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# eLife's transparent reporting form

We encourage authors to provide detailed information within their submission to facilitate the interpretation and replication of experiments. Authors can upload supporting documentation to indicate the use of appropriate reporting guidelines for health-related research (see <a href="EQUATOR Network">EQUATOR Network</a>), life science research (see the <a href="BioSharing Information">BioSharing Information</a> <a href="Resource">Resource</a>), or the <a href="ARRIVE guidelines">ARRIVE guidelines</a> for reporting work involving animal research. Where applicable, authors should refer to any relevant reporting standards documents in this form.

If you have any questions, please consult our Journal Policies and/or contact us: editorial@elifesciences.org.

### Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

As this is an ancient DNA study, sample size was limited by sample availability and the success of obtaining useable DNA from these samples. Samples were chosen to represent individuals from the major centres of their former distribution, spanning as wide a time period as possible. In total, 131 samples were collected and DNA extracted, 75 samples were sent for sequencing, with 41 one of these passing the read processing filtering to be used in analysis. NB: of these samples 6 were previously published but were part of the total dataset of Thomas et al. and so have been included in the sample numbers. This information is provided in the Methods and results sections and summarised in Supplementary File 1a and 1b.

### **Replicates**

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:



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Replications were not performed due to the nature of the samples/study. However, a consensus sequence for each individual was only called for mitochondrial genome sites that were covered by at least three independent sequence reads from the respective individual.

## Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

The extensive statistical analyses are described concisely in the methods section of the main document and in detail in the supplementary material.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

#### **Group allocation**

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

For reconstruction of temporal population structure, the samples were grouped by age and randomization tests were used to validate the results. Specific information is provided in Appendix 2 and Appendix 6.

## Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:



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All relevant data tables are available in the supplementary material. All sequence data used in the analysis has been submitted to GenBank and will be made public following the publication of the study.