

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 <i>P</i> 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	Software: BWA-v.0.6.1a, contamMix v1.0-10,
Data analysis	HaploGrep2, hapROH, ADMIXTURE v1.3.0, smartpca v18160, PLINK1.9, ADMIXTOOLS v6.0, ancIBD, HapNe version 1.20230726

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](#)

Genotype data for individuals included in this study can be obtained from the Harvard Dataverse repository through the following link (<https://doi.org/10.7910/DVN/QGNMRH>). The DNA sequences reported in this paper are deposited in the European Nucleotide Archive under accession number PRJEB81467. Other newly reported data such as radiocarbon dates and archaeological context information are included in the manuscript and supplementary files

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	The ancient individuals studied here were all analyzed with the goal of minimizing damage to their skeletal remains, with permission from local authorities in each location from which they came. Every sample is represented by stewards such as archaeologists or museum curators, who are either authors or thanked in the Acknowledgments. Open science principles require making all data used to support the conclusions of a study maximally available, and we support these principles here by making fully publicly available not only the digital copies of molecules (the uploaded sequences) but also the molecular copies (the ancient DNA libraries themselves, which constitute molecular data storage). Those researchers who wish to carry out deeper sequencing of libraries published in this study should make a request to corresponding author D.R. We commit to granting reasonable requests as long as the libraries remain preserved in our laboratories, with no requirement that we be included as collaborators or co-authors on any resulting publications.

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

Ecological, evolutionary & environmental sciences study design

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

Study description	Genetic analyses were performed on DNA data generated from ancient human skeletons and from samples from present-day populations. Population genetic statistics, primarily testing historical relationships by measuring allele-sharing patterns across populations, were computed using genome-wide SNP genotypes.
Research sample	We generated genome-wide data from 291 never-before-reported ancient individuals, and increased quality data from 63 individuals for whom data have previously been reported.
Sampling strategy	If available and accessible, we prioritized sampling petrous bones, taking bone powder from the cochlea by sandblasting and milling, or directly drilling into the cochlea after physical surface cleaning, or drilling through the cranial base to minimize damage to intact skulls. If we could not sample from the cochlea, we sought to sample a tooth, prioritizing the cementum layer after physical surface cleaning. If neither a cochlea nor a tooth was available, we sought to sample a dense cortical bone, which we analyzed by drilling and collecting powder after physical surface cleaning. For some samples that could not leave the museum, we sampled on site, either drilling directly into the cochlea, the tooth root, or bone after physical surface removal. We sometimes dislodged auditory ossicles during sandblasting or drilling into the cochlea. When this happened during the cleaning procedure, we generally stopped the destructive sampling and collected the ossicle(s).
Data collection	DNA from the ancient remains was extracted, sequenced, and processed into genotype calls.
Timing and spatial scale	Ancient individuals lived from 6400-2000 BCE in Russia and the Danube region..
Data exclusions	The newly reported data come from 803 ancient DNA libraries, of which 195 failed screening.
Reproducibility	All attempts to reproduce were successful.
Randomization	No randomization was possible because this was a study of past processes which are not repeatable.
Blinding	No blinding was possible because knowledge of dates and locations was critical to analysis.
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
☒	☐ 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	The skeletal remains analyzed here were all analyzed here are all from museum collections or other archaeological archives. The samples we analyzed with permission of sample custodians and formal permission of appropriate local authorities.
Specimen deposition	The skeletal samples are under the stewardship of the co-authors or museum collections. Samples may be accessed by their skeletal code listed in Online Table 1.
Dating methods	We report 198 new radiocarbon dates using standard methods.
☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	The skeletal remains analyzed here were all analyzed with permission from local authorities in each location from which they came. Every sample is represented by stewards such as archaeologists or museum curators, who are either authors or thanked in the Acknowledgments.

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