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Correction to Redfern *et al.* (2017) 'Written in Bone': New Discoveries about the Lives of Roman Londoners, *Britannia* 48, 253–77

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ABSTRACT

In 2017, ancient DNA analysis of the Harper Road burial from Southwark (London) found that the individual had male chromosomes. Now analysis has discovered that the individual had female chromosomes, data which match the osteological estimation of sex and the interpretation of the grave-goods.

Keywords: Harper Road; ancient DNA; bioarchaeology; London

INTRODUCTION

The Harper Road burial (HR79) is one of the few very early Roman inhumations from London (A.D. 50–70), and is unique in the area for the range of local and imported grave-goods accompanying the burial, including a British torc and Claudio-Neronian pottery.[1] In 2017, Redfern and colleagues undertook analysis of the individual and grave-goods, and part of that work involved ancient DNA (aDNA) analysis of the human remains using a permanent molar tooth.[2] The osteological analysis estimated the skeleton's sex to be female, and examination of the grave-goods also suggested that many of the objects were more likely to belong to the burial of a woman. In contrast, the aDNA analysis concluded that the person's chromosomes were male (XY), and the burial was interpreted to be that of a person who identified as a woman. More recently, using those published data, Power (2020)

[1] Cotton 2008; Redfern *et al.* 2021.
[2] Redfern *et al.* 2017.

has used it to explore how intersex and non-binary burials are identified and interpreted in the Roman world. As part of wider work for a new museum display about prehistoric London, we undertook further aDNA extraction, and used the most current bioinformatic methods to research the individual's genomic information.[3]

MATERIALS AND METHODS

Ninety milligrams of bone powder were drilled from the petrous portion of the left temporal bone of the Harper Road skeleton following the minimally invasive method of Sirak *et al.* (2017). Established ancient DNA techniques[4] were applied to the bone powder for the preparation of sequencing libraries in the specialised ancient DNA cleanroom facility at The Francis Crick Institute. DNA extraction and single stranded library preparation was performed on automated Agilent Bravo Workstations following standard protocols.[5] The libraries were amplified with unique double index combinations and screened for endogenous DNA on the HiSeq4000 platform.

Downstream analysis was carried out through the nf-core/eager v.2.3 pipeline.[6] Mainly, a 35bp cutoff was set before alignment against the hs37d5 assembly in BWA.[7] Duplicates were removed via Dedup[8] and DNA damage was estimated with DamageProfiler.[9] Genetic (karyotypic) sex was estimated using a previously published approach, restricting to sequences with a mapping quality of at least 30.[10]

RESULTS

Results of shallow sequencing of the Harper Road aDNA library are presented in TABLE 1, with the number of sequences generated which aligned to the human genome being 127,000, which exceeds the 100,000 suggested by Skoglund and colleagues[11] needed for a robust identification. Endogenous content (percentage of sequences aligning to the human genome rather than environmental contaminants) was moderate (21.81 per cent). A high proportion of human sequences (25.70 per cent) showed patterns of C to T damage characteristic of ancient DNA, consistent with authentic ancient DNA.

TABLE 1. RESULTS OF A SHALLOW NEXT GENERATION SHOTGUN SEQUENCING RUN OF THE HARPER ROAD (HR79) ANCIENT DNA LIBRARY.

Sample ID	Sample type	Site name	Skeleton no.	No. of sequences	No. of human sequences	Endogenous content	C to T damage	Karyotypic sex
C10725	Left temporal bone	Harper Road	311	656,433	127,000	19.35%	25.70%	XX

[3] This was wide ranging and included, amongst others, appearance (e.g. hair colour) and genetic population group (haplotype). This work is ongoing and will be published elsewhere (Booth and Redfern pers. comm. 2023).
[4] Gansauge *et al.* 2020; Rohland *et al.* 2018.
[5] Gansauge *et al.* 2020; Rohland *et al.* 2018.
[6] Yates *et al.* 2021.
[7] Li and Durbin 2009.
[8] Peltzer *et al.* 2016.
[9] Neukamm *et al.* 2021.
[10] Skoglund *et al.* 2013.
[11] Skoglund *et al.* 2013.

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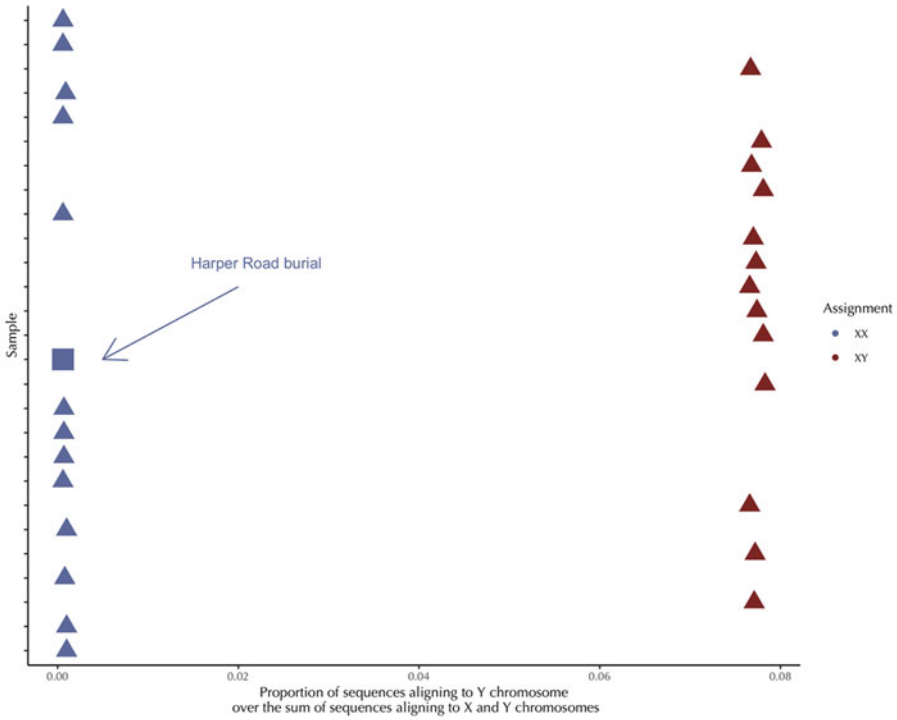

FIG. 1. Results of genetic (karyotypic) sex estimation for ancient individuals analysed in the Ancient Genomics Laboratory at the Francis Crick Institute, including the Harper Road burial (HR 79).

Genetic (karyotypic) sex was estimated based on ratios of DNA reads aligning to the X and Y sex chromosomes. Assessments of the sequencing data indicated that the Harper Road skeleton carried two X-chromosomes and was therefore from a female (FIG. 1). In the shallow screening data 4,773 sequencing reads aligned to the X-chromosome and just 3 to the Y-chromosome. Given that degradation in the form of cytosine deamination was observed, it is hard to imagine this result being due to female contamination of a male individual, but to confirm this further we restricted to sequences with clear evidence of cytosine deamination and a PMD score of at least 1[12] and observed 1,426 alignments to chromosome X but none to chromosome Y. The presence of a Y chromosome can therefore be excluded.

DISCUSSION AND CONCLUSIONS

Our analysis of DNA from the Harper Road skeleton conflicts with the result presented by Redfern and colleagues,[13] whose DNA analysis suggested that the skeleton came from a male (XY karyotype) individual. We used different methods and there could be various reasons why our results differ. The method of sex estimation employed by Redfern and colleagues[14] used

[12] Skoglund *et al.* 2013.
[13] Redfern *et al.* 2017.
[14] Redfern *et al.* 2017.

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targeted DNA hybridisation enrichment to retrieve sequences of particular genes on the Y-chromosome, whereas our approach was more comprehensive in examining reads aligning to all parts of the X or Y-chromosomes,[15] allowing for correction for the small number of spurious alignments to the Y chromosome that are often observed. The approach we use here is well established and is the standard method of estimating genetic sex in ancient samples. Therefore, the female sex estimate given here is more robust and should supersede the male estimate previously published.[16] The sequencing data from this individual is freely available at the European Nucleotide Archive: ENA Accession: PRJEB65282 (Sample number ERS16280561).

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