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Last updated by author(s):	May 3, 2020	

# **Reporting Summary**

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

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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
$\boxtimes$	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.
$\boxtimes$	A description of all covariates tested
X	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
$\times$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$	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

No specific software was used for data collection. All software used in this study is listed below.

Data analysis

All software used in this work is publicly available. Corresponding publications are cited in the main text and supplementary material. List of software and respective versions:

CASAVA v1.8.2

AdapterRemoval v2.1.3

bwa v0.7.10

bwa mem 0.7.10

picard tools v1.127

bamUtil v1.0.14

samtools v1.3.1 GATK v3.3.0

pysam 0.7.4 (python module)

bedtools 2.27.1

mapDamage2.0

contamMix v1.0-5

SHRiMP 2.2.3

YFitter v0.2

Haplogrep 2.0

FineSTRUCTURE

ANGSD v0.915

IBDseq v.r1206

PRANK v.150803 pathPhynder

BEASTv1.8.2

chmutzi v.1.5.4
leaf v2
dmixtools v4.1
GSrelate v.1
LOBETROTTER
EAD
RIMUS v1.9
link and v1.9
DMIXTURE v1.3
AxML-8.1.15
npEff
PAdes-3.9.0
3.2.3
hana
eagle v4.1
ython 2.7.12
erl v5.22.1
ALIB
igTree v.1.4.4

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

### Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence data are available at the European Nucleotide Archive under accession number PRJEB37976.

# Field-specific reporting

Please select the one belo	w that is the best fit for your research	. If yo	ou are not sure, read the appropriate sections before making your selection.
X Life sciences	Behavioural & social sciences		Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

Sample size

We did not rely on statistical methods to predetermine sample sizes. Sample sizes in ancient population genetic studies are limited by the number of samples yielding endogenous DNA proportions amenable to whole genome sequencing.

Data exclusions

We selected 442 samples for whole-genome sequencing, out of all (n=528) screened samples, based on their endogenous content and low contamination estimates. These criteria are described in detail in Supplementary Notes 2 and 3. Furthermore, closely related individuals were excluded from analyses requiring population allele frequencies.

Replication

We did not attempt to specifically replicate experimental findings. But we note that samples from the same population carry similar genetic signatures. Moreover, genome-wide data allows for the analysis of multiple realisations of the sample history, by studying hundreds of thousands of SNP sites.

Randomization

We did not implement any randomization as no experimental groups or effect sizes were measured in this study.

Blinding

No blinding techniques were implemented, as experimental group assignment is not relevant for population genetic studies of this kind.

### 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 Methods n/a Involved in the study n/a Involved in the study ☑ Antibodies ☑ ChIP-seq ☑ Eukaryotic cell lines ☑ Flow cytometry ☑ Palaeontology ☑ MRI-based neuroimaging ☑ Animals and other organisms ☑ Human research participants

Clinical data