI Appendix*, Table S1).

Results

Anzick-1, Anzick-2, and two antler rods were AMS radiocarbon dated following Ezee-filtered collagen (AG), ultrafiltered collagen (AF), and nonroutine chemistry (NRC) protocols as described in *Methods* (Fig. 2 and Table 1). Results show that while measurements within the SR-7599, SR-7602, and SR-8147 datasets are generally consistent with each other, the NRC-02 (HYP) date for SR-8151 (Anzick-1), at 10,915 ± 50 B.P. or 12,905–12,695 cal B.P. at 95.4% confidence (OxA-X-2739-54), is considerably older than those produced using the other three methods and falls within the date range of the two antler rods at 95.4% confidence (*SI Appendix*, Table S2). Moreover, for all collagen samples, collagen yield, C:N, and %C values obtained are within the ORAU's accepted

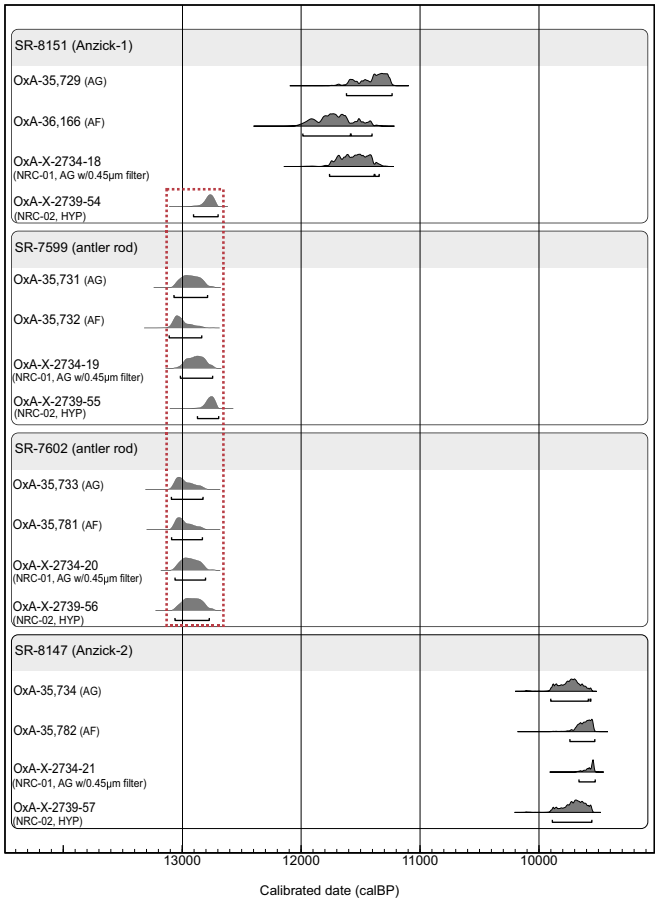

Fig. 2. Oxford-obtained AMS results (calibrated) for the site of Anzick, arranged according to sample (SR-8151, -7599, -7602, and -8147) and pretreatment protocol used (AG, AF, NRC-01, and NRC-02). This figure illustrates how the dates obtained for the Clovis assemblage (antler rods) and the HYP-derived measurement for Anzick-1 (OxA-2739-54), fall within the 95.4% confidence range of each other (red, dashed square).

Table 1. Radiocarbon determinations obtained for bones from the Anzick site at the ORAU

Sample no.	P-code	OxA-	%Yield	%C	C:N	δ^{13}C, ‰	δ^{15}N, ‰	Date, B.P.	±	Cal B.P. (95.4% confidence range)
SR-8151 (Anzick-1)	AG	35,729	13.7	32.8	3.5	−17.9	12.9	9,945	55	11,620–11,235
	AF	36,166	N/A	41.4	3.3	−17.3	12.1	10,110	55	11,990–11,400
	NRC-01 (AG with 0.45-μm filter)	X-2734-18	6.7	40.5	3.3	−17.6	11.4	10,045	40	11,765–11,340
	NRC-02 (HYP)	X-2739-54	N/A	34.7	5.4	−22.4	13.7	10,915	50	12,905–12,695
SR-7599 (antler rod)	AG	35,731	17.6	36.1	3.3	−19.3	2.8	11,065	55	13,070–12,785
	AF	35,732	N/A	41.4	3.2	−19.5	2.8	11,145	55	13,110–12,835
	NRC-01 (AG with 0.45-μm filter)	X-2734-19	12.1	42.3	3.2	−19.2	3.2	11,020	45	13,105–12,740
	NRC-02 (HYP)	X-2739-55	N/A	41.3	5.2	−21.2	3.3	10,900	50	12,875–12,690
SR-7602 (antler rod)	AG	35,733	21.0	32	3.4	−18.8	4.7	11,120	55	13,095–12,820
	AF	35,781	N/A	42.9	3.2	−19.1	3.3	11,120	50	13,090–12,830
	NRC-01 (AG with 0.45-μm filter)	X-2734-20	12.0	42.8	3.2	−18.1	4.0	11,070	45	13,060–12,800
	NRC-02 (HYP)	X-2739-56	N/A	31.2	5.4	−21.4	3.9	11,050	55	13,065–12,770
SR-8147 (Anzick-2)	AG	35,734	15.6	31.7	3.2	−18.7	10.6	8,750	40	9,905–9,560
	AF	35,782	N/A	43.9	3.2	−19.1	9.5	8,655	40	9,740–9,530
	NRC-01 (AG with 0.45-μm filter)	X-2734-21	9.0	42.5	3.2	−18.1	10.1	8,615	35	9,665–9,525
	NRC-02 (HYP)	X-2739-57	N/A	35.1	5.2	−23.2	11.4	8,730	45	9,890–9,555

The pretreatment code (P-code) denotes the preparative chemistry protocol used: AG, AF, NRC-01 (AG with 0.45-μm filter), and NRC-02 (HYP) (*Methods*). %Yield is the percent yield of extracted collagen as a function of the starting weight of the bone sample analyzed, %C is the percentage of carbon in the combusted sample, and C:N is the atomic weight ratio of carbon to nitrogen. AF and NRC-02 protocols were performed on a fraction of the same bone sample that was first treated as AG, so there are no collagen yield values for AF and NRC-02 methods. Stable isotope ratios of C and N are expressed in per mille relative to VPDB and AIR at a measurement precision of ±0.2 and 0.3‰, respectively. For all NRC-02 (HYP) dates, the background carbon derived from the HPLC separation has been subtracted using a correction calculation described in ref. 28. Calibration was done using the OxCal 4.3 software (34) and the IntCal13 calibration curve (35).

ranges—greater than 1% (weight), 2.9–3.5, and 30–50% (weight), respectively (33). These data indicate good collagen preservation and low levels of contamination. C:N values measured for all four NRC-02 (HYP) dates are slightly higher than the theoretical C:N value of HYP (5.0). Tests performed in our laboratory after the preparation of the Anzick material revealed that these higher values are likely due to the difference between the weight of the HYP samples—estimated based on the peak area measured during the HPLC separation (28)—and the weight of the internal standards (alanine) used during the elemental analysis, having no significant effect on AMS measurement and the dates obtained (*SI Appendix*, Table S3).

Discussion

The chronometric data obtained for samples SR-7599, SR-7602, and SR-8147, within each dataset, are in good agreement with each other and previously published results (*SI Appendix*, Table S2). This suggests that (*i*) the pretreatment protocols used in this and other dating efforts were equally efficient in removing contaminants from the material, thus producing comparable results with no discernible trend or pattern, or (*ii*) the samples were not significantly contaminated with exogenous carbon and therefore produced reliable results regardless of the method used to pretreat them. The Anzick-1 dataset, however, does not show the same consistency (*SI Appendix*, Table S2). The HYP date (OxA-X-2739-54), 10,915 ± 50 B.P. or 12,905–12,695 cal B.P. at 95.4% confidence, is considerably older than the AG, AF, and NRC-01 dates (Fig. 2 and Table 1), and agrees with previously published glutamic acid (AA-2979; 10,820 ± 100 B.P. or 12,960–12,565 cal B.P. at 95.4% confidence) and glycine (AA-2981; 10,940 ± 90 B.P. or 13,020–12,700 cal B.P. at 95.4% confidence) determinations (*SI Appendix*, Table S2). This suggests that the sample was likely contaminated with modern carbon that less rigorous collagen-treating protocols did not eliminate or detect—C:N and %C values were acceptable—just as previously published bulk collagen dates hinted (with the exception of CAMS-35912 at 11,550 ± 60 B.P. or 13,490–13,265 cal B.P. at 95.4% confidence, which was likely contaminated with ^{14}C-depleted carbon during pretreatment and considered highly anomalous). Because no more material remains (*Statement Regarding Legal and Ethical Issues*), identification of the contaminant through further analysis is impossible. As it stands, the HYP date suggests that Anzick-1 is temporally coeval with the antler rods measured. This implies that the individual is indeed associated with the Clovis artifact assemblage and dates within the Clovis period (5).

Conclusion

The site of Anzick contains the only known potential Clovis burial. The ^{14}C age discrepancy between the male infant, Anzick-1, and the Clovis assemblage found within the same archaeological context, has brought the association between the two into question. To solve this, we obtained multiple radiocarbon measurements for Anzick-1; Anzick-2, an unassociated, geologically younger individual; and two antler rods from the Clovis assemblage. Each sample was dated four times, following the use of different pretreatment methods: collagen filtration (AG), collagen ultrafiltration (AF), collagen filtration with a 0.45-μm syringe filter (NRC-01), and HYP extraction using prep-HPLC (NRC-02). This comprehensive approach showed that there is strong agreement between the Anzick-1 HYP date (10,915 ± 50 B.P. or 12,905–12,695 cal B.P. at 95.4% confidence; OxA-X-2739-54) and all those obtained for the antler rods (12,990–12,840 cal B.P. at 95.4% confidence). The results therefore suggest that Anzick-1 is temporally coeval with the antler rods, associated with the Clovis assemblage, and dates within the Clovis period (5).

Methods

All samples were processed at the ORAU, using four different preparative protocols. The first, coded AG, is a routine procedure that entailed the

decalcification, alkali wash, reacidification, gelatinization, and filtration of the bone sample using previously cleaned 9-mL Ezee filters (Elkay), per ref. 33. The second, coded AF, is a routine protocol that entailed the ultrafiltration (using Vivaspin 15- to 30-kDa MWCO) of the gelatin following Ezee filtration (33). The third, coded NRC-01, was the first of the nonroutine procedures and involved the use of nonsterile, single-use, disposable Millex syringe filters with a membrane porosity of 0.45 μm, instead of the routinely used Ezee filters (pore size of 45–90 μm) (36). The Millipore 0.45-μm syringe filters were flushed twice with MilliQ deionized water and once with 0.5 M hydrochloric acid before use. These particular filters were the same T.W.S. (CAMS and AA) used on his preparation of the material; keeping a higher degree of consistency between dating efforts. The fourth protocol, coded NRC-02, involved the separation of underivatized amino acids from ultrafiltered, hydrolyzed collagen using prep-HPLC, and the collection of HYP for dating. A full description of this protocol can be found in Deviese et al. (28). Collected collagen and HYP samples were dried, combusted, graphitized, and AMS-dated as per Brock et al. (33).

Statement Regarding Legal and Ethical Issues

Ethical and legal issues surround research of Native American human remains in the United States. The Anzick site was discovered on private land and the human remains recovered have not been under the control of a federally funded museum or federal agency, and thus the Native American Graves Protection and Repatriation Act does not apply. Under Montana state law, unmarked human burials are not considered abandoned. Advice provided to the project by members of the Montana State Burial Board, however, confirmed that because no claimant has made a request for the remains, the human remains from the Anzick site remain under the control of the landowners, the Anzick family. However, to ensure that Native American concerns were addressed, in 2013, Native American groups with reservations in the surrounding area of the Anzick site were informed about our work. Our research included the sampling of the Anzick-1 and Anzick-2 human remains for ancient DNA (aDNA) studies and radiocarbon dating. Samples collected for aDNA analysis went to the Center for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen (Copenhagen, Denmark). Samples for radiocarbon analysis were sent to the ORAU, University of Oxford (Oxford, United Kingdom). The aDNA study was published in 2014 (11). The purpose of the radiocarbon dating was to resolve discrepancies between previously reported ages for the human remains and the Clovis artifacts using a technique known as specific–amino-acid dating. These samples were delayed in processing due to technical and personnel issues at Oxford and were processed in 2017, with the results reported here. All samples supplied to the ORAU have been consumed. With the support of the Anzick family and the research team, all human remains of Anzick-1 and Anzick-2 were reburied during a Native American ceremony in June 2014.

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