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# The Ancestry and Affiliations of Kennewick Man 

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#### Abstract

Kennewick Man, referred to as the Ancient One by Native Americans, is a male human skeleton discovered in Washington state (USA) in 1996 and initially radiocarbon-dated to 8340-9200 calibrated years $\mathrm{BP}^{1}$. His population affinities have been the subject of scientific debate and legal controversy. Based on initial study of cranial morphology it was asserted that Kennewick Man was


[^0]neither Native American nor closely related to the Claimant Plateau tribes of the Pacific Northwest, who claimed ancestral relationship and requested repatriation under the Native American Graves Protection and Repatriation Act (NAGPRA). The morphological analysis was important to judicial decisions that Kennewick Man was not Native American and that therefore NAGPRA did not apply. Instead of repatriation, additional studies of the remains were permitted ${ }^{2}$. Subsequent craniometric analysis affirmed Kennewick Man to be more closely related to circumpacific groups such as the Ainu and Polynesians than he is to modern Native Americans ${ }^{2}$. In order to resolve Kennewick Man's ancestry and affiliations, we have sequenced his genome to $\sim 1 \times$ coverage and compared it to worldwide genomic data including the Ainu and Polynesians. We find that Kennewick Man is closer to modern Native Americans than to any other population worldwide. Among the Native American groups for whom genome wide data is available for comparison, several appear to be descended from a population closely related to that of Kennewick Man, including the Confederated Tribes of the Colville Reservation (Colville), one of the five tribes claiming Kennewick Man. We revisit the cranial analyses and find that, as opposed to genomic-wide comparisons, it is not possible on that basis to affiliate Kennewick Man to specific contemporary groups. We therefore conclude based on genetic comparisons that Kennewick Man shows continuity with Native North Americans over at least the last eight millennia.

The skeleton of Kennewick Man was inadvertently discovered in July of 1996 in shallow water along the Columbia River shoreline outside Kennewick, Washington. On several visits to the locality over the following month, some 300 bone elements and fragments were collected, ultimately comprising $\sim 90 \%$ of an adult male human skeleton ${ }^{3}$. The initial assessment of this individual was that he was a historic-period Euro-American, based largely on his apparently "Caucasoid-like"3 cranium, along with a few artefacts found nearby (later proved not to be associated with the skeletal remains). However, radiocarbon dating subsequently put the age of the skeleton in the Early Holocene ${ }^{1}$. The claim that Kennewick Man was anatomically distinct from modern Native Americans in general, and in particular from those tribes inhabiting northwest North America ${ }^{4}$, sparked a legal battle over the disposition of the skeletal remains. Five tribes who inhabit that region requested the remains be returned to them for reburial under the Native American Graves Protection and Repatriation Act (NAGPRA). The US Army Corps of Engineers, which manages the land where Kennewick Man was found, announced their intent to do so. That in turn prompted a lawsuit to block the repatriation ${ }^{2,5}$, and generated considerable scientific controversy as to Kennewick Man's ancestry and affinities (e.g. ${ }^{3,6-9}$ ). The lawsuit ultimately (in 2004) resulted in a judicial ruling in favour of a detailed study of the skeletal remains, the results of which were recently published ${ }^{2}$.

These studies provide important details on, for example, Kennewick Man's life history, refine his antiquity to $8358 \pm 21{ }^{14} \mathrm{C}$ yrs BP or to within a two sigma range of $8400-8690 \mathrm{cal}$ BP, and demonstrate that the body had been intentionally buried and had eroded out shortly before discovery ${ }^{2}$. They also include anatomical and morphometric analyses, which confirm earlier studies that Kennewick Man resembles circumpacific populations, particularly the Ainu and Polynesians ${ }^{2,10}$; that he has certain "European-like morphological" traits ${ }^{2}$; and that he is anatomically distinct from modern Native Americans ${ }^{2}$. These results are interpreted by most as indicating that Kennewick Man was a descendant of a population that migrated
earlier than, and independently of, the population(s) that gave rise to modern Native Americans ${ }^{2}$.

However, those recent studies did not include DNA analysis. Herein, we here present the genome sequence of Kennewick Man in order to resolve his ancestry and affinities with modern Native Americans. There were several prior efforts to recover genetic material from Kennewick Man ${ }^{11}$, but none were successful.

We obtained $\sim 1 \times$ coverage of the genome, from 200mg of metacarpal bone specimen (Supplementary Information 1) using previously published methods ${ }^{12,13}$. The endogenous DNA content was between $0.4 \%$ and $1.4 \%$ for double-stranded and single-stranded libraries respectively (Supplementary Information 2). Average fragment length was 53.6 bp and exhibited damage patterns typical of ancient DNA, with excessive deamination of cytosine towards the ends of the fragments (Supplementary Information 2). Similarly, patterns of DNA decay agree with published expectations ${ }^{14}$, and display an estimated molecular halflife corresponding to 3,670 years for 100 bp molecules (Supplementary Information 3). The mitochondrial genome was sequenced to $\sim 71 \times$ coverage and is placed at the root of haplogroup X2a (Extended Data Fig. 1, Supplementary Information 2), and the Ychromosome haplogroup is Q-M3 (Extended Data Fi.g 2, Supplementary Information 5); both uniparental lineages are found almost exclusively among contemporary Native Americans ${ }^{15,16}$. We used the X chromosome to conservatively estimate contamination to be $2.5 \%$, which is within the normal range obtained observed in genomic data from ancient human remains ${ }^{17}$, and we further show this contamination to be of European origin (Supplementary Information 4).

We compiled an autosomal reference data set consisting of published SNP array data ${ }^{18-23}$ as well as new data generated from one of the claimant tribes, the Colville (Supplementary Information 10). Due to high levels of recent admixture in many Native American populations, we masked European ancestry from the Native Americans (Supplementary Information 6). No masking was done on the Kennewick Man. When we compare Kennewick Man with the worldwide panel of populations, a clear genetic similarity to Native Americans is observed both in principal components analysis (PCA) and using $f_{3}$ outgroup statistics (Fig1 a,b). In particular, we can reject the hypothesis that Kennewick Man is more closely related to Ainu or Polynesians than he is to Native Americans, as seen in a D-statistic based test where all Native Americans including Kennewick Man and the Clovis age Anzick-1 ${ }^{12}$ genomes fail to reject any tree of the type ((CHB,Ainu/Polynesian), (Native American,Karitiana)) (Extended Data Fig. 3). Model-based clustering using ADMIXTURE ${ }^{24}$ shows that Kennewick Man has ancestry proportions most similar to those of other Northern Native Americans (Fig 1c, Supplementary Information 7), especially the Colville, Ojibwa, and Algonquin. Considering the Americas only, $f_{3}$-outgroup and D-test analyses show that Kennewick Man, like the Anzick-1 child, shares a high degree of ancestry with Native Americans from Central and South America, and that Kennewick Man also groups with geographically close tribes including the Colville (Fig2a,b; Extended Data Fig. 4). Despite this similarity, Anzick-1 and Kennewick Man have dissimilar genetic affinities to contemporary Native Americans. In particular, we find that Anzick-1 is more closely related to Central/Southern Native Americans than is Kennewick Man (Extended

Data Fig. 5). The pattern observed in Kennewick Man is mirrored in the Colville, who also shows a high affinity with Southern populations (Fig 2c), but are most closely related to a neighbouring population in the dataset (Stswecem'c; Extended Data Fig. 4c). This stands in contrast to other populations such as the Chipewyan, who are closer to Northern Native Americans rather than to Central/Southern Native Americans in all comparisons (Fig 2d; Extended Data Fig. 4d).

Our results are in agreement with a basal divergence of Northern and Central / Southern Native American lineages as suggested from the analysis of the Anzick-1 genome ${ }^{12}$. However, the genetic affinities of Kennewick Man reveal additional complexity in the population history of the Northern lineage. The finding that Kennewick is more closely related to Southern than many Northern Native Americans (Extended Data Fig. 4) suggests the presence of an additional Northern lineage that diverged from the common ancestral population of Anzick-1 and Southern Native Americans (Fig. 3). This branch would include both Colville and other tribes of the Pacific Northwest such as the Stswecem'c, who also appear symmetric to Kennewick with Southern Native Americans (Extended Data Fig. 4). We also find evidence for additional gene flow into the Pacific Northwest related to Asian populations (Extended Data Fig. 5), which likely post-dates Kennewick Man. We note that this gene flow could originate from within the Americas, for example in association with the migration of paleo-Eskimos or Inuit ancestors within the past 5 thousand years ${ }^{25}$, or the gene flow could be post colonial ${ }^{19}$.

We used a likelihood ratio test to test for direct ancestry of Kennewick Man for two members of the Colville tribe who show no evidence of recent European admixture. This test allows us to determine if the patterns of allele frequencies in the Colville and the Kennewick man are compatible with direct ancestry of the Colville from the population to which the Kennewick Man belonged, without any additional gene-flow. As a comparison we also included analyses of four other Native Americans with high quality genomes: two Northern Athabascan individuals from Canada ${ }^{25}$ and two Karitiana individuals from Brazil ${ }^{12,13}$. While the test rejects the null hypothesis of direct ancestry with no subsequent gene-flow in all cases, it only does so very weakly for the Colville tribe members (Table 1, Supplementary Information 8). This findings can be explained as: (i) The Colville individuals are direct descendants of the population to which the Kennewick Man belonged, but subsequently received some relatively minor gene-flow from other American populations within the last $\sim 8.5$ thousand years, in agreement with our findings above; (ii) The Colville individuals descend from a population that $\sim 8.5$ thousand years was slightly diverged from the population which the Kennewick Man belonged or (iii) a combination of both.

It has been asserted that "...cranial morphology provides as much insight into population structure and affinity as genetic data" ${ }^{2}$. However, although recent and previous craniometric analyses have consistently concluded that Kennewick Man is unlike modern Native Americans, they disagree regarding his closest population affinities, the cause of the apparent differences between Kennewick Man and modern Native Americans, and whether the differences are historically significant (e.g. represent an earlier, separate migration to the Americas), or simply represent intra-population variation ${ }^{2,3,7,10,26-28}$. These inconsistencies
are likely due to the difficulties in assigning a single individual when comparing to population-mean data, without explicitly taking into account within-population variation. Reanalysis of W. W. Howells' worldwide modern human craniometric data set ${ }^{29}$ (Supplementary Information 9) shows that biological population affinities of individual specimens cannot be resolved with any statistical certainty. While our individual-based craniometric analyses confirm that Kennewick Man tends to be more similar to Polynesian and Ainu peoples than to Native Americans, Kennewick Man's pattern of craniometric affinity falls well within the range of affinity patterns evaluated for individual Native Americans (Supplementary Information 9). For example, the Arikara from North Dakota (the Native American tribe representing the geographically closest population in Howells' data set to Kennewick), exhibit with high frequency closest affinities with Polynesians (Supplementary Information 9). Yet, the Arikara have typical Native-American mtDNA haplogroups ${ }^{30}$, as does Kennewick Man. We conclude that the currently available number of independent phenetic markers is too small, and within-population craniometric variation too large, to permit reliable reconstruction of the biological population affinities of Kennewick Man.

In contrast, block bootstrap results from the autosomal DNA data are highly statistically significant (Extended Data Fig. 3), showing stronger association of the Kennewick man with Native Americans than with any other continental group. We also observe that the autosomal DNA, mtDNA and Y-chromosome data all consistently show that Kennewick Man is directly related to contemporary Native Americans and thus shows genetic continuity within the Americas over at least the past 8 thousand years. Identifying which modern Native American groups are most closely related to Kennewick Man is not possible at this time, since our comparative DNA database of modern peoples is limited, particularly for NativeAmerican groups in the United States. However, we find that among the groups for which we have sufficient genomic data the Colville, one of the Native American groups claiming Kennewick Man as ancestral, show close affinities to that individual or at least to the population to which he belonged. Additional modern descendants could be identified as more Native American groups are sequenced. Finally, it is clear that Kennewick Man differs significantly from the Anzick child who is more closely related to the modern tribes of Mesoamerica and South America ${ }^{12}$, possibly suggesting an early population structure within the Americas.

## METHODS

We extracted DNA from a 200mg bone fragment from Kennewick Man, and built both single and double stranded DNA libraries, which were sequenced on the Illumina HiSeq platform (Supplementary Information sections 1, 2). We performed DNA damage analyses and estimated decay rates to verify authenticity; additionally we estimated contamination on both nuclear and mitochondrial DNA (Supplementary Information sections 2, 3, 4). For the nuclear contamination we developed a model to identify the most likely source population (Supplementary Information section 4). Both mitochondrial and Y-chromosome haplogroup were determined (Supplementary Information sections 2, 5). To resolve the ancestry of Kennewick Man, we performed PCA, outgroup $f_{\mathcal{3}^{-}}$and $D$-statistics, as well as ADMIXTURE analyses on a panel of published SNP array data that was collected and
curated from worldwide populations with suggested relationship to Kennewick Man (Supplementary Information sections 6, 7), in addition to data generated from members of the Colville Tribe (Supplementary Information section 1). Individual and tribal consent was obtained for all study participants, and the National Committee on Health Research Ethics in Denmark had no comments on the design (H-3-2012-FSP21). We tested if Kennewick Man belonged to a population ancestral to the Colville Tribe and estimated their divergence time (Supplementary Information section 8). Lastly, we reanalysed the craniometric data for Kennewick Man, and compared it to both individual samples and population mean data (Supplementary Information section 9).

## Extended Data



Extended Data Fig. 1. Phylogenetic tree of mitochondrial haplogroup X including Kennewick Man
A median-joining network of Genbank sequences from haplogroup X was constructed as described in the supplemental material. Haplogroup names are indicated by bold dark grey boxes, sequences of Native American origin are in light green background. GenBank accession numbers are shown in boxes at branch tips.
a



Extended Data Fig. 2. Y-chromosome haplogroup
a, Phylogenetic tree including representative sequences of haplogroup $P$, the clade that includes haplogroups Q and R. Kennewick Man shares ancestry with orange branches. Each branch is labelled with an integer index and, in brackets, the number of SNPs that define it. b, Counts of SNPs from each branch of the tree, stratified by Kennewick Man genotype (ancestral in blue, derived in orange) and mutation type ( $\mathrm{C} \rightarrow \mathrm{T}$ and $\mathrm{G} \rightarrow \mathrm{A}$ transitions coloured more lightly). Branch 19 was omitted to preserve scale; the Kennewick genotype was ancestral at all 145 sites for which read data were available.


Extended Data Fig. 3. D-statistic based test for Ainu and Polynesian affinity to Han Chinese and Native Americans
Test of the type $\mathrm{D}((\mathrm{CHB}, \mathrm{Ainu} /$ Polynesian $),(\mathrm{X}$, Karitiana $)$ ), where X is an Arctic or Native American population, including Kennewick Man and Anzick-1. Values equal to 0 , has population X being closer to Karitiana than either CHB or Ainu/Polynesian. Thick and thin whiskers represent 1 and 3 standard errors, respectively.


| Reg | ion |
| :---: | :---: |
| $\bigcirc$ | West Europe |
| $\rightarrow$ | East Asia |
| $\rightarrow$ | Siberia |
| $\rightarrow$ | Arctic |
|  | North America |
| $\rightarrow$ | Central America |
| - | South America |

Extended Data Fig. 4. Similarity between Kennewick Man and Anzick-1 as well as Colville Test of D((YRI,Kennewick/Anzick-1/Colville/Chipewyan),(X,Karitiana)), to illustrate similarities between Kennewick Man and Anzick-1, and the Colville tribe. Thick and thin whiskers represent 1 and 3 standard errors, respectively.


Extended Data Fig. 5. Affinity of Kennewick Man and Anzick-1 to Native American populations Test of $\mathrm{D}((\mathrm{YRI}, \mathrm{X}),($ Kennewick,Anzick-1)) to distinguish different affinities of the ancient samples, and $\mathrm{D}((\mathrm{YRI}, \mathrm{CHB}),(\mathrm{X}$, Karitiana $))$ to test for recent Asian gene-flow. Thick and thin whiskers represent 1 and 3 standard errors, respectively.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Genetic affinities between Kennewick Man and a panel of World-wide populations a, principal components analysis (PCA) projecting Kennewick Man and Anzick-1 onto a set of out-of-Africa populations. b, heat map of $f_{\mathcal{3}}$-outgroup statistics between Kennewick Man, Native Americans, Siberians and additional populations with suggested relationship to Kennewick Man (in squares). Warmer colours indicate higher allele sharing. c, admixture proportions for world-wide set of population, including masked Native American, Anzick-1 and Kennewick, shown at $\mathrm{K}=14$.


Figure 2. Shared ancestry among samples within the Americas
Heat maps of $f_{3}$-outgroup statistics testing (YRI; Native Americans, X ), where X is
Kennewick Man (a), Anzick-1 (b), Colville (c) or Chipewyan (d). Warmer colours indicate higher allele sharing.

Figure 3. Illustration of Native American population history
Depicted is a population tree consistent with the broad affinities between modern and ancient Native Americans. Kennewick Man and the Anzick-1 child are indicated with blue and green stars respectively. Red dashed arrows indicate gene flow (1) of Asian-related ancestry with tribes of the Pacific Northwest and (2) between Colville and neighboring tribes.

Table 1

## Direct ancestry test

$c_{1}$ is the probability of coalescence in the Kennewick lineage and $c_{2}$ is the probability of coalescence in the reference population lineage. A value of $c_{1}=0$ corresponds to direct Kennewick ancestry of the reference population with no subsequent gene-flow. Smaller likelihood ratios indicate less evidence against direct Kennewick ancestry

|  | Coalescence probability in <br> Kennewick lineage $\left(\boldsymbol{c}_{\mathbf{1}}\right)$ | Coalescence probability in <br> reference lineage $\left(\boldsymbol{c}_{\mathbf{2}}\right)$ | $\mathbf{2 \times L o g}$ Likelihood ratio <br> $\mathbf{o f} \mathbf{H}_{\mathbf{0}}: \boldsymbol{c}_{\mathbf{1}}=\mathbf{0} \mathbf{~ v s .} \mathbf{H}_{\mathbf{A}}: \boldsymbol{c}_{\mathbf{1}}>\mathbf{0}$. |
| :--- | ---: | ---: | ---: |
| Colville 2 | 0.015 | 0.072 | 19.41 |
| Colville 8 | 0.007 | 0.097 | 3.93 |
| Athabascan 1 | 0.048 | 0.073 | 505.52 |
| Athabascan 2 | 0.056 | 0.097 | 807.69 |
| B116 (Karitiana) | 0.040 | 0.140 | 423.87 |
| HGDP00998 <br> (Karitiana) | 0.040 | 0.170 | 446.30 |


[^0]:    \#Corresponding author: ; Email: ewillerslev@snm.ku.dk DATA SHARING
    The Kennewick Man genome sequence is available at SRS937952. The Colville SNP chip data can be used for scientific confirmation of our findings (with regard to the groups direct ancestry to Kennewick Man). It cannot be used for any other purpose. The Colville data in this study are available under data access agreement with E.W.

    AUTHOR CONTRIBUTION
    EW headed the project. EW and TWS initiated the work. MR conducted the lab work. MR, MS, AA, TSK, JVMR, GDP, MEA, IM, HJ, and RN conducted the DNA analyses. CPEZ and MPL conducted the morphological analyses. EW, DJM, and MR wrote the paper with contributions from all the authors. CONFLICT OF INTEREST STATEMENTS
    CDB is a founder of Identify Genomics. He is also on the Scientific Advisory Board (SAB) of Identify, Etalon, Personalis, and Ancestry.com. He is a former MAB member of InVitae. None of these organizations played a role in the design or conduct of the work presented here.

