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DNA from Pre-Clovis Human Coprolites in Oregon, North America

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The timing of the first human migration into the Americas and its relation to the appearance of the Clovis technological complex in North America ca. 11-10.8 thousand radiocarbon years before present (^{14}C ka B.P.) remains contentious. We establish that humans were present at Paisley 5 Mile Point Caves, south-central Oregon, by 12,300 ^{14}C yr. B.P., through recovery of human mtDNA from coprolites, directly dated by accelerator mass spectrometry. The mtDNA corresponds to Native American founding haplogroups A2 and B2. The dates of the coprolites are >1000 ^{14}C years earlier than currently accepted dates for the Clovis-complex.

The timing, route, and origin of the first human migration into the Americas remain uncertain. Some archaeological (*1*) and genetic [reviewed by (*2*)] evidence has been used to argue for a settlement by 30 ka (calendar) or even earlier, but both lines of evidence remain controversial. The most widely accepted dates of occupation relate to the Clovis complex, ca. 11-10.8 ^{14}C ka B.P. (~12.9-12.8 ka), a distinct technology that appears to have originated and spread throughout North America in as little as 200 to 300 years (*3*).

The oldest directly dated human osteological remains from the Americas are no more than 11,000 ^{14}C yr. B.P. (~12.9 ka) *(3, 4)* and appear to be congruent with the "Clovis-first" model for colonization (*5,6*). However, this theory is complicated by Monte Verde, in southern Chile, that contains artifacts dated to ~12,000 ^{14}C yr. B.P. (~13.9-13.8 ka), that exhibit little technological connection to Clovis (*7*). Although a number of pre-Clovis occupation sites have been reported from North America (e.g. *8*), their age and cultural origins remain controversial, primarily due to the lack of directly dated human remains or artifacts (*9*).

Here, we present evidence for human presence in North America prior to the Clovis-complex, through the identification and genetic profiling of coprolites directly dated to 12,300 ^{14}C yr. B.P. (~14.27-14.0 ka) at the Paisley 5 Mile Point Caves in south-central Oregon (Fig. 1A). The Paisley Caves are wave-cut shelters located on the highest shoreline of Pluvial Lake Chewaucan, which once filled the Summer Lake-Chewaucan-Lake Abert basins (Fig. 1A). As the lake level fell since the LGM (*10*, *11*), the caves began filling with Aeolian transported silt and sand, gravel, roof spall, and organic material (bones, coprolites, plant remains, and artifacts) deposited by humans and animals. Sheltered from moisture, these extremely dry deposits contain perishable human artifacts; manufactured threads of sinew and plant fibers, hide, basketry, cordage, rope, and wooden pegs as well as animal bones and diverse kinds of feces, in an unbroken stratigraphic sequence spanning the late Pleistocene and Holocene (*12*). Stone tool and debitage assemblages are small, suggesting that site occupations were generally brief. Pleistocene assemblages contain few chronologically diagnostic artifacts. The few projectile point fragments recovered are morphologically consistent with lanceolate, Western Stemmed, and foliate types common in the Younger Dryas (10.2-10.7 ^{14}C ka B.P.) and early Holocene archaeological sites of the region. However, stratigraphic distribution of artifacts and radiocarbon dating of likely

butchered bones suggests that the site may have been occupied as early as 12.4 and 12.0 ^{14}C ka B.P.

Fourteen coprolites recovered from the lowest levels of Paisley Caves are morphologically human (based on size, shape, constituents, and color). In initial screening using a multiplex PCR and minisequencing assay, all 14 were positive for human mtDNA (*13*). This result is not surprising as the coprolites had not been excavated under sterile conditions, and could be expected to contain DNA derived from the excavation team (*14*, *15*). Although all of the coprolites contained single nucleotide polymorphisms (SNPs) diagnostic to European populations (consistent with contamination), independently generated cloned and pyrosequenced PCR products from six samples reproducibly yielded SNPs diagnostic to Native American founding mtDNA haplogroups (Hgs) A2 and B2, (Table 1, supporting online text, fig. S1, tables S1, S5, S7, S9) (*16*). The absence of PCR amplifiable Native American mtDNA in the remaining 8 samples may be due to differences in DNA survival across specimens, the possibility that these coprolites are non-human, or more likely, that the ratio of contaminant to endogenous DNA was too great for endogenous mtDNA detection using our techniques.

Human ancient DNA studies are extremely prone to contamination that may occur during sampling or subsequent laboratory analyses (*14*, *15*). To exclude laboratory-based contamination, the Hgs A and B results were independently confirmed using several different DNA typing and sequencing methods (*13*), in multiple laboratories (Copenhagen Denmark, Uppsala Sweden and Leipzig Germany) (Table 1). Furthermore, to ensure that the Hgs A and B results are not the result of contamination during the excavation, we mitotyped the 55 individuals (students, instructors, and visitors) that had been present at the site during the two-season excavation (*13*). Additionally, we mitotyped the 12 researchers present in the principal aDNA laboratory (*13*). This was performed regardless of whether an individual played an active role in the excavation or in the genetic analysis of the coprolites. For example, although 55 people had been present at the archaeological site, only 14 were actively involved in the excavation of the six coprolites reported here, and only two of these could have come in contact with all six specimens. The results show that none of the individuals tested are the sources of the Hgs A and B mtDNA (*13*) (table S4). As the coprolites had been stored in sealed plastic bags from the time of excavation until the genetic analyses were undertaken, it is difficult to explain the results by other sources of post-excavation contamination. To provide additional confirmation for a human origin of the coprolites, we submitted part of the four oldest samples (as material allowed) for protein residue (cross-over immunoelectrophoresis) and reconstitution analyses (trisodium phosphate solution analyses) (*13*). The results of these tests confirmed the results of the morphological and genetic analyses that the coprolites are of human origin (Table 1, supporting online text).

Leaching of DNA from younger to older stratigraphic layers may be a problem in relatively wet, temperate cave settings (*17*). Although it is unlikely that leaching would be able to provide cross-over immunoelectrophoresis false positives, due to the relative large amounts of proteins needed, we conducted two additional tests for DNA leaching. Wood rat (*Neotoma lepida*) fecal pellets are a major constituent of the strata in the Paisley 5 Mile Point Caves, and were found in direct contact with the six coprolites in question (table S9). Thus, we screened the six coprolites for *Neotoma* mtDNA (*13*). To account for any possible differences in *Neotoma* species inhabiting the caves in the past, we used primers designed to amplify mtDNA from all members of the genus. Control *Neotoma* fecal samples from the caves gave positive results for *Neotoma* DNA (table S6). However, all six coprolites testing positive for human DNA tested negative for *Neotoma* DNA. We additionally screened 14 control sediment samples and two long bones morphologically identified as *Spermophilus lateralis* (golden-mantled ground squirrel) recovered from around the coprolites, for the Hg A and B SNPs (*13*) (table S6). Although eight out of the 16 samples were positive for human mtDNA (presumably derived from the excavation team), none of the samples were positive for the Native American SNPs (table S6). Additionally, primers specific for *S. lateralis* gave positive results for the two long-bones, demonstrating that DNA survived in non-coprolite specimens from the Paisley Caves for long times also. The results strongly suggest that leaching of DNA is not a concern in the Paisley Caves, and are in agreement with empirical and theoretical evidence suggesting that significant amounts of liquid water are required to move free DNA molecules between strata (*17*, *18).*

Three of the six coprolites also contained canid 16S mtDNA with high similarity to red fox (*Vulpes vulpes*, 1 substitution difference), coyote (*Canis latrans*, 1 substitution difference) or domestic dog or wolf (*Canis familiaris* or *Canis lupus*, 100% match) (Table 1) (*13*). In light of the non-genetic tests showing a human origin of the coprolites and the findings of diverse canid bones in the strata, the most likely explanations for these results are that humans may have eaten canids, or that canids living in the caves during periods of non-human occupation urinated directly onto human feces.

Accelerator mass spectrometry (AMS) radiocarbon dating of bone collagen from a camelid astragalus, recovered in stratigraphic association (Stratum LU1b) with three of the oldest human coprolites, produced an age of 12,300 ^{14}C yr. B.P. (Fig. 1B). To ensure reliable ages of the coprolites, the

five specimens from the deepest layers were submitted for direct dating by accelerator mass spectrometry (AMS) at two independent laboratories; Beta Analytic Inc. (Florida, USA), and the Oxford Radiocarbon Accelerator Unit (University of Oxford, UK). Although each laboratory used different methodologies (*13*), all specimens except one (sample 1294-PC-5/6B-40), produced consistent dates, ranging from approximately 1300 to 12,300 ^{14}C yr. B.P. and three of the coprolites pre-dating 11,000 ^{14}C yr. B.P. (Table 1 and Fig. 2). Thus our data show that humans were present in North America before the Clovis-complex. Analyses of complete Native American mtDNA genomes implies that the origin of Hgs A2 and B2 originated at 13.9±2.0 and 16.5±2.7 ka, respectively (*16*). The coprolites at Paisley may thus derive from among the earliest members of these haplogroups. The Paisley Caves lacks lithic tool assemblages, thus the cultural and technological association of the early site occupants, and their relationship to the later Clovis technology, are uncertain.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1154116/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Tables S1 to S9
Cloned DNA Sequence Alignments

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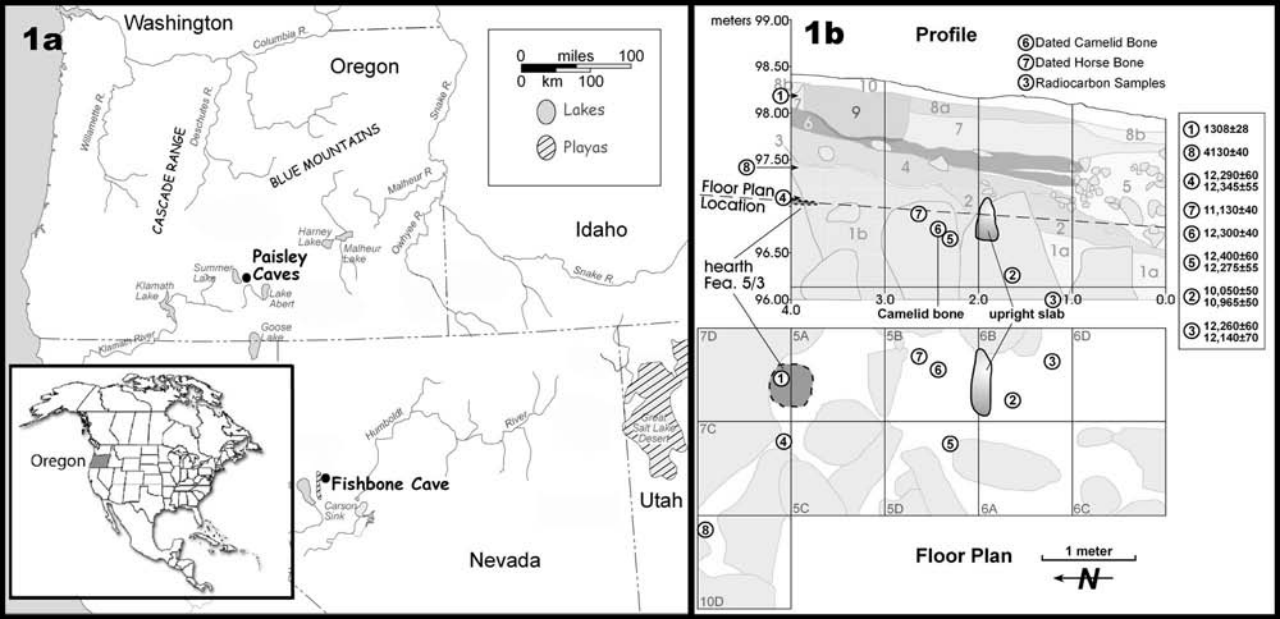
Fig. 1. Geographical and stereographical information of Paisley 5 Mile Point Caves. (**A**) The location of Paisley 5 Mile Point Caves in Oregon and location of Oregon in the United States (insert). (**B**) Horizontal, vertical, and stratigraphic distribution of five of the human coprolites. Sample 1374-PC-1/2A-28 (Table 1) was excavated from another cave (Cave 1) thus is not shown. Also indicated are dated camelid astragalus, horse phalange, and sample 8, a coprolite found out of context indicating some stratigraphic disturbance. For further details see supporting online text.

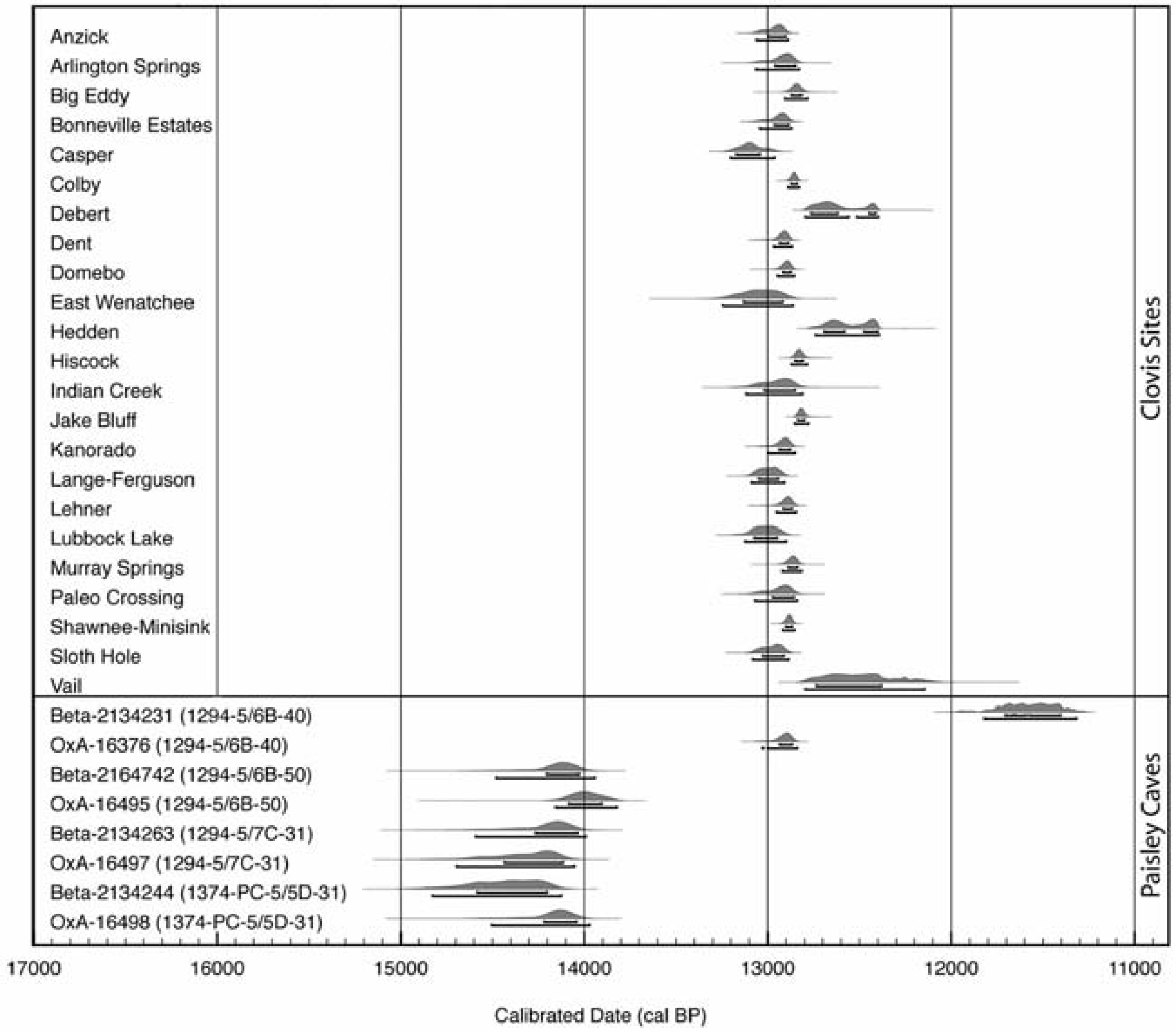
Fig. 2. Calibrated radiocarbon determinations from the four oldest coprolites excavated at Paisley 5 Mile Point Caves obtained using INTCAL04 (*19*) and the OxCal4.0 software (*20*). Mean dates for Clovis sites reported by Waters and Stafford (*3*) are also included for comparison. The calibrated results for Paisley Caves shows that the oldest are ~1000 ^{14}C years older than the earliest Clovis dates.

Table 1. Results of mitochondrial DNA, non-genetic analysis, and AMS dating of the six coprolites identified as of Native American origin.

Sample	mtDNA			AMS dates (conventional ^{14}C years BP)		Cave	Fig. 1B[g]	CIE[h]	TP[i]
	Hg	16S[e]	Site of replication	Beta Analytic Inc.	Oxford University				
1294-PC-5/7D-4	B2[a]	*C. latrans*	Uppsala	Not tested	1,308 ± 28	5	1	-	n/a
1374-PC-1/2A-28	B2[b]		Uppsala	6,640 ± 40	6,608 ± 35	1		-	n/a
1294-PC-5/6B-40	B2[b]	*C. lupus/ familiaris*[f]	Uppsala	10,050 ± 50	10,965 ± 50	5	2	Human	n/a
1294-PC-5/6B-50	A2[c]	*V. vulpes*	Uppsala	12,260 ± 60	12,140 ± 70	5	3	Human	Human
1294-PC-5/7C-31	B[d,j]		Uppsala/ Leipzig	12,290 ± 60	12,345 ± 55	5	4	Human	Human
1374-PC-5/5D-31[k]	B2[b]		Uppsala	12,400 ± 60	12,275 ± 55	5	5	-	Human

Mitochondrial DNA haplogroups (Hgs) identified using different techniques across laboratories: [a]Copenhagen SNaPshot, Uppsala Pyrosequenced, Uppsala cloned, [b]Copenhagen SNaPshot, Copenhagen cloned, Uppsala Pyrosequenced, Uppsala cloned and sequenced, [c]Copenhagen SNaPshot, Uppsala Pyrosequenced,[d]Copenhagen SNaPshot, Uppsala Pyrosequenced, Leipzig cloned and sequenced. While Hgs A and B is based on independently replicated results sub-Hgs A2 and B2 is in general based on single laboratory analyses. For details see supporting online text. [e]Canid sequences detected using generic mammalian 16S mtDNA primers. [f]Sequences are indistinguishable over genetic marker. [g]Sample identification in Fig. 1B. [h]Result of cross-over immunoelectrophoresis analysis. (-) no result recovered due to poor protein preservation in sample (see supporting online text for more discussion). [i]Result following reconstitution in trisodium phosphate solution. (n/a) sample not assessed. [j]Insufficient DNA was available to further resolving the haplogroup. [k]Fig. S1.


1a
Washington
Oregon
Idaho
Nevada
Utah
Columbia R.
Willamette R.
Deschutes R.
Snake R.
CASCADE RANGE
BLUE MOUNTAINS
Malheur R.
Owyhee R.
Harney Lake
Malheur Lake
Summer Lake
Paisley Caves
Lake Abert
Klamath Lake
Klamath River
Goose Lake
Humboldt River
Great Salt Lake Desert
Fishbone Cave
Carson Sink
0 miles 100
0 km 100
Lakes
Playas
Oregon
1b
Profile
meters 99.00
98.50
98.00
97.50
96.50
96.00
4.0
3.0
2.0
1.0
0.0
Camelid bone
upright slab
Floor Plan Location
hearth Fea. 5/3
6 Dated Camelid Bone
7 Dated Horse Bone
3 Radiocarbon Samples
1 1308±28
8 4130±40
4 12,290±60 12,345±55
7 11,130±40
6 12,300±40
5 12,400±60 12,275±55
2 10,050±50 10,965±50
3 12,260±60 12,140±70
7D
5A
5B
6B
6D
7C
5C
5D
6A
6C
10D
Floor Plan
1 meter
N


Anzick
Arlington Springs
Big Eddy
Bonneville Estates
Casper
Colby
Debert
Dent
Domebo
East Wenatchee
Hedden
Hiscock
Indian Creek
Jake Bluff
Kanorado
Lange-Ferguson
Lehner
Lubbock Lake
Murray Springs
Paleo Crossing
Shawnee-Minisink
Sloth Hole
Vail
Clovis Sites
Beta-2134231 (1294-5/6B-40)
OxA-16376 (1294-5/6B-40)
Beta-2164742 (1294-5/6B-50)
OxA-16495 (1294-5/6B-50)
Beta-2134263 (1294-5/7C-31)
OxA-16497 (1294-5/7C-31)
Beta-2134244 (1374-PC-5/5D-31)
OxA-16498 (1374-PC-5/5D-31)
Paisley Caves
17000
16000
15000
14000
13000
12000
11000
Calibrated Date (cal BP)