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An analysis of sexual dimorphism using geometric morphometrics of the femur and tibia: the use of GM in assessing sex of fragmented remains

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AN ANALYSIS OF SEXUAL DIMORPHISM USING GEOMETRIC MORPHOMETRICS OF THE FEMUR AND TIBIA: THE USE OF GM IN ASSESSING SEX OF FRAGMENTED REMAINS

by

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AN ANALYSIS OF SEXUAL DIMORPHISM USING GEOMETRIC MORPHOMETRICS OF THE FEMUR AND TIBIA: THE USE OF GM IN ASSESSING SEX OF FRAGMENTED REMAINS

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ABSTRACT

This project analyzes the sexual dimorphism of the femur and tibia using geometric morphometrics. The study sample includes 250 individuals of known sex and age at death with complete, non-damaged, non-pathological skeletal remains from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. Ages range from 19-96 for males (mean=56.92 years) and 29-97 for females (mean=59.48 years). A combination of landmarks and semi-landmarks were collected on the proximal and distal epiphyses of each bone using a Microscribe, which helps capture the overall size and shape variation present in the sample. Only individuals from one population, White, where analyzed in order to eliminate population variation bias. Classification rates for males and females for the proximal femur were 80.8% and 78.4% respectively, for the distal femur 92.6% and 89.6% respectively, for the proximal tibia 80.8% and 83.2% respectively, and the distal tibia 81.6% and 80.8% respectively, all with a $p<0.0001$. These rates created a classification model for which epiphysis gave the most accurate assessment of sex: the distal femur,

followed by the proximal tibia, then the distal tibia, and lastly the proximal femur. This study indicates the knee joint is the most dimorphic, followed by the ankle and then the hip. The results fall in line with another study indicating the knee is more sexually dimorphic in a modern White population (Spradley and Jantz 2011), though in contrast to their results this study found the distal femur was more dimorphic than the proximal tibia. This method indicates that in comparison to standard measurements, geometric morphometrics may provide a more reliable method for sex estimation when used, specifically on the knee. Certain landmarks were then selected based on the standard taphonomic process of coffin wear and postmortem damage (Pokines and Baker 2014) for exclusion to determine the usability of the method on fragmented or damaged skeletal remains. When combinations of landmarks were removed, the distal femur still possessed the highest classification rates with over 80% accuracy.

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LIST OF ABBREVIATIONS

BU .... Boston University

CVA...Canonical Variates Analysis

DFA...Discriminant Function Analysis

GM..Geometric Morphometrics

GPA...Generalized Procrustes Analysis

PCA ....Principal Components Analysis

INTRODUCTION

The identification of an unknown individual is important in a growing number of specialized fields in biological anthropology. The discipline of forensic anthropology utilizes the estimation of age, sex, stature, ancestry, pathology, and the manner of death, if available, to aide in the identification of an unknown individual in a medicolegal investigation (Dirkmaat *et al.* 2008). Bioarchaeology studies population composition as it existed in prehistoric and historic times using the methods listed above, where the assessment of individuals found in the archaeological record is a valuable tool for reconstructing the past (Knudson *et al.* 2008). In both forensic anthropology and bioarchaeology, when attempting to assess the remains of an unknown individual, one of the first questions addressed is whether they are male or female. There have been many studies assessing the sexual dimorphism, or the variation in shape and, to a larger extent size, that exists between males and females among modern *Homo sapiens* (Albanese 2013; Bernal *et al.* 2009; Bruzek 2002; Buttner *et al.* 2001; Giles and Elliot 1963; Howells and Hotelling 1936; Klales *et al.* 2012; Mall *et al.* 2000, 2001; Phenice 1969; Pretorius *et al.* 2006; Purkait 2003, 2005; Walker 2008; Washburn 1948). This study utilizes a relatively new method of shape analysis to assess sexual dimorphism in anatomically modern humans.

There are a several elements of the adult human skeleton known to be sexually dimorphic, such as the os-coxae and cranium, which are scaled, measured, or qualitatively analyzed for the shape and size variation that exists between males and females (Bruzek 2002; Gonzalez *et al.* 2009; Phenice 1969; Walker 2008). Since the development of geometric morphometrics (reviewed in Slice 2005), sexual dimorphism has been quantified in numerous skeletal elements, including the pelvis and cranium (Bastir *et al.* 2009; Bidmos *et al.* 2010; Buttner *et al.* 2001; Bruce and Maclaughlin 1985; Cardini *et al.* 2012; Frutos 2005; Gehring *et al.* 2000; Pretorius *et al.* 2006; Purkait 2003). Geometric morphometrics is utilized to quantify shape variation, and since its development has become a tool in forensic analysis due to its innovative use of landmark data transcribed into a three-dimensional space that can better assess differences ascribed to sexual dimorphism (Bytheway and Ross 2010).

In the 1960's and 70's, biometricians began using multivariate statistical methods of analysis to describe patterns of shape variation over a wide range of different groups (Kendall 1977; Lagler *et al.* 1962; reviewed in Adams *et al.* 2004). These early attempts combined multivariate statistics and quantitative morphology, which was innovative for the time given the field had previously been dominated by qualitative assessments, but there were still several difficulties that remained (Adams *et al.* 2004; Zelditch *et al.* 2012). Many methods of size correction were proposed, which would allow for comparisons between different sized specimens, but there was little agreement and a lack of standardization on which method of correction should be used (Adams *et al.* 2004; Bookstein 1989). These different size correction methods usually resulted in slightly different results, therefore making the analyses impossible to compare. Another issue was that the homology of linear distances, or the consistency of length between two points, was difficult to assess because many of the distances analyzed were not defined by consistently recognizable points (Adams *et al.* 2004). Some of these issues, particularly those relating to standardization of methods and the location of postcranial landmarks, are still an issue within the field of morphometric analysis to this day (Adams *et al.* 2004; Bookstein 1989; Zelditch *et al.* 2012).

In efforts to address some of the issues presented by traditional morphometrics, landmark-based geometric morphometric methods have recently been established. They begin with the collection of two- or three-dimensional coordinates of biologically definable landmarks that accurately capture the shape in question across a sample of a given population. The variation within the sample can then be mathematically analyzed to determine the statistical significance, if any, of the variation present among groups in the sample (Adams *et al.* 2004; Bookstein 1991; Slice 2005). Landmarks are grouped around a centroid, which is a mathematical determination defined by the distribution of each landmark's coordinates to an equidistant point in space (Lewton 2012). This eliminates the issue of size correction mentioned above because this type of analysis, known as a Procrustes Fit or Generalized Procrustes Analysis (GPA), is a standardized method for rearranging the collected landmarks around a central point to better distinguish the variation present. GPA is used to compare data across individuals within a sample by orienting the data using translation, scaling, and rotation (Zelditch *et al.* 2012).

By the early 1990's, the advantages of geometric morphometrics to more accurately describe the size and shape variation present within a sample were widely understood, and biologists used landmark and outline methods to address a wide range of hypotheses (Adams *et al.* 2004; Bookstein 1991; Klingenberg and McIntyre 1998). The advances in morphometric analyses since its development in the 1960's have been based on a better understanding of the mathematical methods involved, which is a major improvement in the way statistical analyses are utilized in relation to scientific research. Due to the broad applicability of geometric morphometrics, the analysis of sexual dimorphism in modern humans has long been studied but can now more accurately be described statistically using this method.

The present study analyzes the sexual dimorphism of both proximal and distal epiphyses of long bones in the lower limb using geometric morphometrics tested on a sample of known sex and age at death individuals from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. Two skeletal elements were analyzed, the femur and tibia, using a series of pre-selected landmarks that were digitally recorded using a Microscribe digitizer. The landmarks were selected for the purpose of sufficiently summarizing the size and shape of each epiphysis (Holliday *et al.* 2010). Each bony epiphysis is individually assessed for its usefulness in estimating sex within the given sample, and then compared to determine the best usable epiphysis based on this method. The second aspect of this research was to reevaluate the data using subsets of landmarks on each epiphysis to determine the classification rates of possible fragmentary or damaged remains. A comparison between the complete set of epiphyseal landmarks and the subsets was conducted to analyze the usability of this method for assessing the sex of skeletal fragments. Given the size and shape variation that is already established as existing between males and females of anatomically modern *Homo sapiens* (Albanese 2013; Asala 2001; Bidbos *et al.* 2010; Bernal *et al.* 2009; Bruzek 2002; Buttner *et al.* 2001; Bytheway and Ross 2010; Cardini *et al.* 2012; Decker *et al.* 2011; Phenice 1969; Walker 2008), and the superior ability of geometric morphometrics to more accurately depict overall size and shape variation within a sample (Adams *et al.* 2004; Slice 2005; Zelditch *et al.* 2012), this study hypothesizes that a geometric morphometric approach will yield high classification rates between males and females using the epiphyses of the femur and tibia. Though a decreased classification rate is experienced when using fewer landmarks, sexual dimorphism is still assessable using GM, proving this method's usefulness in both archaeological and forensic contexts where damaged or fragmented remains may be encountered.

LITERATURE REVIEW

Scholarly consensus indicates the os-coxae as the skeletal element that provides the most reliable sex determination due to its dimorphic nature, specifically in modern adult *Homo sapiens* (Bruzek 2002; Gonzalez *et al.* 2009; Howells and Hotelling 1936; Phenice 1969; Klales *et al.* 2012; Steyn *et al.* 2009; Walker 2005; Washburn 1948). Typically, sex estimation has been approached qualitatively, such as the widely utilized Phenice and Walker methods (Phenice 1969; Walker 2008). The issue of subjectivity, however, always arises when dealing with the use of qualitative methods of sex assessment. In response to this, other methods for sex estimation have been developed, specifically the use of standard and nonstandard measurements of long bones, which decreases the amount of subjectivity seen with qualitative methods, but have varying rates of success themselves (Agritmis *et al.* 2006; Asala 2001; Buttner *et al.* 2001; Holland 1991; Milner *et al.* 2012; Purkait 2003).

Arguably Howells and Hotelling were the first to examine the variation that exists in the pubic bone between males and females after questions about possible morphological adaptations arose surrounding the ability for women to give birth (Howells and Hotelling 1936). Their study stemmed from the observation that "women possessing masculine forms of pelves were prone to encounter certain obstetrical complications during birth," meaning that women with os-coxae more similar in shape and size to men were experiencing childbirth difficulties (Howells and Hotelling 1936, p.105). The authors found a relationship between the size of the greater sciatic notch and the overall size of the pelvic inlet, indicating that the smaller the notch, the less space available for childbirth (Howells and Hotelling 1936). The authors examined a group of dried os-coxae of American Indians in the collection of the American Museum of Natural History. Measurements were taken and found that sex differences were significant in the sample (Howells and Hotelling 1936).

Sherwood Washburn took measurements of 300 adult skeletal os-coxae, specifically the pubis and ischium, of known race and sex from the collection of the Hamann Museum of Anatomy and Comparative Anthropology (Washburn 1948). The author found the pubic to be longer in human females than males, and that the “ischium-pubis index alone will sex over 90% of skeletons, provided that they belong to one major racial group” (Washburn 1948, p. 206). Washburn’s work indicated that the length of the pubis in relation to the ischium would accurately estimate sex at a rate of 90%, but Washburn used males below pubertal age and claimed the method worked. Anthropologists now understand sexing of pre-pubertal individuals is not recommended due to high inaccuracies, calling into question the rates acquired by Washburn (Buikstra and Ubelaker 1994).

It was then Phenice who developed one of the most widely used methods for sex estimation of the adult human os-coxae (Phenice 1969). The author examined the morphology of the pubic bone for three traits, specifically the ventral arc, the subpubic concavity, and medial aspect of the ischio-pubic ramus

(Phenice 1969). Each of these traits are scored independently based on their appearance, and the majority score indicates the individual's sex (Phenice 1969). The author examined 275 individuals of both White and Black ancestry from the Terry Skeletal Collection and found that, using all three morphologies, the estimation of sex was more than 95% accurate (Phenice 1969). Known as the Phenice method for sex estimation, this study was groundbreaking in that it provided a means to estimate sex based on pelvic morphology, where other methods had only studied collections for measurement variations between males and females.

Since Phenice's groundbreaking research, other anthropologists have developed various methods for qualifying or visually describing sexual dimorphism. One such study was conducted by Jaroslav Bruzek (2002), who examined five characteristics of the os-coxae and scored them as either male, indeterminate, or female based on their morphology. All five traits, when combined, offer a classification rate of 95%, but only 60-80% when traits were individually assessed (Bruzek 2002). This method, however, is not widely utilized because it was only tested on a single European sample and if the os-coxae is damaged, this method would prove difficult in the assessment of sex.

Walker (2005) visually assessed the greater sciatic notch, which appears alongside Phenice's method in *Standards for Data Collection from Human Skeletal Remains* (Buikstra and Ubelaker 1994). This text is a biological anthropologist's manual for widely accepted methods of skeletal data collection.

The method uses a scoring system to analyze the morphology of the greater sciatic notch, where a score of one is more female while a score of five is more male (Walker 2005). This method claims an 80% accuracy rate, though 35% of specimens from the sample had an indeterminate morphology (Walker 2005). Though widely accepted, this method is also susceptible to subjectivity and interobserver variation, which, as previously mentioned, is a common issue when using scoring methods for sex estimation.

Klales, Ousley and Vollner (2012) have since updated the Phenice method for sex estimation by adapting the qualitative aspect and adding a quantitative assessment of sex to their research. The authors analyzed the three traits of the Phenice method, and instead of the three possibilities they developed a five-score method (Klales *et al.* 2012). Each trait is scored independently, and then inputted into an excel spreadsheet created by the authors, which calculates a probability for being either male or female based on the scores assigned (Klales *et al.* 2012). The authors found that their method provided a classification accuracy of 86.2%, but again, since it is based on a subjective scoring system, the amount of subjectivity has yet to be assessed in subsequent research (Klales *et al.* 2012).

Several analysts have attempted looking at other skeletal elements for sex estimation, as well as means of establishing quantifiable, and therefore less subjective, methods for identifying sex. One such individual is Ruma Purkait (2003, 2005), who examined three measurements of the proximal femur to determine sex based on the assumption that males use the muscles in this area more heavily than females and therefore the sites of these muscle attachments will be larger and provide greater measurements than in females. Though Purkait (2005) found all three measurements provided an 86.4% classification rate, the sample was heavily biased towards males and only focused on one population in central India. What is important to take away from Purkait's (2005) research is the use of quantifiable metric data to estimate sex, which removes a substantial amount of subjectivity from the analysis.

Another area strongly analyzed for its sexual dimorphism in modern *Homo sapiens* is the skull. Giles and Elliot (1963) analyzed eleven measurements of the cranium using discriminant function analysis (DFA) to correctly identify males and females within a sample. What is arguably most important about this study is its quantitative method and application of DFA to estimate sex. The authors explain DFA as a method for assigning an individual in a sample into one or more groups on the basis that some number of variables characteristic of each group is represented by each individual within the sample (Giles and Elliot 1963). This method will assign an individual to one group or another based on information available and variation present (Giles and Elliot 1963). When assigning a sex, the number of groups is two, and the variables are based on the method of analysis, be it measurements, or in the case of the current study, skeletal landmarks. The discriminant function, therefore, is the derived linear function that best distinguishes the two groups. This method of analysis will be discussed further in the next chapter.

Walker (2008) used a qualitative method to score five cranial traits, specifically the nuchal crest, the mastoid process, the supra-orbital margin, the supra-orbital ridge/glabella, and the mental eminence. This qualitative method for sex estimation was adapted from the original 1970 publication by Aşcadi and Nemeskeri for the purposes of inclusion in *Standards* (1994). Walker (2008) found an 88% accuracy rate utilizing discriminant function analysis and argued this method better than the best logistic regression model with the pelvis, which was only 78% accurate (Walker 2008).

Standard measurements of post-cranial elements have also proven useful in the assessment of sex. Albanese (2013) collected standard and non-standard measurements of the clavicle, humerus, ulna, and radius, and developed equations that were tested using four independent samples where classification rates for sex estimation ranged between 87.4-97.5%. Mall *et al.* (2001) took standard measurements of the humerus, ulna, and radius, and found a significant difference between males and females, with a correct classification rate ranging from 88.49-94.93% using either a single measurement or a combination of measurements, respectively. Purkait (2003) analyzed four measurements of the femoral head using discriminant function analysis, and claimed morphology correctly classified sex with 92.1% accuracy. These studies indicate that sexual dimorphism is quantifiable in postcranial elements besides the os-coxae, which shows there is variation between adult males and females throughout the skeleton, both statistically significant qualitatively and quantitatively.

Subsection One: Geometric Morphometrics

When analyzing variation in size and shape of skeletal elements, variance can be indicative of several factors, including but not limited to function, growth, and development (Zelditch *et al.* 2012). By studying these variations, significant inferences about the population or populations in question can be deduced (Bastir *et al.* 2009; Bernal *et al.* 2009; Bookstein 1991; Bookstein *et al.* 2012; Holliday *et al.* 2010; McKeown and Schmidt 2012; Pretorius *et al.* 2006; Slice 2005, 2007; Zelditch *et al.* 2012).

Geometric morphometrics is used to quantify biological shape using landmark data (Bookstein 1991; McKeown and Schmidt 2012; Slice 2005; Zelditch *et al.* 2012). Multivariate morphometrics originated from the measurement of human proportions, also known as anthropometry, which has a long history dating back to the ancient Egyptians (Slice 2005). The field of biological anthropology arguably got its start due to the desire for physical evidence of the variation within and between the so-called races of man in the form of measurements of lengths, breadths, and heights of anatomical structures (McKeown and Schmidt 2012; Slice 2005). Anthropometry has since developed to compare, rather than segregate, differences within and between groups (Little and Kennedy 2010). The traditional approach to morphometric analysis involves the application of multivariate statistics to measurements, angles, or distance ratios (Slice 2005). These traditional methods, however, failed to recognize all of the geometric information about the biological structures they were analyzing. Without this information, morphometric analyses may neglect important morphology, and therefore may produce biased or inaccurate results (Slice 2005, 2007; Zelditch *et al.* 2012). Shape analysis plays an important role in many kinds of biological studies, and for the purposes of this study one of those areas in more recent years has been the analysis of sexual dimorphism (Zelditch *et al.* 2012).

Modern technological innovations, such as the Microscribe digitizer, have improved the application of geometric morphometric analyses by allowing researchers to quickly collect three-dimensional coordinates which capture size and shape data of biological forms. Due to the three-dimensional aspect of geometric morphometric analysis, it is arguably the most effective means by which to quantify differences of morphology (Holliday *et al.* 2010; McKeown and Schmidt 2012; Slice 2005; Zelditch *et al.* 2012). The use of geometric morphometrics is becoming increasingly popular because it is a quantified statistical analysis based on landmark data (Bastir *et al.* 2009; Bookstein *et al.* 2012; Bernal *et al.* 2009; Holliday *et al.* 2010; Pretorius *et al.* 2006).

The method, however, is not without its issues, some of which include the subjective nature of landmark collection on post-cranial elements that can result in high error rates when studies are recreated. Other problems arise because it is difficult to define and standardize a set of axes to record landmarks, for example the use of axes with respect to anatomically recognized positions, such as Frankfurt horizontal (Slice 2007). These axes can actually increase the amount variation due to the fact that they are selected based on a presumed consistency among all individuals within a population or species. This is slightly rectified by the fact that coordinate data are collected with respect to some arbitrary axes that are unique in regards to the elements under study (Slice 2005). These arbitrary axes can then be collected around a centroid in order to better analyze the possible variation present within a sample or between samples.

Landmarks, or Cartesian coordinates, address many of the problems of distances and angles that arise with typical anthropometry measurements (McKeown and Schmidt 2012; Slice 2005). When used, landmarks more accurately describe the overall shape and size of an object and have the ability to include morphological variation that may be overlooked when using standard measurements. Standard measurements are simple distances between point A and B, whereas landmarks can be selected that capture the curvature or shape of an object. Figure 1 depicts just this, where strategically placed landmarks more accurately describe the curvatures that exists in a skull compared with standard measurements. Each landmark is a point in three-dimensional space with an X, Y, and Z coordinate. The collection of landmarks can be done by recording locations on the specimen using specially designed hardware, such as the previously mentioned Microscribe, or by using software where 2-dimensional images or 3-dimensional scans, such as Computed Typography (CTs) (Slice 2005). Software used in conjunction with the Microscribe directly inputs the coordinates into Excel, which can then be formatted for multiple statistical analysis programs depending on the purpose of the research.

Each landmark can be classified as either Type I, Type II, or Type III (Bookstein 1991). Type I anatomical landmarks are defined with respect to specific intersections of tissues, such as triple points of suture intersections on the cranium or the location of a tubercle (Bookstein 1991; Slice 2005; Stevens and Vidarsdottir 2008). Type II landmarks are maximum points of curvature associated with local structures, usually with biomechanical function like the curvature on a facet, which are the types of landmarks used in this study (Bookstein 1991; Stevens and Vidarsdottir 2008). Lastly, Type III landmarks are extreme points, like endpoints of maximum length or breadth, but unlike Type II, Type III landmarks are defined with respect to a distant structure, such as the other end of a measurement (i.e. point A opposite of point B) (Bookstein 1991; Slice 2005; Stevens and Vidarsdottir 2008). The two- and three-dimensional locations of Types I and II are defined with respect to local morphology, while Type III are "deficient" in that they contain meaningful information only with respect to its relation with another structure (Slice 2005).

There have been several studies analyzing sexual dimorphism based on multivariate analyses and geometric morphometrics, including assessments of the orbit; the mandibular ramus; and greater sciatic notch, and shape of the os-coxae (Bytheway and Ross 2010; Gonzalez *et al.* 2009; Mall *et al.* 2001; Pretorius *et al.* 2006).

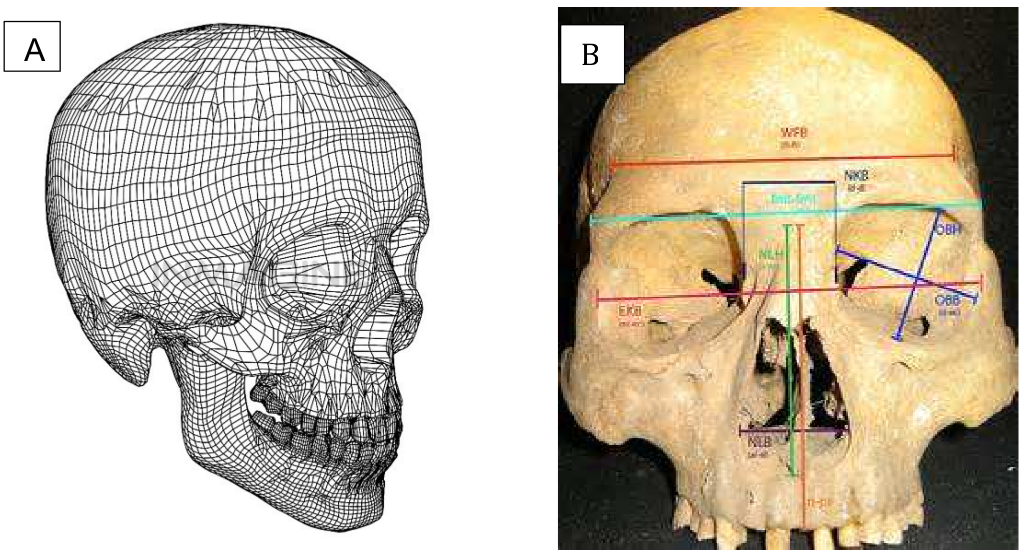
Bytheway and Ross (2010) used geometric morphometrics of the os-coxae to estimate sex in an African American and European American sample, where European Americans were correctly sexed with 98% accuracy, and African Americans with close to 100% accuracy. Gonzalez *et al.* (2009) also utilized geometric morphometrics to analyze the sexual dimorphism of the os-coxae from two-dimensional figures and had a 90.1-93.4% classification accuracy rate. Pretorius *et al.* (2006) assessed three different morphologic characteristics using geometric morphometrics in a sample of South African Blacks- the shape of the greater sciatic notch, mandibular ramus flexure, and shape of the orbits- to determine their usability in estimating sex. The orbit was the least dimorphic with a classification rate of 51.51%, followed by the greater sciatic notch at 90.27%, and lastly the mandibular ramus flexure at 95.14% (Pretorius *et al.* 2006).

This study aims to utilize a geometric morphometric approach that is suggested to be more statistically accurate at quantifying sexual dimorphism on four skeletal traits located on two elements. The majority of landmarks used in this study have been presented in three other articles (Bastir et al 2009; Bytheway and Ross 2010; Stevens and Vidarsdottir 2008). Stevens and Vidarsdottir (2008) use Type II and III landmarks to assess the shape of the distal femur and proximal tibia, in order to analyze the morphological change in shape that occurs in the knee with advancing age. Though the scope of their research is different in that they were looking for age-related morphological variation and this research is interested in sex-based variation, the landmarks used by Stevens and Vidarsdottir (2008) accurately described the overall size of the bony knee joint, which is why many of them were applied to the current study. Recently, Brzobohata *et al.* (2014) examined sex classification rates using landmarks on the proximal and distal tibia epiphyses on a sample from Czech populations from two centuries. The landmarks from their study greatly align with those adapted from Stevens and Vidarsdottir (2008) on the proximal tibia and the ones created by the author on the distal tibia for the purposes of this research, and therefore lead to the conclusion that the landmarks utilized in this study accurately describe the overall size of the proximal and distal epiphyses of the tibia.

By using Type II landmarks as described above, the current study analyzes both epiphyses of the femur and tibia. The landmarks utilized are defined using anatomical terminology in tables 1-4 in the next chapter. The studies presented in this chapter indicate sexual dimorphism is better quantified using geometric morphometrics compared with standard measurements, and the current research examines skeletal areas not typically analyzed for the estimation of sex. Geometric morphometrics can be a valuable tool for assessing sex of postcranial elements of skeletal remains belonging to unknown individuals, as it is currently done by the program 3D-ID with the skull (Slice and Ross 2009). By using elements not normally quantified for size variation, this study is of importance when analyzing skeletal remains found in both forensic and archaeological situations as well as in determining if the method can accurately classify fragmentary remains.

Figures

<u>Figure 1: Cranial Wireframe vs Standard Measurements</u>



http://www.inmagine.com/spl013/spl013548-photo

http://blogs.wlc.edu/anthropos/2010/02/03/study-of-prehistoric-wisconsin-crania/

MATERIALS AND METHODS

The data for this study was collected from femora and tibiae drawn from the William M. Bass Donated Skeletal Collection at the University of Tennessee, Knoxville. Landmarks were collected from 250 individuals (males=125/ females=125) of known sex, age at death, and ancestry. Individual elements in the study sample were not damaged and free from pathological defects. Only left-sided elements were used in this sample. The ages of males ranged from 19-96 with a mean of 56.92 years and females ranged from 29-97 with a mean of 59.48 years. The landmarks used in this study were chosen based on previous research (Asala 2001; Harmon 2007; Holliday *et al.* 2010; Steven and Vidarsdottir 2008), which helped guide the author to determine usable landmarks that encompass size variation existing on both proximal and distal epiphyses of the femur and tibia. The landmarks for the distal femur and proximal tibia epiphyses were adapted from Stevens and Vidarsdottir (2008), which assessed morphological changes in the shape of the knee in relation to age. Since the aim of this research was to grasp the overall size variation that exists between males and females rather than the changes in shape that occur with age, not all the landmarks presented in their paper were utilized in this research.

Landmark data were collected using a Microscribe G2X. The Microscribe connects directly to a personal computer, and has an associated hand switch. The Microscribe has a 360 degree movable digitizing arm with a stylus on the end that allows the researcher to precisely collect landmark data on a variety of surfaces (Immersion 2002). Microscribe Utility Software (MUS) is free and can be downloaded onto any computer, and allows for the transfer of data from the Microscribe into Excel, where the X, Y, and Z coordinates are directly inputted. When the hand switch is clicked, the software logs the X, Y, and Z coordinate in the designated column for that landmark into Excel, and then automatically moves to the next row to prepare for the collection of the next landmark coordinate.

It is imperative that the landmarks collected for each epiphysis occur in a specific order. This is important for later statistical analyses, and when landmarks are removed to test the method for the usability on fragmented remains, following the pre-specified order is critical. The author selected the order of landmark collection before arriving at the Bass Collection to optimize time and efficiency. After each day, data were copied from Excel and formatted into a Text (.txt) document, one for each epiphysis, as specified for the statistical analysis programs used in this research study. At the end of the allotted time in Tennessee, data from two hundred fifty individuals had been collected, formatted into the appropriate .txt file, and ready for statistical analysis.

A subsample of fifteen individuals were selected at random to calculate the error of the collection of landmarks selected for this project. Landmarks for each epiphysis of the individuals selected were collected three times in a row before moving to the next individual. The calculation of error is discussed in detail below and analyzed in the Discussion.

After all landmark data were collected and preliminary statistical analyses conducted, several landmarks were selected for removal to determine the usability of the method on fragmented remains. Landmark removal was conducted to best reflect the taphonomic process of coffin wear and postmortem breakage, common phenomenon which affect lower limb elements (Pokines and Baker 2014).

Decomposition is a well-understood process that follows a predictable trajectory culminating in skeletonization (Clark *et al.* 1997; Damann and Carter 2014; Marks *et al.* 2009; Reed 1958; Wilson-Taylor 2013). The phenomenon of coffin wear occurs as decomposition progresses through the established stages, where bony projections of the skeleton are the first to be exposed. This causes them to come into contact with the surrounding environment before other surfaces of the skeleton (Pokines and Baker 2014). As this contact occurs, damage to the cortical layer of bone exposing the underlying trabecular bone can be observed as a pattern of postmortem breakage. As decompositional processes continue, bones settle at different rates. According to Pokines and Baker (2014), coffin wear affects posterior aspects of certain elements such as the femur and tibia that remain fixed in place. Specifically, the os-coxae and femora are unlikely to move because of their overall structure that, in anatomical position, remain fixed (Pokines and Baker 2014). This is of particular interest in relation to femora, because if they are less likely to move from their original position, then it is likely the femoral head will be protected from the process of coffin wear because it is encased in the acetabulum of the os-coxae, which is also unlikely to move. Though the femoral head and neck may remain intact, the greater and lesser trochanters are likely to suffer damage due to their anatomical position in relation to the coffin floor (Pokines and Baker 2014). Because of this, these associated landmarks were removed for the first run. On the distal femur, damage is typically seen on the most lateral aspects, depending on the position of the body when placed in the coffin, and on the posterior aspects as well. Pokines and Baker (2014) provide imaging of coffin wear and indicate areas of interest when considering observed damage.

The author of this study participated in the Unidentified Persons Project in San Bernardino, California, sponsored by the Institute for Field Research. This project is a forensic anthropology and forensic archaeology field school, in which excavations of unknown individuals from the County cemetery Potter's field are conducted in order to complete updated biological profiles and submit samples for DNA analysis in attempts to identify those individuals. Most of those excavated during the field school season passed away in the 1930's and 40's and were buried in standard coffins. Because of this experience, the author has firsthand knowledge of coffin wear taphonomy, which confirmed the reasoning for the removal of landmarks. Given the amount of time since deposition, many of the elements had settled and resettled, specifically femora, where the proximal ends were exposed and suffered postmortem damage. In some instances the articular surface of the proximal tibia was protected due to its articulation with the distal femur, but often the posterior region of the proximal tibia had suffered damage.

Several runs were completed removing different combinations of landmarks on each epiphysis, which will be discussed further. On the proximal femur, the first run consisted of removing the landmarks located on the trochanters, specifically the most superior point of the greater trochanter (landmark 12), the most anterior point of the greater trochanter (landmark 13), the most lateral point of the greater trochanter (landmark 17), and the most posterior point of the lesser trochanter (landmark 14). These landmarks were selected for exclusion due to their likelihood of exposure during decomposition. These areas are most likely to come into contact with external surfaces, causing damage to the bony projections and therefore making these landmarks unusable in this type of analysis (Pokines and Baker 2014). With these landmarks removed, all that remains are those on the femoral head and neck. If an individual is left to decompose without disturbance, the femoral head is left in situ within the acetabulum of the os-coxae, therefore protecting it from taphonomic processes. The second run of removing landmarks on the proximal femur excluded landmarks on the head and trochanters, incorporating the previously mentioned taphonomic damage with that which might be seen on the thin regions of the cortical bone of the femoral head. Landmarks 1-7, 12-14, and 17 were excluded from the second run. Theoretically, if this sort of specimen was present in an archaeological or forensic context, both the trochanters and the femoral head would be damaged, leaving only the neck and head/neck intersection present for analysis.

On the distal femur, the first run consisted of removing landmarks associated with the most medial and lateral points (landmarks 7 and 8) as well as the most posterior points of each condyle (landmarks 14 and 16). As a body decomposes, these medial, lateral, and posterior projections of the knee are most likely to come into contact with external surfaces and become damaged (Pokines and Baker 2014). If a body is left undisturbed while it decomposes, the articulation with the tibia and patella can protect the other landmarks, for a time, from becoming damaged. Once damaged, however, landmarks are not usable with this method of assessing sexual dimorphism. The second run of removing landmarks on the distal femur excluded the same four landmarks above, as well as the most anterior points of both condyles (landmarks 11 and 12), and the most inferior points of both condyles (landmarks 13 and 15). Since epiphyseal cortical bone is thinner in anatomically modern humans, these regions are more susceptible to processes that would cause damage, and therefore it is reasonable to assume that the four landmarks excluded from the first run for the distal femur would be damaged first during standard decompositional processes, while the rest of the landmarks would be protected by articulation with other skeletal elements. However, as more time passes without discovery, disarticulation and scattering bony elements can be observed, therefore exposing the other landmarks included in this second run to damaging processes, i.e. the most inferior points (normally protected by the tibia) and the most anterior points (normally protected by the patella) of both condyles.

The first run on the proximal tibia consisted of excluding landmark 1 (most anterior point of tibial tuberosity), and landmarks 9, 10, 11, and 12 (all landmarks associated with the intercondylar eminence). Since the intercondylar eminence can easily get damaged when in contact with the distal femur, and since the anterior aspect of the tibial tuberosity is so close to the external surface of the body, these points can arguably be the first to obtain damage due to the taphonomic process of coffin wear. Therefore, the landmarks included for analysis were those of the epiphysis/neck intersections, and the most anterior, medial, posterior, and lateral points of the tibial plateau. The second run consisted of removing landmark 1 (tibial tuberosity), and landmarks 5-8 (most anterior, medial, posterior, and lateral points of the tibial plateau). The delicate nature of the proximal tibia often leaves exfoliation of the cortical bone around the tibial plateau, especially in areas that come into contact with other skeletal elements or surfaces as decomposition progresses (Pokines and Baker 2014). Therefore, the landmarks included were those of the epiphysis/neck intersections and the intercondylar eminence, on the chance it does not get damaged during standard taphonomic processes. A third run was conducted by the author on the proximal tibia due to visible dimorphism present within the sample during data collection. The intercondylar eminence alone seemed to be variable in size between males and females, so the third run was executed to determine if there was any statistical significance behind the variation present. Therefore, all landmarks were excluded except those of the intercondylar eminence (landmarks 9-12).

On the distal tibia, two runs were conducted. The first run consisted of excluding landmarks 5, 7 (the most laterally projecting points of the fibular notch), and 12 (the most inferior point on the medial malleolus). Because the medial malleolus is very near the exterior surface of the body, and the projections of the fibular notch regularly come into contact with the fibula, it can be assumed that these landmarks will obtain damage as decomposition of the body progresses. The second run on the distal tibia excluded landmarks 8, 10, 11, and 14 (most posterior point of epiphysis, most posterior and inferior point of medial malleolus, most medial point of epiphysis, and most anterior point of epiphysis). As has been previously mentioned, the thinning of cortical bone along epiphyses can acquire damage somewhat easily, and due to this landmarks along these areas may become damaged and therefore not usable in this method of analysis. As decomposition progresses and skeletal elements are disarticulated, the areas most likely to come into contact with external surfaces will become damaged first, such as those associated with landmarks excluded in the second run for the distal tibia.

Subsection One: Statistical Analyses

Two statistical analysis software programs were used for the purposes of this research project. Morphologika v2.5 was utilized to capture several of the figures (Figures 2-7) of the graphical results of Generalized Procrustes Analysis and Principal Components Analysis, because it accurately depicts the 3-dimensional nature of the data collected as well as the variation that exists within the sample. The other statistical analyses were completed in MorphoJ 1.06a. MorphoJ is programmed to accept .txt files formatted for Morphologika, so the same files were used for each program.

Two statistical processes are critical to understanding what is occurring during the geometric morphometric analysis, which will now be discussed in detail. Once all landmark data is collected, there needs to be a method for grouping the landmarks around a theoretical central point in order to best assess the variation that exists within the sample. Generalized Procrustes Analysis (GPA), or procrustes fit analysis, is used for several reasons. It is used to group all landmarks in a sample around an arbitrary designated central point, or centroid. (Rohlf and Slice 1990; Slice 2005, 2007; Zelditch *et al.* 2012). GPA is also used as a means of scaling landmarks without changing the position of the landmarks in relation to each other (Slice 2005, 2007; Zelditch *et al.* 2012). Lastly, GPA is used to rotate, if necessary, each specimen in relation to the centroid so the sample can best be analyzed comparatively (Klingenberg 2010; Klingenberg and Monteiro 2005; Lewton 2012; McKeown and Schmidt 2012;

Slice 2005, 2007; Zelditch *et al.* 2012). In other words, GPA is used to orient the landmarks of each specimen in a standard position, in order to better determine the variation within the sample. GPA is used by both Morphologika and MorphoJ to visually identify variation, which can be seen in Figure 2 and Figure 3. Figure 2 is what the original data of the proximal femur looks like when first inputted into Morphologika, and Image 2 is after GPA has been conducted.

Once GPA has been performed, the second analysis, called Principal Components Analysis (PCA), can be conducted (Adams *et al.* 2004; McKeown and Schmidt 2012; Slice 2005, 2007; Zelditch *et al.* 2012). This type of analysis is used to rotate data and plots new axes through it based on the variation present in the sample, which more accurately describes the variation compared to the original axes. PCA is used to simplify the variation present by replacing the original variables with new ones, called principal components (PCs). These new variables are linear combinations of the original variation and are independent, and when compared each PC represents a percentage of the overall variation present in the sample (Klingenberg 2010, 2011; McKeown and Schmidt 2012; Slice 2005, 2007; Zelditch *et al.* 2012). In other words, each variable, be it a measurement or a coordinate, within a sample can be plotted on an axis or set of axes. PCA simplifies the data within a sample by regrouping the data that is closely correlated into smaller groups of variables. The first few PCs will statistically represent the most variability that exists in the sample and the higher the PC the more representative of the variation between groups (Klingenberg

2010, 2011; Slice 2005, 2007; Zelditch *et al.* 2012). The overall goal is to represent the data's variability in the first few PCs, which would signify a high correlation within the sample between groups, and in the case of this study variation between males and females. The PCA outputs for each epiphysis can be seen in Figures 4-7.

Morphologika allows for the analysis of both PCA and GPA. Because this project analyzes size variation between males and females, it is important to use full tangent space projection, which is standard in PCA, as well as Procrustes from space (Morphologika2, 2010). Using Procrustes from space allows shape as well as size variations to be analyzed, which the latter is significantly important when assessing sexual dimorphism (McKeown and Schmidt 2012; Slice 2005; Zelditch *et al.* 2012). Full tangent space projection is specifically in reference to shape space, because geometric morphometric analysis is primarily between shape variations through the scaling of specimens around a single origin. Therefore the use of Procrustes from space is for the analysis of shape as well as size (Morphologika2, 2010). In other words, since GPA analysis involves scaling, the variations examined through PCA are shape rather than form, which includes size, variations. If size is also to be examined, as is the desired effect of analyzing sexual dimorphism, this can be achieved by carrying out an analysis of form by selecting the appropriate option for PCA, which adds in centroid size as a variable (Morphologika2, 2010). PCA in MorphoJ does not allow for a form analysis, which is why Canonical Variates Analysis (CVA) is used when dealing with this program, as discussed below.

In MorphoJ, after a Procrustes fit has been performed, a Covariance Matrix must be generated. Simply put, covariance is an association between variables (Zelditch *et al.* 2012). The covariance matrix allows for a general understanding of the variation present within multiple dimensions. No other analyses in MorphoJ can be performed until a covariance matrix is conducted.

MorphoJ allows for further analysis, which not only separates males and females based on variation present, but can also determine the successful rate of classification into a group based on this variation within the sample using both Canonical Variates Analysis (CVA) and Discriminant Function Analysis (DFA), respectively. CVA simplifies and describes the differences of the groups present and creates mathematical functions that are used for assigning individuals in the sample to different groups (McKeown and Schmidt 2012; Slice 2005; Zelditch *et al.* 2012). There are many similarities between CVA and PCA. Like PCA, CVA is used to create a new coordinate system (canonical variates, CVs). The various CVs are “new shape variables that maximize the separation between groups relative to the variation within groups,” (Klingenberg 2010, p. 625). This means that CVA reorganizes the data based on the variation present (Adams *et al.* 2013; Klingenberg 2010, 2011; Klingenberg and Monteiro 2005; McKeown and Schmidt 2012; Zelditch *et al.* 2012). CVs, however, are the direction in which groups are best separated based on the variation present, whereas PCs just account for the variation present within the sample (Adams *et al.* 2013; Klingenberg 2010, 2011; Klingenberg and Monteiro 2005; McKeown and Schmidt 2012; Zelditch *et al.* 2012).

For the purposes of this research, when using MorphoJ, instead of conducting PCA, which lacks the ability to select for form (size and shape analysis), CVA is a more beneficial because it separates groups best, and can be used with DFA to calculate classification rates, which is the ultimate goal when testing the usability of the method for sex estimation. Since there are only two groups, males and females, that need to be separated using CVA, only one CV is necessary and ever given from MorphoJ.

After the Covariance Matrix is generated, although PCA is an option in MorphoJ, it does not account for accurate variation because it is only analyzing shape and not form, which is excluding half the definition of sexual dimorphism. PCA is therefore not executed in MorphoJ but rather it is in Morphologika, and CVA is substituted for PCA in MorphoJ. Figures 4-7 are the graphical representation of the Morphologika PCA outputs using PCs 1 and 2, which account for the majority of the variation present in the sample.

The last aspect is the discriminant function analysis (DFA or DF), which can only be conducted in MorphoJ once CVA has been performed. By definition, DFA is the combination of variables that separates two groups (Jantz and Ousley 2005; McKeown and Schmidt 2012; Zelditch *et al.* 2012). This last analysis is required to assess the number of correctly classified individuals into any specified group within a sample given the variation present. This step therefore gives the percentage of correctly classified males and females within the sample, which will be presented and discussed in the following chapters. The correct number classified divided by the total for each group gives the correct classification rate.

There are four significant outputs of CVA and DFA performed in MorphoJ: eigenvalue, Mahalanobis distances among groups, Procrustes distances among groups, and p-values. The eigenvalue provides the smallest set of axes necessary for a matrix defined by the CVs (Pavlicev *et al.* 2009; Zelditch *et al.* 2012). The eigenvectors, or PCs/CVs of a covariance matrix, and the eigenvalue corresponding to these eigenvectors, gives the variance for that axis (Pavlicev *et al.* 2009; Zelditch *et al.* 2012). A relatively large eigenvalue, therefore, will represent significant variation present, and the closer to zero the less variation is present (Klingenberg and Monteiro 2005). The Mahalanobis distance among groups is the “squared distance between two means divided by the pooled sample variance-covariance matrices” (Zelditch *et al.* 2012, p. 461). This distance can be used to separate groups within a single population (McKeown and Schmidt 2012; Zelditch *et al.* 2012). The larger the distance, the better the separation. The Procrustes distances among groups refers to the “sum of squared distances between corresponding points of two superimposed shapes after one shape has been centered on the other and rotated to minimize that sum of squares,” (Zelditch *et al.* 2012, p. 464). Because it is proportionate to the fixed centroid, which is 1, this value will not exceed 1 (Zelditch et al 2012). In other words, the Procrustes distance among groups refers to the location of each point in relation to the centroid and separates groups based on the distances of corresponding points in the different groups analyzed. Lastly, p-values represent a statistically significant probability, where a lower p-value indicates a higher likelihood that the observed data is true and less likely due to random chance (Sullivan 2012). If a p-value is low, then the variation present is statistically significant, and typically statistics are run at a 95% confidence interval, which would be a p-value< 0.05. Anything below this indicates stronger than 95% confidence (Sullivan 2012).

It should be briefly noted that in MorphoJ, once a Procrustes Fit has been conducted, landmarks can be selected for exclusion from the analysis, which is necessary for the second aspect of this research project. Once all the normal analysis had been conducted, specific landmarks were removed based on standard taphonomic processes, as discussed in detail above.

Subsection Two: Calculation of Error

Ideally, landmarks are selected for three criteria: having the same location, provide adequate coverage of the morphology, and can be found repeatedly and reliably (Bookstein 1991; Zelditch *et al.* 2012, p. 25-29). To have the same location, or to be homologous, indicates that the points on the specimen correspond to that point on all individuals. The second criterion is selfexplanatory as the use of certain landmarks are seeking to determine a comprehensive shape and size of the desired item. The third criterion is the basis for this section and is arguably the most important because it is directly correlated with calculating error in any study of morphometrics.

There are several limitations of landmark-based analysis, but primarily is the concern of identifying specific landmark location across all individuals (Chollet *et al.* 2013). If landmarks are difficult to locate they contribute to measurement error (Bookstein 1991; Slice 2005; Zelditch *et al.* 2012). These landmarks can be difficult to locate for two reasons. Landmarks, specifically Types II and III, are defined in terms of a change in curvature, so locating these landmarks must be determined visually. This curvature, however, may vary among individuals of the same species or even between sides on the same individual (Zelditch *et al.* 2012). This is partially addressed in the current research by only using lefts among the entire sample. The rest, however, becomes an issue because it will produce some subjectivity into the sample, but the best way to analyze just how much subjectivity is by the assessment of intra-observer error, or the error rates that are produced by the re-collection of landmarks across a subsample of specimens from the original sample by the primary observer.

Unlike the other analyses conducted in this study, the calculation of error is done directly with the original data in Excel. A subsample of fifteen individuals were selected at random to recollect the landmarks a total of three times, henceforth referred to as trials 1, 2, and 3, once all data from the original sample had been collected. Since each landmark has an X, Y, and Z coordinate in three-dimensional space, the first step for calculating error was to take the average of each X, Y, and Z coordinate from trials 1, 2 and 3 for each landmark, giving a mean X, Y, and Z value for each landmark (Xm, Ym, and Zm). The next step was calculated the mean shape centroid X, Y, and Z value for each individual in the subsample. The shape centroid is the center point of the collection of landmarks, and the mean shape centroid is calculated by taking the average of all the mean X values for each individual, which is then repeated for the Y and Z values for each individual. Then the distance of each trial from the mean for each original landmark value, also known as the trial deviation or Euclidean distance, was determined (Lewton, 2012; McNulty 2005; von Cramon-Taubadel *et al.*2007). This was done by taking the square root of the sum of the difference between the original X, Y, and Z value and the mean X, Y, and Z value. The equation for this is $\sqrt{((x1 - xm)^2 + (y1 - ym)^2 + (z1 - zm)^2\ )}$ where x1, y1, and z1= the original X, Y, or Z coordinate, and xm, ym, and zm= the mean X, Y, or Z value calculated from all three trials (von Cramon-Taubadel *et al.* 2007). Once the Euclidean distance, or trial deviation, was calculated for each trial, the mean landmark deviation was calculated by taking the average of each trial deviation for each landmark. The next step was calculating the distance from the landmark consensus to the centroid, which is the same equation listed above, but instead of using the original X, Y, and Z values, the difference between the mean X, Y, and Z values and mean shape centroid X, Y, and Z values were used, making the equation $\sqrt{((xm - MSCx)^2 + (ym - MSCy)^2 + (zm - MSCz)^2)}$ for each landmark of each individual. The landmark percentage error was then calculated by dividing the mean landmark deviation by the distance from the landmark consensus to centroid and multiplying it by 100. Lastly, to determine the overall error rate for each landmark, the average of landmark percentage error for each landmark was determined, giving the average landmark error across the subsample.

Tables

Table 1: Proximal Femur Landmarks

1. Most superior point of femoral head	Type II
2. Most anterior point of femoral head	Type II
3. Most inferior point of femoral head	Type II
4. Most posterior point of femoral head	Type II
5. Most superior point of fovea capitis	Type II
6. Most interior point of fovea capitis	Type II
7. Most inferior point of fovea capitis	Type II
8. Most superior point of femoral neck	Type II
9. Most anterior point of femoral neck	Type II
10. Most inferior point of femoral neck	Type II
11. Most posterior point of femoral neck	Type II
12. Most superior point of greater trochanter	Type II
13. Most anterior point of greater trochanter	Type II
14. Most posterior point of lesser trochanter	Type II
15. Anterior point of head-neck intersection	Type II
16. Posterior point of head-neck intersection	Type II
17. Most lateral point of greater trochanter	Type II

Table 2: Distal Femur Landmarks

1. Central point of intercondylar fossa on ridge	Type II
2. Most lateral point of intercondylar fossa of medial condyle	Type II
3. Center point of intercondylar fossa	Type II
4. Most medial point of intercondylar fossa of lateral condyle	Type II
5. Medial condyle, most superior and posterior point of tibial facet on ridge	Type II
6. Lateral condyle, most superior and posterior point of tibial facet on ridge	Type II
7. Most medial point of medial condyle (from posterior)	Type II
8. Most lateral point of lateral condyle (from posterior)	Type II
9. Medial epiphysis/neck intersection	Type II
10. Lateral epiphysis/neck intersection	Type II
11. Patellar surface- most anterior point on lateral condyle	Type II
12. Patellar surface- most anterior point on medial condyle	Type II
13. Most inferior point on medial condyle	Type II
14. Most posterior point on medial condyle	Type II
15. Most inferior point on lateral condyle	Type II
16. Most posterior point on lateral condyle	Type II
17. Anterior epiphysis/neck intersection	Type II
18. Posterior epiphysis/neck intersection	Type II
19. Most interior point of divot between condyles on articular surface	Type II
20. Most anterior and lateral point on lateral condyle	Type II
21. Most anterior and lateral point on medial condyle	Type II

Table 3: Proximal Tibia Landmarks

1. Most anterior point of tibial tuberosity	Type II
2. Lateral epiphysis/neck intersection	Type II
3. Posterior epiphysis/neck intersection	Type II
4. Medial epiphysis/neck intersection	Type II
5. Most anterior point of tibial plateau	Type II
6. Most medial point of tibial plateau	Type II
7. Most posterior point of tibial plateau	Type II
8. Most lateral point of tibial plateau	Type II
9. Most superior point of medial intercondylar eminence	Type II
10. Most superior point of lateral intercondylar eminence	Type II
11. Most inferior point of medial intercondylar eminence on articular surface	Type II

12. Most inferior point of lateral intercondylar eminence on articular surface	Type II

Table 4: Distal Tibia Landmarks

1. Lateral epiphysis/neck intersection	Type II
2. Anterior epiphysis/neck intersection	Type II
3. Medial epiphysis/neck intersection	Type II
4. Posterior epiphysis/neck intersection	Type II
5. Most lateral point of anterior fibular notch projection	Type II
6. Most medial point of fibular notch	Type II
7. Most lateral point of posterior fibular notch projection	Type II
8. Most posterior point of epiphysis	Type II
9. Most posterior and medial point of medial malleolus	Type II
10. Most posterior and inferior point of medial malleolus	Type II
11. Most medial point of medial malleolus (most medial point of epiphysis)	Type II
12. Most inferior point of medial malleolus	Type II
13. On talar articular surface, most superior point (next to malleolus)	Type II
14. Most anterior point of epiphysis	Type II
15. Most medial point of fibular notch of talar surface (on ridge)	Type II

Figures

Figure 2: Proximal Femur Landmarks Before GPA

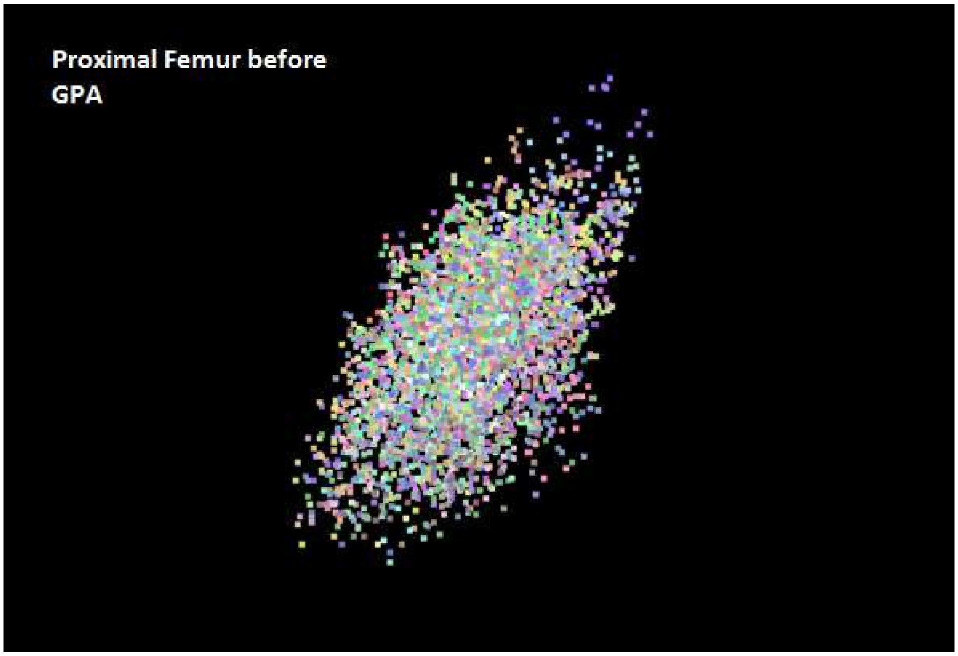


Figure 3: Proximal Femur Landmarks after GPA

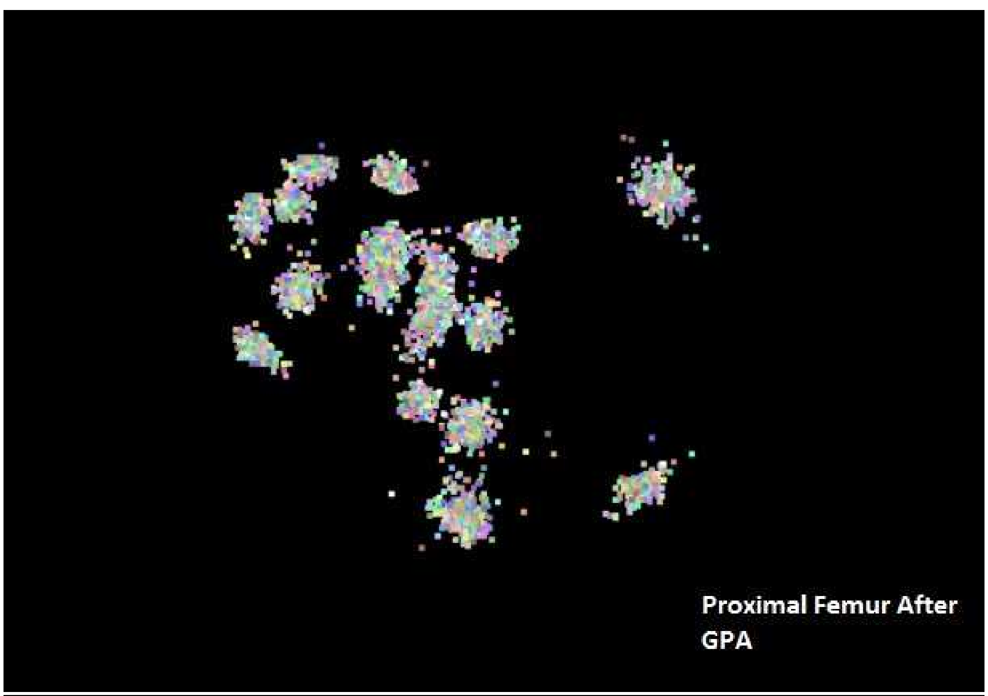


Figure 4: PCA of Proximal Femur

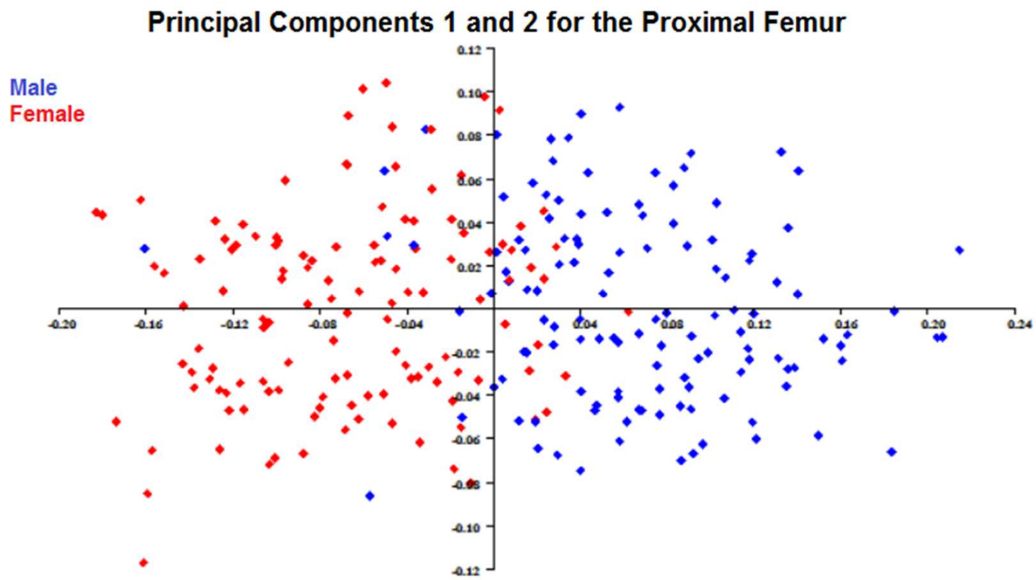


Figure 5: PCA of Distal Femur

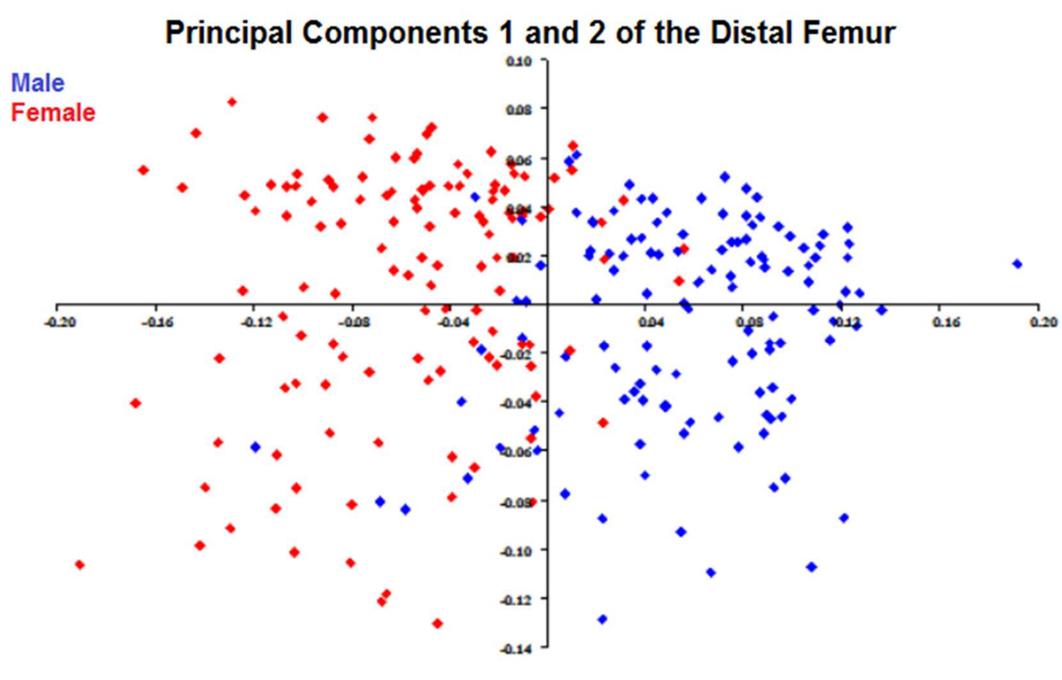


Figure 6: PCA of Proximal Tibia

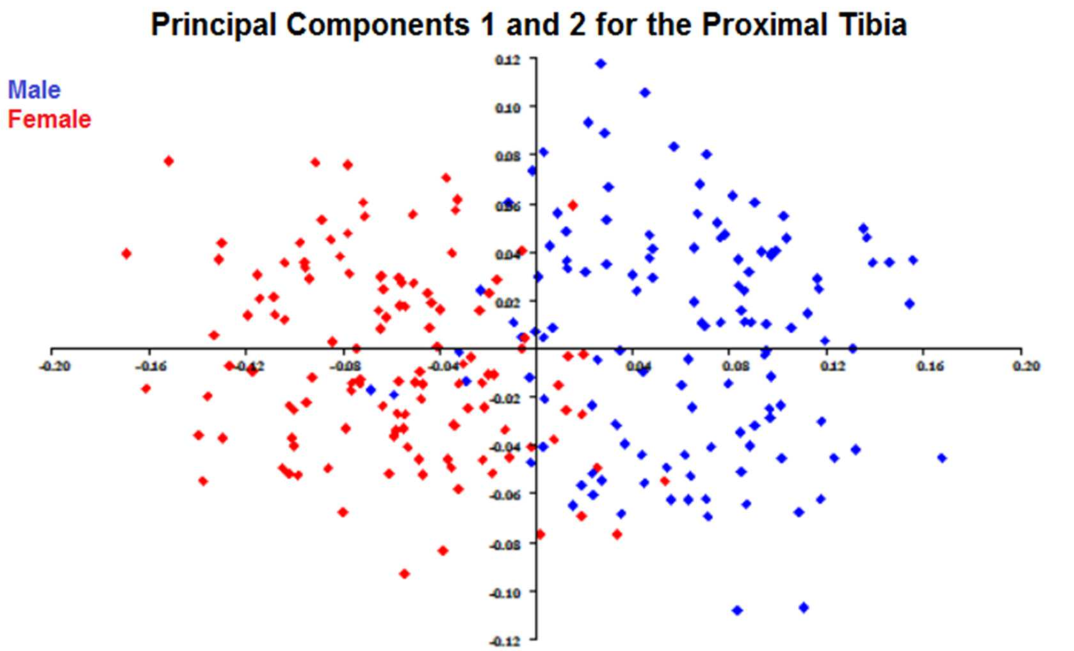
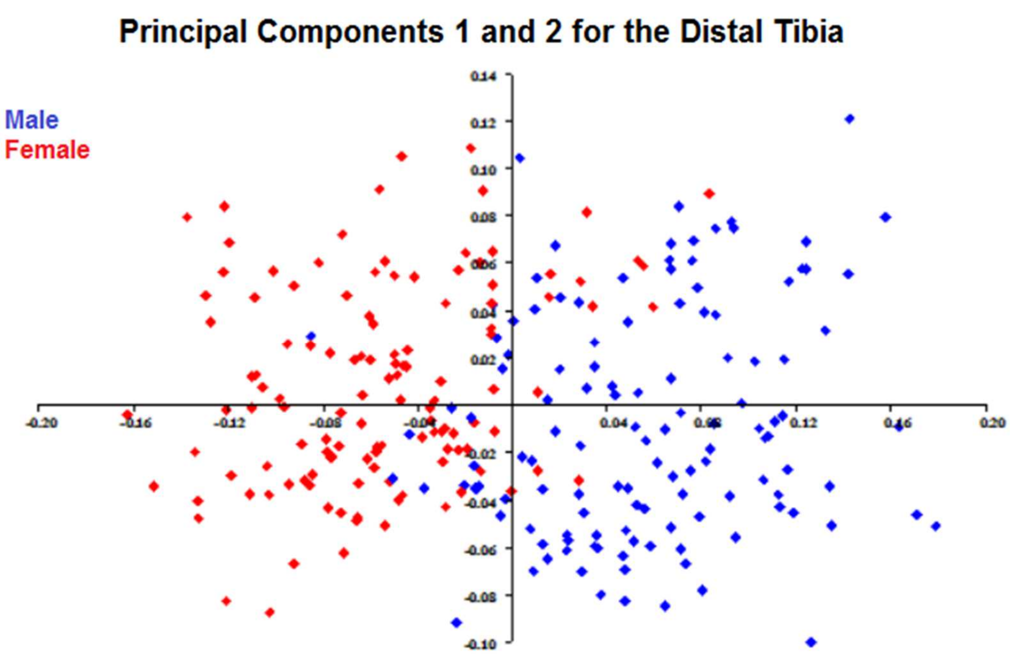


Figure 7: PCA of Distal Tibia



RESULTS

Proximal Femur

A discriminant function analysis (DFA) was conducted on the data for the proximal femur (for a detailed explanation, see previous chapter). There were 98 females that were correctly classified based on the DFA out of 125, indicating 27 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 78.4% for females on the proximal femur using the combination of landmarks listed in the previous chapter. There were 101 males correctly classified based on DFA out of 125, indicating 24 were incorrectly classified as male given the variation present in the sample. This gives a correct classification rate of 80.8% for males on the proximal femur using the combination of landmarks listed in the previous chapter. Figure 8 refers to the discriminant function separation of males and females on this element.

The CVA examined the variation between the two groups, which is scaled by the inverse of the within-group variation. CVA eigenvalue for the proximal femur was 0.59553408, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.5372, the Procrustes distances among groups is 0.0214, and the p-value <0.0001 (Table 9). Figure 8 is a graphical representation of the variation between males and females based on the landmarks for the proximal femur using DFA, and is a bimodal distribution of the slightly higher classification rate of males over females given the slightly higher distribution of males along the X axis.

Distal Femur

Results are next presented for the distal femur. Using the same method outlined above, there were 112 females correctly classified out of 125 based on the DFA, which indicates 13 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 89.6% for females on the distal femur using the landmarks outlined in the previous chapter. 115 males were correctly classified out of 125 based on the DFA, which indicates 10 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 92.0%, which is the highest classification achieved in this study. Figure 9 refers to the discriminant function separation of males and females on this element.

CVA Eigenvalues for the distal femur was 1.73890364, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 2.6268, the Procrustes distances among groups is 0.0289, and the p-value<0.0001 (Table 9). Figure 9 is a graphical representation of the bimodal distribution between males and females based on the landmarks for the distal femur using DFA.

Proximal Tibia

Using the same method outlined above, there were 104 females correctly classified out of 125 based on the DFA, which indicates 21 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 83.2% for females on the proximal tibia using the landmarks outlined in the previous chapter. 101 males were correctly classified out of 125 based on the DFA, which indicates 24 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 80.8% for males using the specified landmarks on the proximal tibia. Figure 10 refers to the discriminant function separation of males and females on this element. The proximal tibia was the second highest classification epiphysis in this sample, and leads to the conclusion that the knee joint shows the most sexual dimorphism out of the epiphyses used in this study.

CVA Eigenvalues for the proximal tibia was 0.71781046, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.6877, the Procrustes distances among groups is 0.0314, and the p-value<0.0001 (Table 9). Figure 10 is a graphical representation of the variation between males and females based on the landmarks used on the proximal tibia using DFA.

<u>Distal Tibia</u>

Using the same method outlined above, there were 101 females correctly classified out of 125 based on the DFA, which indicates 24 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 80.8% for females on the distal tibia using the landmarks outlined in the previous chapter. 102 males were correctly classified out of 125 based on the DFA, which indicates 23 were incorrectly classified based on the size and shape variation present in the sample. This gives a correct classification rate of 81.6% for males using the specified landmarks on the distal tibia. Figure 11 refers to the discriminant function separation of males and females on this element.

CVA Eigenvalues for the distal tibia was 0.79322095, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.7741, the Procrustes distances among groups is 0.0264, and the p-value<0.0001 (Table 9). Figure 11 is a graphical representation of the variation between males and females based on the landmarks used on the distal tibia using DFA.

Subsection One: Removal of Landmarks

Two statistical runs were conducted per epiphysis, mimicking different taphonomic processes outlined in the previous chapter.

<u>Proximal Femur</u>

On the proximal femur, the first run consisted of removing the landmarks located on the trochanters, specifically the most superior point of the greater trochanter (landmark 12), the most anterior point of the greater trochanter (landmark 13), the most lateral point of the greater trochanter (landmark 17), and the most posterior point of the lesser trochanter (landmark 14). With these landmarks removed, all that remains are those consisting of the proximal femoral head and neck.

When the aforementioned landmarks were removed on the proximal femur, there were 98 females correctly classified out of 125 based on the DFA, which indicates 27 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 78.4% for females on the proximal femur using the landmarks selected based on standard taphonomic processes. 97 males were correctly classified out of 125 based on the DFA, which indicates 28 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 77.6% for males using the specified landmarks on the proximal femur. CVA Eigenvalues for proximal femur was 0.42267853, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.2951, the Procrustes distances among groups is 0.0263, and the p-value<0.0001 (Table 10).

The second run of removing landmarks on the proximal femur included the exclusion of landmarks on the head and trochanters, incorporating the previously mentioned taphonomic damage with that which might be seen on the thin regions of the cortical bone of the femoral head. Landmarks 1-7, (most superior, anterior, inferior, posterior landmarks and fovea capitis landmarks) 12-14, and 17 were excluded from the second run. Theoretically, these landmarks would the only ones available on the proximal femur if both the trochanters and the femoral head were damaged, leaving only the neck and head/neck intersection present for analysis. When these landmarks were removed, there were 80 females correctly classified out of 125 based on the DFA, which indicates 45 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 64.0% for females on the proximal femur using the landmarks selected based on standard taphonomic processes. 79 males were correctly classified out of 125 based on the DFA, which indicates 46 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 63.2% for males using the specified landmarks on the proximal femur. CVA Eigenvalues for proximal femur was 0.09098901, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 0.6009, the Procrustes distances among groups is 0.0282, and the p-value of 0.0322 (Table 10).

Distal Femur

On the distal femur, the first run consisted of removing landmarks associated with the most medial and lateral points (landmarks 7 and 8) as well as the most posterior points of each condyle (landmarks 14 and 16). When these landmarks were removed from the analysis of the distal femur, 106 females were correctly classified out of 125 based on the DFA, which indicates 19 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 84.8% for females on the distal femur using the landmarks selected based on standard taphonomic processes. 104 males were correctly classified out of 125 based on the DFA, which indicates 21 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 83.2% for males using the specified landmarks on the distal femur. CVA Eigenvalues for proximal femur was 1.03205011, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 2.0237, the Procrustes distances among groups is 0.0256, and the p-value<0.0001 (Table 10).

The second run of removing landmarks on the distal femur excluded the same four landmarks above, as well as the most anterior points of both condyles (landmarks 11 and 12), and the most inferior points of both condyles (landmarks 13 and 15). When these landmarks were excluded from analysis, 101 females were correctly classified out of 125 based on the DFA, which indicates 24 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 80.8% for females on the distal femur excluding certain landmarks based on standard taphonomic processes. 102 males were correctly classified out of 125 based on the DFA, which indicates 23 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 81.6% for males using the specified landmarks on the distal femur. CVA Eigenvalues for proximal femur was 0.66939607, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.6298, the Procrustes distances among groups is 0.0237, and the p-value<0.0001 (Table 10).

Proximal Tibia

On the proximal tibia, three runs were performed. The first run consisted of excluding landmark 1 (most anterior point of tibial tuberosity), and landmarks 9, 10, 11, and 12 (all landmarks associated with the intercondylar eminence). When these landmarks were excluded from analysis, 93 females were correctly classified out of 125 based on the DFA, which indicates 32 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 74.4% for females on the proximal tibia excluding certain landmarks based on standard taphonomic processes. 93 males were also correctly classified out of 125 based on the DFA, which indicates 32 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This again gives a correct classification rate of 74.4% for males using the specified landmarks on the proximal tibia. CVA Eigenvalues for proximal tibia was 0.39007504, with a %Variance of 100.00 and a Cumulative%

of 100.00. The Mahalanobis distances among groups is 1.2441, the Procrustes distances among groups is 0.0336, and the p-value<0.0001 (Table 10).

The second run consisted of removing landmark 1 (tibial tuberosity), and landmarks 5-8 (most anterior, medial, posterior, and lateral points of the tibial plateau). When these landmarks were excluded from analysis, 92 females were correctly classified out of 125 based on the DFA, which indicates 33 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 73.6% for females on the proximal tibia excluding certain landmarks based on standard taphonomic processes. 86 males were correctly classified out of 125 based on the DFA, which indicates 39 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 68.8% for males using the specified landmarks on the proximal tibia. CVA Eigenvalues for proximal tibia was 0.26970920, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.0345, the Procrustes distances among groups is 0.0334, and the p-value<0.0001 (Table 10).

A third run was conducted by the author on the proximal tibia due to visible dimorphism present within the sample when data was being collected. Due to this, all landmarks were excluded except those of the intercondylar eminence (landmarks 9-12). 68 females were correctly classified out of 125 based on the DFA, which indicates 57 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 54.4% for females on the proximal tibia excluding certain landmarks based on standard taphonomic processes. 69 males were correctly classified out of 125 based on the DFA, which indicates 56 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 55.2% for males using the specified landmarks on the proximal tibia. CVA Eigenvalues for proximal tibia was 0.01008206, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 0.2000, the Procrustes distances among groups is 0.0080, and the p-value of 0.7821 (Table 10).

Distal Tibia

Lastly, two runs were conducted on the distal tibia. The first run consisted of excluding landmarks 5, 7 (the most laterally projecting points of the fibular notch), and 12 (the most inferior point on the medial malleolus). When these landmarks were removed, 102 females were correctly classified out of 125 based on the DFA, which indicates 23 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 81.6% for females on the distal tibia excluding certain landmarks based on standard taphonomic processes. 100 males were correctly classified out of 125 based on the DFA, which indicates 25 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 80.0% for males using the specified landmarks on the distal tibia. CVA Eigenvalues for the distal tibia was 0.67241550, with a %Variance of 100.00 and a Cumulative% of 100.00. The Mahalanobis distances among groups is 1.6334, the Procrustes distances among groups is 0.0268, and the p-value<0.0001 (Table 10).

The second run on the distal tibia excluded landmarks 8, 10, 11, and 14 (most posterior point of epiphysis, most posterior and inferior point of medial malleolus, most medial point of epiphysis, and most anterior point of epiphysis). When these landmarks were excluded from analysis, 91 females were correctly classified out of 125 based on the DFA, which indicates 34 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 72.8% for females on the distal tibia excluding certain landmarks based on standard taphonomic processes. 93 males were correctly classified out of 125 based on the DFA, which indicates 32 were incorrectly classified based on the size and shape variation present in the sample after the selected landmarks were removed. This gives a correct classification rate of 74.4% for males using the specified landmarks on the distal tibia. CVA Eigenvalues for distal tibia was 0.50404739, with a %Variance of 100.00 and a Cumulative% of 100.00. The

Mahalanobis distances among groups is 1.4142, the Procrustes distances among groups is 0.0279, and the p-value<0.0001 (Table 10).

Subsection Two: Calculation of Error

As discussed in the previous chapter, the average landmark error was calculated for each landmark across a subsample of fifteen individuals to determine the rate of intraobserver error in repeatedly locating landmarks. For the majority of landmarks used in this study, the average error rate was less than 5%, with only a few being slightly above 5%. The average landmark error rates are listed below in tables 5-8 and discussed in the next chapter.

Tables

Table 5: Average Landmark Error for the Proximal Femur

Landmark Number	Average Landmark Error
1	1.92865
2	4.193594
3	5.111236
4	3.279599
5	1.255459
6	1.588629
7	1.427218
8	2.181618
9	3.755189
10	5.180857
11	5.435263
12	1.296253
13	1.659791

14	1.319937
15	4.615005
16	7.323438
17	0.662869

Table 6: Average Landmark Error for the Distal Femur

Landmark Number	Average Landmark Error
1	1.931724
2	3.18749
3	5.315808
4	2.957744
5	2.841299
6	2.231203
7	1.627236
8	2.30673
9	3.127855
10	2.820477
11	1.497704
12	1.741046
13	1.792662
14	1.836396
15	1.770867
16	2.907447
17	1.235047
18	3.403396
19	2.389313
20	3.012948
21	2.658262

Table 7: Average Landmark Error for the Proximal Tibia

Landmark Number	Average Landmark Error
1	2.928192
2	4.552663
3	3.03573
4	3.132548
5	3.074643

6	1.864726
7	1.91056
8	2.824446
9	1.602418
10	1.533035
11	3.102261
12	2.471326

Table 8: Average Landmark Error for the Distal Tibia

Landmark Number	Average Landmark Error
1	3.808933
2	3.978985
3	5.624696
4	5.012185
5	1.421176
6	3.829352
7	2.517226
8	2.722306
9	2.379695
10	1.270518
11	1.970509
12	0.831342
13	3.595165
14	4.069173
15	1.576537

Figures

Figure 8: Discriminant Function Separation Proximal Femur

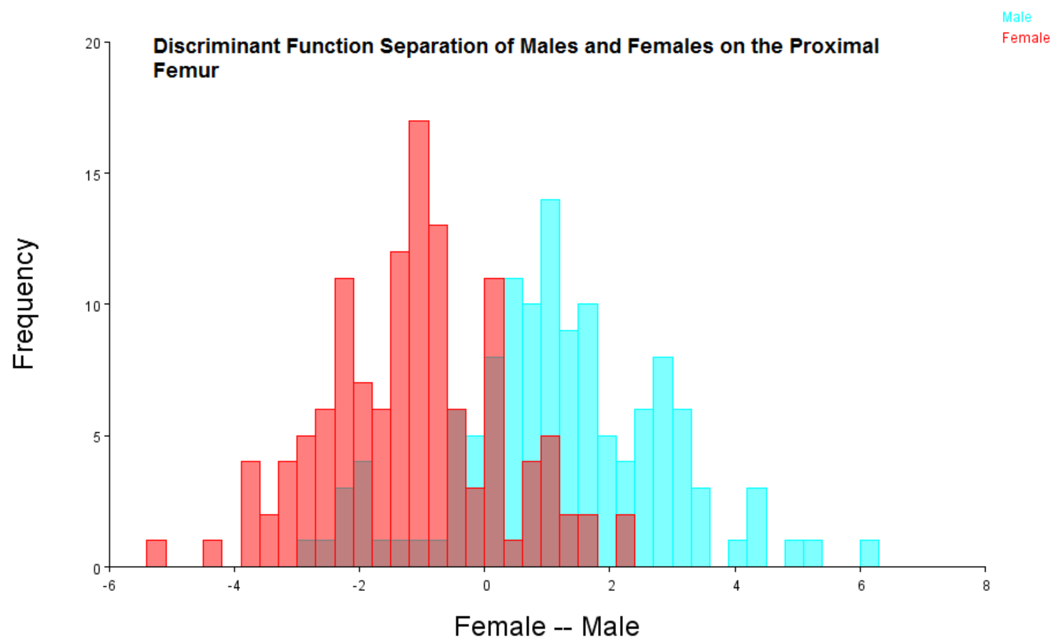


Figure 9: Discriminant Function Separation Distal Femur

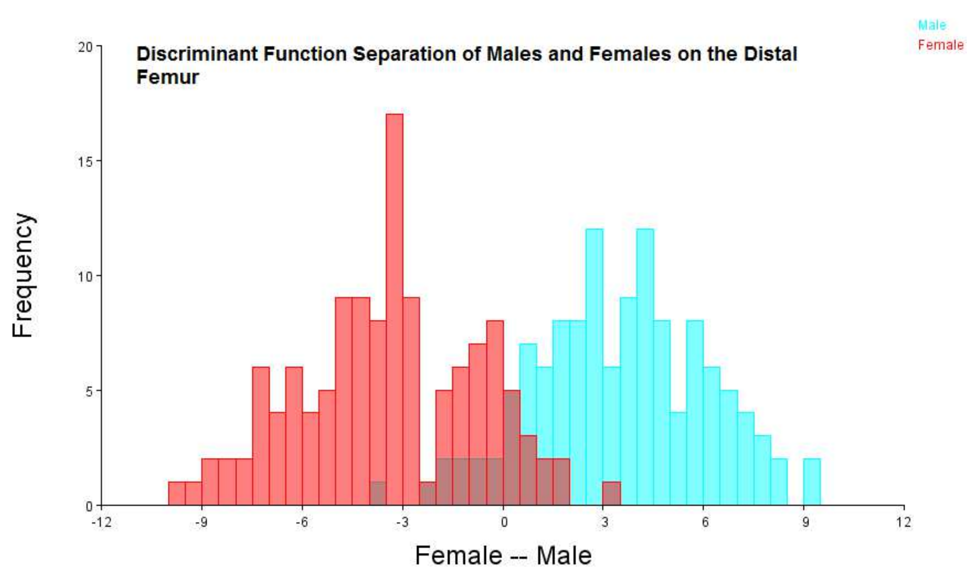


Figure 10: Discriminant Function Separation Proximal Tibia

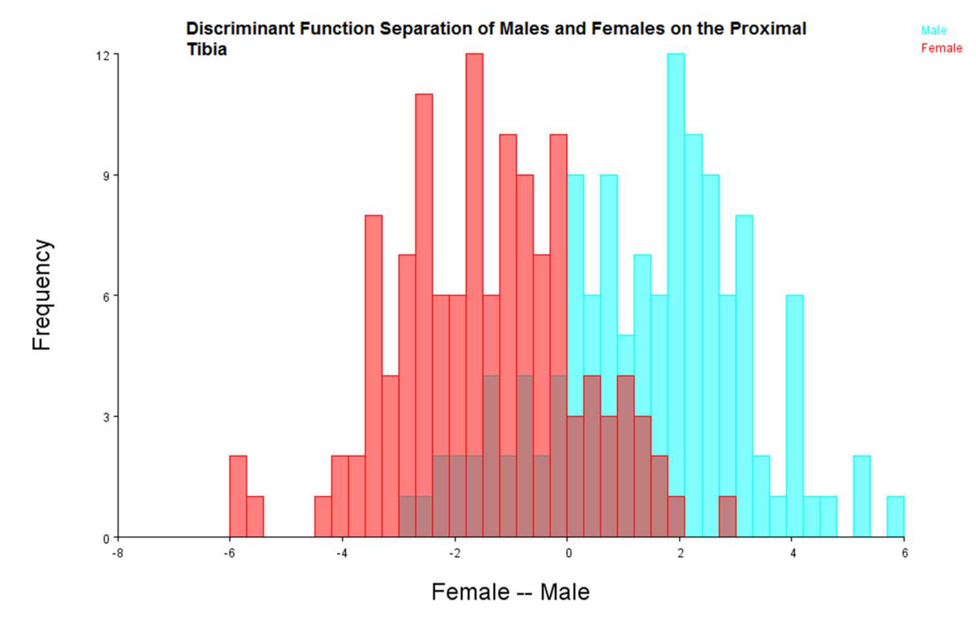
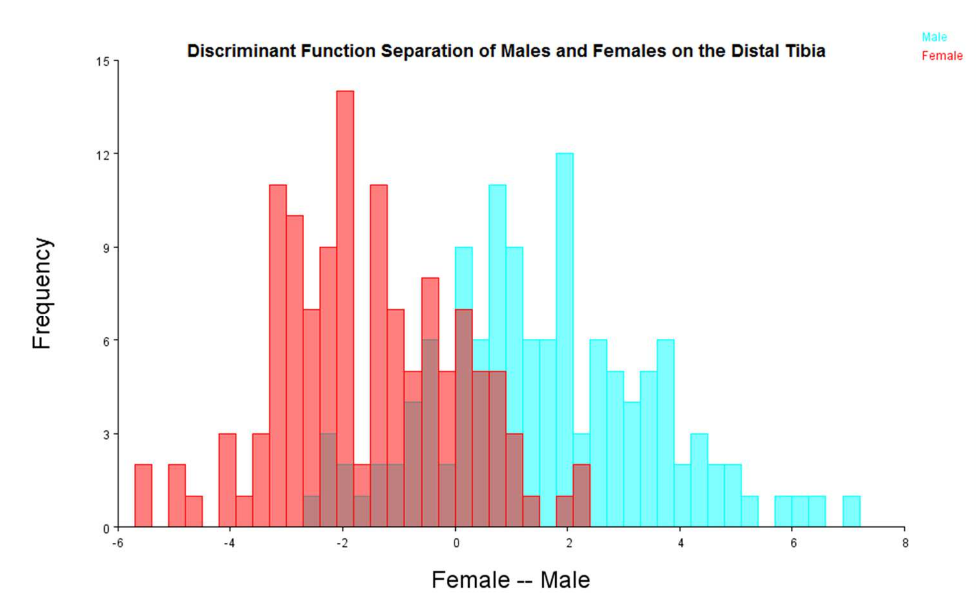


Figure 11: Discriminant Function Separation Distal Tibia



DISCUSSION

Given the results presented in the previous chapter, the most sexually dimorphic epiphysis, using the original group of landmarks established throughout this study, was the distal femur, with a correct classification rate of 89.6% for females and 92.0% for males. This was followed by the proximal tibia, with a correct classification rate of 83.2% and 80.8% for females and males, respectively. The third most sexually dimorphic epiphysis was the distal tibia, with a correct classification rate of 80.8% and 81.6% for females and males, respectively. This was closely followed by the proximal femur, with a correct classification rate of 78.4% and 80.8% for females and males, respectively.

When analyzing these classification rates, several trends immerge. For the proximal femur, these statistics indicate that, when all of the selected landmarks are used, the classification rates for males in slightly higher than females, but a correct classification rate of more than 75% is expected when tested on an unknown individual. For the distal femur, these statistics indicate that, when all of the selected landmarks are used, the classification rates for males is slightly higher than females, but a correct classification rate of 90% is expected when tested on an unknown individual given that classification rates for both males and females using the distal femur are at or greater than 90%. For the proximal tibia, these statistics indicate that, when all of the selected landmarks are used, the classification rates for males in slightly lower than females, but a correct classification rate of more than 80% is expected when tested on an unknown individual. Though classification rates are not as high as with the distal femur, this epiphysis in conjunction with the distal femur indicate that either epiphysis will give you a high classification of sex if only the knee was found in a forensic or archaeological circumstance. For the distal tibia, these statistics indicate that, when all of the selected landmarks are used, the classification rates for males in slightly higher than females, but a correct classification rate of more than 80% is expected when tested on an unknown individual.

When looking at the results, there were higher classification rates for males on all epiphyses except the proximal tibia. This could be due to several factors. There may be a larger size differentiation in these epiphyses across all populations or just in this sample that is more accurately described using geometric morphometrics. If ranking sexual dimorphism based on sex, for females it would be the order described above, but for males it would be the distal femur, followed by the distal tibia, and then the proximal femur and tibia are tied at 80.8% correct classification rates. For males, analyzing sex using any of the epiphyses gives a correct classification rate over 80%, while for females all but the proximal femur give correct classification rates over 80%.

These results indicate that there is a significant morphological size variation between males and females in the knee joint, and more research needs to be conducted to truly determine why this may be. It is the author's opinion that the morphology of the hip, which has previously been studied for its sexually dimorphic nature and discussed in the Literature Review chapter of this writing, is impacting the morphology of the knee. More specifically, the well-known Q-angle is having a significant impact on the size of the knee joint, which has not previously been analyzed. In relation to this, it is also surprising that the proximal femur had the lowest classification rates, given the well-known sexual dimorphism of the associated pelvic area. This could be due to the fact that the wider pelvis in females is having more of an effect on the knee due to variation in biomechanical properties of this area between the sexes.

The next element of this study was the removal of landmarks and the determination of classification rates left for analysis. For the first run on the proximal femur, when the selected landmarks were removed, the classification rates for males in slightly lower than females, but a correct classification rate of more than 75% is expected when tested on an unknown individual. As was expected, when specific landmarks are removed that help accurately describe the overall size of the proximal epiphysis of the femur, i.e. the landmarks on the greater and lesser trochanters, a lower classification was experienced, but since the classification is above the 75^{th} percentile, this method still gives a valuable method of assessing sex of an unknown individual. For the second run on the proximal femur, when the selected landmarks were removed, the classification rates for males is slightly lower than females, but a correct classification rate of more than 60% is experienced for both males and females within the sample. As was expected, when specific landmarks are removed that help accurately describe the overall size of the proximal epiphysis of the femur, i.e. the landmarks on the greater and lesser trochanters as well as the femoral head, a lower classification was experienced. Given the classification rate is only slightly above 50% for the second run, these landmarks used independently of the others are not a successful indicator of sex.

For the first run on the distal femur, when the selected landmarks were removed, the classification rates for males is slightly lower than females, but a correct classification rate of more than 80% is experienced for both males and females within the sample. As was expected, when specific landmarks are removed that help accurately describe the overall size of the distal epiphysis of the femur, i.e. the landmarks on the medial, lateral, and posterior aspects of the condyles, a lower classification was experienced, but given the relatively high classification rate, despite losing these landmarks this method can still be utilized to assess sex. For the second run on the distal femur, when the selected landmarks were removed, the classification rates for males is slightly higher than females, but a correct classification rate of more than 80% is experienced for both males and females within the sample. Again, when specific landmarks are removed that help accurately describe the overall size of the distal epiphysis of the femur, i.e. the landmarks on the medial, lateral, anterior, inferior, and posterior aspects of the condyles, a lower classification was experienced, but given the relatively high classification rate that was still obtained with the included landmarks, this method is significant for the assessment of sex when remains may be damaged or fragmentary.

For the first run on the proximal tibia, when the selected landmarks were removed, the classification rates for males and females is equal, and a correct classification rate of more than 70% is experienced. As was expected and previously experienced, when specific landmarks are removed that help accurately describe the overall size of the proximal epiphysis of the tibia, i.e. the landmarks on the intercondylar eminence and the tibial tuberosity, a lower classification was experienced, but given the relatively high classification rate, despite losing these landmarks this method can still be utilized to assess sex. For the second run on the proximal tibia, when the selected landmarks were removed, the classification rates for males is slightly lower than that for females, and a correct classification rate of more than 65% is experienced for both males and females within the sample. As was expected and previously experienced, when specific landmarks are removed that help accurately describe the overall size of the proximal epiphysis of the tibia, i.e. the most anterior, lateral, posterior and medial landmarks of the tibial plateau and the tibial tuberosity, a lower classification was experienced. If these landmarks were damaged, this method alone is not necessarily the best for accurately assessing sex of an unknown individual. For the third run on the proximal tibia, when the selected landmarks were removed, specifically all but those on the intercondylar eminence, the classification rates for males is slightly higher than that for females, and a correct classification rate of more than 50% is expected when tested on an unknown individual. But given that these rates are barely above 50% and the p-values is high, the intercondylar eminence alone is not statistically significant for estimating sex. Therefore, the results suggest that there is little sexual dimorphism in the size and shape of the intercondylar eminence, despite what appeared to the observer as visual dimorphism.

Lastly, for the first run on the distal tibia, when the selected landmarks are removed, the classification rates for males is slightly lower than females, and a correct classification rate of or more than 80% is experienced for both males and females within the sample. Interestingly, when these specific landmarks are removed that help accurately describe the overall size of the distal epiphysis of the tibia, i.e. the landmarks on the medial malleolus and the lateral projections of the fibular notch, there was no change in classification rates. This indicates that, despite seemingly adding to the overall size of the distal tibia, these landmarks are not required to accurately assess sex, and if not available there will be no decrease in classification rates. For the second run on the distal tibia, when the selected landmarks are removed, the classification rates for males is slightly lower than females, and a correct classification rate of or more than 70% is experienced for both males and females within the sample. As was expected and previously experienced, when specific landmarks are removed that help accurately describe the overall size of the distal epiphysis of the tibia, i.e. the most anterior, posterior and medial landmarks, a lower classification was experienced. Though not the strongest correlation that has been experienced in this study, if these landmarks were damaged on an unknown individual, a statistically significant assessment of sex could be determined on the distal tibia alone.

Given these results, the distal femur is better at assessing sex even when landmarks are removed, followed by the proximal femur, then the proximal tibia, and lastly the distal tibia, but this is all dependent on which landmarks are damaged due to standard taphonomic processes. Any combination of these landmarks can be removed and the epiphysis reexamined, but these particular combinations were chosen to be the most likely observable in relation to damage seen when dealing with how taphonomy can alter bone in both a forensic and archaeological setting.

When selected landmarks were removed from each epiphysis mimicking standard taphonomic processes, there were several that discernably lowered classification rates. When analyzing these specific landmarks, it can be stated that these areas are responsible for more size variation due to sexual dimorphism, and therefore these areas are more sexually dimorphic than others. For the proximal femur, the landmarks on the trochanters (12, 13, 14, and 17) did not have a significant impact on classification rates, which remained in the high 70^{th} percentile. This indicates that the majority of size variation for this epiphysis within this sample exists in the femoral head and neck. When the landmarks of the femoral head and trochanters were removed (1-7, 12-14, and 17), leaving the landmarks of the neck and head/neck intersection, the classification rates decreased to the low 60th percentile. In other words, there is still a noticeable size and shape variation between males and females in the femoral neck region, but the combination of the femoral head and neck will give a more accurate classification rate. For future studies, the use of more landmarks in these locations may posit an even higher classification rate between males and females.

When selected landmarks were removed from the epiphysis of the distal femur, specifically the most medial, lateral and posterior landmarks (7, 8, 14, and 16), classification rates were still in the lower 80th percentile. This indicates these landmarks are not significant for their size variation between males and females. When the most anterior points of each condyle and the most inferior points of each condyle (11, 12, 13, and 15) were also removed along with the aforementioned four, the classification rates were still in the lower 80th percentile. This indicates that, despite the loss of these landmarks, the majority of size and shape variation exists in the remaining landmarks not removed, more specifically the points of the intercondylar fossa (1-6), the epiphysis/neck intersection points (9, 10, 17, and 18), and the antero-lateral points of each condyle (20-21). Future research should concentrate on exploring these areas with more landmarks to determine whether a higher classification rate could be obtained.

Selected landmarks were removed from the proximal tibia, specifically the point of the tibial tuberosity and the points associated with the intercondylar eminence (1, 9-12). These points were chosen because of their delicate and protruding nature. The classification rates were in mid-70th percentile, indicating they do not drastically affect the classification rate based on size variation between males and females within this sample. When the landmarks around the tibial plateau were removed (1, 5-8), the classification rates decreased to the low 70th and high 60th percentiles for females and males, respectively. This indicates that the majority of variation exists in the epiphysis/neck intersections and the outline of the tibial plateau. A third run removing landmarks was conducted to see if the visual variation between males and females on the intercondylar eminence observed by the author was statistically significant. When all landmarks were removed except those related to the intercondylar eminence, the classification rate was barely above 50% and the p-value high, indicating this area, though visually dimorphic, is not statistically significant for assessing sexual dimorphism. Future research should focus on the areas mentioned above, with a concentration on the outline of the tibial plateau and epiphysis/diaphysis intersection, which may indicate a higher classification rate if more landmarks are added to these areas.

Lastly, landmarks were removed from the distal tibia epiphysis, specifically the most laterally projecting points of the fibular notch (5, 7) and the most inferior point on the medial malleolus (12). The classification rates were in the low 80th percentile, indicating these landmarks do not significantly impact the classification rates and are therefore not strongly correlated with sexual dimorphism. The second run removed the most posterior point of the epiphysis, most posterior and inferior point of the medial malleolus, the most medial point of the epiphysis, and the most anterior point of the epiphysis (8, 10, 11, and 14). The classification rates were in the lower 70^{th} percentile, indicating these locations contain more variation between males and females than the most laterally projecting areas described above. Future research should focus on these areas as well the remaining areas, specifically the epiphysis/diaphysis intersections, the landmarks on the malleolus, and the outline of the articular surface. More landmarks in these areas might posit higher classification rates of males and females, and indicate these areas are more indicative of sexual dimorphism than those where landmark removal did not greatly affect classification rates.

What is arguably most noteworthy about this section of the current research study is that, when landmarks were removed, three of the four epiphyses analyzed still had statistically significant classification rates of the epiphysis/diaphysis intersection points. These area have previously never been considered as being indicative of sexual dimorphism, and this may be attributed to the biomechanical function of the thicker cortical bone in these areas in comparison to the thinner areas of the epiphyses.

The reason behind determining error rates is two-fold: it can determine the replicability of the method in terms of locating the same landmarks across a sample, and it can help determine the usability of the method. In both instances, a high error rate indicates the landmarks used are not easily located and the method should be approached with caution. The location of post-cranial landmarks is known to be troublesome, and the lack of standardization of post-cranial landmarks makes the calculation of error rates a very important aspect of every study done on post-cranial remains (Smith and Boaks 2014). For the purposes of this particular study, there was only enough time allotted to calculate the intra-observer error rates by locating the landmarks described in the previous two chapters across a subsample of individuals a total of three times. The specific error rates are presented in tables 5-8. What deserves mentioning is the relatively low intra-observer error rates across all landmarks used in this study. The majority of landmarks had 5% or less error rates, with only six landmarks above 5% and less than 6%, and only one landmark slightly above 7%. These error rates are well within acceptable ranges and posit that this study is viable for replicability. Future research would involve using multiple data collectors to determine the inter-observer error rates.

In determining the applicability of this method of analyzing sexual dimorphism, data from the Databank for Forensic Anthropology in the United States (FDB), 1962-1991 (Jantz and Moore-Jansen 2000) was compared to data presented in this paper. Specifically, the percentages of standardized measurements of the femur and tibia that could not be taken for the FDB were compared to determine which landmarks would not be available for collection in the same set of cases.

When analyzing the FDB for missing measurements of the femur, the following percentages were observed. In 29.3% of cases, the maximum length of the femur could not be taken, which would exclude the most superior landmark on the femoral head (proximal femur 1) and the most inferior landmark on the medial condyle (distal femur 13) (Jantz and Moore-Jansen 2000). In 35.6% of cases, the femoral bicondylar length could not be taken, which would exclude the landmarks just described as well as the most inferior landmark on the lateral condyle as well (distal femur 15) (Jantz and Moore-Jansen 2000). In 35.7% of cases the maximum diameter of the femoral head could not be taken, which would exclude at least two of the four landmarks associated with the femoral head (Jantz and Moore-Jansen 2000). In 40.6% of cases, the epicondylar breadth of the femur could not be taken, which would exclude the most lateral and medial landmarks on the distal epiphysis (7 and 8) (Jantz and Moore-Jansen 2000).

When analyzing the FDB for missing measurements of the tibia, the following percentages were observed. In 34.0% of cases, the tibial length could not be taken, which would exclude the most inferior point on the medial malleolus (distal tibia 12), but it should be noted that this measurement already excludes the intercondylar eminence so the lack of this measurement does not affect the collection of landmarks on the proximal tibia (Jantz and Moore-Jansen 2000). In 44.3% of cases, the maximum distal breadth of the tibia could not be taken, which would exclude the most lateral points of the fibular notch projection (distal tibia 5 and 7) and the most medial point of the distal tibia epiphysis on the medial malleolus (distal tibia 11) (Jantz and Moore-Jansen 2000). In 44.7% of cases, the maximum proximal breadth of the tibia could not be taken, which would exclude the most medial and lateral points of the tibial plateau (proximal tibia 6 and 8) (Jantz and Moore-Jansen 2000).

When looking at the proximal femur landmarks in comparison to the measurements missing from the FDB, approximately 35% of landmark collection may be compromised in a real forensic circumstance, depending on the missing measurement (i.e. the maximum length or femoral head diameter). Given the relatively few measurements associated with the landmarks selected in this research, this estimate is just that, an estimate, and more landmarks may be compromised depending on damage accrued by any given skeletal element. For the distal femur, approximately 40% of most lateral and medial landmarks may be compromised, though again, this is just an estimate. For the proximal tibia, approximately 45% of medial and lateral tibial plateau landmarks may be compromised, and for the distal tibia, approximately 44% of the most medial and lateral landmarks may be compromised.

Another way of comparing the FDB information with the current study was to examine the percentage of missing elements or sections of elements. The femur was missing in 38.8% of cases and the tibia was missing in 39.1% of cases (Jantz and Moore-Jansen 2000). The femoral head was missing in 48.7% of cases (Jantz and Moore-Jansen 2000). The femoral greater trochanter was missing in 50.1% of cases (Jantz and Moore-Jansen 2000). The distal femur was missing in 50.1% of cases (Jantz and Moore-Jansen 2000). The proximal tibia was missing in 52.9% of cases (Jantz and Moore-Janse 2000). The distal tibia was missing in 53.4% of cases (Jantz and Moore-Jansen 2000).

It should be noted that the measurements that could not be taken are from the elements that were present as opposed to the elements or parts of elements that were not available. For the purposes of this discussion, the comparison should focus on what measurements could not be taken on the elements that were present. It is important to keep in mind that in real forensic situations, epiphyses of both the femur and tibia were absent in roughly 50% of cases. The FDB does not specify whether the percentages of missing elements were from the right or left, whereas the percentages of measurements that could not be taken did specify which side they were from. Because of this, the percentages of missing measurements listed above were from all lefts given that the data in this research study were collected from all lefts in order for a more accurate comparison. Of the femora and tibiae where measurements could not be taken, in all cases the percentages were less than 50%, indicating that more than 50% of measurements could be taken. It can then be assumed that, of femora and tibiae present in actual forensic cases, landmark data could potentially be collected on more than 50% of elements. If these elements were in fact just damaged, it is also possible that some landmark data could be collected, and that being the case this method for sex assessment would be a viable contribution to those forensic cases.

A second area for comparison is with another study that determined sexually dimorphic classification rates for White and Black populations using data from the FDB (Spradley and Jantz 2011). This study used standard measurements reported in the FDB to create sectioning points for each measurement and using these created classification rates for each measurement which was population specific. The authors found that, for modern White individuals, the proximal tibia epiphyseal breadth had a classification rate of 90%, while the femoral head diameter and femoral epicondylar breadth had classification rates of 88%, and the tibia distal epiphyseal breadth had a classification rate of 78%. The current research study found that the distal femur had the highest classification rate, over 90% for males, indicating that, when compared with standard measurements, geometric morphometrics more accurately describes the overall size of an object in question. Though the classification rates from the Spradley and Jantz (2011) study are comparable to those presented here, future research with additional landmarks concentrated on the areas of epiphyses that may be more dimorphic between males and females could yield higher classification rates and therefore determine beyond a doubt that geometric morphometrics is superior for the estimation of sex.

As with any study analyzing a single population from one collection, several biases are introduced. Analysis of a single population automatically introduces population bias, where the results from this study cannot be applied to another population. Since the data presented here were collected on a single population (i.e. modern European-Americans), it is limited in comparison to other populations. Another form of bias is that of age. A master list of individuals available for analysis from the William M. Bass Donated Skeletal Collection was given to the author before data collection began. This list contained the age-at-death of each individual in the collection, and the majority of individuals are middle aged or elderly. The author collected data from as many younger individuals as were available to limit age bias, but this introduces selection bias. The only way to avoid selection bias is to do random sampling, but given the nature of the age distribution of the collection, this would have almost certainly introduced significant age bias. In order to limit sex bias as well, the selection of an even number of males and females across a broad age range was conducted, but this also contributed to the selection bias aforementioned. The last bias that has been discussed in previous chapters is that of landmark collection. Since there was a time limitation on data collection, only one observer was used during this study. When only one data collector is used, this introduces landmark collection bias in reference to intraobserver error. For future research, the use of multiple observers would more accurately analyze the replicability of this method by determining interobserver error and limit landmark collection bias.

There are multiple areas in which this study could be expanded for future research. As previously discussed, more landmarks could be added to the areas that statistically display more variation in size between males and females. The addition of multiple populations, both modern and archaeological, would be extremely beneficial in determining the applicability of this method of analyzing sexual dimorphism across populations and temporal differences between populations. The expansion of geometric morphometric analysis on not just epiphyses but also diaphyses would be an important contribution in the cases where only the diaphysis survives or when the epiphyses are too damaged for landmark collection.

Geometric morphometric analysis has developed into a valuable method of size and shape variation that is applicable to multiple fields. Its usability in forensics is only just beginning to be explored, and while the uses for morphometric analysis are arguably limitless. The application of this particular research could be applied to studies concerning morphological adaptation over time in the form of possible sexually dimorphic secular change, as well as clinical analyses concerning pathological conditions that may be affected by sexual dimorphism.

APPENDIX

Tables

Table 9: Statistical Output Before Landmark Removal

Epiphysis	Re-Classification Rate	P-value	Eigenvalue	Mahalanobis Distance	Procrustes Distance
Proximal Femur	Males: 80.8% Females: 78.4%	<0.0001	0.596	1.537	0.021
Distal Femur	Males: 92.0% Females: 89.6%	<0.0001	1.739	2.627	0.029
Proximal Tibia	Males: 80.8% Females: 83.2%	<0.0001	0.718	1.688	0.031
Distal Tibia	Males: 81.6% Females: 80.8%	<0.0001	0.793	1.774	0.026

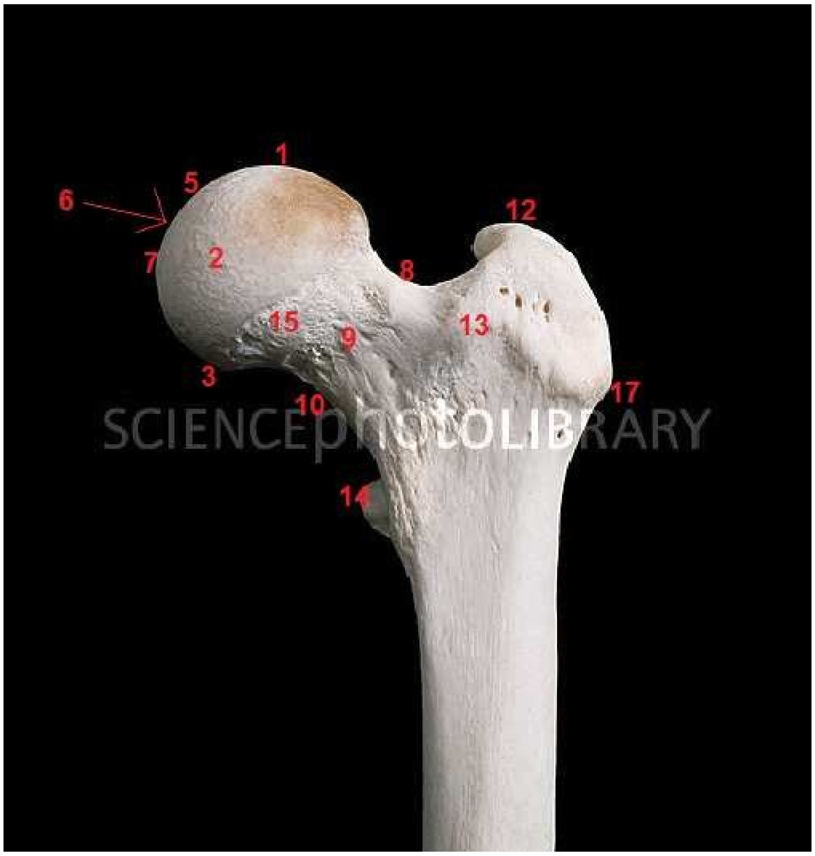
Table 10: Statistical Output After Landmark Removal

Run #	Re-Classification Rate	P-value	Eigenvalue	Mahalanobis Distance	Procrustes Distance
Prox. Femur 1	Males: 77.6% Females: 78.4%	<0.0001	0.423	1.295	0.026
Prox. Femur 2	Males: 63.0% Females: 64.0%	0.0322	0.091	0.601	0.028
Distal Femur 1	Males: 83.2% Females: 84.8%	<0.0001	1.032	2.024	0.026

Distal Femur 2	Males: 81.6% Females: 80.8%	<0.0001	0.669	1.630	0.024
Prox. Tibia 1	Males: 74.4% Females: 74.4%	<0.0001	0.390	1.244	0.034
Prox. Tibia 2	Males: 68.8% Females: 73.6%	<0.0001	0.270	1.035	0.033
Prox. Tibia 3	Males: 55.2% Females: 54.4%	0.7821	0.010	0.200	0.782
Distal Tibia 1	Males: 80.0% Females: 81.6%	<0.0001	0.672	1.633	0.027
Distal Tibia 2	Males: 74.4% Females: 72.8%	<0.0001	0.504	1.414	0.028

Figures

Figure 12: Proximal Femur Landmarks



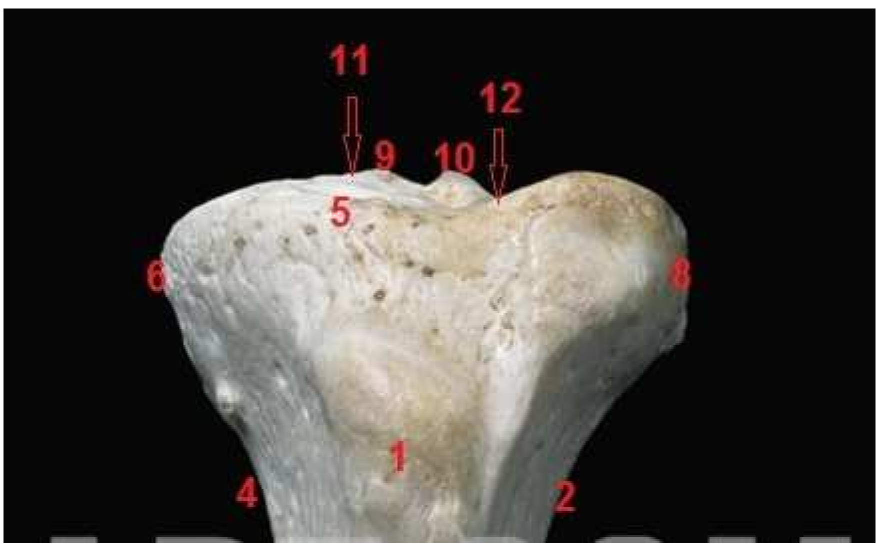
http://www.sciencephoto.com/media/455288/enlarge

*Not pictured: Landmarks 4, 11, and 16

Figure 13: Distal Femur Landmarks

http://www.pediatric-orthopedics.com/Topics/Bones/Femur/femur.html

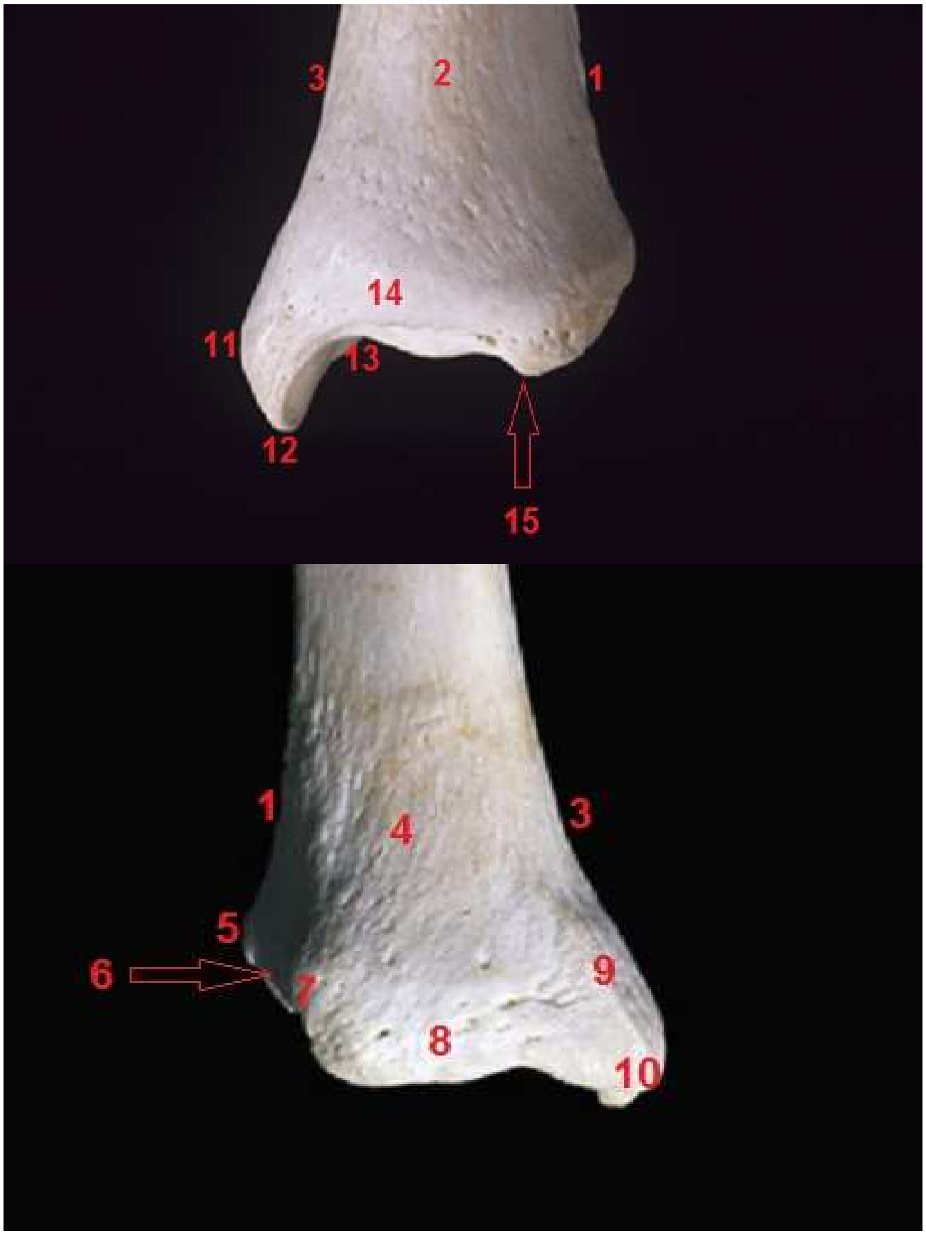
Figure 14: Proximal Tibia Landmarks



http://www.art.com/gallery/id--a238146/posters_p6.htm

*Not pictured: Landmarks 3 and 7

Figure 15: Distal Tibia Landmarks



http://www.art.com/gallery/id--a238146/posters_p6.htm

LIST OF JOURNAL ABBREVIATIONS

Acta Zool ....Acta Zoologica

Am J Phys Anth ....American Journal of Physical Anthropologists

Am Midl Nat..American Midland Naturalist

Evol Biol ....Evolutionary Biology

For Sci Int .... Forensic Science International

Homo-J... Homo- Journal of Comparative Human Biology

Int J Legal Med...International Journal of Legal Medicine

Int J of Osteoarch ....International Journal of Osteoarchaeology

Ital J Zool.. Italian Journal of Zoology

J Anat .... Journal of Anatomy

J For Sci ....Journal of Forensic Science

S Afr J Sci... South African Journal of Science

Syst Biol ....Systematic Biology

Syst Zool ....Systematic Zoology

Yrbk Phys Anth...Yearbook of Physical Anthropology

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age, sex, stature, ancestry, and pathology/trauma. Took and submitted samples for DNA analysis. Worked closely with the San Bernardino Sheriff's Department because individuals in the cemetery are still part of active investigations

Bioarchaeology Field School- Cluj- Napoca, Romania *July 2012-Aug. 2012*

- Organization: Archaeological Techniques and Research Center (Archaeotek)
- Program Coordinator: Andre Gonciar
    - Studied the ancient Noua culture from Cluj-Napoca, Romania, reconstructed skeletal remains from archaeological excavations, created biological profiles of the available sample and conducted a paleopathological assessment based on non-destructive macroscopic analysis

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    - Geometric morphometric surface analyses of the femoral head of Tragulid and Bovid species to determine morphological adaptations to correlate ecology with their physical traits *Sept. 2012- March 2013*
    - Angle analyses of the astragalus bone of extinct Dorcatherium and various Tragulid and Bovid species using ImageJ to determine morphology adaptations in attempts to reconstruct the paleoenvironment in which they lived *Sept. 2011- Dec. 2011*
    - Analysis of sexual dimorphism in humans using geometric morphometrics of the femoral head and neck *March- June 2011*

- Advisor: Professor Stuart Smith, University of California, Santa Barbara
    - Analysis of fatty acid isotopic ratio composition on ancient Nubian and Egyptian pottery sherds to reconstruct the paleodiet of the two civilizations and comparing for cross-cultural similarities and differences *Jan.-June 2012*
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- Creating a biological profile of human skeletal remains
- Maceration
- Geometric Morphometrics
- Differentiating between human/non-human osseous materials
- Differentiation and sequencing of taphonomic events
- Basic crime scene processing, documentation, and evidence collection
- MorphoJ, Morphologika, ImageJ
- Analysis and reconstruction of pottery sherds
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