**[For SUPPLEMENTARY MATERIAL]**

**High levels of consanguinity in a child from Paquimé, Chihuahua, Mexico**

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**Ancient DNA laboratory work**

Ancient DNA (aDNA) from burial 23-8 was collected as part of the Reich and Snow Labs’ collaboration and the Proyecto de Investigación de Poblaciones Antiguas en el Norte y Occidente de México (PIPANOM). Under the collaboration agreement, individuals from Paquimé to be sampled for aDNA were brought to the Snow Laboratory (University of Montana) where they underwent initial processing. This involved producing powder from the petrous/cochlea or teeth; the powder was then divided, with half retained by the Snow lab for furthering processing to obtain mitochondrial (mtDNA) data and the other half sent to the Reich Lab (Harvard University) for processing to obtain genome-wide aDNA data. The Reich Lab received 0.05 grams of cochlea powder for processing; we describe the procedure below.

The powder that arrived at the Reich Lab was turned into an extract through a series of previously described steps to isolate, clean and amplify the DNA (Dabney *et al.* 2013; Rohland *et al.* 2015). In brief, the DNA was converted into a form that could be sequenced using a double-stranded library preparation protocol, which involved a partial pre-treating with the enzyme uracil-DNA glycosylase (UDG) to reduce the characteristic cytosine-to-thymine errors in aDNA at all but the terminal positions in the reads. Following Fu *et al*. (2013a, 2015), approximately 1.2 million single nucleotide polymorphisms (SNPs) were targeted, sequenced on a HiSeq X10 Illumina instrument and mapped to the human genome reference sequence hg19 as previously described (Mathieson *et al.* 2015). For the whole genome data, an allele at each position was determined by randomly sampling a single position. For the mtDNA, a consensus sequence was built aligning to the Reconstructed Sapiens Reference Sequence genome (Behar *et al.* 2012).

Sequencing statistics are presented in Table S1.

**Table S1. Analytical processes and results**

|  |  |
| --- | --- |
| **Information** | **Value** |
| Skeletal Code | 23-8 |
| Skeletal element | cochlea |
| 14C: Material used for radiocarbon measurement | XAD amino acids |
| 14C: Fraction Modern | 0.9255 ± 0.0017 |
| 14C: D14C (‰) | -74.5 ± 1.7 |
| 14C: Uncalibrated radiocarbon date (radiocarbon years BP) | 620 ± 15 |
| 14C: δ13C (‰) | -11.27 |
| 14C: δ15N (‰) | 10.82 |
| 14C: %C | 14.73 |
| 14C: %N | 5.35 |
| 14C: C:N ratio | 3.21 |
| 14C: Lab Code | PSUAMS-10865 |
| 14C: OxCal mu (years) | 604 |
| 14C: OxCal sigma (years) | 30 |
| 14C: 94.5% confidence interval for calibrated date | cal AD 1301–1397 |
| 14C: Calibration Program | OxCal 4.4.2 and IntCal20 (Reimer *et al.* 2020; Bronk Ramsey 2021) |
| Genetic (methodology): Library ID | S22220.Y1.E2.L1 |
| Genetic (methodology): Individual ID | I22220 |
| Genetic (methodology): amount of powder used in DNA extraction (mg) | 39 |
| Genetic (methodology): Extraction Method | (Rohland *et al.* 2018); Dabney Buffer, silica beads, robotic |
| Genetic (methodology): Library preparation | double-stranded, partially UDG-treated |
| Genetic (methodology): amount of powder in library (mg) | 7.8 |
| Genetic (methodology): Library Preparation Method | (Rohland *et al.* 2015); silica coated magnetic beads and 7x volume PB (Qiagen) for cleanups, Bst2.0 in smaller volume, 100ul total PCR volume, higher primer concentration, SPRI cleanup of PCR |
| Genetic (methodology): Target enrichment performed | simultaneous enrichment of ~1.2M SNPs and mtDNA (Fu *et al.* 2013a, 2015) |
| Genetic (methodology): Sequencing platform for 1240k capture data | HiSeq X10: 2x101 reads, 2x7 bp to read out the indices |
| Genetic (shotgun sequencing of unenriched library): percentage mapping to the human reference genome hg19 | 0.038 |
| Genetic (shotgun sequencing of unenriched library): mean length of sequences | 53.9 |
| Genetic (enriched sequences mapping to the reference mtDNA genome sequences rsrs (Behar *et al.* 2012): average coverage | 149.7 |
| Genetic (enriched sequences mapping to the reference mtDNA genome sequences rsrs): mean sequence length | 58.4 |
| Genetic (enriched sequences mapping to the reference mtDNA genome sequences rsrs): fraction of C-to-T damage in last base | 0.068 |
| Genetic (enriched sequences mapping to the reference mtDNA genome sequences rsrs): match to consensus sequence 95% CI using contamMix version 1.0-12 (Fu *et al.* 2013a) | [0.994, 1.000] |
| Genetic (enriched sequences mapping to the reference mtDNA genome sequences rsrs): mtDNA haplogroup based on haplogrep 2 (Weissensteiner *et al.* 2016) | C1b |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): average coverage at 1.15M autosomal targeted SNPs | 0.454593 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): targeted autosomal SNPs covered at least once | 395547 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): mean sequence length | 63.1 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): fraction of C-to-T damage in last base | 0.07 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): targeted X chromosome SNPs covered at least once | 9273 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): targeted Y chromosome SNPs covered at least once | 6461 |
| Genetic (enriched sequences mapping to targeted nuclear genomic positions): genetically determined sex | M |

*Contamination estimation*:We used several methods to assess evidence of contamination for burial 23-8. We measured the rate of damage in the first nucleotide and found that burial 23-8 did not have less than 3% cytosine to thymine substitution rate in the first nucleotide. We found no evidence of contamination in mtDNA with *contamMix* (Fu *et al.* 2013b), and no evidence of X chromosome contamination with ANGSD (Korneliussen *et al.* 2014).

**Table S2. Contamination statistics for burial 23-8.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ANGSD SNPs** | **ANGSD MOM point estimate** | **ANGSD MOM Z-score** | **ANGSD MOM 95% CI truncated at 0** | **mt coverage** | **mt damage last base** | **mt consensus match** | **mt consensus match 95CI** |
| 480 | 0.007 | 1.376 | 0.000, 0.016 | 149.7 | 0.068 | 0.999266 | 0.994, 1.000 |

*Uniparental markers:*For mtDNA, consensus sequences were created with samtools and bcftools version 1.31 using majority rule and then using HaploGrep2 with Phylotree version 17; the consensus match was 0.994. The Y chromosome haplogroup was determined using the individual sequence data (BAM-file). We filtered out reads with mapping quality <30 and bases with base quality <30 and trimmed the first and last 2bp of each sequence to remove potential errors due to characteristic aDNA damage. We made a haplogroup determination based on the most derived mutation using the nomenclature of the International Society of Genetic Genealogy (ISOGG) (http://www.isogg.org) version ISOGG v15.73 (2020) notation using Yfitter (Jostins *et al.* 2014).

*Genetic sex:* Genetic sex was determined by computing Y = the number of sequences overlapping SNPs on the Y chromosome (Y) and X = the number of sequences overlapping SNPs on the X chromosomes (X). We then computed the ratio R=Y/(X+Y). Using this calculation, genetic males have vales of R>0.35; burial 23-8’s R=0.411.

**Demographic modelling**

*Principal Component Analysis*: Principal component analysis was performed using smartPCA in EIGENSOFT (Patterson *et al*. 2012). We used the default parameters except for “lsqproject: YES” and “shrinkmode: YES” and performed PCA on modern Indigenous populations from the Americas including: Chane, Guarani, Huichol, Mixtec, Piapoco, Pima, Quechua and Zapotec (Reich *et al.* 2012; Lazaridis *et al.* 2014; Skoglund *et al.* 2015; Barbieri *et al.* 2019). We then projected published ancient individuals (Malaspinas *et al.* 2014; Raghavan *et al.* 2014, 2015; Rasmussen *et al.* 2015; Kennett *et al.* 2017, 2022; Lindo *et al.* 2017; de la Fuente *et al.* 2018; Moreno-Mayar *et al.* 2018a & b; Posth *et al.* 2018; Scheib *et al.* 2018; Schroeder *et al.* 2018; Flegontov *et al.* 2019; Bongers *et al.* 2020; Nakatsuka *et al.* 2018, 2020; Nägele *et al.* 2020; Nieves-Colón *et al.* 2020; Capodiferro *et al.* 2021; Fernandes *et al.* 2021; Popović *et al.* 2021; Tiesler *et al.* 2022) and burial 23-8 on to the principal components determined using present-day individuals.

*ADMIXTURE*: We used the ancient and modern populations above, along with European (America; The 1000 Genomes Project Consortium 2015), Africa (Mbuti; Bergström *et al.* 2020) and South American (Surui, Karitiana; Bergström *et al.* 2020) populations to examine admixture. We first used PLINK2 (Chang *et al*. 2015) to prune the dataset using the –geno 0.7 option to remove all sites that had less than 70% of the samples with a called genotype. We then ran unsupervised ADMIXTURE (Alexander *et al.* 2009) with K=2 to 10 with 10 replicates for each K. We selected K=9 for Figure 6 because of its usefulness for visual discrimination of ancestry components and because validation errors for K2–10 were not significantly different.

*f-statistics*: We used the *qp3pop* package in ADMIXTOOLS version 6.0 to compute *f3*-statistics. We computed outgroup *f3*-statistics of the form *f3(Pop1, Pop2; Mbuti)* to measure the shared genetic drift between burial 23-8 (population 1) and Americas populations (population 2).

**Runs of homozygosity analyses**

We used *hapROH* (version 0.1a8; https://pypi.org/project/hapROH/) to identify runs of homozygosity (ROH) (Ringbauer *et al.* 2021). We used the 1000 Genomes Project haplotype panel as the reference panel with 5008 global haplotypes. We then analysed the ancient and present-day data of individuals with at least 300 000 SNPs covered to identify ROH longer than 4 centimorgans (cM). We used the default settings of hapROH for all analyses. All published individuals from the Americas that had high enough coverage to calculate ROH are provided in Table S7 (Rasmussen *et al.* 2014, 2015; Posth *et al.* 2018; Scheib *et al.* 2018; Flegontov *et al.* 2019; Bongers *et al.* 2020; Nakatsuka *et al.* 2018, 2020; Nägele *et al.* 2020; Fernandes *et al.* 2021; Kennett *et al.* 2022; Tiesler *et al.* 2022). Individuals with the highest long ROH are summarised in Table S3.

**Table S3. Americas individuals with the highest measured long RoH.**

|  |  |
| --- | --- |
| **Individual ID # and location** | **Sum total RoH segments >20cm** |
| I0748 (California)1 | 499.06 |
| **Burial 23-8 (Paquimé)** | **270.40** |
| I5320 (Alaska)2 | 248.39 |
| SN-44 (California)3 | 247.85 |
| I11968 (California)1 | 241.17 |
| SN-11 (California)3 | 226.22 |
| I0308 (Argentina)4 | 162.07 |
| I13322 (Caribbean)5 | 137.28 |
| I23708 (California)1 | 133.78 |
| I0042 (Peru)6 | 133.77 |
| I7966 (Caribbean)5 | 130.04 |
| I11557.SG (California)1 | 108.32 |
| I10758 (Caribbean)5 | 96.31 |

(1Nakatsuka *et al.* 2023; 2Flegontov *et al.* 2019; 3Scheib *et al.* 2018; 4Posth *et al.* 2018; 5Fernandes *et al.* 2021; 6Nakatsuka *et al.* 2018). Note: expected summed long RoH values from inbreeding of siblings: 625 cM, first cousins: 125 cM, second cousins: 30 cM, third cousins: 15 cM (from Ringbauer *et al*. 2021).

**Radiocarbon dating**

As noted in the main manuscript, burial 23-8 was buried beneath a post that had been dated through dendrochronology. Despite this, we felt it necessary to directly date burial 23-8, especially considering the issues that have been reported with the dendrochronology of Paquimé (Dean & Ravesloot 1993; Lekson 2015). To obtain a date, we removed a small piece of bone from the same petrous (left) that was sampled for aDNA and sent it to the Pennsylvania State University AMS laboratory. Our first sampling attempt failed, but a second attempt from the left petrous was successful. The C and N isotope values are provided in Table S4.

**Table S4. Radiocarbon data for burial 23-8. Penn State University lab # PSUAMS-10865.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **14C age (BP)** | **±** | **δ13C (‰)** | **δ15N (‰)** | **%C** | **%N** | **C:N** |
| 620 | 15 | -11.26727104 | 10.81560855 | 14.7303342 | 5.350976343 | 3.211636307 |

**Strontium analysis**

Strontium analysis was performed at the Johnson Mass Spectrometry Laboratory at New Mexico State University, which has performed strontium ratio analysis for archaeological fauna remains from the same region (e.g. Semanko & Ramos 2022).

To minimise the impact of destructive sampling, we used the remaining powder from the cochlea sampled from burial 23-8 for Strontium (Sr) analysis. While there has been limited research on the lack of turnover of Sr in cochlea bone, what has been done (Harvig *et al.* 2014; Cavazzuti *et al.* 2019; Kootker & Laffoon 2022) showed that it does represent childhood strontium ratios, presumably for the same reason that cochlea bone is so good at preserving aDNA (Pinhasi *et al.* 2015). In any case, the young age of the individual means that there would have been insufficient time for the Sr ratio to have been changed from a birth location other than Paquimé to the value for Paquimé, especially since the strontium ratio was at the centre of the Paquimé range. Moreover, a large sample of fauna bone strontium ratios from the site from the same study as the human tooth ratios bracket the local human ratios (Offenbecker 2018). This makes it clear that bone ratios are not subject to meaningful diagenesis in this locality and time frame. Thus, while the child’s strontium ratio comes from bone and not tooth enamel, like the other human samples, they are clearly comparable, and the child was both born and lived his short life at Paquimé.

Once in the Johnson laboratory the powder was cleaned; 50mg of pre-powdered bone mass was dissolved using doubly distilled, 6N hydrochloric acid (HCl). The powder was placed in a 15ml Teflon container with approximately 5ml of doubly distilled 6N HCl. The sample was placed on a hotplate at ~180°C overnight.

After digestion, the sample was dried, redissolved in 1.0ml of 2.5N HCl, and centrifuged. Approximately 0.5ml was loaded onto 22ml pyrex columns containing approximately 2ml of 200–400 mesh cation exchange resin. Doubly distilled and calibrated 2.5N HCl was used to obtain pure Sr following the procedures of Ramos (1992). Purified Sr solutions were then dried in preparation for loading and analyses using thermal ionisation mass spectrometry (TIMS).

Sr was loaded onto pre-outgassed and clean rhenium filaments with phosphoric acid and tantalum oxide. Sr isotopes were analysed using a VG Sector 54 thermal ionisation mass spectrometer and five Faraday collectors in dynamic mode with 88Sr=3.0V. Sr isotopes were normalised to 86Sr/88Sr=01194 and corrected for any rubidium present during the analysis. NBS987 Sr carbonate was 0.710275 ± 0.000010.

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