

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	BWA version 0.7.15 and other bioinformatics tools and data workflows (https://github.com/DReichLab/ADNA-Tools and https://github.com/DReichLab/adna-workflow). A detailed description of the data preprocessing steps is given in the Methods section of the manuscript.
Data analysis	We deposited the Code for analyzing the data and producing the figures in this manuscript at https://github.com/hringbauer/punic_aDNA . Relevant published software we used (including version): anclBD version 0.5, hapROH version 0.63, SeqPrep 1.1, smartpca version 18150, ADMIXTURE version 1.3.0, qpAdm version 1201, Phylotree version 17, Yfull version 8.09, HaploGrep2 version 2.1.1, contamMix version 1.0-12, ANGSD version 0.923, OxCal version 4.4.4, SAMtools 1.3.1, pmdtools 0.60, PLINK V1.9, PONG V1.5

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw DNA sequences are deposited in the European Nucleotide Archive under the accession number XXX. Other newly reported data such as radiocarbon dates and archaeological context information are included the manuscript and supplementary files. Processed genotype data for individuals newly sequenced in this study can be obtained from the Harvard Dataverse repository through the following link (XXX).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size The sample size and geographic distribution is determined by accessibility of relevant osteological remains with sufficient aDNA preservation; it is not something we can choose a priori. We report new ancient DNA data from many contexts where ancient DNA has not previously been reported, analyzing all samples with sufficient aDNA preservation. Although even more powerful inferences could have been made if sample sizes were larger, we can make many meaningful new inferences with the available samples.

Data exclusions We excluded samples that did not fall within the geographic scope of the study. After collecting genetic data, we excluded individuals from the analysis dataset as described in the Methods section entitled "Determination of ancient DNA authenticity." Specifically: "We determined ancient DNA authenticity based on five criteria. First, we required that the lower bound of the 95% confidence interval for contamination from ANGSD (if we were able to compute it) was <1%. Second, we required that the upper bound of the 95% confidence interval for match rate to mitochondrial consensus sequence (if we were able to compute it) was >95%. Third, we required that the average rate of cytosine-to-thymine errors at the terminal nucleotide for all sequences passing filters was >3% for double-stranded partially UDG-treated libraries³⁹ and >10% for single-stranded USER-treated libraries and double-stranded non-UDG-treated libraries (the latter libraries are all from previously published data that we reanalysed here). Fourth, we required the ratio of sequences mapping to the Y chromosome to the sum of sequences mapping to the X and Y chromosome for the 1240K data to be less than 3% (consistent with a female) or >35% (consistent with a male). Fifth, to report an individual we required the number of SNPs covered at least once to be at least 5,000 (for most actual population genetic analyses, we required at least 20,000).

Replication Only a single library can be made from each extract aliquot so no replication from the same extract is possible. For the individuals with more than one library (15x two libraries per sample, 3x three, 1x four, 1x five), we confirmed in all cases that the libraries were from the same individuals. Another measure of replication also derives from the fact that the ancestry distributions in individuals from the same periods tended to be very similar. As a result of this, key findings in this study are not dependent on single samples.

Randomization Historical studies are retrospective rather than prospective -- and the actual trajectory of human history has occurred only once -- so

Randomization	randomization of the data into independent processes is not possible. The manuscript discusses a caveat about possible biases due to non-random sampling.
Blinding	Co-analysis of the genetic and archaeological data was central to the study, so we could not be blind to the sample identity.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	We describe the provenance of all archaeological specimens in Supplementary Data 1 and the "Archaeological Site Descriptions", a document that for each Archaeological site lists the 1) General Location and Chronology, 2) Excavation history, 3) Description of cemeteries and 4) Relevant references.
Specimen deposition	The bone and tooth parts that remain after analysis for ancient DNA are under the stewardship of the archaeologists and cultural institutions from which they were sampled. At present, they are either already returned to the sample stewards or they are stored on long-term loan at the ancient DNA laboratories where they were analysed. They can be re-examined upon request to the sample stewards. Researchers who wish to replicate analyses from this study or gather new data on the libraries generated for this study are welcome to make a request for aliquots of those libraries to corresponding author David Reich who will fulfill all reasonable requests
Dating methods	We obtained 111 accelerator mass spectrometry-based dates on bone for 99 distinct human remains in specialized C14 laboratories at Pennsylvania State University (82 measurements), the D-REAMS laboratory at the Weizmann Institute of Science (24), Oxford (four), and CIRAM (one). We report conventional radiocarbon ages and standard errors (Supplementary Data 1B). We used the calibration curve IntCal20 to calibrate conventional radiocarbon dates (see Methods). Eleven individuals were analyzed by two different labs, with date ranges from the two labs being highly concordant in all cases. For these individuals, we used the R_combine method to combine date ranges.
☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	All human skeletons analysed in this study were sampled with written permission of the stewards of the skeletons and every individual is represented by at least one co-author. Researchers who wish to obtain further information about specific individuals should write to the corresponding authors and/or the authors who provided the archaeological contextualisation for those individuals whose names are specified in the "Archaeological Site Descriptions".

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Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A