

RECENT ADVANCES IN SEX IDENTIFICATION OF HUMAN SKELETAL REMAINS IN SOUTH AFRICA

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Keywords:
discriminant function analysis; forensic anthropology; geometric morphometric; metrical method; molecular method; morphological method; sex estimation; South Africa

Dates:

Received: 22 Apr. 2010
Accepted: 03 Oct. 2010
Published: 15 Nov. 2010

How to cite this article:
Bidmos MA, Gibbon VE, Štrkalj G. Recent advances in sex identification of human skeletal remains in South Africa. *S Afr J Sci.* 2010;106(11/12), Art. #238, 6 pages. DOI: 10.4102/sajs.v106i11/12.238

ABSTRACT

We review methods of sex estimation from human skeletal remains in South Africa within the forensic context. Sex is one of the key variables in obtaining a biological profile of the individual or population whose remains are analysed. Sex estimation based on the morphological characteristics of skeletal elements is population specific and thus the establishment of regional criteria is one of the imperatives for modern forensic anthropology. A literature review was carried out wherein the available methods of sex identification (morphological, metrical, geometric morphometrics and molecular) from South African skeletal material were critically examined. The approaches to sex estimation based on bone morphology have a long and productive history in South Africa. A number of approaches providing accurate results on the local populations have been developed. Research in molecular sex determination methods is still in its infancy in South Africa and the first innovative studies appeared only in recent years. While each of the four methods analysed is bounded by a number of constraints, they seem to complement each other and provide the best results when applied in conjunction with each other.

INTRODUCTION

Forensic anthropology is a relatively new scientific discipline, born at the intersection of physical anthropology and forensic medicine. It is usually defined as the application of physical anthropology methodology and techniques within the medico-legal context.¹ Although considerable advances have been made within the discipline in recent years, numerous challenges still face its practitioners.

The roots of forensic anthropology can be traced to research in the late 19th century, such as Alphonse Bertillon's anthropometric system for human identification and the dubious discipline of criminal anthropology.² Much closer to modern practices are several pioneering studies for identifying human remains carried out by anatomists and anthropologists such as Thomas Dwight and Harris Hawthorne Wilder.² The rise and development of the discipline was strongly accelerated with the publication of Wolton M. Krogman's 1939 *Guide to the Identification of Human Skeletal Material*³ and by significant involvement of physical anthropologists in the identification of victims from the Second World War. More recently, as noted by İşcan⁴, the establishment of a forensic anthropology section at the 1987 meeting of the International Association of Forensic Sciences in Vancouver played a major role in facilitating the interchange of information and research data amongst practitioners as well as the professionalisation of the discipline. These, he argued, led to an explosion in the number and quality of publications in the form of books and scientific papers, on subjects relating to the identification of people from human skeletons.⁴ Technological advances, together with methodological improvements in physical anthropology and related disciplines, have contributed to the growth of the discipline and shaped its current structure.

The importance of modern forensic anthropology cannot be overemphasised. In criminal cases, war atrocities and a wide variety of large-scale disasters, human remains encountered by forensic experts are often highly decomposed and fragmentary, requiring a battery of different interpretative techniques.^{5,6,7} Teeth and bones, being composed of tissues more resistant than any others to the effects of degradation, are of utmost importance in the process and often serve as a key tool in forensic identification.⁸ Thus, although a considerable amount of work within the discipline focuses on the soft tissues, forensic anthropology is most often applied to the identification and study of the human skeleton – forensic osteology. As a result, special attention in forensic anthropology has been given to the development and understanding of bone analysis and osteometric standards.⁹

When skeletal remains are found, it is necessary to reconstruct a biological profile in order to understand the demographics of the population and the individual represented.^{5,8} This includes estimating age, sex, ancestry and stature. However, numerous constraints face forensic anthropologists. These stem not only from the often fragmented nature and poor preservation of the remains analysed, but also from the complexities of human biology. Many parameters and interpretative models are applicable within certain limits. Thus, many variables have been shown to be population specific and their application to populations other than those they are derived from is strongly discouraged.^{10,11,12,13,14,15,16} As a result, it is imperative for forensic anthropologists in different geographical regions to develop applicable standards for local populations.⁹

Sex estimation from skeletal remains is crucial in the identification of human remains, as it halves the number of possible matches.¹⁷ Furthermore, other biological reconstruction variables, such as age at death, rely on the knowledge of sex of the individual. The aim of this paper is to provide a review of research on sex estimation or identification from skeletal material carried out in South Africa and its application to local populations.

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Forensic anthropology in South Africa

Since the publication of Tobias's seminal paper,^{18,19} the field of forensic anthropology has grown rapidly in South Africa. This growth has accelerated in recent years, with major teaching and research centres being developed at several institutions. Thus, forensic anthropology is now offered as a course or a module at some of the leading universities in the country, including the Universities of the Witwatersrand, Pretoria and Cape Town. The development of forensic anthropology is facilitated by the development of collections of skeletons of known origin at the Universities of Stellenbosch, Cape Town, Pretoria²⁰ and especially the Witwatersrand, which houses the world-famous Raymond A. Dart Collection of Human Skeletons.²¹

At present, the Department of Anatomy of the University of Pretoria is playing a leading role in this field, as its researchers analyse a significant number of cases for the South African Police Service. The unit, under the leadership of Prof. Maryna Steyn has also produced a guide on forensic anthropology in CD format,²² which is an extremely useful tool for students, teachers and researchers of the subject.

As a result of the complexity of the microevolutionary processes mediated by a complicated history in South Africa, this region is populated by a number of biologically heterogeneous populations. For this reason, the establishment of regionally specific standards for the interpretation of skeletal remains is necessary. In 1997, Steyn et al.⁹ noted that most of the standards used for human identification in South Africa were derived from international sources, without local standards, which led to problems for accurately identifying individuals from skeletal material. In response to these observations, studies were conducted for the purpose of establishing local standards for human identification in South African populations from crania and postcranial skeletons. This procedure should become continuous in South Africa, with equal importance given to the verification of these results, a practice very few researchers have adhered to.^{23,24,25} In addition, it is strongly advised that data be collected from as many components of the human skeleton as possible.⁵

RESEARCH ON SEX ESTIMATION OR IDENTIFICATION

Sex is defined as a 'biological category based upon reproductive attributes and roles in sexually reproducing species' which consequently may be used in the 'classification of individuals into categories based on types of gamete production'²⁶. Traditionally, physical anthropologists have used two methods of skeletal sex estimation, namely morphological (non-metrical) and metrical, including geometric morphometrics. They ultimately rely on the mild, but still detectable sexual dimorphism found within *Homo sapiens*. To traditional approaches based on bone morphology, molecular methods focusing on DNA analysis have been added with the recent 'molecular' revolution in life sciences. While all methods have their strengths and their areas of applicability, their efficacy is constrained by a number of elements.

Morphological or non-metrical method

The morphological method involves the visual observation of sexual traits on bones that exhibit sexual dimorphism. The most commonly used bones are found in the skull and pelvis.⁵ The recognition of these traits by an experienced observer can produce an accurate classification of sex.

The pelvis, which is designed to allow for parturition in females, appears to be the most reliable for sex determination.^{5,17} In South Africa, Steyn et al.²⁷ used the geometric morphometric technique in the assessment of the usefulness of the greater sciatic notch for the estimation of sex in a South African population and concluded that this trait is not reliable for sex estimation.

Previously, De Villiers^{28,29} used 14 non-metrical features of the skull of indigenous South Africans for sex estimation. She

included the glabellar prominence, superciliary eminences, superior orbital margin, inferior margin of nasal aperture, supramastoid crest, mastoid process, zygomatic arch and shape of the chin, among others. All of the features showed sexual dimorphism and provided a useful guide for sex estimation using the skulls of indigenous South Africans.

In 1996, Loth and Henneberg³⁰ introduced a new morphological trait for the estimation of sex in South African populations, namely the mandibular ramus flexure, which had an average accuracy between 91% and 99%. This study seems to have attracted more attention and discussion (in terms of supporting and contradictory reports) than any other in the history of forensic anthropology in South Africa. Soon after the publication of the study, the reliability of the method was challenged by Koski³¹, who concluded that the ramus flexure is not only a difficult trait to identify on radiographs, but is also not a useful morphological trait for sex estimation. However, studies using other samples have produced results in favour and support of this method. Indrayana et al.³² and Balci et al.³³ confirmed the reliability of the mandibular ramus flexure as a good indicator of sex using Indonesian (Javanese) and Turkish samples, respectively.

Despite the support for this method by some, many researchers have conducted work that disputes the accuracy of the mandibular ramus flexure for sex estimation. Donnelly et al.³⁴ obtained a low accuracy in sex classification using skeletal samples of Native American, Hispanic, European and African Americans. Hill³⁵, Haun³⁶ and Kemkes-Grotenthaler et al.³⁷ also came to the same conclusion as Koski³¹ and Donnelly et al.³⁴, rejecting the mandibular ramus flexure as a good morphological trait for estimating sex. None of the aforementioned studies were conducted using samples from South Africa where the original study was carried out. It was not until 2005 that Oettlé et al.²⁴ carried out the first and only study to date using samples from the Raymond A. Dart and Pretoria Bone collections. They used a more reliable technique of geometric morphometric analysis and came to the conclusion that the mandibular ramus flexure is not a good indicator for sex determination. Although there is still no general consensus on the reliability of this method, all indications from the aforementioned research point to the weakness of this trait in the assignment of sex.

Although the morphological method can produce valuable results and is ideal for quick and preliminary assessments, it relies largely on the experience and level of expertise of the scientist and therefore involves a significant level of subjectivity.^{27,37} To reduce the influence of subjectivity through the utilisation of multiple measurements (the metrical method), attempts have been made at estimating sex from bones that do not display obvious sexual differences.

Metrical method

The metrical method involves subjecting a suite of measurements of the bones to various forms of metrical analyses including the Student's *t*-test, indices, use of demarking and identification points and discriminant function analysis. Discriminant function analysis proved to be the most reliable approach and is therefore the most widely used metrical method. The use of the metrical approach in the estimation of sex is more structured than morphological evaluation and does not require vast experience on the part of the scientist. Furthermore, it can be repeated to validate the obtained results. However, inter- and intra-observer errors are well documented with the use of the metrical method.

Discriminant function analysis is a statistical method that was developed in 1936 by Ronald A. Fisher, as reported by Thieme and Schull³⁸ and since then it has been widely used in forensic science for the purpose of sex estimation. This method explores the differences between groups by determining which combination of variables can best predict group membership. It therefore requires a suite of measurements be taken on a bone in order to ascertain which measurements or combination of

measurements are the best predictor of sex. Usually, more than one measurement is required in order to obtain a high degree of accuracy for the discriminant function equation. It becomes difficult to use this technique in situations where the measurements with the best prediction of sex are not measurable on the bone. In addition, discriminant function equations are population specific and, as such, equations derived for one population cannot be used on other, unrelated groups. These equations are also affected by temporal change and therefore require revision from time to time.

De Villiers subjected 36 variables of the skulls and 15 of the mandibles of Black South Africans^{28,29} to metrical analysis and concluded that, while all measured variables showed sexual dimorphism, the mandibular measurements showed the greatest sexual dimorphism. Earlier, Giles and Elliot³⁹ and Kajanoja⁴⁰ had obtained acceptably high average accuracies from discriminant function equations derived from measurements of the crania of Americans and Finns, respectively. In the first study of its kind in South Africa, Rightmire⁴¹ subjected 32 measurements of the crania of Zulu and Sotho people to discriminant function analysis. The function thus obtained could be used in the correct assignment of sex in 90.6% of cases. Kieser and Groeneveld⁴² used the same technique on four measurements and two indices of the skulls of Black South Africans and they reported an average accuracy between 78% and 91%. Interestingly, they did not present any discriminant function equations for sex estimation, which made their study unusable for forensic work. In an attempt to address this deficiency, Franklin et al.⁴³ studied crania of indigenous South Africans obtained from the Raymond A. Dart Collection. On each of these crania, 12 measurements were obtained from mathematically transformed three-dimensional data. These measurements were then used in the derivation of six discriminant function equations with average accuracies in correct sex estimation that ranged between 79% and 83%. While Franklin et al.⁴³ used a microscribe (not always readily available in South Africa) in the acquisition of the data used in their study, Dayal et al.⁴⁴ measured 21 variables of the skulls of 120 indigenous South Africans using traditional anthropometric instruments like sliding and spreading callipers, with reproducible results. The results of the latter study (with accuracies between 80% and 85%)⁴⁴ compared well with those of the former (with accuracies of 77% – 80%),⁴³ with both studies presenting useful equations for sex estimation in indigenous South Africans.

Prior to the studies of Franklin et al.⁴³ and Dayal et al.⁴⁴, Steyn and İşcan⁴⁵ measured 12 variables on the skulls of 91 White South Africans obtained from the Dart and Pretoria collections. They presented five discriminant function equations with accuracies between 80% and 86%. Steyn and İşcan⁴⁵ observed that the cranial measurements had a better, albeit slight, predictability of sex than mandibular measurements – a finding in contrast to earlier observations made by De Villiers^{28,29}.

Forensic and physical anthropologists from all over the world have presented equations for sex estimation using discriminant function analysis on bones of the postcranial skeleton. In South Africa, similar efforts have also been made to derive population specific equations, with varying degrees of success based on average accuracy: humerus (95% – 96%),⁴⁶ radius and ulna (76% – 86%),⁴⁷ pelvis (72% – 86%),⁴⁸ patella (77% – 85%,⁴⁹ 78% – 85%),⁵⁰ talus (80% – 88%,⁵¹ 80% – 89%)¹⁶ and calcaneus (73% – 92%).⁵² However, the femur (68% – 91%) remains the most studied bone for sex estimation in South Africa.^{13,53,54,55}

Steyn and İşcan¹³ subjected six femoral and seven tibial measurements of White South Africans to discriminant function analysis. They obtained an average accuracy that ranged from 86% to 91%. In 2004, Asala et al.⁵⁵ evaluated the usefulness of the fragments of the femur in sexing. Five measurements from the proximal and three from the distal ends of the femur were subjected to discriminant function analysis. Univariate analysis

produced an average accuracy of between 68% and 83%, while a higher degree of accuracy (83% – 85%), expectedly, was obtained from the multivariate analyses. The study also showed that the proximal end of the femur is more dimorphic compared to the distal end. Asala et al.⁵⁵ proposed an explanation based on two factors, namely, the role of the femur in the transmission of body mass and the greater mobility of the hip joint. It was shown that these factors differ between sexes.^{56,57}

Geometric morphometrics

Despite the abundant body of knowledge on sex estimation from bones using non-metric methods, the use of morphological features still poses a great challenge to physical and forensic anthropologists.⁵⁸ The most notable challenge faced by practitioners of this field is the complex relationship between sex and its effect on the size and shape of skeletal elements.⁵⁸ While the traditional metric methods have demonstrated increased accuracy as described above, it is impossible to appreciate and quantify shape differences, because this variation may not be captured by the span of the callipers.⁵⁹ It is against this backdrop that geometric morphometrics was developed in order to assist forensic anthropologists to get a better understanding of the relationship between the size and shape of features of bones and how quantification of these features can assist in sex estimation.⁶⁰

In one of the earliest studies using this technique in South Africa, Franklin et al.⁶¹ used the microscribe to acquire data from 96 landmarks of 332 crania of indigenous South Africans with the view to ascertain the usefulness of this technology in sex determination for forensic purposes. The obtained accuracy from the use of these landmarks (87%) compared well with those obtained from studies that were based on the use of traditional linear techniques (77% – 80%)⁴³ and 80% – 85%)⁴⁴. In addition, Franklin et al.⁶¹ reported that geometric morphometrics are a useful tool that can be used to demonstrate highly significant sexual dimorphism in a studied sample with respect to size and shape variation. They reported that the most sexually dimorphic feature of the cranium is the bizygomatic breadth.⁶¹

In another study, Franklin et al.⁶² further demonstrated the versatility of geometric morphometrics in forensic anthropology when they acquired data from the mandible of sub-adult indigenous South Africans. Even though the method has been credited with great versatility, the results of this study indicated that the sub-adult mandible of the studied population group does not display sexual dimorphism. In 2008, Franklin et al.⁶³ examined 225 mandibles of adult indigenous South Africans using the same technique as the previous studies. From the subsequent analysis of data from 38 bilateral three-dimensional landmarks through the use of a microscribe, Franklin et al.⁶³ were able to demonstrate sexual dimorphism of the studied landmarks, with the condyle and ramus being the most consistently dimorphic part of the mandible. The cross-validated sex classification accuracy in the pooled data (83.1%) was similar to that presented by Steyn and İşcan⁴⁵ (81.5%) using traditional linear metrical methods.

While a high degree of accuracy could be produced by all three methods – morphological, metrical and geometric morphometrics – the applicability of these methods is severely constrained if the remains are fragmented and key morphological features cannot be observed and measured, as well as in cases where the remains are juvenile and have yet to develop detectable sex-specific traits.

Molecular methods

With the invention of the polymerase chain reaction (PCR) in the 1980s, researchers began to extract DNA from skeletal tissue.^{64,65,66,67} Genetic information retrieved from skeletal material provides the opportunity for personal identification, especially in forensic cases (such as criminal cases, mass graves and accidents), where molecules can often be the only method of identification.

Founded in 1996, the International Commission on Missing Persons locates and identifies people that have disappeared during armed conflict or human rights violations.⁶⁸ In the past seven years, this organisation and others like it have led the way for major improvements in DNA extraction and amplification methods from bone.^{62,69,70} Genetic sex identification is done through the isolation and amplification of a gene or genes in the sex chromosomes (X and Y). The most commonly used sex determining genes are the sex determining region Y (SRY locus), zinc finger protein (ZF) and the amelogenin (AMEL) genes.

In South Africa, molecular sex determination from skeletal tissue is still an emerging area of study and only ZF and AMEL genes have been used for sex identification. Pillay and Kramer⁷¹ presented a new method for sex determination from teeth using the ZF gene, which is located on both sex chromosomes and can be co-amplified in one PCR reaction. They extracted DNA from modern teeth using phenol-chloroform extraction. However, as a result of PCR inhibition, this method is not ideal for forensic, archaeological or ancient samples.

The AMEL gene has been used in a molecular sex-typing system in numerous studies involving the use of skeletal material.^{72,73,74,75,76,77,78,79,80,81} The structure and properties of this gene make it a very good candidate gene for sex identification from complicated forensic material, such as highly fragmented, burnt, juvenile and foetal remains where sex cannot be estimated with traditional morphometric methods.^{74,82}

The AMEL gene has also been used for sex identification in South Africa. Urbani et al.⁸³ used the gene to determine sex and to assess the level of molecular degradation produced by varying high temperatures. This study was conducted to examine the remains of forensic victims of fire and to determine at what temperatures human teeth can still retain intact, useable DNA for identification. In addition, Gibbon et al.⁸² developed two methods of molecular sex determination using the AMEL gene. One of these methods isolates ten sex-specific polymorphic sites and the other method additionally isolates an indel (six base pairs). These methods have been used on archaeological materials from the Raymond A. Dart collection.^{82,84}

The silica column DNA extraction methods or variations thereof are preferred, followed by an enzymatic process of EDTA and proteinase K, when sampling from skeletal tissue, as the result is high quality DNA. Both the South African Police Service and Gibbon and colleagues⁸² use a silica extraction method, followed by isolation of the AMEL gene for identifying sex in human forensic samples.

The methods of bone and tooth extraction have traditionally been destructive to skeletal remains, which has made it difficult to gain access to important collections of human skeletal remains. Another development in South Africa was a mechanically minimally invasive method of bone sampling from the intercondylar fossa of the femur, optimal for molecular analyses.^{85,86} This method preserves the physical properties of the skeletal remains, allowing them to be continually used for morphometric analysis.

Molecular sex determination has certain advantages over morphometric methods, as it is not affected by individual variation in the size and architecture of skeletal material. It can be used to determine sex from foetal and juvenile remains and is not restricted by physical fragmentation in the same way, requiring only one complete bone or tooth for sex determination. However, molecular methods are limited by a number of constraints. Molecular contamination is a major concern but can usually be avoided through the implementation of strict laboratory measures. Molecular preservation of the specimen poses the greatest challenge for molecular methods, because of the difficulty in predicting the level of preservation. Numerous environmental factors can induce molecular degradation and

thus severely impair the process of obtaining DNA for forensic analysis. Additionally, molecular methods can be costly and thus their use is often restricted to forensic material where other methods are not useful.

CONCLUSION

In recent years, the field of forensic anthropology has been developing rapidly. South African forensic anthropology has followed and contributed to these advances through the development of local standards for the interpretation of skeletal remains, as well as to knowledge applicable outside of the region. This is also the case with the estimation or identification of sex, a critical step in the interpretation of skeletal remains.

Research shows that both traditional morphometric and molecular methods of sex estimation or determination have their limitations. The awareness of these is of utmost importance in the forensic interpretation of human remains. However, these methods also have numerous applications and furthermore they complement each other. Ideally, when it is viable, in an attempt to reconstruct as much of the biological profile as possible, all of the available methods for sex determination should be used.

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