**[For SUPPLEMENTARY MATERIAL]**

**Unveiling Bishop Teodomiro of Iria Flavia? An attempt to identify the discoverer of St James’s tomb through osteological and biomolecular analyses (Santiago de Compostela, Galicia, Spain)**

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**1. Skeletal Inventory of Individual NCS200**

* Cranium (*calvaria*): The skull is the best-preserved bony element. Both parietal bones, the frontal bone, both temporal bones, and part of the occipital bone are preserved, although there is a loss of the *foramen magnum*. However, along with the occipital bone, the right temporal and parietal bones are fractured and incomplete. Taphonomic changes can be observed as a result of abrasion/erosion after the burial of the remains. In addition, there are marks of recent blows, possibly caused during the excavation and exhumation carried out in 1955. Presence of Wormian bones in the occipital suture.
* Cranium (*splanchnocranium*): Maxillae (right and left) are incomplete and fragmented. Teeth 41 and 42 were found separately (fall post mortem), which were used for aDNA analysis. Although no teeth were preserved in situ in the left maxillary fragment, the first and second molars were lost perimortem, while the two premolars, the canine, and both incisors were lost post mortem. A fragment of the right maxilla was found, showing the perimortem loss of the second molar (17), with the first molar (16), the two premolars (14 and 15), the canine (13), and the second incisor (12) falling out post-mortem. Presence of an abscess in the left maxilla.
* Cranium (*mandible*): Mandible is fragmented and incomplete. Teeth 41-43 on the right side and 31-38 on the left side are preserved in situ. Presence of abscesses and tartar.
* Vertebrae: Only 1 vertebral body with part of the arch (cervical), and fragments of cervical arch (cervical and thoracic) are preserved.
* Ribs: Few fragments belonging to the ribs are preserved (n = 5).
* Clavicle: Two fragments corresponding to the left clavicle and one fragment corresponding to the right clavicle.
* Scapula: Three small fragments from the left scapula (coracoid process).
* Arms: They are preserved, although very fragmentary and incomplete, especially with the loss of the proximal and distal epiphyses. The presence of the humerus, ulna, and radius of both arms have been identified. None of the elements are complete enough to make measurements, and therefore, estimates of stature and/or sex.
* Legs: They are preserved, although very fragmentary and incomplete, especially with the loss of the proximal and distal epiphyses. The presence of the femur, tibia, and ulna of both legs has been identified. None of the elements are complete enough to make measurements, and therefore, estimates of stature and/or sex. Periostitis has been observed in the metaphysis of the left tibia.
* Calcaneus: Fragment of the right calcaneus.

Minimum number of bone elements identified = 39 (Number of bone fragments = 90)

**2. Radiocarbon dating**

Oxford Radiocarbon Accelerator Unit (ORAU), Oxford, UK

The human bone (rib fragment) of NCS200 was radiocarbon dated at the Oxford Radiocarbon Accelerator Unit (ORAU). Prior to extensive sampling of human skeletal remains we screened small (3–5mg) sub-samples of drilled bone powder by measuring the elemental nitrogen concentration. This is a useful proxy for protein, and therefore presence of collagen in the bone (Brock *et al.* 2010; Jacob *et al.* 2018). Samples with >~0.5% N were passed for full collagen extraction treatment for AMS dating (ORAU pretreatment code AF). The methods used are outlined in Brock *et al*. (2010). Briefly, collagen was extracted using an acid-base-acid procedure followed by gelatinisation and lyophilisation (Brock *et al*. 2010). The extracted gelatin was filtered using pre-cleaned Vivaspin™30kD MWCO ultra-filters (Brown *et al.* 1988; Higham *et al.* 2006). Ultrafiltration removes low molecular weight contaminants and produces a better purified collagen fraction as indicated by improved C:N atomic ratios and carbon mass on combustion. The filtered collagen was freeze-dried and combusted in a CHN analyser linked in continuous flow mode to a Europa isotope ratio mass spectrometer (EA-CFIRMS) using a He carrier gas. δ13C and δ15N values, nitrogen and carbon content, and bone C:N atomic ratios were determined. The purified CO2 was then reduced to graphite using H2 in a reaction catalysed by 2mg of a Fe powder at 560°C for 6 hr. The graphite was pressed into an Al target holder prior to radiocarbon measurement using AMS (Bronk Ramsey *et al.* 2004). We tested the reliability of dating bone with collagen yields of this size and the models showed that none were outliers. All other analytical parameters measured, including the carbon to nitrogen atomic ratio, were acceptable. We therefore consider the results to be robust. The calibration and the calibration curve were performed by OxCal V4.3.2 Bronk Ramsey (2017): r.5: IntCarl13 atmospheric curve (Reimer *et al.* 2013).

**3. δ13C and δ15N analysis of bone collagen**

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The diet of past humans can provide information on their social status and origin (Pérez-Ramallo *et al.* 2022). Variation in stable carbon isotopes (δ13C) in terrestrial ecosystems is primarily influenced by the photosynthetic pathway used by plants at the base of the food chain (Smith & Epstein 1971). This leads to distinct, non-overlapping δ13C values, with C3 plants (e.g. trees, shrubs, temperate grasses and crops such as wheat) ranging between approximately -24 to -36‰ (global average of -26.5‰) (Vogel & van der Merwe 1977; Ambrose & Norr 1993), and C4 plants (e.g. tropical grasses and crops such as maize, millet and sugar cane) ranging between approximately -9 to -17‰ (global average of -12‰) (Vogel & van der Merwe 1977; Ambrose & Norr 1993). Due to a different source of CO2 in marine ecosystems, marine plants have δ13C values more similar to C4 plants. Meanwhile, CAM plants (Crassulacean acid metabolism, e.g. succulents) have δ13C values overlapping and between both C3 and C4 plants. These δ13C distinctions are passed up the food chain into the tissues of consumers with an enrichment of 0.5–2‰ in δ13C between trophic levels (Vogel & van der Merwe 1977; Ambrose & Norr 1993). Stable nitrogen isotope (δ15N) values for terrestrial and aquatic animals vary with trophic level, increasing by +3–6‰ with each trophic level (Deniro & Epstein 1981). Longer foodchains and differences in nitrogen sources lead to, on average, higher δ15N values among marine and freshwater consumers, although their δ13C is far more variable (Schoeninger & DeNiro 1984; Dufour *et al.* 1999).

Bone samples (approx. 1g) were broken into small pieces and adhering soil was removed by abrasion using a sandblaster. Samples were demineralised by immersion in 0.5M HCl for 1–7 days. Once demineralisation was complete, samples were rinsed three times with ultra-pure H2O. The residue was gelatinised in pH3 HCl at 70°C for 48 hours and the soluble collagen solution Ezee-filtered to remove insoluble residues (Brock *et al.* 2013). Samples were lyophilised in a freeze dryer for 24 hours. Where sufficient material was available approximately 1.0mg of the resulting purified collagen was weighed in duplicate into tin capsules for analysis. The δ13C and δ15N ratios of the bone collagen were determined using a Thermo Scientific Flash 2000 Elemental Analyser coupled to a Thermo Delta V Advantage mass spectrometer at the Isotope Laboratory, MPI-GEA, Jena. Isotopic values are reported as the ratio of the heavier isotope to the lighter isotope (13C/12C or 15N/14N) as δ values in parts per mill (‰) relative to international standards, Vienna Pee Dee Belemnite (VPDB) for δ13C and atmospheric N2 (AIR) for δ15N. Results were calibrated against international standards (IAEA-CH-6 Sucrose, IAEA-N-2 Ammonium Sulfate and USGS40 L-Glutamic Acid): USGS40 13Craw = -26.4 ± 0.1, 13Ctrue = -26.4 ± 0.0, 15Nraw = -4.4 ± 0.1, 15Ntrue = -4.5 ± 0.2; IAEA-N-2 15Nraw = 20.2 ± 0.1, 15Ntrue = 20.3 ± 0.2; IAEA-CH-6 13Craw = -10.9 ± 0.1, 13Ctrue = -10.8 ± 0.0.

Replicate analyses of standards suggest that machine measurement error is ± 0.1‰ for δ13C and ± 0.1‰ for δ15N. Overall measurement precision was studied through the measurement of repeat extracts from a fish gelatin standard (n = 20, ± 0.1‰ for δ13C and ± 0.1‰ for δ15N). The bone collagen can be affected by contamination that modifies the amino acid composition. The ratio of carbon and nitrogen stable isotopes serves to verify the purity and the preservation of the collagen, establishing a range between 2.9–3.6 to be acceptable, which is the value that can be found in the collagen of a fresh bone (DeNiro 1987). The elemental mass percentages may be around 34.8±8.8% for the carbon and between 11 and 15% for the nitrogen (van Klinken 1999). External factors that affected the bone remains can alter these percentages (e.g. humid acids or salts) (van Klinken 1999). The collagen yield is the percentage of collagen extracted from the bone and serves as an indicator of bone preservation quality. Fresh bone contains approximately 20% collagen. Diagenesis can lead to a loss of collagen in the bone, to the point where the isotopic signature obtained from a low-yield sample is no longer representative of its original isotopic signature. The filtration of the sample helps to avoid residues but can be the cause of loss of yield (around 40 to 60%) (Jay 2005). Ambrose & Norr (1993) established a limit of 1.2%. However, van Klinken (1999) determined a minimum of between 0.5–1.0% for archaeological bones.

**4. δ18Oap and δ13Cap analysis in tooth enamel**

Max Planck Institute for Geoanthropology (formerly Max Planck Institute for the Science of Human History), Jena, Germany

The δ18O of human dental enamel bioapatite (δ18Oap) is closely related to the δ18O of groundwater and precipitation, which varies as a product of geographical factors such as distance from the coast, altitude and local climate (Webb *et al.* 2014). The conversion of tooth enamel carbonate δ18O into drinking water δ18O values can be performed (e.g. Longinelli 1984; Levinson *et al*.1987). This should, however, be applied with caution as errors and uncertainties are associated with the conversion (Lightfoot & O’Connell 2016). In addition, human δ18O can be impacted by climatic changes through time as well as drink and food processing (e.g. fermentation). Intra-population variability can also be significant (Lightfoot & O’Connell 2016). Tooth enamel δ13Cap provides more completed diet contributions for the period of enamel formation (including carbohydrates, proteins and lipids) (Yoder 2012).

We used a tungsten drill to carefully remove enamel from specific clean locations. This process involved powdering enamel from the entire crown to analyse for a long-term average signal. The resulting enamel powder was collected and placed in a 1.5mL Eppendorf tube. The samples were treated with 1mL of 1% NaClO for 60 minutes, rinsed with deionised water, mixed, and centrifuged a total of 3 times. Samples were then treated again with 0.1M for 10 minutes, followed by rinsing with deionised water, mixed, and centrifuged again 3 times, followed by the removal of all the water. Enamel samples were then frozen and placed in the freeze dryer for 4 hours. From each sample, 3.5mg was weighed into 12ml borosilicate glass vials and sealed with rubber septa. The vials were then flushed/filled with helium at 100ml/min for 10 minutes. Followingreaction with 100% phosphoric acid, gases evolved from the samples were analysed to stable carbon and oxygen isotopic composition using a Thermo Gas Bench 2 connected to a Thermo Delta V Advantage Mass Spectrometer at the Department of Archaeology, Max Planck Institute for Geoanthropology. Stable oxygen (δ18O) and carbon isotope (δ13C) values were calibrated against international standards (IAEA NBS 18, IAEA 603, IAEA CO8) registered by the International Atomic Energy Agency: IAEA NBS 18: δ13C -5.014 ± 0.032‰, δ18O -23.2±0.1‰; IAEA 603: δ13C +2.46±0.01‰, δ18O -2.37±0.04‰; IAEA CO8: δ13C -5.764±0.032‰, δ18O 22.7±0.2‰; and USGS44: δ13C = ~ -42.1‰. Replicate analyses of standards suggest that machine measurement error is ± 0.1‰ for δ13C and ± 0.2‰ for δ18O. Overall measurement precision was studied through the measurement of repeat extracts from a bovid tooth enamel standard (n = 20, ± 0.2‰ for δ13C and ± 0.4‰ for δ18O).

Based on the δ13Cap carbonate results, we equate the average of bulk dietary intake following the diet-consumer fractionation of Ambrose & Norr (1993) (approximately 9.5‰) (δ13Capcoll), and another with the enamel-bone apatite adjustment following Warinner & Tuross (2009) (approximately 2.3‰).

**5. DNA extraction**

Centre for Palaeogenetics (CPG), Stockholm University, Sweden

The tooth was sampled in the ancient DNA (aDNA) facilities at the Centre for Palaeogenetics (CPG), Stockholm University (Sweden). The sample was decontaminated prior to analysis with a 0.5% sodium hypochlorite solution and UV irradiated (6 J/cm2 at 254 nm). The root tip of the tooth was cut with a multitool drill (Dremel) to get approximately 80 to 150mg of bone powder/root tip and placed in an Eppendorf tube in 1ml of pre-digestion buffer (0.45M EDTA pH 8.0) at 37oC in a hybridisation oven. After 30 minutes the supernatant was removed to reduce the microbial and exogenous DNA. Following this pre-digestion step, 1ml extraction buffer (0.45M EDTA pH 8.0 and 0.25mg/ml of proteinase K) was added to all the samples and they were incubated at 37oC in the hybridisation oven for 1 day until all root tip was dissolved. The extraction was conducted following Dabney *et al*. (2013), with 1ml of digested extract being combined with 13ml of binding buffer containing 5M guanidine hydrochloride, 40% (vol/vol) isopropanol, 0.05% Tween-20 and 90mM sodium acetate (pH 5.2). 50ml silica columns (Roche, High Pure Viral Nucleic Acid Large Volume Kit) were used for purification and the DNA was eluted in 45μl of EB buffer (Qiagen).Blank controls were used during every step of extraction, library preparation and amplification.

*Library preparation and sequencing*

20μl of extract was used to prepare blunt-end ligation DNA libraries coupled with P5 and P7 adapters and indexes as described in Meyer & Kircher (2010). The amplification reactions had a total volume of 50μl, with 5μl DNA library, and the following in final concentrations: 1 X AmpliTaq Gold Buffer, 2.5mM MgCl2, 250μM of each dNTP, 2.5 U AmpliTaq Gold (Thermo Fisher Scientific, Waltham, MA), and 200nM of each of the index primers (Meyer & Kircher 2010). The optimal number of PCR cycles for library amplification was determined with qPCR. PCR was done with the following conditions: an activation step at 94°C for 10 min followed by 8–13 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 45s, and a final elongation step of 72°C for 10 min. Four amplification reactions with the same indexing primer were made for each library to increase complexity. Finally, the amplified libraries were pooled and purified with AMPure XP beads (Agencourt; Beckman Coulter, Brea, CA), and the fragment size and concentration were checked using BioAnalyzer with the High Sensitivity Kit (Agilent Technologies, Cary, NC).

*Processing and alignment of sequencing reads*

Purified libraries were pooled in equimolar concentration and sequenced on NovaSeq 6000 at the SciLife Sequencing Centre in Stockholm. Sequencing reads were demultiplexed according to the pair of indexes of each sample sequence. Cutadapt v. 2.3 was used for trimming adapters and FLASH v. 1.2.11 for merging of fastq reads. The reads were mapped against the human reference genome build 37 (hg19) using the Burrows-Wheeler Algorithm, as implemented by BWA v. 0.7.10, (Li & Durbin 2009) with the following parameters: aln (-l 16500 -n 0.01 -o 2). Next, a slightly modified version of FilterUniqueSAMCons.py (Kircher 2012) was used to condense the reads with identical start and end position into a consensus read. Finally, reads shorter than 35 base pairs and reads with less than 90% consensus with the reference were filtered out using percidentity\_threshold.py (Skoglund *et al.* 2012). Due to the low coverage of the ancient sample the sequencing depth was too low to call diploid genotypes. Therefore, we built pseudo-haploid genomes by randomly choosing one read at each heterogenous position with minimum mapping quality 30 and a base quality of 30.

*Data validation*

We used several approaches to validate the dataset. Firstly, all libraries yielded short read lengths (~70 bp), and patterns of cytosine deamination were estimated using PMDtools (Skoglund *et al.* 2014) (Table 1). We have applied one method based on the mitochondrial DNA to estimate contamination in our sample (Green *et al.* 2008). In addition, we used the 'Contamination' program in ANGSD following Korneliussen *et al*. (2014) v.0.911 to estimate X-chromosome contamination (as described in Rasmussen *et al.* 2021). Contamination estimates are shown in Table 1. Finally, sex was assigned following the method described in Skoglund *et al*. (2013) (Table 1).

*PathPhynder*

We determined the Y chromosome haplogroup using pathPhynder v.1a (Martiniano *et al.* 2022) (Table 1). The ‘BigTree’ Y chromosome dataset (included with pathPhynder) was used as the reference phylogeny, the minimum base quality was set at 20, and maximum tolerance was set both to default 3 and 100. Both “default” and “-m transversions” filtering modes were used, which generated comparable results.

*Population genomics datasets*

We trimmed 10bp at the end of each read in our ancient individual to remove the ancient damage patterns characteristic of ancient and degraded DNA. Next, we merged the ancient individual with a 54.1.p1 version of the Allen Ancient DNA Resource (AADR) dataset, https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypespresent-day-and-ancient-dna-data, where duplicate and related individuals were removed. Ancient individuals from Spain (n= 93) from the Iron Age to the late medieval period (Olalde *et al.* 2019; Patterson *et al.* 2022) where used as comparative data in the population genetic analyses.

*Principal components analysis*

Principal components analysis was performed on modern reference data using the smartpca module in EIGENSOFT (v.6.0.1) (Patterson *et al.* 2006). No outlier iterations were performed, and the parameters “ldregress: 200” and “ldposlimit: 100000” were used to perform LD regression. The results were plotted with R (R Core Team 2021).

*Admixture estimations*

Unsupervised ADMIXTURE v.1.3 (Alexander *et al.* 2009) was run on 93 ancient samples and 460 modern individuals from the dataset "1240K+HO" from the AADR (v. 54.1.p1). The dataset was pruned for linkage disequilibrium between markers using Plink v1.90 – indep-pairwise 200 25 0.4 (Purcell *et al.* 2007). The results were parsed, aligned and plotted with PONG (Behr *et al.* 2016).

**References**

Alexander, D.H., J. Novembre & K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* 19: 1655–64. https://doi.org/10.1101/gr.094052.109

Ambrose, S.H. & L. Norr. 1993. Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate, in J.B. Lambert & G. Grupe (ed.) *Prehistoric human bone*: 1–37. Berlin: Springer.

Behr, A.A., K.Z. Liu, G. Liu-Fang, P. Nakka & S. Ramachandran. 2016. pong: fast analysis and visualization of latent clusters in population genetic data. *Bioinformatics* 32: 2817–23. https://doi.org/10.1093/bioinformatics/btw327

Brock, F., T. Higham & C. Bronk Ramsey. 2010. Pre-screening techniques for identification of samples suitable for radiocarbon dating of poorly preserved bones. *Journal of Archaeological Science* 37: 855–65. https://doi.org/10.1016/j.jas.2009.11.015

Brock, F., V. Geoghegan, B. Thomas, K. Jurkschat & T.F.G. Higham. 2013. Analysis of bone “collagen” extraction products for radiocarbon dating. *Radiocarbon* 55: 445–63. https://doi.org/10.1017/S0033822200057581

Bronk Ramsey, C. 2017. Methods for summarizing radiocarbon datasets. *Radiocarbon* 59: 1809–33. https://doi.org/10.1017/RDC.2017.108

Bronk Ramsey, C., T. Higham & P. Leach. 2004. Towards high-precision AMS: progress and limitations. *Radiocarbon* 46: 17–24. https://doi.org/10.1017/S0033822200039308

Rasmussen, K.L. *et al*. 2021. Investigations of the relics and altar materials relating to the apostles St James and St Philip at the Basilica dei Santi XII Apostoli in Rome. *Heritage Science* 9. https://doi.org/10.1186/s40494-021-00481-9

Brown, T.A., D.E. Nelson, J.S. Vogel & J.R. Southon. 1988. Improved collagen extraction by modified Longin method. *Radiocarbon* 30: 171–77. https://doi.org/10.1017/S0033822200044118

Dabney, J. *et al*. 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences* 110: 15758–63. https://doi.org/10.1073/pnas.1314445110

Deniro, M.J. 1987. Stable isotopy and archaeology. *American Scientist* 75:182–91.

Deniro, M.J. & S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* 45: 341–51. https://doi.org/10.1016/0016-7037(81)90244-1

Dufour, E., H. Bocherens & A. Mariotti. 1999. Palaeodietary implications of isotopic variability in Eurasian lacustrine fish. *Journal of Archaeological Science* 26: 617–27. https://doi.org/10.1006/jasc.1998.0379

Green, R.E. *et al*. 2008. A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. *Cell* 134: 416–26. https://doi.org/10.1016/j.cell.2008.06.021

Higham, T., C. Bronk Ramsey, I. Karavanić, F.H. Smith & E. Trinkaus. 2006. Revised direct radiocarbon dating of the Vindija G1 Upper Paleolithic Neandertals. *Proceedings of the National Academy of Sciences* 103: 553–7.https://doi.org/10.1073/pnas.0510005103

Jacob, E., D. Querci, M. Caparros, C. Barroso Ruiz, T. Higham & T. Devièse. 2018. Nitrogen content variation in archaeological bone and its implications for stable isotope analysis and radiocarbon dating. *Journal of Archaeological Science* 93: 68–73. https://doi.org/10.1016/j.jas.2018.02.019

Jay, M. 2005. Stable isotope evidence for British Iron Age diet. Inter- and intra-site variation in carbon and nitrogen from bone collagen at Wetwang in East Yorkshire and sites in East Lothian, Hampshire and Cornwall. Unpublished PhD dissertation, University of Bradford.

Kircher, M. 2012. Analysis of high-throughput ancient DNA sequencing data, in B Shapiro & M. Hofreiter (ed.) *Ancient DNA: methods and protocols* (Methods in Molecular Biology 840): 197–228. Totowa (NJ): Humana. https://doi.org/10.1007/978-1-61779-516-9\_23

Korneliussen, T.S., A. Albrechtsen & R. Nielsen. 2014. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics* 15: 356. https://doi.org/10.1186/s12859-014-0356-4

Levinson, A.A., B. Luz & Y. Kolodny. 1987. Variations in oxygen isotopic compositions of human teeth and urinary stones. *Applied Geochemistry* 2: 367–71. https://doi.org/10.1016/0883-2927(87)90021-7

Li, H. & R. Durbin. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25: 1754–60. https://doi.org/10.1093/bioinformatics/btp324

Lightfoot, E. & T.C. O’connell. 2016. On the use of biomineral oxygen isotope data to identify human migrants in the archaeological record: intra-sample variation, statistical methods and geographical considerations. *PLoS ONE* 11: e0153850. https://doi.org/10.1371/journal.pone.0153850

Longinelli, A. 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochimica et Cosmochimica Acta* 48: 385–90. https://doi.org/10.1016/0016-7037(84)90259-X

Martiniano, R., B. De Sanctis, P. Hallast & R. Durbin. 2022. Placing ancient DNA sequences into reference phylogenies. *Molecular Biology and Evolution* 39. https://doi.org/10.1093/molbev/msac017

Meyer, M. & M. Kircher. 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols* 2010. https://doi.org/10.1101/pdb.prot5448.

Olalde, I. *et al*. 2019. The genomic history of the Iberian Peninsula over the past 8000 years. *Science* 363: 1230–34. https://doi.org/10.1126/science.aav4040

Patterson, N., A.L. Price & D. Reich. 2006. Population structure and eigenanalysis. *PLoS Genetics* 2: e190. https://doi.org/10.1371/journal.pgen.0020190

Patterson, N. *et al*. 2022. Large-scale migration into Britain during the Middle to Late Bronze Age. *Nature* 601: 588–94. https://doi.org/10.1038/s41586-021-04287-4

Pérez-Ramallo, P. *et al*. 2022. Multi-isotopic study of the earliest mediaeval inhabitants of Santiago de Compostela (Galicia, Spain). *Archaeological and Anthropological Sciences* 14: 214. https://doi.org/10.1007/s12520-022-01678-0

Purcell, S. *et al*. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics* 81: 559–75. https://doi.org/10.1086/519795

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/

Reimer, P.J. *et al.* 2013. IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* 55: 1869–87. https://doi.org/10.2458/azu\_js\_rc.55.16947

Schoeninger, M.J. & M.J. Deniro. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica et Cosmochimica Acta* 48: 625–39.

https://doi.org/10.1016/0016-7037(84)90091-7

Skoglund, P. *et al.* 2012. Origins and genetic legacy of neolithic farmers and hunter-gatherers in Europe. *Science* 336: 466–69. https://doi.org/10.1126/science.1216304

Skoglund, P., J. Storå, A. Götherström & M. Jakobsson. 2013. Accurate sex identification of ancient human remains using DNA shotgun sequencing. *Journal of Archaeological Science* 40: 4477–82. https://doi.org/10.1016/j.jas.2013.07.004

Skoglund, P., B.H. Northoff, M. V. Shunkov, A.P. Derevianko, S. Pääbo, J. Krause & M. Jakobsson. 2014. Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proceedings of the National Academy of Sciences* 111: 2229–34. https://doi.org/10.1073/pnas.1318934111

Smith, B.N. & S. Epstein. 1971. Two categories of 13C/12C ratios for higher plants. *Plant Physiology* 47: 380–84. https://doi.org/10.1104/pp.47.3.380

van Klinken, G.J. 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science* 26: 687–95. https://doi.org/10.1006/jasc.1998.0385

Vogel, J.C. & N.J. van der Merwe. 1977. Isotopic evidence for early maize cultivation in New York State. *American Antiquity* 42: 238–42. https://doi.org/10.2307/278984

Warinner, C. & N. Tuross. 2009. Alkaline cooking and stable isotope tissue-diet spacing in swine: archaeological implications. *Journal of Archaeological Science* 36: 1690–97. https://doi.org/10.1016/j.jas.2009.03.034

Webb, E.C., C.D. White & F.J. Longstaffe. 2014. Investigating inherent differences in isotopic composition between human bone and enamel bioapatite: implications for reconstructing residential histories. *Journal of Archaeological Science* 50: 97–107. https://doi.org/10.1016/j.jas.2014.07.001

Yoder, C. 2012. Let them eat cake? Status-based differences in diet in medieval Denmark. *Journal of Archaeological Science* 39: 1183–93. https://doi.org/https://doi.org/10.1016/j.jas.2011.12.029