SUPPLEMENTAL INFORMATION FOR

Female bone physiology resilience in a past Polynesian outlier community

Justyna J. Miszkiewicz1,2*, Hallie R. Buckley3, Michal Feldman4,5,6, Lawrence Kiko7, Selina Carlhoff6, Kathrin Naegele6, Emilie Bertolini8, Nathalia R. Dias Guimarães1, Meg M. Walker1, Adam Powell9, Cosimo Posth4,5,6, Rebecca L. Kinaston3,10,11

1School of Archaeology and Anthropology, Australian National University, Canberra, Australia
2School of Social Science, University of Queensland, St Lucia, Australia
3Department of Anatomy, Otago School of Biomedical Sciences, University of Otago, Dunedin, New Zealand
4Archaeo- and Palaeogenetics Group, Institute for Archaeological Sciences, University of Tübingen, Tübingen, Germany
5Senckenberg Centre for Human Evolution and Palaeoenvironment, University of Tübingen, Tübingen, Germany
6Department of Archaeogenetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
7The Solomon Islands National Museum, Honiara, Solomon Islands
8Department of Archaeogenetics, Max Planck Institute for the Science of Human History, Jena, Germany
9Department of Human Behavior, Ecology and Culture, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
10Centre for Social and Cultural Research, Griffith University, Queensland, Australia
11BioArch South, Waitati, New Zealand

*CORRESPONDENCE: j.miszkiewicz@uq.edu.au (ORCID: 0000-0002-9769-2706)
**Table 1.** Descriptive summary of data differences between the left and right femora. SD: standard deviation, SE mean: standard error mean.

| VARIABLES | FEMUR SIDE | N  | MEAN  | SD    | SE MEAN |
|-----------|------------|----|-------|-------|---------|
| Femur length (mm) | Right | 14 | 426.86 | 43.05 | 11.50 |
|  | Left | 9  | 429.44 | 39.55 | 13.18 |
| Midshaft circumference (Circ) (mm) | Right | 50 | 92.32 | 8.26 | 1.17 |
|  | Left | 19 | 93.58 | 4.63 | 1.06 |
| Posterior cortical width (Ct.W) (mm) | Right | 50 | 9.48  | 2.20 | 0.31 |
|  | Left | 19 | 10.37 | 1.52 | 0.35 |
| Vascular porosity (V.Po) adjusted by Ct.W (unitless) (V.Po/Ct.W) | Right | 50 | 2.10  | 0.78 | 0.11 |
|  | Left | 18 | 1.77  | 0.52 | 0.12 |
| V.Po adjusted by Circ (unitless) (V.Po/Circ) | Right | 50 | 20.47 | 5.75 | 0.81 |
|  | Left | 18 | 19.77 | 4.87 | 1.15 |
| Haversian canal:secondary osteon area ratio (H.Ar/On.Ar) (unitless) | Right | 14 | 8.08  | 1.24 | 0.33 |
|  | Left | 7  | 7.57  | 1.89 | 0.72 |
| Osteon population density (OPD) adjusted by Ct.W robusticity index (Ct.W_RI) (OPD/Ct.W_RI) | Right | 8  | 5.87  | 1.23 | 0.43 |
|  | Left | 4  | 5.50  | 1.14 | 0.57 |
| OPD adjusted by Circ robusticity index (Circ_RI) (OPD/Circ_RI) | Right | 8  | 6.39  | 1.04 | 0.37 |
|  | Left | 5  | 6.59  | 1.16 | 0.52 |
| Circ_RI | Right | 14 | 22.48 | 3.91 | 1.04 |
|  | Left | 9  | 21.97 | 3.08 | 1.03 |
| Ct.W_RI | Right | 14 | 2.43  | 0.65 | 0.17 |
|  | Left | 9  | 2.47  | 0.45 | 0.15 |

**Table 2.** Summary of data differences between the left and right femora tested using an independent samples $t$-test. There were no statistically significant differences in all variables, so both femoral sides were pooled for our analyses. DF: degree of freedom, DIFF.: difference, SE: standard error.

| VARIABLES | $t$  | DF | $p$  | MEAN DIFF. | SE DIFF. | 95% CONFIDENCE INTERVAL OF THE DIFF. | LOWER | UPPER |
|-----------|-----|----|-----|------------|----------|-------------------------------------|-------|-------|
| Femur length mm | -0.145 | 21 | 0.886 | -2.59 | 17.84 | -39.68 to 34.51 |
| Circ mm | -0.626 | 67 | 0.534 | -1.26 | 2.01 | -5.27 to 2.76 |
| Ct.W mm | -1.606 | 67 | 0.113 | -0.88 | 0.55 | -1.98 to 0.21 |
| V.Po/Ct.W | 1.640 | 66 | 0.106 | 0.33 | 0.20 | -0.07 to 0.72 |
| V.Po/Circ | 0.463 | 66 | 0.645 | 0.70 | 1.52 | -2.34 to 3.74 |
| H.Ar/On.Ar | 0.743 | 19 | 0.466 | 0.51 | 0.68 | -0.92 to 1.94 |
| OPD/Ct.W_RI | 0.502 | 10 | 0.627 | 0.37 | 0.74 | -1.27 to 2.01 |
| OPD/Circ_RI | -0.320 | 11 | 0.755 | -0.20 | 0.61 | -1.56 to 1.16 |
| Circ_RI | 0.329 | 21 | 0.745 | 0.745 | 0.51 | 1.54 to -2.70 |
| Ct_W_RI | -0.146 | 21 | 0.885 | 0.885 | -0.04 | 0.25 to -0.55 |
EXTENDED DNA METHODS

The DNA processing was conducted in dedicated aDNA facilities at the Max Planck Institute for the Science of Human History, Jena, Germany. For each individual, the dense part within the petrous portion of the temporal bone was drilled for DNA sampling [1]. DNA was extracted from around 50 mg of the sampled powder following published protocols [2]. To prepare the extract for next-generation sequencing a 25-ul aliquot was processed to produce a double-stranded and double-indexed Illumina DNA library following [3, 4]. To prevent that post-mortem deamination damages would be mistaken as authentic sequences in downstream analysis, damage caused by cytosine deamination was partially removed using uracil-DNA glycosylase and endonuclease VIII as described in [5]. Damage was retained in the two terminal positions to be later used for estimating the fraction of deaminated reads [5]. The DNA libraries were subsequently amplified using Herculase II Fusion DNA polymerase according to the manufacturer’s protocol. All libraries were directly shotgun single-end sequenced on an Illumina HiSeq 4000 platform (1 × 75 + 8 + 8 cycles). To control for potential laboratory contamination, blank extractions and library preparations were included for each sample batch.

The sequenced reads were binned (demultiplexed) allowing for one mismatch per index. The multiplexed libraries were than processed using the EAGER (v 1.92.54) pipeline [6]. As part of the pipeline, the Illumina adapter sequences were clipped off and the reads were filtered, retaining only reads longer than 30 base pairs using AdapterRemoval (v2.2.0) [8]. The clipped and filtered reads were mapped against the human genome reference hg19 using the BWA aln/samse alignment software (v0.7.12), with a stringency parameter of 0.01, seeding off (-l 16,500), and only retaining reads with Phred-scaled mapping quality scores higher than 30 [8]. Duplicate reads were removed using DeDup v0.12.2 [6]. To authenticate the ancient DNA library, levels of DNA deamination post-mortem damage were measured using mapDamage (v2.0) [9] and compared to the expected values in similar libraries prepared from ancient skeletal elements. Two terminal positions of each fragment were then masked to exclude DNA damage from following analyses [10].

Due to the low number of sequences yielded for each library, the genetic sex was inferred using two independent approaches. Both approaches aim to determine the copy number of each sex chromosome by calculating the number of reads mapping to sex- and autosomal chromosomes. Since genetic females have two copies of the X-chromosome and two copies of each autosomal chromosome, their X-chromosome coverage is expected to be comparable to the autosomal one. However, males have only one copy of the X-chromosome and one of the Y-chromosome and therefore the coverage of each of their sex chromosomes is expected to be half of the autosomal one.

The first method uses the mapping counts across a total of around 1.24 million genome-wide SNPs that were ascertained since they are informative for population history studies [11-13]. However, they can also be useful to estimate genetic sex [14]. For this purpose, the reads mapping to each ascertained position were counted using SAMtools and averaged for each chromosome using an inhouse script [15]. The Y- and the X- chromosome average coverages were each normalized using the average autosomal coverage. Then the normalized Y- and the X- chromosome average coverages were compared and used for the sex assignment.

The second approach was specifically designed for low-covered shotgun genomes and has been shown to confidently estimate genetic sex for libraries with as little as 1,000 mapping reads. In contrast to the first method, here the average coverage is estimated across the entire X- and the entire autosomal- chromosome sequences of the human reference hg19 (and not
on specific positions). The ratio between the X and the autosomal average coverages is calculated and used for the sex assignment as described in [16].

References:

1. Pinhasi, R. et al. DNA yields from the inner ear part of the human petrous bone. *PLOS ONE* **10**, e0129102 (2015).
2. Dabney, J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 15758–15763 (2013).
3. Meyer, M., Kircher, M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, pdb.prot5448 (2010).
4. Kircher, M., Sawyer, S., Meyer, M. Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**, e3 (2012).
5. Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., Reich, D. Partial uracil–DNA–glycosylase treatment for screening of ancient DNA. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **370**, 20130624 (2015).
6. Peltzer, A. et al. EAGER: Efficient ancient genome reconstruction. *Genome Biol.* **17**, 60 (2016).
7. Schubert, M., Lindgreen, S., Orlando, L. AdapterRemoval v2: Rapid adapter trimming, identification, and read merging. *BMC. Res. Notes* **9**, 88 (2016).
8. Li, H., Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009).
9. Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P.L.F., Orlando, L. mapDamage2.0: Fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics* **29**, 1682–1684 (2013).
10. Jun, G., Wing, M. K., Abecasis, G. R., Kang, H. M. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. *Genome Res.* **25**, 918–925 (2015).
11. Fu, Q. et al. DNA analysis of an early modern human from Tianyuan Cave, China. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 2223–2227 (2013).
12. Haak, W. et al. Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature* **522**, 207–211 (2015).
13. Mathieson, I. et al. Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499–503 (2015).
14. Fu, Q., et al. The genetic history of ice age Europe. *Nature* **534**, 200-205 (2016).
15. Li, H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27**(21), 2987-2993 (2011).
16. Mittnik, A., Wang, C. C., Svoboda, J., Krause, J. (2016). A molecular approach to the sexing of the triple burial at the Upper Paleolithic Site of Dolní Věstonice. *PloS one*, **11**, e0163019.
SI Table 3 (continues p. 6). Matching of sex results based on gross anatomical methods and those supported by aDNA. There was total n = 69, total n of mismatches = 6, total n of matches = 42, which results in 88% success rate of sex estimation using both methods. aDNA was not available (n/a) for n = 21 individuals.

| ID    | Estimated sex | Genetic sex approach 1 (all positions; X/auto ratio) | Genetic sex approach 2 (1240K positions; X/Y ratio) | Mismatch |
|-------|---------------|-----------------------------------------------------|---------------------------------------------------|----------|
| B178  | Female        | Male                                                 | Male                                              | x        |
| B54   | Female        | Female                                              | Female                                            |          |
| B83   | Female        | n/a                                                  | n/a                                               | n/a      |
| B91   | Female        | Female                                              | Female                                            |          |
| B13   | Female        | n/a                                                  | n/a                                               | n/a      |
| B140  | Female        | Female                                              | Female                                            |          |
| B21   | Female        | Male                                                 | Male                                              | x        |
| B48   | Female        | Male                                                 | Male                                              | x        |
| B63   | Female        | Female_low_certainty                                 | Female                                            |          |
| B115  | Female        | Female                                              | Female                                            |          |
| B121  | Female        | Male                                                 | Male                                              | x        |
| B150  | Female        | Female                                              | Female                                            |          |
| B84   | Female        | Female                                              | Female                                            |          |
| B141  | Female        | n/a                                                  | n/a                                               | n/a      |
| B139  | Female        | Female                                              | Female                                            |          |
| B3    | Female        | Female                                              | Female                                            |          |
| B41   | Female        | n/a                                                  | n/a                                               | n/a      |
| B71   | Female        | n/a                                                  | n/a                                               | n/a      |
| B103  | Female        | Female                                              | Female                                            |          |
| B163  | Female        | Female                                              | Female                                            |          |
| B159  | Female        | Female_low_certainty                                 | Female_low_certainty                              |          |
| B6    | Female        | n/a                                                  | n/a                                               | n/a      |
| B65   | Female        | Female                                              | Female                                            |          |
| B79   | Female        | n/a                                                  | n/a                                               | n/a      |
| B23   | Female        | n/a                                                  | n/a                                               | n/a      |
| B15   | Female        | Male                                                 | Male                                              | x        |
| B25   | Female        | n/a                                                  | n/a                                               | n/a      |
| B38   | Female        | n/a                                                  | n/a                                               | n/a      |
| B109  | Female        | Female_low_certainty                                 | Female                                            |          |
| B59   | Female        | Female                                              | Female                                            |          |
| B152  | Female        | Female                                              | Female                                            |          |
| B3    | Female        | n/a                                                  | n/a                                               | n/a      |
| B110  | Female        | Female                                              | Female                                            |          |
| B160  | Female        | n/a                                                  | n/a                                               | n/a      |
| 105-1 | Female        | n/a                                                  | n/a                                               | n/a      |
| 105-2 | Female        | n/a                                                  | n/a                                               | n/a      |
| B180  | Female        | Female                                              | Female                                            |          |
| B45   | Female        | n/a                                                  | n/a                                               | n/a      |
| B95   | Female        | Female                                              | Female                                            |          |
| B69   | Female        | Female                                              | Female                                            |          |
|   |   |   |   |
|---|---|---|---|
| B44 | Female | Male | Male |
| B149 | Male | Male | Male |
| B68 | Male | Male | Male |
| B195 | Male | n/a | n/a |
| B108 | Male | Male | Male |
| B42 | Male | Male | Male |
| B73 | Male | Male | Male |
| B85 | Male | n/a | n/a |
| B126 | Male | Male | Male |
| B145 | Male | Male | Male |
| B169 | Male | Male | Male |
| B148 | Male | n/a | n/a |
| B177 | Male | Male | Male |
| B179 | Male | Male | Male |
| B194 | Male | n/a | n/a |
| B1 | Male | Male | Male |
| B181 | Male | Male | Male |
| B104 | Male | Male | Male |
| B176 | Male | Male | Male |
| B189 | Male | Male | Male |
| B196 | Male | Male | Male |
| B24 | Male | n/a | n/a |
| B133 | Male | Male | Male |
| B87 | Male | Male | Male |
| B14 | Male | Male | Male |
| B173 | Male | n/a | n/a |
| B182 | Male | Male | Male |
| B22 | Male | Male | Male |
**SI Table 4.** Statistically significant results of correlations of bone histological markers compared between the sex and age-at-death groups. H.Ar: Haversian canal area, On.Ar: osteon area, OPD: osteon population density per mm², V.Po: density of canals/pores per mm², Rho: Spearman’s Rho test. *p < 0.5, **p < 0.01, ***p < 0.001.

| CORRELATIONS       | Test statistic | n  | p       |
|--------------------|----------------|----|---------|
| **Histology correlations across the sample** |                |    |         |
| H.Ar and On.Ar     | $Rho = 0.435$  | 21 | 0.049*  |
| On.Ar and OPD      | $Rho = -0.670$ | 21 | <0.001*** |
| V.Po and OPD       | $Rho = 0.531$  | 20 | 0.016**  |