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## The genome of a late Pleistocene human from a Clovis burial site in western Montana

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### Author statement

Acknowledging the complex ethical and legal issues surrounding the research of Native American human remains in the United States, we have striven not only to comply with federal and state law but also to proactively consult with Native American tribes. The Anzick burial site was discovered on private land and the remains recovered have not been in control of a federally funded museum or federal agency, and thus the Native American Graves Protection and Repatriation Act (NAGPRA) 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 since no claimant has made a request for the remains, the human remains from the Anzick burial site remain under the control of the landowners, the Anzick family. However, to ensure that Native American concerns were addressed, we have informed nine Native American groups with reservations in the surrounding area of the Anzick site about our work. EW and SD visited in person the cultural representatives of the Northern Cheyenne, Crow, Black Feet and Salish and Kootenai tribes. SD was in direct contact with the Lakota, Rocky Boys, Assiniboine, Gros Ventre and Chippewa cultural representatives. We received no objections to our research from these groups; however, many of the tribal representatives requested reburial of the remains. The Anzick family is working with Crow member, SD, towards an intertribal reburial ceremony, with the Crow acting as the lead tribe in these efforts.

### Author contributions

EW, SLA, MW and TWS conceived the project. EW headed the project. EW and MR designed the research project setup. RN supervised the bioinformatical and population genetic analyses. SMD helped with ethics and contact to local communities. SLA and MW provided access to the Anzick-1 sample and the archaeological context, with input from SSW and DM. TWS performed AMS <sup>14</sup>C dating, stable isotope analyses and provided geochemical and geochronological assessments. SLA and JS performed initial mtDNA screening experiments. Elk extracts were processed by PDH and IB. Ancient DNA extractions and library constructs for shotgun sequencing, preparation for sequencing was done by MR. OEC prepared the two modern genomes. MR and SR did initial bioinformatics and mapping of the ancient sample. Mapping of modern samples, and genotype calls was done by SR, with input from TSK. SR, TSP and SB provided super computing resources. OEC and SR did phasing and ancestry painting. MA and MC did half-life estimates, with input on geology from TWS. DNA damage patterns were done by MR and LO. mtDNA consensus and damage estimate was done by ASM. IM and AA performed the X chromosome contamination estimates, error rate estimates and ABBA-BABA analyses. GDP did chrY analysis. MaM did ADMIXTURE analysis. KT, MK and MaM did mtDNA characterization. PS did *f*<sub>3</sub>-statistics on SNP array data and tested Native American population models using *D*-statistics. MD performed *TreeMix* analysis, and genome-wide *f*<sub>3</sub>-statistics. RN and MD developed and implemented the ancestry test. MR, SLA, MW, PS, MD, RN and EW wrote the majority of the manuscript with critical input from TWS, MA, ASM, SR, IM, AA, GDP, KT, MaM, RG, VG, RY, PDH, OEC, MC, FB, AM and the remaining authors.

### Author Information

Sequence data (fastq files) for Anzick-1 is available for download through NCBI SRA accession number SRX381032. Additionally, alignments and genotype calls are available for download, <http://www.cbs.dtu.dk/suppl/clovis/>. Raw reads (fastq files) and alignments (BAM files) for the two modern genomes sequenced in this study are available for demographic research under data access agreement with E.W. (ewillerslev@snm.ku.dk). The *Cervus elaphus* sequences are available under GenBank accession KF906070, KF906071 and KF906072.

The authors declare no competing financial interests.

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## Abstract

Clovis, with its distinctive biface, blade and osseous technologies, is the oldest widespread archaeological complex defined in North America, dating from 11,100 to 10,700 <sup>14</sup>C years BP (13,000 to 12,600 calendar years BP)<sup>1,2</sup>. Nearly fifty years of archaeological research point to the Clovis complex as having developed south of the North American ice sheets from an ancestral technology<sup>3</sup>. However, both the origins and genetic legacy of the people who manufactured Clovis tools remain debated. It is argued that these people ultimately derived from Asia and were directly related to contemporary Native Americans<sup>2</sup>. An alternative, Solutrean, hypothesis posits that the Clovis predecessors immigrated from Southwestern Europe during the Last Glacial Maximum (LGM)<sup>4</sup>. Here, we report the genome sequence of a male infant (Anzick-1) recovered from the Anzick burial site in western Montana. The human bones date to 10,705±35 <sup>14</sup>C years BP (CAMS-80538; *c.* 12,707–12,556 calendar years BP) and were directly associated with Clovis tools. We sequenced the genome to an average depth of 14.4× and show that the gene flow from the Siberian Upper Palaeolithic Mal'ta individual<sup>5</sup> into Native American ancestors is also shared by the Anzick-1 individual and thus happened prior to 12,600 years BP. We also show that the Anzick-1 individual is more closely related to all indigenous American populations than to any other group. Our data are compatible with the hypothesis that Anzick-1 belonged to a population directly ancestral to many contemporary Native Americans. Finally, we find evidence of a deep divergence in Native American populations that pre-dates the Anzick-1 individual.

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The only known Clovis burial and associated mortuary assemblage was found in the Americas at the Anzick site, Montana, in 1968<sup>6–8</sup> (Figure 1a,b). Here, *c.* 100 stone tools and 15 osseous tool fragments (Figure 1d,e) (at least one made of elk; SI3.4) that are technologically consistent with artefacts of the Clovis complex<sup>9,10</sup> were found in direct association with the partial fragmentary remains of an infant child (Anzick-1). The human remains were found directly below the Clovis artefacts and were covered with red ochre. Bone from the skeleton was directly dated using XAD-collagen to 10,705±35 <sup>14</sup>C years BP (CAMS-80538) or 12,707–12,556 calendar years BP, close to the end of the Clovis time period<sup>1,7</sup> (Figure 1c).

Initial genetic screening of the Anzick-1 skeletal remains using PCR coupled with cloning and Sanger sequencing yielded a mtDNA haplogroup assignment of D4h3a (SI3). The D4h3a haplogroup was verified and further characterized in the subsequent shotgun sequencing of Anzick-1 (SI12, Extended Data Figure 2). D4h3a is one of the rare mtDNA lineages specific to Native Americans, it is distributed along the Pacific coast in North and South America among contemporary populations<sup>11</sup> and is also present in ancient specimens<sup>12</sup>. Its current distribution has been interpreted as evidence for an early coastal migration route<sup>11</sup>. Our findings of this mtDNA haplogroup inland in the oldest skeleton from the Americas mtDNA typed to date question such interpretation and underscores that current distributions of genetic markers are not necessarily indicative of the movement or distribution of peoples in the past<sup>3</sup>. The Anzick-1 D4h3a does not carry any of the polymorphisms that define the several sub-groups of the haplogroup<sup>13</sup> and is thus placed at the root of D4h3a. Our finding implies that the origin of the D4h3a branch is likely to be at the upper bound of the previously obtained estimate of 13,000±2600 calendar years BP<sup>14</sup>, or possibly even older.

Shotgun sequencing of the Anzick-1 DNA revealed a highly variable endogenous human DNA content across different extracts, even those recovered from the same bone fragment (0.5%–28.2%, SI4, Extended Data Table 3). This suggests significant influence of microenvironments in long-term DNA preservation across the Anzick-1 bone fragments<sup>15</sup>. We obtained an average sequencing depth of 14.4× coverage. Based on the observed DNA fragment length distribution, we computed molecular half-life estimates<sup>16</sup> for a 100-bp fragment to be 4,852 and 5,137 years for nuDNA and mtDNA, respectively. These are consistent with predictions based on estimated burial temperature and geographical location (SI7). Nucleotide misincorporation patterns were consistent with increased *post-mortem* cytosine deamination at DNA fragment termini<sup>17</sup> (SI8). The mtDNA data provided a contamination estimate with a 95% credibility interval of 0.15%–0.38%<sup>18</sup> (SI9). As the Anzick-1 individual was found to be male, we were also able to quantify contamination levels based on the heterozygosity levels seen on the X chromosome<sup>19</sup>, which resulted in similarly low contamination estimates (<1.2%, SI11). Characterisation of the error rate yielded an overall estimate of 0.84% (SI10). Per-library error rates as well as nucleotide type-specific error rates are shown in Extended Data Figure 1c; as expected, the error estimates are driven by deamination related DNA-damage errors.

We determined the Y-chromosome haplogroup to be Q-L54\*(xM3) and, along with 15 previously analysed Y-chromosome sequences<sup>20</sup>, we constructed a tree to illustrate the phylogenetic context within haplogroup Q (SI13, Extended Data Figure 2). Confining our analyses to transversion SNPs, we leveraged the date of Anzick-1 to estimate a divergence time between haplogroups Q-L54\*(xM3) and Q-M3, two of the major founding Y-chromosome lineages of the Americas, of *c.* 16,900 years ago (95%: 13,000–19,700, SI13).

We assessed the genome-wide genetic affinity of the Anzick-1 individual to 143 contemporary non-African human populations by computing outgroup  $f_3$ -statistics<sup>21</sup>, which are informative on the amount of shared genetic drift between an individual and other populations. The data set included 52 Native American populations, for which genomic segments derived from recent European and African admixture have been excluded<sup>22</sup>. We

found that the Anzick-1 individual showed a significantly closer affinity to all 52 Native American groups than to any extant Eurasian population (Figure 2). The same conclusion was reached from admixture clustering analyses (SI14, Extended Data Figures 3), and when using  $D$ -statistics based on genomic sequence data (SI16).

Interestingly, the Anzick-1 individual showed less shared genetic history with seven Northern Native Americans from Canada and the Arctic, including three Northern Amerind-speaking groups (“NA” group), and the Central American Yaqui (SI15, Figure 2), than with 44 Native populations from Central and South America (“SA” group) (Figure 2). This finding was further supported by allele frequency based  $D$ -statistics, which strongly rejects the hypothesis of a population tree with topology [Anzick-1, [NA, SA]]. However, a tree assuming a branching pattern of [NA, [Anzick-1, SA]] could not be rejected (SI15, Figure 3).

We investigated two possible scenarios that could explain this pattern: (*i*) a basal diversification of the NA and SA lineages that pre-dates the Clovis period, with the Anzick-1 individual belonging to the SA lineage (Figure 3c). (*ii*) the Anzick-1 individual is basal to both lineages, but the lineages diverged at a time close to *c.* 12,600 years ago, and the NA group received subsequent gene-flow from other, perhaps Siberian, populations (Figure 3b). This latter hypothesis of additional pulses of gene flow has been suggested for the four Na-Dene and Eskimo-Aleut-speaking groups by a previous study, whereas the three Northern Amerind groups and Yaqui have been found to carry exclusively ‘First American’ ancestry<sup>22</sup>.

We analysed 19 different published Siberian populations and found that allele frequency based  $D$ -statistics in all cases, with the exclusion of Naukan, were compatible with a diversification pattern of [Siberian, [NA, SA]], with no evidence for gene-flow into the NA group (SI15), a pattern that agrees with previously published results<sup>22</sup>. This suggests that model (*i*) is more likely, and that the structure between Native American lineages pre-dates the Anzick-1 individual and thus appears to go back to pre-Clovis times.

We used outgroup  $f_3$ -statistics to evaluate the shared genetic history between all Native American populations and the Anzick-1 genome, the 24,000-year-old human from Mal’ta, Siberia<sup>5</sup> and the 4,000-year-old Saqqaq Palaeo-Eskimo from Greenland<sup>19</sup>. We again found a closer relationship between Anzick-1 and all Native Americans (SI15, Extended Data Figure 5). Together with the fact that Anzick-1 shows the same relative affinity to Western- and Eastern Eurasians, this suggests that the gene-flow from the Mal’ta lineage into Native Americans happened before the NA and SA groups diverged.

Next, we addressed the relationship of the Anzick-1 genome to whole-genome sequences from contemporary humans, including two novel genomes from Karitiana and Mayan individuals, and from the ancient Saqqaq sample<sup>19</sup>. To explore the question of direct ancestry, we developed a novel method to assess the amount of genetic drift experienced by each of a pair of populations after their divergence, without making any assumptions about demography (SI17). If the Anzick-1 individual belonged to a population directly ancestral to a sampled contemporary population, the amount of genetic drift on the Anzick-1 branch

should be zero, as it would have experienced no genetic drift since the “divergence” with the sampled population. We find that the data are compatible with the Anzick-1 individual belonging to a population that is directly ancestral to the two South American Karitiana samples, as is the case for the Mayan, after masking the latter for recent European admixture (Figure 4a,b). In contrast, there is a positive amount of drift on both lineages when comparing Anzick-1 to Saqqaq, Europeans, Asians and Pacific Islanders. This shows that the Anzick-1 sample has diverged from populations from outside the Americas. Furthermore, in agreement with the SNP analyses, *TreeMix*<sup>23</sup> (Figures 4c and S18) and outgroup  $f_3$ -analyses<sup>5,21</sup> (Extended Data Figure 5 and 6), the Anzick-1 sample is genetically more closely related to Central and South Americans than to any other populations, including the Saqqaq individual from Greenland. After masking the Mayan genome for recent European admixture, *TreeMix* places the Anzick-1 individual in a position in the tree compatible with the hypothesis that it is ancestral to both Mayan and Karitiana, with Anzick-1 exhibiting virtually no drift on its branch since its divergence from other populations (Figure 4c).

We conclude that the male infant, buried *c.*12,600 years ago with ochre-covered Clovis artefacts at the Anzick site, belonged to a meta-population from which many contemporary Native Americans are descended and is closely related to all indigenous American populations. As such, contemporary Native Americans are effectively direct descendants of the people who made and used Clovis tools and buried this child. In agreement with previous archaeological and genetic studies<sup>24,25</sup>, our genome analysis refutes the possibility that Clovis originated via a European (Solutrean) migration to the Americas<sup>4</sup>. Furthermore, the Anzick-1 findings do not support the hypothesis, based on cranial data, that American populations around the time of Clovis were subsequently assimilated by more recent migrants who were the ancestors of contemporary Native Americans<sup>26,27</sup>. The Anzick-1 data thus serves to unify the genetic and archaeological records of early North America, it is consistent with a human occupation of the Americas a few thousand years before Clovis,<sup>2,3,28</sup> and demonstrates that contemporary Native Americans are descendants of the first people to successfully settle the Americas. Our results are also consistent with previous models derived from mtDNA, which imply that Native American populations primarily derive from a single source population, but that there was a secondary movement into northern North America<sup>29</sup>. However, several different scenarios are compatible with an early divergence of the NA and SA groups and analyses of more ancient human remains are needed to further test the findings and interpretations from this single individual and to elucidate the complex colonization history of the Arctic and North American populations.

## Methods Summary

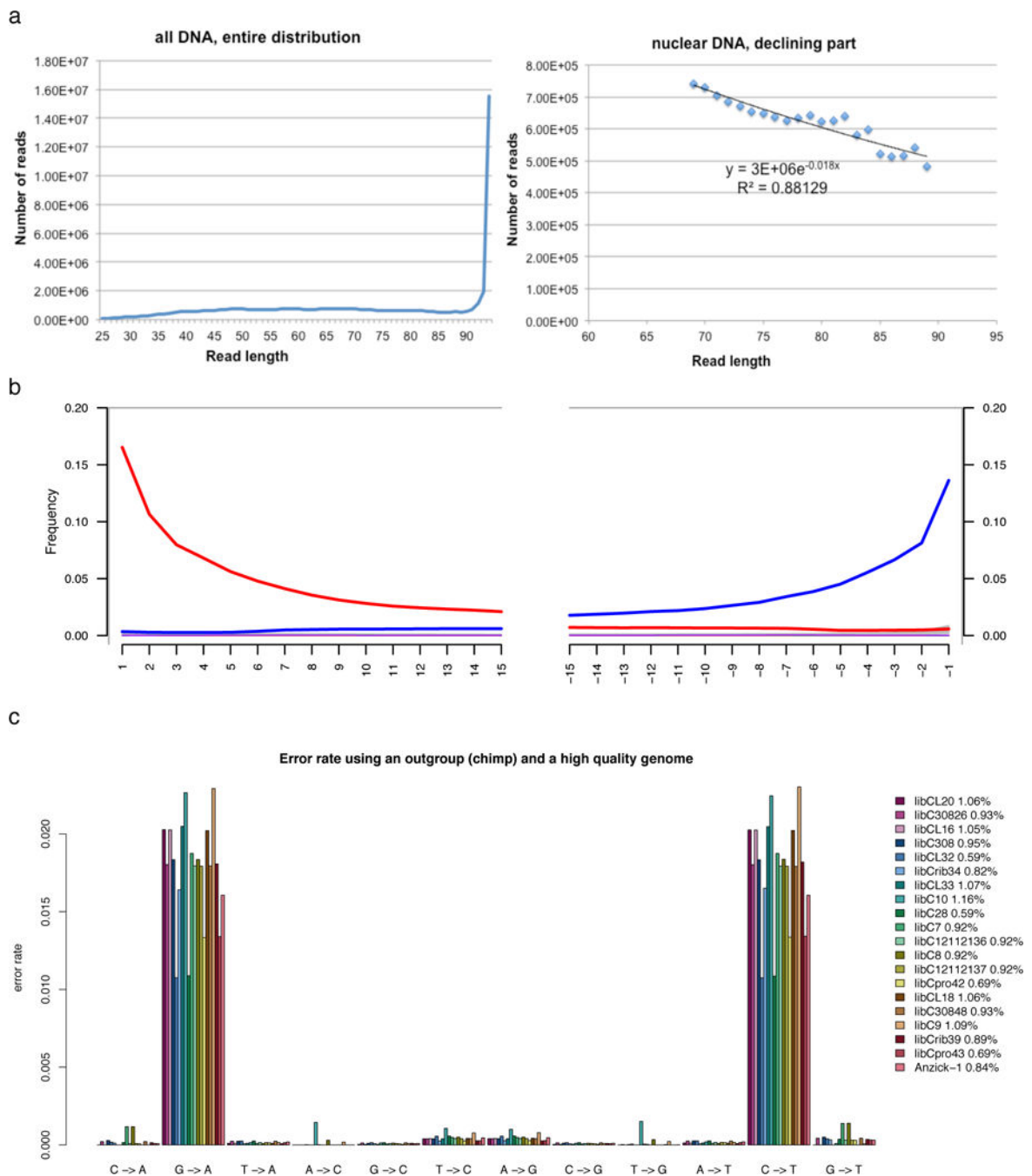
We sampled bone fragments from Anzick-1 skeleton, from the Anzick site in Montana, for ancient DNA and <sup>14</sup>C dating analyses (SI1-2). From DNA extracts we generated Illumina sequencing libraries, which were sequenced on the Illumina HiSeq platform (SI3-4). We verified the authenticity of the ancient DNA through damage patterns and decay rates (SI7-8), and monitored contamination using analyses of both mtDNA and nuclear DNA (SI9-10). To investigate the affinity of the Anzick-1 individual to worldwide modern-day populations we computed outgroup  $f_3$ -statistics<sup>5,21</sup> using a genome-wide SNP data set of 143 groups collected from multiple studies (SI15). To test historical models relating the



Anzick individual to modern-day Native Americans from Northern group (Cree, Ojibwa, and Algonquin) and a Southern group (44 Central- and South American groups) we compute  $D$ -statistics<sup>21</sup> using the full allele frequency information (SI15). To test if the Anzick-1 individual could have belonged to a population ancestral to some of the modern populations, we developed a new Maximum Likelihood (ML) method for estimating the length of the branches of a drift tree with two populations (leaves in the tree) represented by two genomes. The method fits a model with the following parameters: the probability of pairwise coalescence within population 1 ( $c_1$ ), the probability of pairwise coalescence within population 2 ( $c_2$ ), and the probabilities of genotype patterns in the ancestral population, to the observed counts of joint genotype patterns of the two genomes. These parameters are uniquely determined by the observed genotype configurations. Letting the Anzick-1 individual be represented by population 1, the null hypothesis that it belongs to an ancestral population of a specific modern population (population 2) then formally corresponds to a test of the hypothesis  $c_1 = 0$  (SI17).

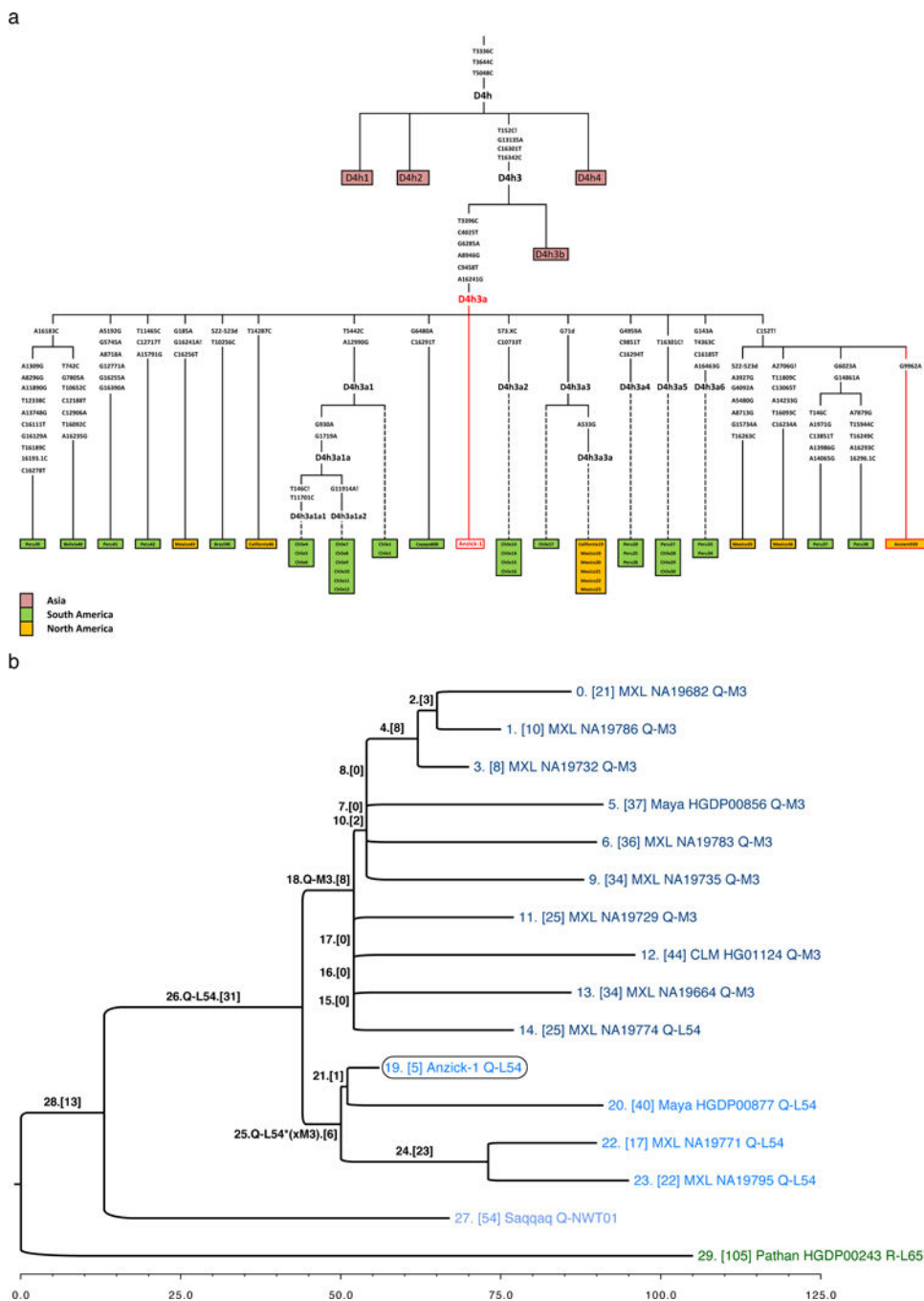


## Extended Data

**Extended Data Figure 1.**

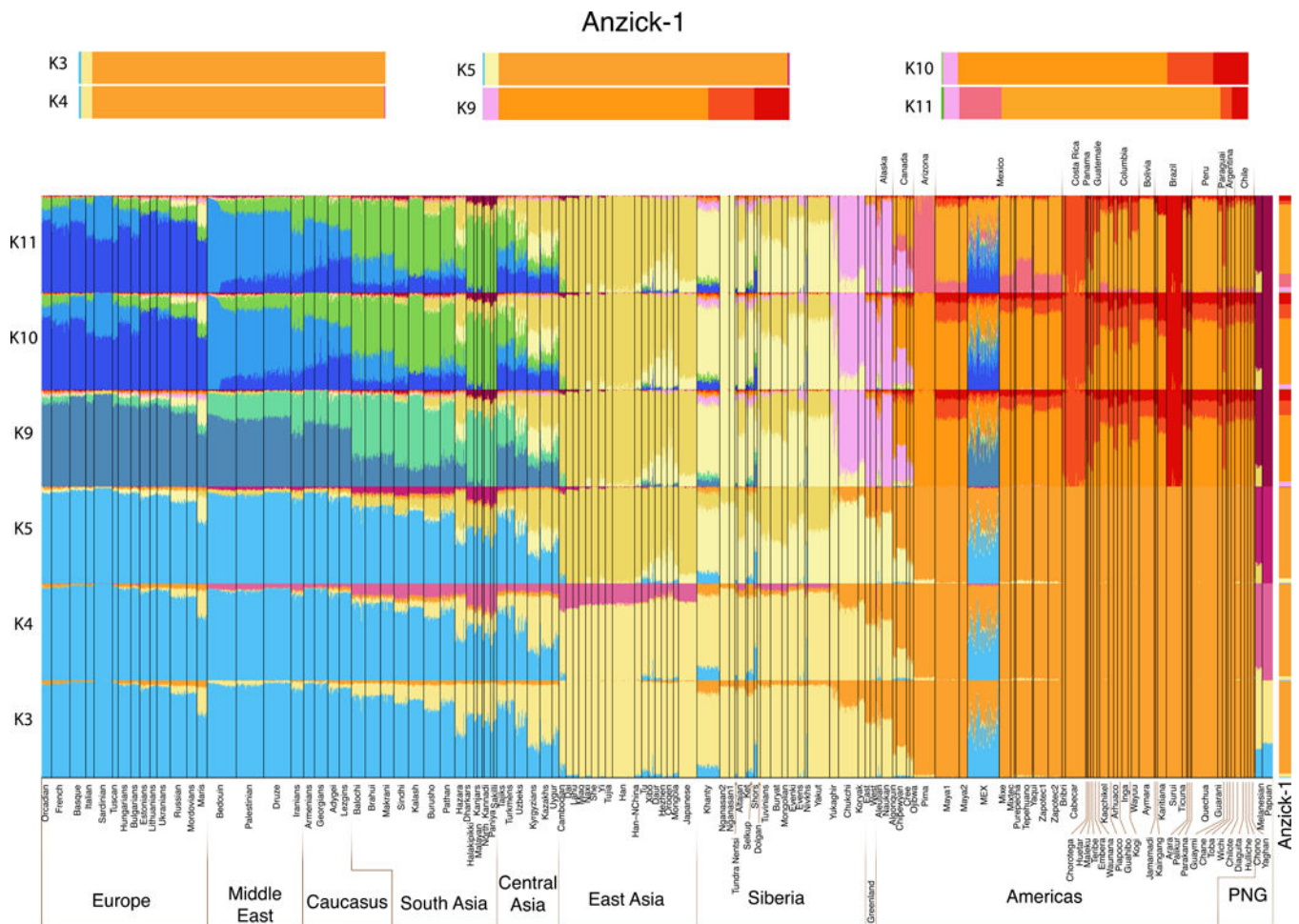
DNA fragmentation, damage and type specific error. **a**, Left, fragment length distribution of the Anzick-1 DNA sequences mapping to a human reference genome. The maximum read length with the applied chemistry on the HiSeq Illumina platform is 94 bp (100 - 6 bp index read), hence the large peak at this length simply represent the entire tail of the distribution. Right, the declining part of the distribution for the nuclear DNA, and the fit to an exponential model. The decay constant ( $\lambda$ ) is estimated to 0.018. **b**, Damage patterns for the

Anzick-1 individual in a random 0.5% subset of all mapped reads. Mismatch frequency relative to the reference as function of read position, C to T in red and G to A in blue. **c)** Type specific error rates for the Anzick-1 sample and the individual libraries. Estimates of overall error rates are given in the right-hand side.



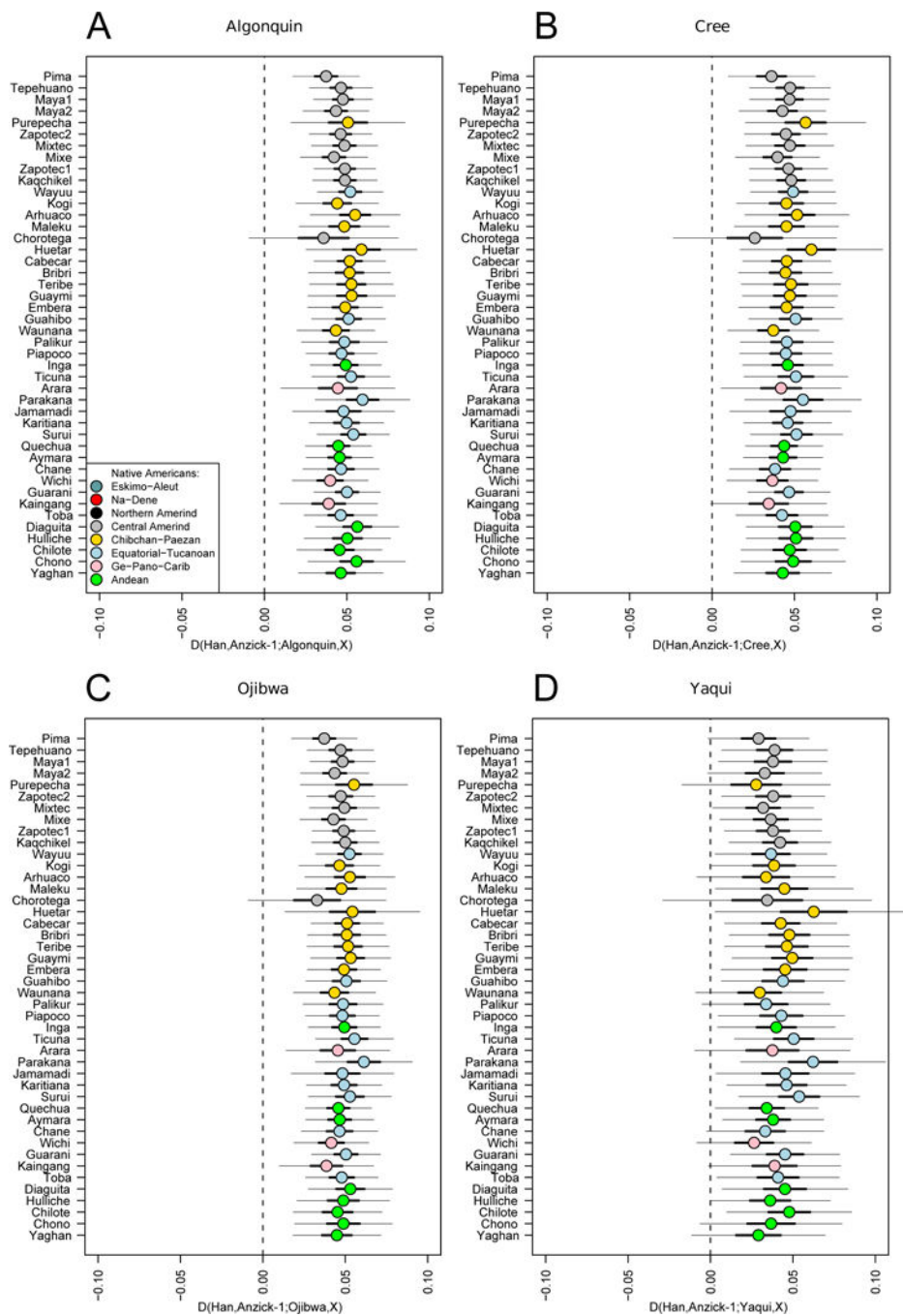
**Extended Data Figure 2.** mtDNA and Y-chromosome subtrees. **a,** Schematic phylogenetic tree of mtDNA haplogroup D4h3 and its sub-branch D4h3a. Mutations from the root of haplogroup D4h are specified

only for haplogroup D4h3a lineage, in case of broken lines only the mutations defining an existing sub-branch has been shown. The haplotypes of Anzick-1, identical with the root haplotype of D4h3a, and ancient full sequence from North-western coast of North America (Ancient939) is indicated in red. Insertions are indicated with “.” followed by a number of inserted nucleotides (X if not specified), deletions are indicated with „d“ and back mutations to ancestral state with „!““. The geographical spread of sub-branches of hg D4h is shown with different colours specified in figure legend. **b**, Placement of Anzick-1 within the Y-chromosome phylogeny. Anzick-1 (circled) represents Y-chromosome haplogroup Q-L54\*(xM3) (blue), which is offset by haplogroup Q-M3 (dark blue). The lineage carried by the ancient Saqqaq Palaeo-Eskimo (light blue) constitutes an outgroup to Q-L54. Each branch is labelled by an index and the number of transversion SNPs assigned to the branch (in brackets). Terminal taxa (individuals) are also labelled by population, ID, and haplogroup. Branches 21 and 25 represent the most recent shared ancestry between Anzick-1 and other members of the sample. Branch 19 is significantly shorter than neighbouring branches, which have had an additional ~12,600 years to accumulate mutations.



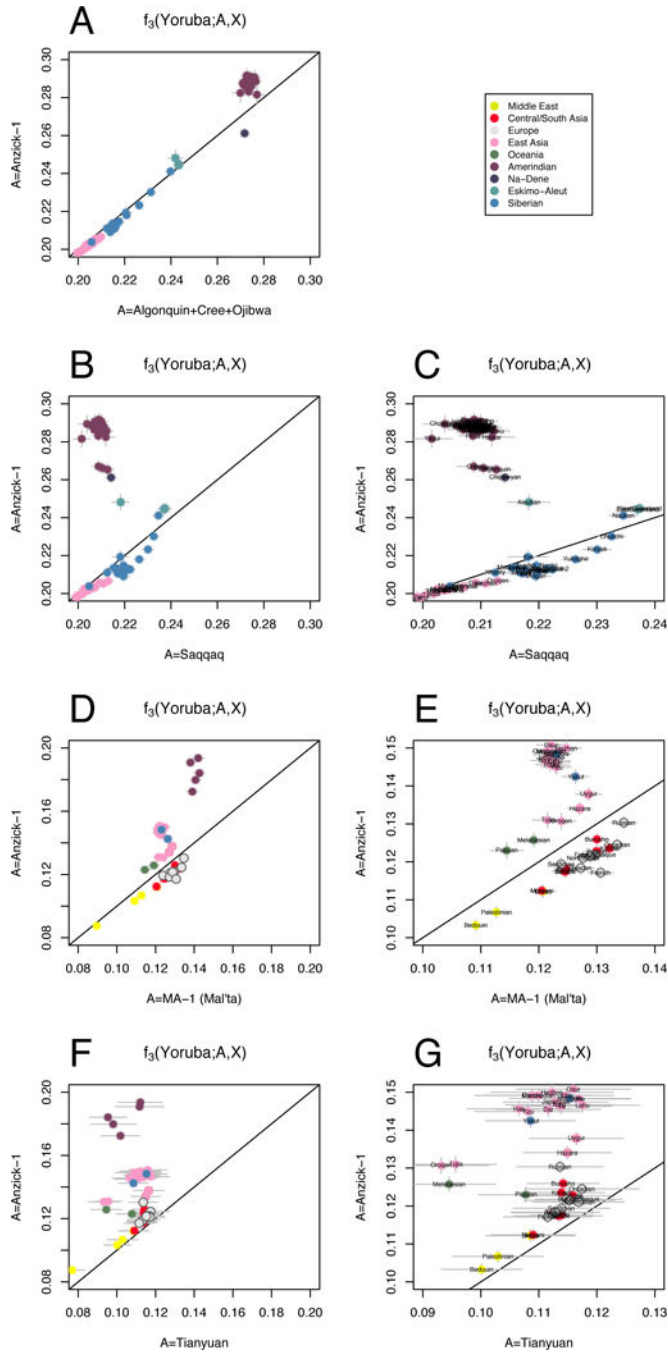
Extended Data Figure 3.

Ancestry proportions of Anzick-1 as determined by ADMIXTURE assuming 3 to 5 and 9 to 11 hypothetical “ancestral” populations or genetic components for a set of 135 extant Eurasian, Oceanian and New World populations. Shown are results from one of the converged runs at each K. We note that the model at K=11 was found to have the best predictive accuracy as determined by the lowest cross validation index values (see SI text). At each K each sample is represented by a stacked vertical bar whereas these of the Anzick-1 are magnified and presented horizontally at the top. Note that irrespective of the number of genetic components assumed the Anzick-1 sample shares all the components present in different contemporary Native American populations.



**Extended Data Figure 4.**

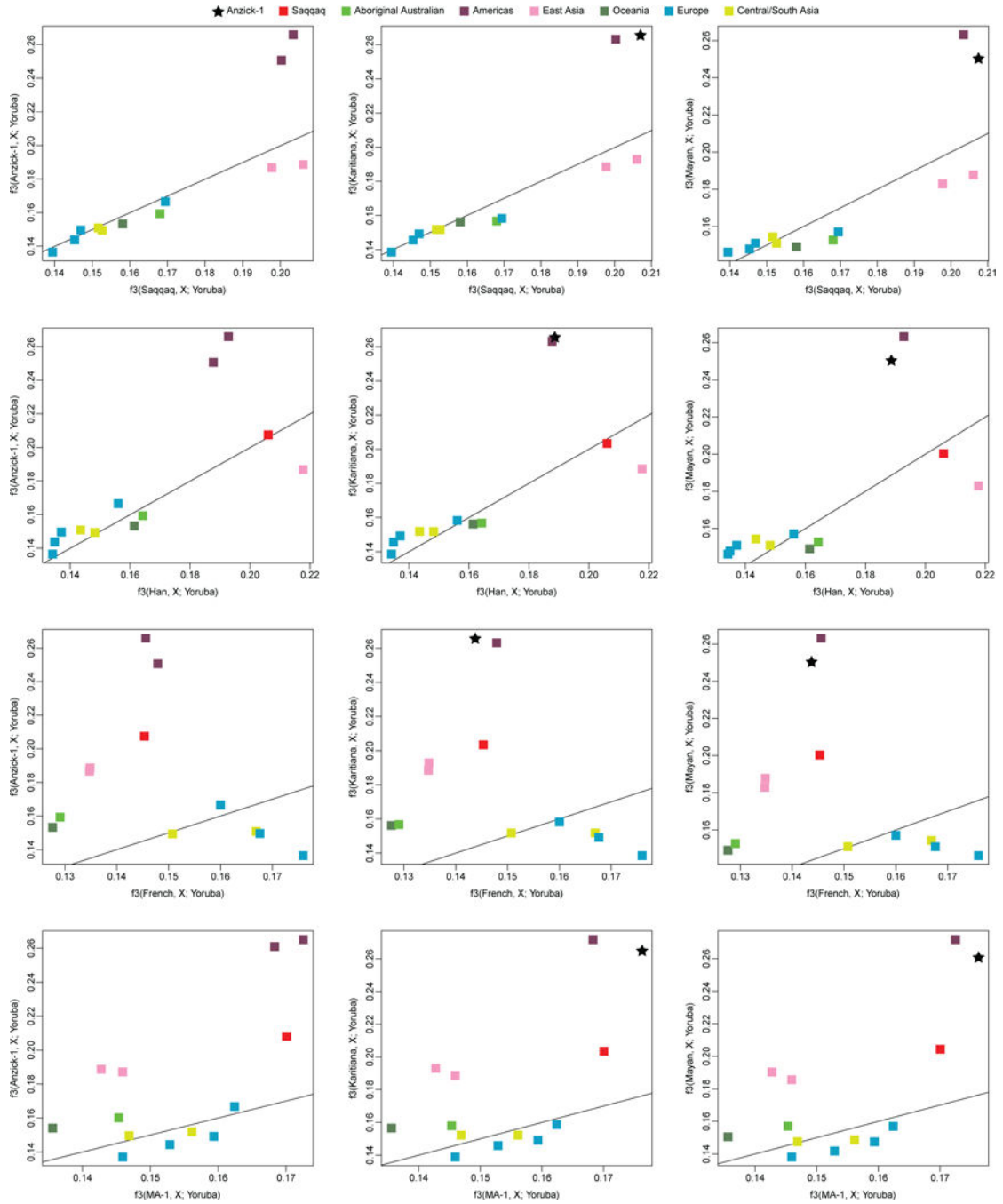
The closer relationship between Anzick-1 and Southern Native Americans compared to Algonquin, Cree, Ojibwa and a Yaqui individual (a–d) is consistent for different 44 Southern and Central Native American populations to Anzick-1. We used the test  $D(\text{Han}, \text{Anzick-1}; \text{Algonquin/Cree/Ojibwa/Yaqui}, \text{Central/Southern Native Americans})$ . Thick and thin whiskers represent 1 and 3 SEs, respectively.



**Extended Data Figure 5.**

Outgroup  $f_3$ -statistics contrasted for different combinations of populations. (A) Shared genetic history with Anzick-1 compared to shared genetic history with the three Northern Amerind-speaking populations. (B and C) shared genetic history with the Anzick-1 individual compared to the ~4,000-year-old Saqqaaq from Greenland. (D and E) Anzick-1 compared to shared genetic history with the 24,000-year-old MA-1 individual from Central Siberia. (F and G) shared genetic history with Anzick-1 compared to shared genetic history with the 40,000 year old Tianyuan individual from China.





**Extended Data Figure 6.**  
 Pairwise outgroup  $f_3$  statistics computed using Saqqaq, Han, French or ancient MA-1 (Mal'ta) on the x-axis and Anzick-1, Karitiana, or Mayan on the y-axis. The black line indicates the  $y=x$  line.



**Extended Data – Table 1**

AMS  $^{14}\text{C}$  and stable isotope analyses of human bone and antler artefacts from the Anzick site, Montana. The previously published  $^{14}\text{C}$  measurements are provided for reference. Some of the analyses were carried out to collect stable isotope and geochemistry data, and thus show no age information. Calibrated Ages using IntCal 09 are given only for  $^{14}\text{C}$  dates completed since 2006 and for chemical fractions considered the highest purity and having the greatest accuracy; calibration of chemically impure chemical fractions is not warranted. CAMS, Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratories, Livermore, California. AA, NSF-Arizona Accelerator Mass Spectrometry Facility, Tucson. SR, Stafford Research, Boulder, Colorado. Beta, Beta Analytic, Florida. – not tested.

AMS LAB NO.	Chemical Fraction Dated	$^{14}\text{C}$ Age, RC yr.± SD	$^{14}\text{C}$ Date, CAL BP (95.4% C.I.)	$\delta^{15}\text{N}$ ‰ (AIR)	$\delta^{13}\text{C}$ ‰ (VPDB)	Collagen Yield (wt%)
<b>Red, Hematite-Stained Petrous Bone (ANZICK-1)</b>						
AA-313A	Decalcified Collagen	8690±310				
AA-313B	Untreated Gelatin	10,500±400				
AA-2978	Aspartic acid	10,240±120				
AA-2982	Alanine	10,370±130				
AA-2980	Hydroxyproline	10,710±100				
AA-2979	Glutamic acid	10,820±100				
AA-2981	Glycine	10,940±90				
CAMS-80535	Decalcified Collagen	10,580±35		+9.1	-17.6	–
CAMS-80536	KOH-Collagen	10,525±35		+8.6	-18.1	–
CAMS-80537	Gelatin	10,610±30		+10.1	-18.0	–
CAMS-80538	XAD-Gelatin	10,705±35	12,722–12,590	–	–	–
Beta-163833	Alkali Collagen	10,780±40	12,746–12,660	–	-21.2	–
SR-8149	KOH-Collagen	–		+10.5	-18.0	22.7
SR-8149	Gelatin	–		+11.1	-17.9	22.7
SR-8151	KOH-Collagen	–		+11.0	-17.9	18.7
SR-8151	Gelatin	–		+11.6	-17.8	18.7
<b>Antler Rods</b>						
Specimen #118/119						
UCIAMS-61661	XAD-Collagen	11,025±30	13,010–12,777	–	–	–
Beta-163832	Alkali Collagen	11,040±60	13,053–12,753	–	-22.5	–
Specimen #122						
Beta-168967	Alkali Collagen	11,040±40	13,040–12,780	–	-18.2	–
	Average, 3-rods	11,035±45	13,039–12,763			

**Extended data – Table 2**

Statistics of the genome sequence data used in the study and high confidence genotype calls.  
 AvgDepth: average depth. Cov >1: fraction of the genome covered with at least 1 read.  
 HomRef: Homozygote reference call, Het: Heterozygote call, HomAlt: Homozygote alternative call.

Sample	Region	Population	Reference	Gender	Avg. Depth	Cov >1X	HomRef	Het	HomAlt
DNK02	Africa	Dinka	Meyer <i>et al</i> , 2012	Male	24.3	0.90	1,356,439,142	1,194,183	653,791
HGDP00456	Africa	Mbuti	Meyer <i>et al</i> , 2012	Male	20.3	0.90	1,333,191,748	1,198,421	741,996
HGDP00521	Europe	French	Meyer <i>et al</i> , 2012	Male	22.6	0.90	1,352,074,026	928,373	611,106
HGDP00542	SE Asia	Papuan	Meyer <i>et al</i> , 2012	Male	21.6	0.90	1,343,127,185	745,786	753,954
HGDP00665	Europe	Sardinian	Meyer <i>et al</i> , 2012	Male	19.9	0.90	1,334,884,400	908,557	606,234
HGDP00778	Han	Han	Meyer <i>et al</i> , 2012	Male	22.3	0.90	1,349,104,846	879,299	668,621
HGDP00927	Africa	Yoruba	Meyer <i>et al</i> , 2012	Male	26.7	0.90	1,361,142,931	1,221,258	664,333
HGDP00998	S. America	Karitiana	Meyer <i>et al</i> , 2012	Male	21.3	0.90	1,343,817,819	667,456	753,001
HGDP01029	Africa	San	Meyer <i>et al</i> , 2012	Male	26.9	0.90	1,362,464,890	1,273,369	774,869
HGDP01284	Africa	Mandenka	Meyer <i>et al</i> , 2012	Male	20.6	0.90	1,338,712,364	1,210,852	646,656
HGDP01307	SE Asia	Dai	Meyer <i>et al</i> , 2012	Male	23.8	0.90	1,350,414,031	879,716	660,662
HGDP00877	S. America	Mayan	This study	Male	13.2	0.90	1,130,447,805	673,358	547,730
B116	S. America	Karitiana	This study	Female	23.4	0.89	1,278,750,988	664,223	684,479
Avar	SC Asia	Avar	Raghavan <i>et al</i> , 2013	Male	12.8	0.83	622,360,039	468,944	299,356
Indian	SC Asia	Indian	Raghavan <i>et al</i> , 2013	Female	15.9	0.89	861,422,992	643,238	389,937
Mari	SC Asia	Mari	Raghavan <i>et al</i> , 2013	Male	12.0	0.87	235,202,480	199,301	120,287
Tadjik	SC Asia	Tajik	Raghavan <i>et al</i> , 2013	Male	16.4	0.88	939,846,482	687,749	431,934
AusAboriginal	SE Asia	Australian Aboriginal	Rasmussen <i>et al</i> , 2011	Male	6.3	0.50	356,126,648	240,732	220,901
Saqqaq	Greenlander	Saqqaq	Rasmussen <i>et al</i> , 2010	Male	12.8	0.79	515,972,797	307,585	306,065
Anzick-1	N. America	Clovis	This study	Male	14.4	0.87	780,953,901	864,870	409,731

**Extended Data – Table 3**

Sequencing and mapping statistics for the Anzick-1 libraries.

Library	Raw Reads	Per cent human reads	Mapped human reads Q30	Final BAM	Per cent clonality
C10	55,495,972	0.5%	289,526	96,136	67%
C121121_36	2,000,862,136	24.6%	491,779,235	116,018,652	76%
C121121_37	1,938,412,150	24.8%	481,682,594	100,596,004	79%
C28	412,441,694	27.8%	114,736,785	89,356,892	22%
C30_8	943,369,850	22.7%	214,367,643	21,016,810	90%
C30_8_26	1,234,827,852	23.5%	289,965,256	18,404,080	94%
C30_8_48	1,116,970,391	23.4%	261,200,675	15,489,743	94%
C7	531,754,382	12.0%	63,602,547	8,025,652	87%
C8	298,045,766	10.5%	31,415,268	7,273,279	77%
C9	247,005,820	0.9%	2,234,390	171,718	92%
CL16	614,007,221	22.1%	135,844,895	28,405,912	79%
CL18	803,188,531	21.6%	173,273,845	35,132,866	80%
CL20	695,954,288	21.7%	150,806,716	29,319,798	81%
CL32	477,347,711	28.2%	134,386,357	108,903,306	19%
CL33	791,303,957	21.4%	169,518,285	25,024,524	85%
Cpro_42	201,998,907	8.3%	16,846,370	3,381,783	80%
Cpro_43	305,882,477	8.3%	25,418,416	2,896,283	89%
Crib_34	323,616,468	0.9%	2,983,594	594,741	80%
Crib_39	281,843,911	0.9%	2,447,883	302,525	88%
Total	13,274,329,484	20.8%	2,762,800,280	610,410,704	78%

**Extended Data – Table 4**

Site patterns and drift estimates for non-African populations vs. the Anzick-1 sample. Top, data based on conditioning on African polymorphism. Bottom, data based on conditioning on African polymorphism and removing sites where a C and T or G and A were observed.

ID	Population	Including all sites					Drift in population	Drift in Anzick-1
		n(AAAA)	n(AaAA)	n(AAAa)	n(AaAa)	n(Aaaa)		
HGDP00877 (masked)	Mayan	123095	112507	38900	76913	1064893	0.0294	0.0000
HGDP00998	Karitiana	222029	162274	77002	111472	1791319	0.1581	0.0000
BI16	Karitiana	196546	150372	66032	105219	1630510	0.1258	0.0000
Saqqaq	Saqqaq	152815	129619	71706	71545	1179386	0.1911	0.0979
HGDP00778	Han	210095	237386	97103	124521	1703214	0.0553	0.1266
HGDP01307	Dai	211459	239636	98327	125610	1713361	0.0554	0.1283
HGDP00542	Papuan	237801	200257	140039	95850	1688750	0.2725	0.1802
Aboriginal	Aboriginal	116797	100838	67763	48350	847186	0.2569	0.1774
HGDP00521	French	212922	265527	112889	122188	1661041	0.0617	0.1890
HGDP00665	Sardinian	211703	261689	116240	118827	1633910	0.0772	0.1984

Including all sites								
ID	Population	n(AAAa)	n(AaAA)	n(AAaa)	n(AaAa)	n(AAAA)	Drift in population	Drift in Anzick-1
Avar	Avar	161692	197302	84076	92078	1262525	0.0653	0.1804
Mari	Mari	67063	81810	32757	40631	547086	0.0397	0.1554
Tadjik	Tadjik	195832	247682	99654	118142	1567438	0.0385	0.1748
Indian	Indian	170384	214686	86426	103309	1371359	0.0382	0.1722

Excluding sites where a C and T or G and A were observed								
ID	Population	n(AAAa)	n(AaAA)	n(AAaa)	n(AaAa)	n(AAAA)	Drift in population	Drift in Anzick-1
HGDP00877 (masked)	Mayan	38605	35517	12138	24518	334804	0.0232	0.0000
HGDP00998	Karitiana	68442	51065	24000	34758	554162	0.1519	0.0000
BI16	Karitiana	60878	47002	20607	33087	509629	0.1216	0.0000
Saqqaq	Saqqaq	46180	39448	21528	21683	358284	0.1869	0.0974
HGDP00778	Han	64920	74011	29884	38635	527612	0.0507	0.1273
HGDP01307	Dai	65190	74986	30472	39117	530308	0.0501	0.1316
HGDP00542	Papuan	73369	62598	43620	29859	522442	0.2693	0.1842
Aboriginal	Aboriginal	34529	30008	20184	14512	250288	0.2544	0.1787
HGDP00521	French	65748	83094	34912	38023	514107	0.0563	0.1915
HGDP00665	Sardinian	65549	81338	35819	36721	506155	0.0749	0.1987
Avar	Avar	49028	60027	25643	27920	384344	0.0657	0.1825
Mari	Mari	19666	24153	9541	12091	160779	0.0334	0.1531
Tadjik	Tadjik	60041	76457	30652	36532	483570	0.0357	0.1758
Indian	Indian	52265	65827	26564	31632	420851	0.0391	0.1728

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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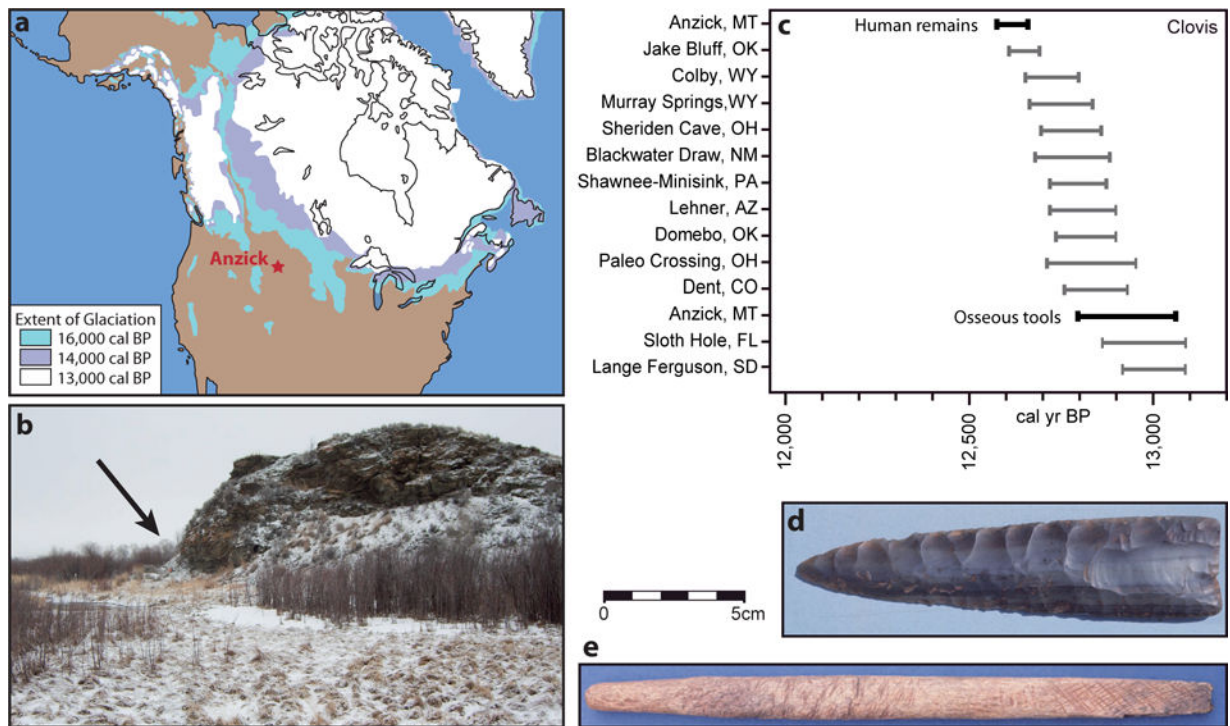
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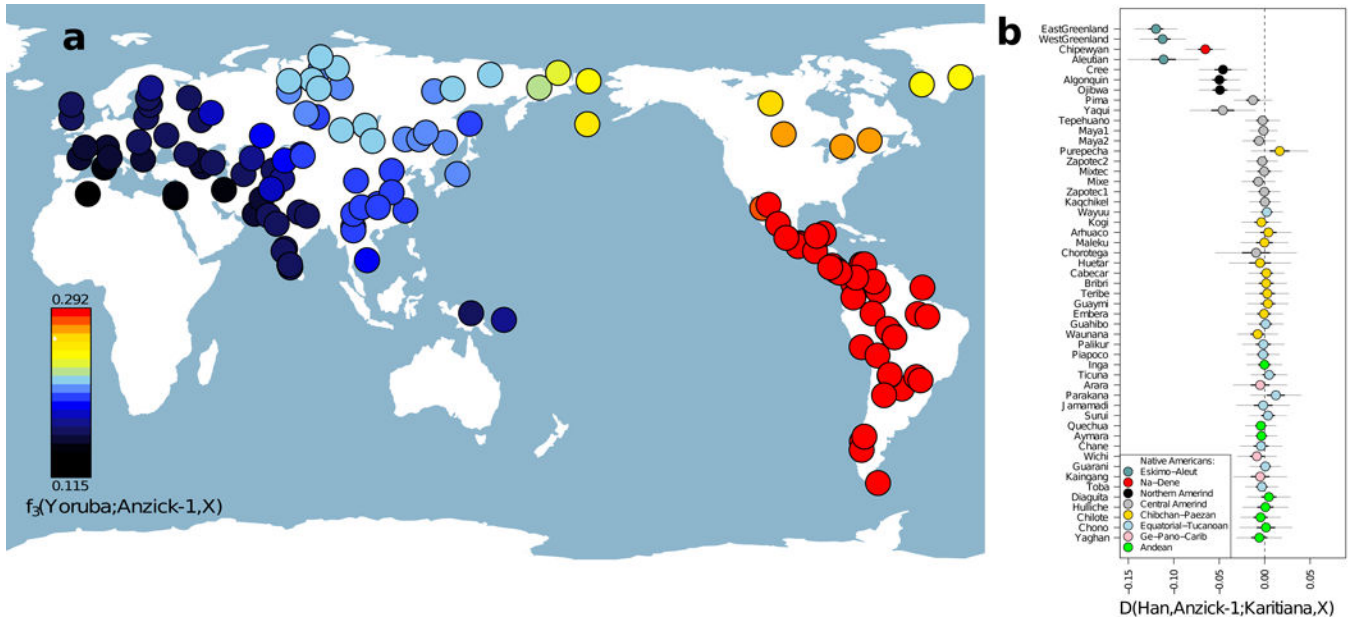
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**Figure 1.**

Geographic and C14 dating overview, and examples of artefacts from the site. **a**, Location of the Anzick site relative to continental glacial positions from 16,000 to 13,000 calendar years before present. **b**, Photograph of the Anzick site. Site is located at the base of the slope at the far left. **c**, Age of the human remains and osseous tools relative to other Clovis sites. **d**, Clovis fluted projectile point from the site. **e**, Clovis osseous rod from the site.





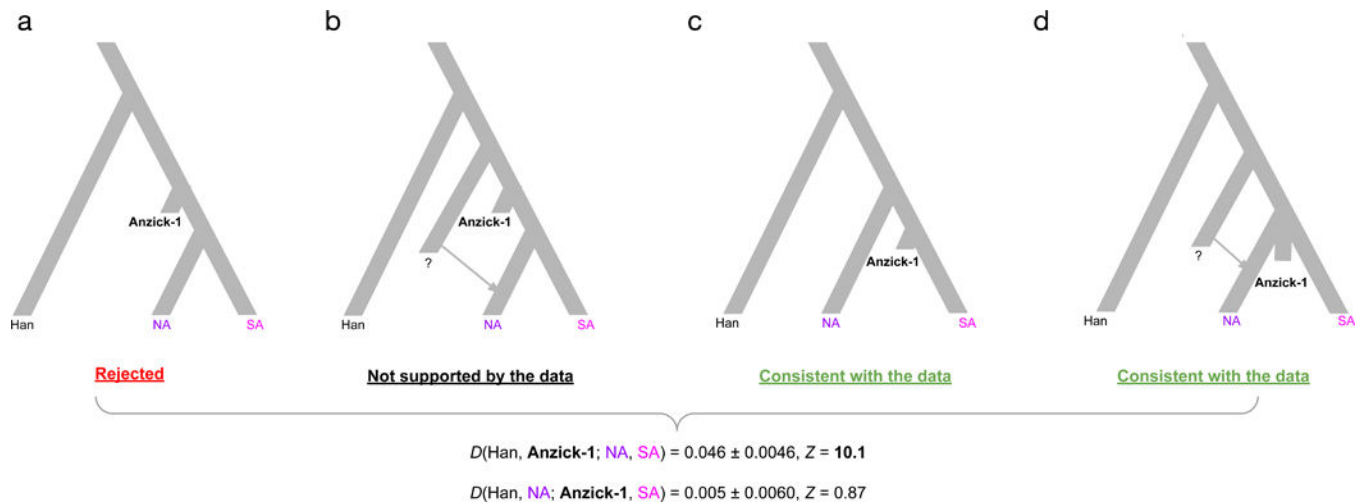
**Figure 2.** Genetic affinity of Anzick-1. **a**, Anzick-1 is most closely related to Native Americans. Heat map representing estimated outgroup  $f_3$ -statistics for shared genetic history between the Anzick-1 individual and each of 143 contemporary human populations outside sub-Saharan Africa. **b**, Anzick-1 is less closely related to Northern Native American populations and a Yaqui individual than to Central and South Native Americans such as the Brazilian Karitiana. We computed a  $D$ -test of the form  $D(\text{Han}, \text{Anzick-1}; \text{Karitiana}, X)$  to test the hypothesis that a second Native American population  $X$  is as closely related to Anzick-1 as the South American Karitiana is. Thick and thin whiskers represent 1 and 3 SEs, respectively.

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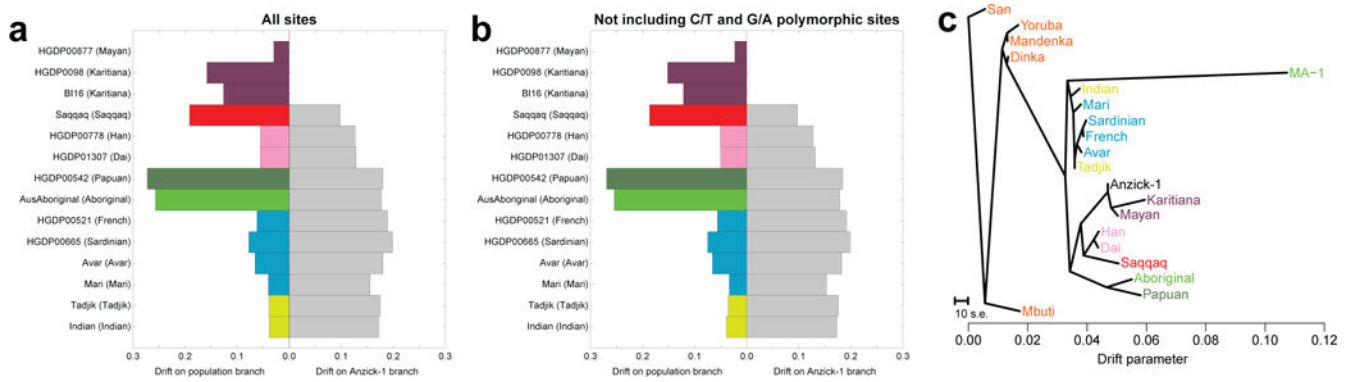
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**Figure 3.**

Simplified schematic of genetic models. Alternative models of the population history behind the closer shared ancestry of the Anzick-1 individual to Central and Southern American (SA) populations than Northern American (NA) populations, see main text for further definition of populations. We find that the data is consistent with a simple tree-like model where NA populations are historically basal to Anzick-1 and SA. We base this conclusion on two  $D$ -tests conducted on the Anzick-1 individual, NA and SA. We used Han Chinese as outgroup. We first tested the hypothesis that Anzick-1 is basal to both NA and SA populations using  $D(\text{Han, Anzick-1; SA, NA})$ . **a**, As in the results for each pairwise comparison between SA and NA populations (Extended Data Figure 4), this hypothesis is rejected. **b**, Next, we tested  $D(\text{Han, NA; Anzick-1, SA})$ , if NA populations were a mixture of post-Anzick-1 and pre-Anzick-1 ancestry, we would expect to reject this topology. **c**, We found that a topology with NA populations are basal to Anzick-1 and SA populations is consistent with the data. **d**, However, another alternative is that the Anzick-1 individual is from the time of the last common ancestral population of the Northern and Southern lineage, after which the Northern lineage received gene flow from a more basal lineage.



**Figure 4.**

Estimated drift and maximum likelihood tree. Site patterns and drift estimates for non-African populations vs. the Anzick-1 sample. **a**, Data based on conditioning on African polymorphism, non-African populations in colour on the left, Anzick-1 in grey on the right. **b**, Data based on conditioning on African polymorphism and removing sites where a C and T or G and A were observed. **c**, Maximum likelihood tree generated by *TreeMix* using the whole genome sequencing data with the Mayan genome masked for European ancestry.