

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 Skeletons contains 36 skeletons sourced from a Chinese cemetery of 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

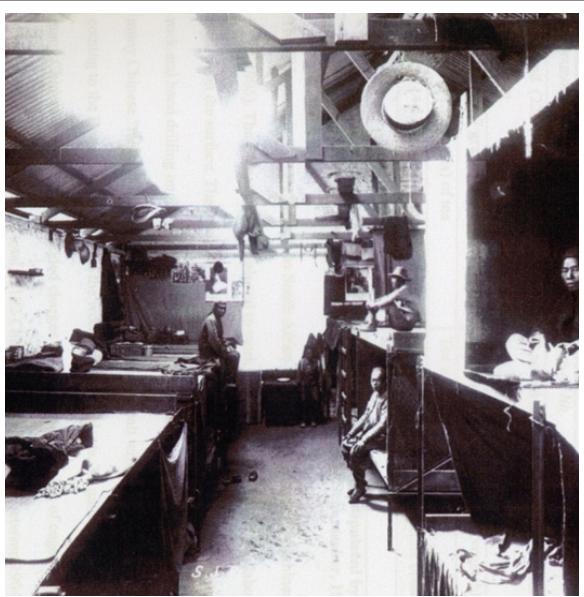
The materials were prepared and methods designed using guidelines for ancient DNA research, to ensure no contamination was introduced during the DNA extraction. As a result, decontamination standards were implemented from the extraction of the skeletal tissue. In order to reduce the risk of sample contamination, 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-

BOX 1**Historical background to Chinese indentured labour on the South African gold mines**

In 1898, the city of Johannesburg was the largest source of gold in the world and, as gold was the basis of international trade, the South African economy relied on this industry.^{7,8,9,10} Mining activities stopped during the Anglo-Boer War (1899–1902), which led to debt accumulation by the mines.^{4,10,11} The war was followed by a major shortage of unskilled labour for the South African gold mines,^{4,5,7,10,12,13} as other industries offered more lucrative employment options for the African workforce. The Chamber of Mines Labour Importation Agency looked to China to solve their labour shortage.⁵ Between 1904 and 1910, men were brought from northern China to work on the South African gold mines.

In 1904, two documents, the Anglo-Chinese Labour Convention and the Transvaal Labour Importation Ordinance, were developed to regulate recruiting, shipping and the conditions of the indentured labour service contract.⁷ The Chinese indentured labourers who were brought to South Africa were employed on 3-year contracts, that could be renewed for an additional 3 years.^{4,5,7,10,14} They were allowed to bring their families (although this was not generally practised) and were provided with cash wages, food, lodging (Figure 1) and clothing.^{4,5} However, as a result of media reports of torture and abuse in the mines, the recruitment of Chinese labour was stopped in 1907.¹⁵ The labourers already employed by the mines were provided time for the completion of their contracts, ending the Chinese involvement within a period of two and a half years.^{5,6,8,10,14} The last shipment of repatriated miners returned to China at the beginning of 1910.^{7,10}

Prior to their arrival on the mines, the Chinese labourers' health was assessed at least twice. Therefore, it may be concluded that, upon arrival in South Africa, these men were of relatively good health.¹⁰ The causes of death of the individuals whose skeletal remains were found are unknown. However, one may hypothesise that the main reasons for fatalities were hazards related to mining, as well as the poor living conditions of the miners.^{4,6,7,10} Injuries and fatalities were frequent amongst the Chinese miners, often caused by mining accidents such as digging and drilling in holes with unexploded dynamite, snapping of cage ropes and inexperience with explosives.^{4,7} In 1904 and 1905, 1167 labourers who were incapacitated as a result of physical problems or disease were repatriated to China.¹⁴ It is estimated that 3192 Chinese miners died on the Witwatersrand between the years 1904 and 1910.^{4,6,7}



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FIGURE 1

A photograph taken at the Simmer & Jack Gold Mining Company in the 1900s, showing Chinese indentured miners' bunk rooms situated inside the compounds

PCR). Each of these was completely equipped with consumables, chemicals, pipettes, reagents and labware.¹⁶ The extraction areas were sterilised with 6% sodium hypochlorite,¹⁷ followed by 70% ethanol. Gloves and protective clothing were worn at all times. To ensure no contaminants infiltrated the sample, negative (distilled water) and positive (blood) controls were carried out in parallel throughout the molecular systems, starting with the bone extraction.

The bone extraction method used was in accordance with Woodward et al.¹⁸ and Gibbon et al.¹⁹ According to this method, a power drill (Bosch PSB 650RE, Johannesburg, South Africa) with a sterile 4.5 mm titanium masonry drill bit was used to create a hole between the lateral and medial condyles in the intercondylar fossa of the femur, for the extraction of

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.¹⁹).

The molecular sex identification method used two independent nested PCR-based systems, as described in Gibbon et al.²⁰ 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 Synthetic DNA Laboratory in the Department of Molecular and Cellular Biology at the University of Cape Town, South Africa. The primers in the first system for the initial round of PCR were: forward primer 5' GTCTCTYYTAATGKAAACAATTGCGAT 3' and reverse primer 3' CCAACCATCAGAGCTTAAACTG 5'. In the second round of PCR, the primers were: forward primer 5' TGTKAACAATTGCATATTGACTTAATC 3' and reverse primer 3' CACTATTCTTACAGAGCCCAGG 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 Gibbon²⁰. 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' GTCTCTYYTAATGKAAACAATTGCGAT 3' and reverse primer 3' CCAACCATCAGAGCTTAAACTG 5'. In the second round of PCR, the primers were: forward primer 5' GGCAACCCTGGTTATATCAACTCA 3' and reverse primer 3' CCATCAGAGCTTAACTGGGAAGC 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.

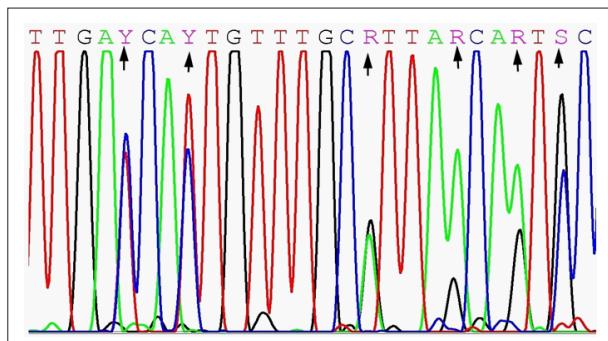


FIGURE 2

Chromatogram of Specimen A997 (male) from the first system showing polymorphic sites (indicated by the arrows) and demonstrating the co-dominance of the X and Y alleles

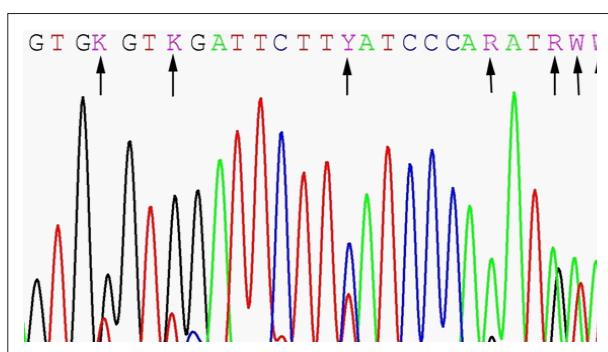


FIGURE 3

Chromatogram of Specimen A1003 (male), showing polymorphic regions and the insertion/deletion represented in the second system (indicated by the arrows), with both the X and Y chromosomes represented

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 samples.²¹ Even though there were few positive results, it must be stipulated that this is not unusual in ancient DNA studies, as each individual specimen will have its own micro-degradation processes. The results obtained here correspond well with other studies that were able to determine sex from 30% to 80% of their analysed samples.^{22,23,24,25}

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.⁶ Based on the fact that Chinese indentured labour

TABLE 1
The condition of the bone, the concentration of DNA obtained and the results of molecular sex determination using both systems for all 31 specimens

Specimen	Condition of bone	Concentration of DNA obtained (ng/µL)	First system result	Second system result	Overall sex determination
A996	Good	44.8		M	M
A997	Good	52.8	M	M*	M
A998	Fair	44.7		M	M
A999	Good	115.7			?
A1000	Good	22.3	M	M	M
A1001	Good	32.8	M		M
A1002	Fair	28.5			?
A1003	Good	24.8	M	M	M
A1004	Fair	31.6		M	M
A1005	Fair	50.0			?
A1006	Fair	222.3			?
A1007	Fair	30.5	M	M	M
A1008	Good	30.8	F	M	M
A1009	Fair	23.6			?
A1010	Fair	43.7			?
A1011	Fair	31.3			?
A1012	Poor	35.8			?
A1013	Good	60.4		F*	?
A1014	Good	51.1		F*	?
A1015	Fair	20.5	M	F*	M
A1016	Fair	44.3	M	M	M
A1017	Poor	19.5			?
A1018	Fair	55.8			?
A1019	Fair	17.1			?
A1020	Fair	1128.6			?
A1022	Fair	14.2		F*	?
A1023	Fair	69.0		F*	?
A1027	Fair	37.9		F*	?
A1028	Fair	306.8	M	M	M
A1030	Fair	24.4	M	M	M
A1031	Fair	28.8		M*	?

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

*Results in the second system that were unconfirmed with sequencing and thus excluded.

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.²⁶ 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 study³ 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



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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