THE SEX PROFILE OF SKELETAL REMAINS FROM A CEMETERY OF CHINESE INDENTURED LABOURERS IN SOUTH AFRICA

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ABSTRACT

For a short period of time in the early 20th century, indentured labourers from China were imported to work on the South African gold mines. The Raymond A. Dart Collection of Human Skeletoneans contains 36 skeletons sourced from a Chinese cemetery on this time period on the site of the old Witwatersrand Deep Gold Mine. An earlier morphometric study on this collection recorded a high number of female individuals. However, the general historical records from the early gold mining era conflict with the results of this study, stating that very few Chinese females were among those to arrive in South Africa. In this study, the sex profile of this collection was analysed using molecular sex identification through the amelogenin gene. Results were obtained for 13 (41.93%) specimens, all of which were determined to be male – data that correspond well with the historical records.

INTRODUCTION

In 1951, a cemetery on the site of the old Witwatersrand Deep Mine was discovered while the Boksburg Municipality in the greater Johannesburg area was laying a main water line.1 The cemetery contained the skeletal remains of indentured miners who had died while working for the mine at the beginning of the 20th century. No records containing the demographic variables of the individuals represented, nor information on the excavation were preserved. However, general records relating to this period of gold mining in South Africa are available. The former Transvaal Provincial Administration allowed the remains to be exhumed and transferred to the Department of Anatomy (now the School of Anatomical Sciences) at the University of the Witwatersrand. Since their excavation, they have been stored and curated as part of the Raymond A. Dart Collection of Human Skeletons (Dart Collection).1,2

The preliminary description and analysis of the remains was carried out by the renowned anthropologist Raymond Dart.1 He noted that each individual was buried with mining apparel. At this time only African and Chinese labourers were employed on the gold mines and the rule of the mines was to bury the deceased of these two groups separately. Dart1 established that the skeletal features of the remains were not African and, through the process of elimination, concluded they must be Asiatic.

In 2004, a morphometric study was carried out on this collection.3 The results for sexing through morphological assessment indicated that 57.14% of the individuals were male and 42.86% female. However, metrical analyses suggested that 78.50% of the sample was male and 21.50% female.3 The results of this study were rather surprising as the relatively high number of female individuals is difficult to reconcile with historical records from the gold mines across South Africa. These records show that, between 1904 and 1907, 63 695 Chinese labourers were imported to work on the gold mines, of whom only 2 were women and 12 were children.4,5,6 Therefore, it seems highly improbable that any female skeletons are a part of the sample housed in the Dart Collection. If the morphometric study provided an accurate result, however, this would call for the reassessment of the historical record and suggest that the demographic data of the gold mines for this period might not be accurate. The aim of this study was to examine the sex profile of the Chinese indentured miners from the Dart Collection, using molecular sex identification from femoral skeletal tissue.

MATERIALS AND METHODS

Specimens

The skeletal remains of Chinese labourers housed in the Dart Collection belong to 36 individuals who died working for a gold mine on the Witwatersrand (see Box 1 for a historical background). During the initial assessment of these skeletons, it was noted that they had varying degrees of preservation, ranging from well preserved to a high level of fragmentation. As a result of fragmentation (specimens A1021, A1025 and A1029), or because both femora were missing (A1024 and A1026), five skeletons were not suitable for use in the present study. A further five specimens had an unusable left femur and, as such, the right femur was used for these individuals (A1009, A1011, A1015, A1023 and A1028). Molecular sex identification was therefore performed on only 31 specimens. Ethical clearance was obtained through the School of Anatomical Sciences Collections Committee.

Sex identification

The materials were prepared and methods designed using guidelines for ancient DNA research, to ensure no contamination was introduced during the DNA extraction. As a result, decontamination standards were implemented from the extraction of the skeletal tissue. In order to reduce the risk of sample contamination, four physically separated laboratories were used, (1) a preparation area, (2) a polymerase chain reaction (PCR) area, (3) a gel electrophoresis area and (4) sequencing areas (post-
approximately 1 g of bone powder. DNA was isolated using a silica membrane DNA extraction kit (QIAamp DNA Micro Kit, Johannesburg, South Africa) to process 40 mg of fine bone powder from each specimen (for details see Gibbon et al.).

The molecular sex identification method used two independent nested PCR-based systems, as described in Gibbon et al.²⁵

In the first system, the final PCR product was analysed with sequencing, while insertion/deletion analysis was used in the second system. All primers were synthesised by the Synthentic DNA Laboratory in the Department of Molecular and Cellular Biology at the University of Cape Town, South Africa. The primers for the first system in the initial round of PCR were: forward primer 5'CCTCTTTAATTGAAATCAGGCAT CAT 3' and reverse primer 3' CACACCTAGAGCTTAAAGC 5'.

In the second round of PCR, the primers were: forward primer 5' TGTAAACATTGCACTATGCTTTAAC 3' and reverse primer 3' CACATTTCTTTACAGGAGCCG 5'. The length of the final PCR product was 199 base pairs.

Following electrophoresis, the PCR product was purified and each sample was analysed twice on the sequencer, once with each of the forward and reverse primers. The samples were sequenced with a Big Dye v3.1 Terminator Sequencing Kit (Applied Biosystems, Johannesburg, South Africa) in a GeneAmp PCR System 9700 Perkin and Elmer/Applied Biosystems PCR machine in Johannesburg, South Africa as per Gibbon²⁵. The classification of sex was analysed by viewing the sequence of each sample in a chromatogram file (Figure 2) using Chromas Lite software (free online software).

In the second system, the primers for the first round of PCR were: forward primer 5' GTCTCYYTTAATGKAAATGCT CAT 3' and reverse primer 3' CAAACCTAGAGCTTAAAGC 5'.

In the second round of PCR, the primers were: forward primer 5' GCCACCGTGGATATCAGCCT CAT 3' and reverse primer 3' CACACCTAGAGCTTAAAGC 5'.

The length of the PCR product for the X chromosome is 188 base pairs and 194 base pairs for the Y chromosome. The PCR product for each sample was analysed using the Bio-Rad Experion DNA 1K system (Johannesburg, South Africa) for insertion/deletion analysis. To verify that the correct gene was amplified, the PCR product was purified using a Qiagen purification kit (Johannesburg, South Africa) and sequenced by Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). The classification of sex was analysed in a chromatogram file using Chromas Lite software (Figure 3) and through band separation on micro-fluidic gel electrophoresis.

RESULTS

In the first system, sex was identified by analysing each sequence for the ten polymorphic regions to determine whether the sequence corresponded to the X and Y chromosomes (male), or if only the X chromosome (female) was represented; 10 (32.25%) of the specimens produced results, 9 of these were male and 1 was female (Table 1). In analysing the polymorphic sites one notable specimen, A1028, had co-dominance of the X and Y chromosome in some polymorphic sites, while, in others, only the X chromosome was visible. As this particular specimen had both the X and Y chromosome, it was classified as a male. In the second system, the specimens that produced a positive result on the Experion station, but which were unconfirmed with sequencing, were omitted. In total, 10 (32.25%) specimens produced results and they were all classified as male.

Specimen A1008 produced odd results that suggested a possible female: an X chromosome in the first system and a mix of polymorphic regions corresponding to both the X and Y chromosomes in the second system (Figure 3). Even though the amplification of the Y chromosome was lower, the presence of the Y chromosome indicated this individual was a male and was diagnosed as such.
Sex profile of skeletal remains of Chinese indentured labourers

DISCUSSION AND CONCLUSION

Using both systems of molecular sex identification, 13 (41.93%) specimens in the sample produced results, all of which indicated that the specimens were male (Table 1). Based on this data, seven (53.84%) specimens in the sample produced the same result in both systems and two specimens produced different results in each system. Results were generated for four specimens using only one method: one using the first system and three using the second system of limited molecular preservation of the DNA molecules in the area of interest on the amelogenin gene. Both systems produced results for the same number of specimens (i.e. 10 [32.25%]). The differences between the results were most likely due to physical and chemical degradation of the DNA molecules in these samples, which disabled the desired binding site of the primers. Therefore, the primers were either not binding, or binding ineffectively, to the DNA, resulting in low or no amplification. The second system provided more results as it has the advantage of amplifying smaller DNA fragments, which are known to preferentially amplify in ancient DNA molecules. The results obtained here correspond well with other studies that were able to determine sex from 30% to 80% of their analysed samples.

These skeletons were all buried at the same location, within the 6-year period when Chinese labour was employed in South Africa. The remains of these individuals were exposed to the same taphonomic conditions and have approximately the same post mortem interval (PMI). Prior to repatriation, many of the labourers submitted urgent appeals for the remains of those who died on the mine to be exhumed and cremated for their return to China, which was granted, provided the graves were older than 12 months. Based on the fact that Chinese indentured labour was employed from 1904 and the final shipment of repatriated miners was in 1910, the PMI for these individuals is 98–104 years. In the ground, DNA degradation is characteristically slower due to the cooler conditions and decreased exposure to oxidative damage. These remains were accessioned for 57 years and thus have been kept at room temperature. So although the PMI is not particularly long for these skeletons, being in storage for 57 years may have negatively influenced the attainment of results. Other unpredictable factors could affect the molecular preservation of each sample, such as the acidity of the soil and all other taphonomic processes. These factors are undocumented and it is thus impossible to assess their role in the attainment of our results.

Data gathered on these 31 Chinese specimens, ranging from the condition of the bone to the DNA concentrations obtained for each specimen, were compiled and compared with the identified sex results (Table 1). Of these, the only factor that was reflective of molecular preservation was the condition of bone. Two specimens were recorded as being in poor condition and neither of these produced a result. While all of the specimens used in this study were intact, they did not all produce results, implying that intactness of the specimen was not necessarily indicative of molecular preservation.

As mentioned previously, a prior morphometric study suggested a high number of female individuals within the assemblage. These findings challenge the historical data, including the Witwatersrand Deep Mine records and the documentation of how the Chinese cemetery was used. In contrast, the present study produced results for 13 individuals, which showed

### TABLE 1

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Condition of bone</th>
<th>Concentration of DNA obtained (ng/µL)</th>
<th>First system result</th>
<th>Second system result</th>
<th>Overall sex determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>A996</td>
<td>Good</td>
<td>44.8</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A997</td>
<td>Good</td>
<td>52.8</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A998</td>
<td>Fair</td>
<td>44.7</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A999</td>
<td>Good</td>
<td>115.7</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1000</td>
<td>Good</td>
<td>22.3</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1001</td>
<td>Good</td>
<td>32.8</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1002</td>
<td>Fair</td>
<td>28.5</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1003</td>
<td>Good</td>
<td>24.8</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1004</td>
<td>Fair</td>
<td>31.6</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1005</td>
<td>Fair</td>
<td>50.0</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
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<td>Fair</td>
<td>222.3</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1007</td>
<td>Fair</td>
<td>30.5</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1008</td>
<td>Good</td>
<td>30.8</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1009</td>
<td>Fair</td>
<td>23.6</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1010</td>
<td>Fair</td>
<td>43.7</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1011</td>
<td>Fair</td>
<td>31.3</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1012</td>
<td>Poor</td>
<td>35.8</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1013</td>
<td>Good</td>
<td>60.4</td>
<td>F*</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1014</td>
<td>Good</td>
<td>51.1</td>
<td>F</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1015</td>
<td>Fair</td>
<td>20.5</td>
<td>M</td>
<td>F*</td>
<td>?</td>
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<tr>
<td>A1016</td>
<td>Fair</td>
<td>44.3</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1017</td>
<td>Poor</td>
<td>19.5</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1018</td>
<td>Fair</td>
<td>55.8</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1019</td>
<td>Fair</td>
<td>17.1</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1020</td>
<td>Fair</td>
<td>112.6</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1022</td>
<td>Fair</td>
<td>14.2</td>
<td>F*</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1023</td>
<td>Fair</td>
<td>69.0</td>
<td>F</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1027</td>
<td>Fair</td>
<td>37.9</td>
<td>F*</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>A1028</td>
<td>Fair</td>
<td>306.8</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1030</td>
<td>Fair</td>
<td>24.4</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>A1031</td>
<td>Fair</td>
<td>28.8</td>
<td>M*</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

M, male; F, female; ?, result not obtained.

*Results in the second system that were unconfirmed with sequencing and thus excluded.
that all were male. Unfortunately, the morphometric study produced only a summary of results and did not provide the sex identification for each individual; thus rendering a full comparison of the results from both studies impossible. Owing to the small number of individuals for which results were obtained in the current study, it cannot be stated with certainty that the results of the morphometric study were incorrect. However, taken together with the historical record, the results of our study strongly suggest that female individuals were not represented in large numbers and, more than likely, not at all in the skeletal remains of the Chinese indentured miners from the Dart Collection of Human Skeletons.

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