nature portfolio

Corresponding author(s): Chris Fowler, Iñigo Olalde, Ron Pinhasi, David Reich

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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 (*n*) 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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 *d*, Pearson's *r*), 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 v0.6.1, HaploGrep2 v2.1.19, contamMix v1.0-10, ANGSD v0.923, NgsRelate, Samtools, bcftools, other bioinformatics tools and data workflows (https://github.com/DReichLab/ADNA-Tools and https://github.com/DReichLab/adna-workflow)
Data analysis	hapROH v0.3

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 aligned sequences are available through the European Nucleotide Archive, accession PRJEB46958 [to go live upon publication]; the genotype dataset is available as supplementary material.

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

Ecological, evolutionary & environmental sciences study design

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

Study description	This study investigates whether or not a large number of individuals buried in the same Neolithic tomb were close biological relatives, and, if they were, how they were related. By comparing these results with archaeological contextual information, such as where each individual was placed in the tomb, we are able to draw detailed inferences about kinship practices. The study collected 74 samples from human skeletal remains for DNA analysis, assessed the biological relationships between those individuals, and situated that alongside the available archaeological data for the site in reaching its conclusions
Research sample	The study collected 74 aDNA samples, largely taken from petrous bones and teeth but also including some post-cranial elements and not known to be from the same individuals, in an attempt to perform as complete as possible as a survey of ancient DNA of individuals buried in Hazleton North chambered cairn. Of these samples, 66 produced viable results. Our genetic analysis indicates that the samples derive from 35 distinct individuals whose remains were found within the tomb; the high rate at which we are obtaining DNA from multiple samples from the same individuals suggests that our sampling is well on its way to achieving its goal of sampling a large fraction of the individuals buried in the tomb. The 35 distinct individuals we identify from DNA can be compared with the osteological estimate of the presence of a minimum number of 41 individuals from the tomb. Archaeological and osteological data is drawn from the original excavation report (Saville 1990) and a PhD study involving a re-examination of the human remains (Cuthbert 2019), both of which are cited in the article. A full catalogue of each skeletal element is available from Corinium Museum on request.
Sampling strategy	The study took a sample from every petrous portion of the temporal bone that was available during the two times the museum was visited (17 in total), and samples from teeth (49 in total), and post-cranial bones (8 in total) designed to cover as many of the individuals buried in the tomb as possible. Petrous bones and teeth were prioritized as they contain the densest concentrations of surviving DNA and so are most likely to yield a result. We never knowingly took multiple samples from the same individual, as our goal was to minimize destruction of skeletal elements while obtaining data from as high a fraction as possible of the individuals in the tomb. Our goal was to sample enough so that we began to saturate the total number of individuals buried in the tomb, as we in fact did by observing that the 66 samples with viable results came from only 35 distinct individuals.
Data collection	The first round of skeletal sampling was performed by a team from the University of Vienna (Olivia Cheronet) in the presence of staff at the Corinium museum. The second round of skeletal sampling was performed entirely by the staff at the Corinium museum. DNA was extracted from using an automated protocol with silica coated magnetic beads and 'Dabney binding buffer'. DNA extracts equivalent to between 6 and 8 mg of powder were converted into either single-stranded or double stranded libraries following automated library preparation. For some samples we built multiple libraries. USER treatment was applied before single-stranded library preparation and partial UDG treatment before double-stranded library preparation. Amplified libraries were enriched using two rounds of consecutive hybridization capture enrichment ('1240k' strategy32,33) targeting 1,233,013 SNPs and the mitochondrial genome or, 'Twist Ancient DNA' , a custom probe panel synthesized by Twist Biosciences. This custom panel targets the very same 1,233,013 SNPs as well as additional SNPs and tiling regions (Twist probes targeting the mitochondrial genome were spiked in) and was performed for only one round of enrichment using reagents and buffers provided by Twist Biosciences. Captured libraries were sequenced either on an Illumina NextSeq500 instrument with 2x76 cycles (2x7 cycles for the indices) or on an Illumina HiSeq X10 with 2x101 cycles (2x7 for the indices). For this study, we restricted all our analysis to the 1,233,013 SNPs in common between '1240k' and 'Twist Ancient DNA' and the mitochondrial genome. Following the same procedure as in Olalde et al. 201936, we trimmed adapter sequences, merged paired-end sequences, aligned to both the human reference genome (hg19) and the mitochondrial genome (RSRS) using BWA v0.6.146 and removed PCR duplicate sequences. The computational pipelines are available on github (https://github.com/DReichLab/ADNA-Tools, https://github.com/DReichLab/adna-workflow).
Timing and spatial scale	The samples were taken during two sampling visits: the first in 2018 and the second in 2021.
Data exclusions	The only excluded sample was an individual that yield less than 5000 single nucleotide polymorphisms (SNPs) covered at least once, which is too little data for meaningful analysis.
Reproducibility	Only a single library can be made from each extract aliquot so no replication from the same extract is possible. However, the data from the 35 individuals came from 156 distinct libraries (range of 1-15 per individual). For the 29 individuals with more than one library, we had internal replication confirming that the libraries were from the same individuals.
Randomization	The sampling attempted to obtain DNA from every individual in the tomb, and so we aimed to analyze a sample from every individual that appeared osteologically distinct as well as additional samples that potentially could be osteologically distinct.

Blinding: Co-analysis of the genetic and archaeological data was central to the study, so we could not be blind to the sample identity.

Did the study involve field work? ☐ Yes ☒ No

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Palaeontology and Archaeology

Specimen provenance: The genetic samples were taken from human remains excavated in the 1981-2 excavations of the long barrow at Hazleton North, England, and subsequently curated at Corinium Museum, Cirencester, England.

Specimen deposition: The human remains are all still curated by Corinium Museum, and are either (a) currently in the Museum or (b) currently on load to Harvard Medical School and scheduled for return to the Museum within 6 months of the completion of their analysis.

Dating methods: No new dates are provided.

☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight: Permission to study the samples was carried out with official permission from the Corinium Museum which curates the samples for scientific study.

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