[Supplementary material]

The introduction of the European fallow deer to the northern provinces of the Roman Empire: a multi-proxy approach to the Herstal skeleton (Belgium)

Fabienne Pigière[1,*], Denis Henrard[2], Naomi Sykes[3], Nathalie Suarez[4] & Gontran Sonet[5]

[1] *School of Archaeology, University College Dublin, Ireland*

[2] *Agence wallonne du Patrimoine, Liège, Belgium*

[3] *Department of Archaeology, University of Exeter, UK*

[4] *Faculté de Philosophie et Sciences Sociales Dpt Histoire, Université Libre de Bruxelles, Belgium*

[5] *Royal Belgian Institute of Natural Sciences, Brussels, Belgium*

[*] *Author for correspondence ✉: fabienne.pigiere@ucd.ie*

Radiocarbon dating and isotopes analysis

Sample pre-treatment

Collagen was extracted from the bones following the Longin method (Longin 1971). A 1% NaOH-wash was introduced between the demineralisation and hydrolisation step for 15 minutes. Hereafter, C:N ratio was analysed and if C:N ratio falls between 2.9 and 3.6 (De Niro 1985; Ambrose 1990) it indicates uncontaminated collagen. If the C:N is higher, the collagen is contaminated and nanofiltration is performed to improve the sample quality. The in-house–developed filtration installation and the used protocol is described by Boudin *et al*. (2015).

Radiocarbon dating

Dried samples were transferred into quartz tubes with CuO and Ag and combusted to CO_2. Graphitisation of CO_2 was carried out using H_2 over a Fe catalyst. Targets were prepared at the Royal Institute for Cultural Heritage in Brussels (Belgium) (Van Strydonck & van der Borg 1990–1991) and ^{14}C concentrations were measured with accelerator mass spectrometry (AMS) at the Royal Institute for Cultural Heritage, Brussels, Belgium (Boudin *et al*. 2015). Results are expressed in pMC (percentage modern carbon) and indicate the percent of modern (1950) carbon corrected for fractionation using the $d^{13}C$ measurement. Calibrations of ^{14}C dates were performed using OxCal 3 and the IntCal13 calibration curve data (Bronk Ramsey 1995, 2001; Reimer *et al*. 2013).

Method of DNA analysis

Four samples of bone powder were collected from three bone fragments identified as fallow deer on the basis of the morphological criteria: 50mg from one distal end of a left tibia, 50mg from one proximal end of a right tibia, and 2 × 50mg from one proximal end of a metacarpal (right anterior leg).

DNA extraction was performed in a DNA lab dedicated to ancient DNA, equipped with UV lamps and under positive air pressure to avoid contamination. Best practices recommended when working with ancient DNA (Gilbert *et al.* 2005; Willerslev & Cooper 2005) were applied. Extraction negatives (samples treated like all others but without any bone powder inside) were included in all experiments. For all extractions, the outer layer of the bone was first removed by scraping off its surface using a structured tooth tungsten carbide cutter attached to a hand rotary tool (8100 8v Max Rotary Tool). After 10 minutes of exposure to UV, bone powder was collected by drilling inside the bone using the hand rotary tool at 5000 rpm with an engraving cutter (1.6mm). For one sample (50mg from the proximal end of the metacarpal), the bone powder was cleaned with 900µl bleach once, and then rinsed five times with 1ml pure water. DNA was then extracted from the bone powder following the protocol of Dabney *et al.* (2013). One short fragment of the 12S ribosomal DNA gene (mitochondrial DNA) was amplified by polymerase chain reaction (PCR) using primers 12SDD-FW and 12SCERV-REV (Fajardo *et al.* 2007). Each PCR consisted of a mix of 25µl with 3µl of DNA template, 1.5mM of Mg^{2+}, 0.2mM of each dNTP, 0.5µM of each primer, 0.03 units/µl Platinum Taq DNA Polymerase and 1 × PCR buffer (Invitrogen, ThermoFisher). The PCR profile consisted of one first step at 4 °C for 3 min, a second step of 40 cycles at 94°C for 30 s, 53°C for 30s, and 72°C for 15s, and a final step at 72°C for 7 min. PCR products were purified using ExoSAP-IT (ThermoFisher) and then sequenced in both directions using the BigDye Terminator v.1.1 Cycle Sequencing Kit (Life Technologies) and an ABI 3130xl Genetic Analyser (LifeTechnologies). Chromatograms, base calling and consensus sequences (of forward and reverse reads) were checked using CodonCode Aligner v. 8.0.2 (CodonCode Corporation).

The consensus of each DNA sequence produced here was used as a query to search for the most similar sequences in the public repository of nucleotide sequences (GenBank) using the program blastn of BLAST (basic local alignment search tool, Altschul *et al.* 1990). Using Mega 7.0.26 (Kumar *et al*. 2016), character-based comparisons were performed with unique 12S haplotype sequences available for the Cervinae subfamily. A phylogenetic analysis using

the maximum likelihood (ML) method was made to reconstruct a tree including all 12S ribosomal sequences of Cervinae retrieved from GenBank (using keywords "12S" and "Cervinae") and overlapping with the DNA sequence obtained here. The 12S sequence of *Elaphodus cephalophus* (accession number in GenBank: DQ873526) was used as an outgroup. Unique haplotypes were extracted from the dataset and used as input for the tool RAxML-HPC2 on XSEDE (8.2.10) (Stamatakis 2014) available on the CIPRES Science Gateway V3.3 portal (Miller *et al*. 2010). No partitioning was made. The General Time Reversible (GTR) model of evolution (Lanave *et al.* 1984) was applied with a gamma distributed rate variation among sites. Finally, bootstrapping was performed using 500 pseudo-replicates. The best tree was visualized and nodes with bootstrap values <70% were collapsed using TreeGraph 2.14.0-771 beta (Stöver & Miller 2010).

References

ALTSCHUL, S.F., W. GISH, W. MILLER, E.W. MYERS & D.J. LIPMAN. 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.

AMBROSE, S.H. 1990. Preparation and characterization of bone and tooth collagen for stable carbon and nitrogen isotope analysis. *Journal of Archaeological Science* 17: 430–51.

BOUDIN, M., M. VAN STRYDONCK, T. VAN DEN BRANDE, H.-A. SYNAL & L. WACKER. 2015. A new AMS facility at the Royal Institute for Cultural Heritage, Brussels, Belgium. *Nuclear Instruments and Methods in Physics Research B* 361: 120–23. https://doi.org/10.1016/j.nimb.2015.04.006

BRONK RAMSEY, C. 1995. Radiocarbon calibration and analysis of stratigraphy: the OxCal program. *Radiocarbon* 37: 425–30. https://c14.arch.ox.ac.uk/oxcal3/oxcal.htm

– 2001. Development of the radiocarbon calibration program. *Radiocarbon* 43(2A): 355–63.

DABNEY, J., M. KNAPP, I. GLOCKE, M.T. GANSAUGE, A. WEIHMANN, B. NICKEL, C. VALDIOSERA, N. GARCÍA, S. PÄÄBO, J.-L. ARSUAGA & M. MEYER. 2013. mtDNA genome from a Middle Pleistocene cave bear. *Proceedings of the National Academy of Sciences of the USA* 110: 15758–63. https://doi.org/10.1073/pnas.1314445110.

DENIRO, M. 1985. Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to palaeodietary reconstruction. *Nature* 317: 806–809. https://doi.org/10.1038/317806a0

FAJARDO, V., I. GONZÁLEZ, I. LÓPEZ-CALLEJA, I. MARTÍN, M. ROJAS, P.E. HERNÁNDEZ, T. GARCÍA & R. MARTÍN. 2007. Identification of meats from red deer (*Cervus elaphus*), fallow

deer (*Dama dama*), and roe deer (*Capreolus capreolus*) using polymerase chain reaction targeting specific sequences from the mitochondrial 12S rRNA gene. *Meat Science* 76: 234–40. https://doi.org/10.1016/j.meatsci.2006.11.004.

GILBERT, M.T.P., H.-J. BANDELT, M. HOFREITER & I. BARNES. 2005. Assessing ancient DNA studies. *Trends in Ecology and Evolution* 20: 541–44. https://doi.org/10.1016/j.tree.2005.07.005.

KUMAR, S., G. STECHER & K. TAMURA. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–74. https://doi.org/10.1093/molbev/msw054.

LANAVE, C., G. PREPARATA, C. SACCONE & G. SERIO. 1984. A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* 20: 86–93. https://doi.org/10.1007/BF02101990

LONGIN, R. 1971. New method of collagen extraction for radiocarbon dating. *Nature* 230: 241–42. https://doi.org/10.1038/230241a0

MILLER, M.A., W. PFEIFFER & T. SCHWARTZ. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, in *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA*: 1–8. Piscataway: IEEE

REIMER, P. *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

STAMATAKIS, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–13. doi:10.1093/bioinformatics/btu033.

STÖVER, B.C. & K.F. MÜLLER. 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. *Bioinformatics* 11: 7. https://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-11-7.

VAN STRYDONCK, M. & K. VAN DER BORG. 1990–1991. The construction of a preparation line for AMS-targets at the Royal Institute for Cultural Heritage, Brussels. *Bulletin Koninklijk Instituut voor Kunstpatrimonium* 23: 228–34.

WILLERSLEV, E. & A. COOPER. 2005. Ancient DNA. *Proceedings of the Royal Society B: Biological Sciences* 272: 3–16. https://doi.org/10.1098/rspb.2004.2813.