

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

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**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 before present (BP) (13,000 to 12,600 calendar years BP)<sup>1,2</sup>. Nearly 50 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 the genetic legacy of the people who manufactured Clovis tools remain under debate. It is generally believed 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 emigrated from southwestern Europe during the Last Glacial Maximum<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 (approximately 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 population<sup>5</sup> into Native American ancestors is also shared by the Anzick-1 individual and thus happened before 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 predates the Anzick-1 individual.**

The only known Clovis burial and associated mortuary assemblage was found in the Americas at the Anzick site, Montana, in 1968 (refs 6–8) (Fig. 1a, b). Here, approximately 100 stone tools and 15 osseous tool fragments (Fig. 1d, e) (at least one made of elk; Supplementary Information section 3.4) 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> (Fig. 1c).

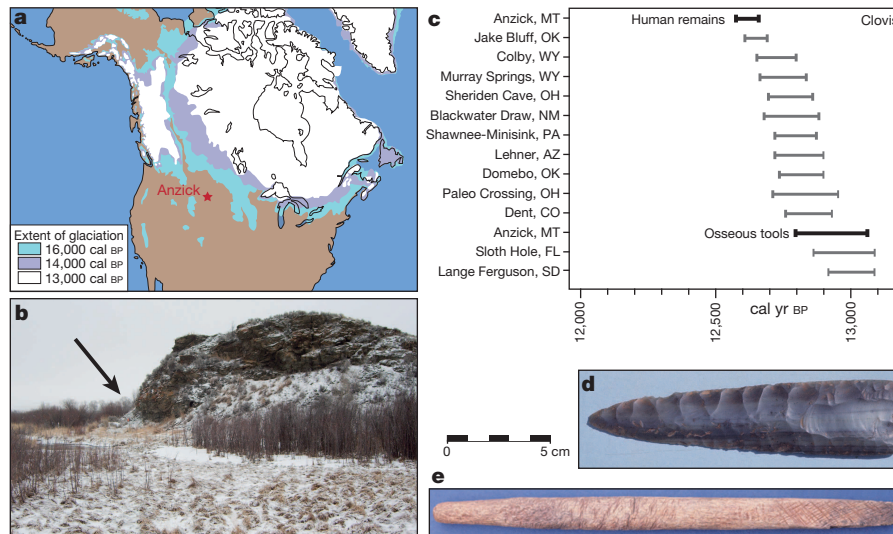
Initial genetic screening of the Anzick-1 skeletal remains using PCR coupled with cloning and Sanger sequencing yielded a mitochondrial DNA (mtDNA) haplogroup assignment of D4h3a (Supplementary Information section 3). The D4h3a haplogroup was verified and further characterized in the subsequent shotgun sequencing of Anzick-1 (Supplementary Information section 12 and Extended Data Fig. 2). D4h3a is one of the rare mtDNA lineages specific to Native Americans, 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 to be mtDNA-typed to date question such interpretation and underscore the view 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 subgroups of the haplogroup<sup>13</sup> and is thus placed at the root of D4h3a. Our finding suggests that the origin of the D4h3a branch is likely to be at the upper bound of the previously obtained estimate of 13,000 ± 2,600 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%; Supplementary Information section 4 and Extended Data Table 3). This suggests a considerable influence of microenvironments on long-term DNA preservation across the Anzick-1 bone fragments<sup>15</sup>. We obtained an average sequencing depth of 14.4× coverage. On the basis of the observed DNA fragment

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**Figure 1 | Geographic and  $^{14}\text{C}$  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 BP (cal BP). **b**, Photograph of the

length distribution, we computed molecular half-life estimates<sup>16</sup> for a 100-base pair fragment to be 4,852 and 5,137 years for nuclear DNA and mtDNA, respectively. These are consistent with predictions based on estimated burial temperature and geographical location (Supplementary Information section 7). Nucleotide misincorporation patterns were consistent with increased post-mortem cytosine deamination at DNA fragment termini<sup>17</sup> (Supplementary Information section 8). The mtDNA data provided a contamination estimate with a 95% credibility interval of 0.15–0.38%<sup>18</sup> (Supplementary Information section 9). As the Anzick-1 individual was found to be male, we were also able to quantify contamination levels on the basis of heterozygosity levels seen on the X chromosome<sup>19</sup>, which resulted in similarly low contamination estimates (<1.2%, Supplementary Information section 11). Characterization of the error rate yielded an overall estimate of 0.84% (Supplementary Information section 10). Per-library error rates as well as nucleotide-type-specific error rates are shown in Extended Data Fig. 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 (Supplementary Information section 13 and Extended Data Fig. 2). Confining our analyses to transversion single nucleotide polymorphisms (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 approximately 16,900 years ago (95% confidence interval: 13,000–19,700; Supplementary Information section 13).

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 statistically significant closer affinity to all 52 Native American groups than to any extant Eurasian population (Fig. 2). The same conclusion was reached from admixture clustering analyses (Supplementary Information section 14 and Extended Data Fig. 3), and when using  $D$ -statistics based on genomic sequence data (Supplementary Information section 16).

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),

Anzick site. The site is located at the base of the slope at the far left (arrow). **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.

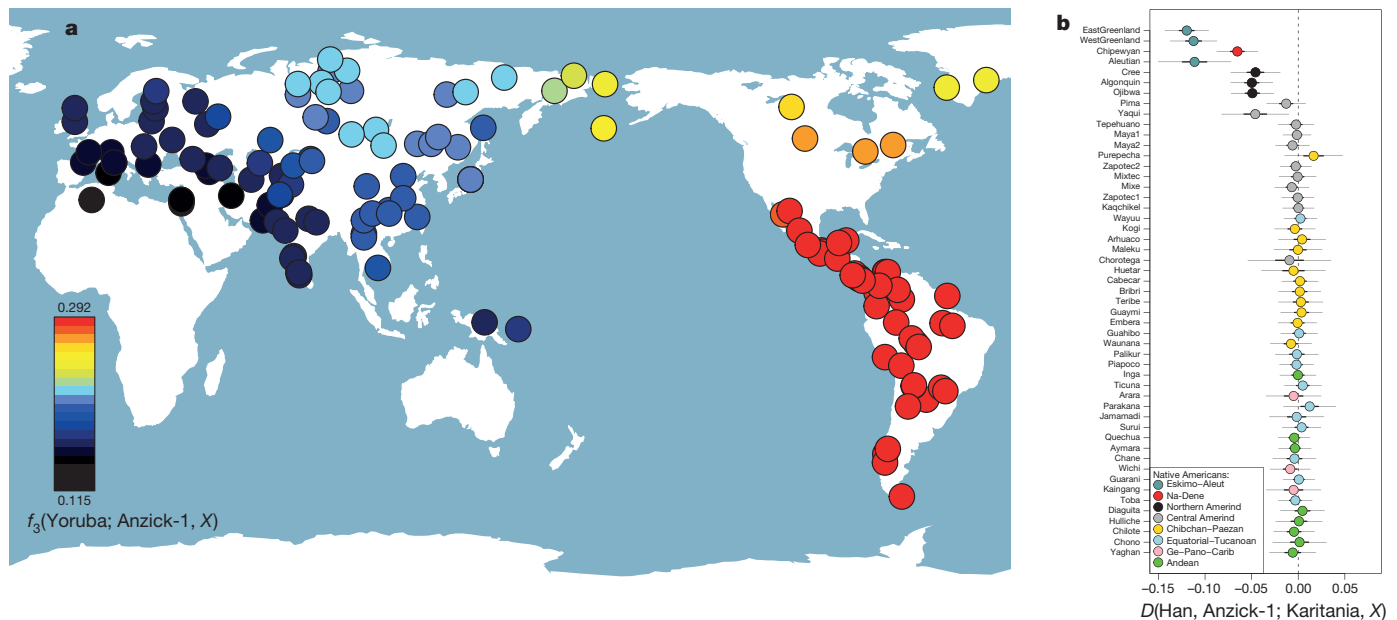
and the Central American Yaqui (Supplementary Information section 15, Fig. 2), than with 44 Native populations from Central and South America ('SA' group) (Fig. 2). This finding was further supported by allele-frequency-based  $D$ -statistics, which strongly reject the hypothesis of a population tree with topology (Anzick-1, (NA, SA)). By contrast, a tree assuming a branching pattern of (NA, (Anzick-1, SA)) was not rejected (Supplementary Information section 15 and Fig. 3).

We investigated two possible scenarios that could explain this pattern: (1) a basal diversification of the NA and SA lineages that predates the Clovis period, with the Anzick-1 individual belonging to the SA lineage (Fig. 3c); and (2) the Anzick-1 individual is basal to both lineages, but the lineages diverged at a time close to approximately 12,600 years ago, and the NA group received subsequent gene-flow from other, perhaps Siberian, populations (Fig. 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 NA 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 (Supplementary Information section 15), a pattern that agrees with previously published results<sup>22</sup>. This suggests that model (1) is more likely, and that the structure between Native American lineages predates 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 sample from Mal'ta, Siberia<sup>5</sup> and the 4,000-year-old Saqqaq Palaeo-Eskimo sample from Greenland<sup>19</sup>. We again found a closer relationship between Anzick-1 and all Native Americans (Supplementary Information section 15 and Extended Data Fig. 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 (Supplementary Information section



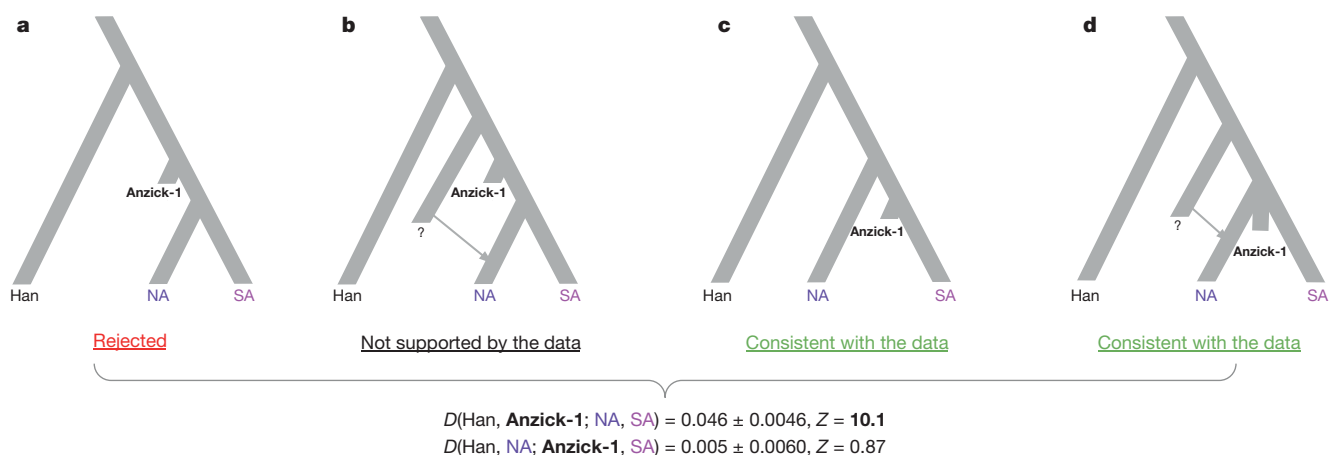
**Figure 2 | Genetic affinity of Anzick-1.** **a**, Anzick-1 is most closely related to Native Americans. Heat map representing estimated outgroup  $f_3$ -statistics for shared genetic history between the Anzick-1 individual and each of 143 contemporary human populations outside sub-Saharan Africa. **b**, 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  $D$ -statistics 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 standard errors, respectively.

17). 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 (Fig. 4a, b). By 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> (Fig. 4c

and Supplementary Information section 18) and outgroup  $f_3$ -analyses<sup>5,21</sup> (Extended Data Figs 5, 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 (Fig. 4c).

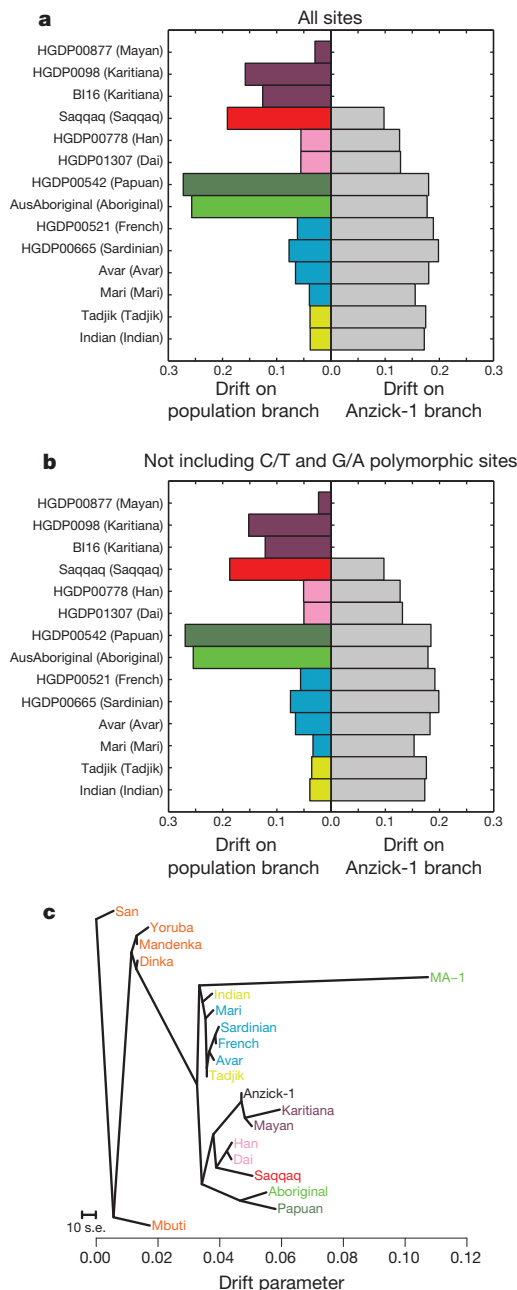
We conclude that the male infant, buried approximately 12,600 years ago with ochre-covered Clovis artefacts at the Anzick site, belonged to a meta-population from which many contemporary Native Americans are



**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 Native American (NA) populations; see main text for further definition of populations. We find that the data are consistent with a simple tree-like model in which 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. **a**, We first tested the hypothesis that Anzick-1 is basal to both NA and SA populations using  $D(\text{Han}, \text{Anzick-1}; \text{NA}, \text{SA})$ . As in

the results for each pairwise comparison between SA and NA populations (Extended Data Fig. 4), this hypothesis is rejected. **b**, Next, we tested  $D(\text{Han}, \text{NA}; \text{Anzick-1}, \text{SA})$ ; 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 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 versus 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. HGDP, Human Genome Diversity Project.

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 settle successfully in 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 the Anzick-1 skeleton, from the Anzick site in Montana, for ancient DNA and <sup>14</sup>C dating analyses (Supplementary Information sections 1, 2). From DNA extracts we generated Illumina sequencing libraries, which were sequenced on the Illumina HiSeq platform (Supplementary Information sections 3, 4). We verified the authenticity of the ancient DNA through damage patterns and decay rates (Supplementary Information sections 7, 8), and estimated contamination using analyses of both mtDNA and nuclear DNA (Supplementary Information sections 9, 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 (Supplementary Information section 15). To test historical models relating the Anzick-1 individual to modern-day Native Americans from a Northern group (Cree, Ojibwa and Algonquin) and a Southern group (44 Central and South American groups), we computed  $D$ -statistics<sup>21</sup> using the full allele frequency information (Supplementary Information section 15). To test whether the Anzick-1 individual could have belonged to a population ancestral to some of the modern populations, we developed a new maximum likelihood 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  $c_1 = 0$  (Supplementary Information section 17).

**Statement regarding legal and ethical issues.** 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 as 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. E.W. and S.M.D. visited in person the cultural representatives of the Northern Cheyenne, Crow, Black Feet and Salish and Kootenai tribes. S.M.D. 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 towards reburial of the human remains from the site.

**Online Content** Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** E.W., S.L.A., M.R.W. and T.W.S. conceived the project. E.W. headed the project. E.W. and M.R. designed the research project setup. R.N. supervised the bioinformatical and population genetic analyses with input from M.J. S.M.D., R.S.M. and T.L.P. helped with ethics and contact to local communities. S.L.A. and M.R.W. provided access to the Anzick-1 sample and the archaeological context, with input from S.S.W. and D.J.M. T.W.S. performed AMS  $^{14}\text{C}$  dating, stable isotope analyses and provided geochemical and geochronological assessments. S.L.A. and J.S. performed initial mtDNA screening experiments. Elk extracts were processed by P.D.H. and I.B. Ancient DNA extractions and library constructs for shotgun sequencing and preparation for sequencing was done by M.R. O.E.C. prepared the two modern genomes. M.R. and S.R. did initial bioinformatics and mapping of the ancient sample. Mapping of modern samples, and genotype calls was done by S.R., with input from T.S.K., A.E., V.M.W. and M.C.L. S.R., T.S.-P. and S.B. provided super computing resources. O.E.C. and S.R. did phasing and ancestry painting, with input from A.E., V.M.W. and M.C.L. M.E.A. and M.C. did half-life estimates, with input on geology from T.W.S. DNA damage patterns were done by M.R. and L.O. mtDNA consensus and damage estimate was done by A.-S.M. I.M. and A.A. performed the X-chromosome contamination estimates, error rate estimates and *D*-statistic analyses on genomic sequence data. G.D.P. conducted Y-chromosome analysis with input from C.D.B. M.M. did ADMIXTURE analysis. K.T., M.K. and M.M. did mtDNA characterization. P.S. did  $f_3$ -statistics on SNP array data and tested Native American population models using *D*-statistics. M.D. performed *TreeMix* analysis and genome-wide  $f_3$ -statistics. R.N. and M.D. developed and implemented the ancestry test. M.R., S.L.A., M.R.W., P.S., M.D., R.N. and E.W. wrote most of the manuscript with input from T.W.S., M.E.A., A.-S.M., S.R., I.M., A.A., G.D.P., K.T., M.M., R.G., V.G., R.Y., P.D.H., O.E.C., M.C., F.B., A.M., L.S. 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 at <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. The *Cervus elaphus* sequences are available under GenBank accessions KF906070, KF906071 and KF906072. Reprints and permissions information is available at [www.nature.com/reprints](http://www.nature.com/reprints). The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to E.W. ([ewillerslev@snm.ku.dk](mailto:ewillerslev@snm.ku.dk)).