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THREE-DIMENSIONAL GEOMETRIC MORPHOMETRIC SEX DETERMINATION AND MODELED FRAGMENTARY ANALYSIS OF THE HUMAN PUBIC BONE

Katherine Scot Baca

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By

KATHERINE SCOT BACA

Master of Arts, University of Montana, Missoula, Montana, 2018

Bachelor of Arts, New Mexico State University, Las Cruces, New Mexico, 2016

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Approved by:

Scott Whittenburg,
Graduate School Dean

Dr. Meradeth Snow, Chair
Department of Anthropology

Dr. Brandon Bridge
Department of Economics

Dr. Kirsten Green
Department of Anthropology

Dr. Aldo Fusaro
Montana State Crime Lab – Missoula

Dr. Ashley Kendell
Department of Anthropology (California State University, Chico)

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Chapter One: Introduction

Sexual dimorphism in the human pelvis is primarily based on the fact that females are able to give birth, while males are not (1,2). This causes shape differences between the male and female pelvis that forensic anthropologists are able to use to differentiate between the two sexes. Traditionally, the analysis methods used to estimate a male or female origin of an unknown bone are based on a visual analysis of specific parts of the pelvis. These visual analysis methods are consistently 90-95% accurate when employed by an experienced forensic anthropologist (1,3). Very few metric methods exist, and those that do are either based on these visual analysis techniques or utilize interlandmark distances for analysis. The goal of the present research is to refine a new method of three-dimensional geometric morphometric analysis in order to predict an unknown bone as male or female, using only the pubic bone.

The pubic bone is a small, rectangular shaped portion of bone at the anterior aspect of the pelvis. Everyone has a right and left pubic bone, meeting in the middle to form the pubic symphysis. Visual analyses to estimate sex using the pubic bone include an analysis of the ischiopubic ramus, the ventral arc, and the general shape of the bone (2). It is known that sexual dimorphism exists in the pubic bone, so it follows that a metric analysis of these shape differences should result in accurate sex predictions. Geometric morphometrics is the metric analysis of shape and can be completed in a two- or three-dimensional technique (4). By utilizing a three-dimensional geometric morphometric shape analysis, it should be possible to gain a much more accurate and complete understanding of the shape differences between male and female pubic bones. The overarching hypothesis of this research is that a geometric morphometric shape analysis of the pubic bone will result in statistically accurate sex determinations on both whole and fragmented human pubic bones and that certain landmarks will be more effective than others in establishing a sex determination.

This research uses a sample from the University of New Mexico Maxwell Museum's Documented Skeletal Collection (5). This collection offers more than 300 nearly complete skeletal individuals, all of which include known demographic information, such as sex, age-at-death, ancestry affiliation, and cause of death. Many also include known information on parity and occupation. All individuals in the collection have died within the last 50 years, making this one of the most modern collections in the US. 213 adult individuals from this sample were utilized for this research, which yielded 378 individual pubic bones (not all individuals had complete right and left pubic bones). Each bone was placed into a rubber vice which held the bone still and in place while data collection took place. A Microscribe digitizer was used to collect eight landmark points from each pubic bone. After the initial data collection on all 378 bones was complete, a random group of 50 individuals (n=100) was re-digitized in order to test the replicability of the method.

The statistical analyses used in this research included a Generalized Procrustes Analysis in order to remove size and orientation from the data; a Principal Components Analysis to create groupings based on similarities in variance within the data; and a series of Discriminant Function Analyses to determine the predictive power of the sample to classify unknown bones as male or female. In order to test the applicability of this method to more realistic fragmented pubic bones, a modeled fragmentary analysis was performed by running Discriminant Functions Analyses on all possible landmark combinations of three landmarks or more. This provided 218 possible landmark combinations that represent fragmented bones missing landmarks. The results of these analyses are presented and discussed in the following papers.

Also conducted as a smaller research project, was an overview of trophy skulls found in Montana over the past few years. Trophy skulls and other retained body parts are generally associated with times of war and summon imaginings of ancient civilizations, such as the Aztec (6). While it is no longer common to keep the skull of one's enemy, archaeological crania are still found, kept, and often displayed (7). The question becomes, how does the forensic anthropologist approach these crania, and where should their final resting place be? This research presents six case reports of unknown individuals whose skull had been found and displayed in some manner. It addresses the oddly high occurrence of trophy skulls in a state of relatively small population (Montana), and analyzes the possible origination of each, determining that modern trophy skulls may not be relics of war, but rather stumbled-upon keepsakes. It is also argued that a change in terminology from trophy skull to souvenir skull be considered in order to better reflect the background and provenience of these crania. Further analysis and discussion of this research can be found in the third of the following articles.

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Chapter Two: Background Literature Review

The use of geometric morphometrics has become increasingly popular in forensic anthropological research (1–3). Geometric morphometrics is, at its most basic level, the metric analysis of shape (4). This method has been used in many biological studies for at least the past three decades, but is relatively new in the field of Forensic Anthropology (4). Geometric morphometrics differ from traditional methods because morphoscopic analysis does not metrically assess bone, and interlandmark distance measurements do not fully capture the shape of a bone. 3D geometric morphometric analysis is still a new technique in Forensic Anthropology, and because of this, there are relatively few studies utilizing the method and there is little standardization among the existing research.

Much of the existing geometric morphometric work focuses on sex and ancestry assessment of the cranium (1,5,6). It has been shown, however, that metric analysis of the post-cranial skeleton can be just as accurate or more accurate in sex estimation as the cranium and it is accepted that the pelvis is the absolute best indicator of sex (7). Further research using 3D techniques on the post-cranial skeleton is needed to help increase accuracy of sex estimation in forensic and archaeologic contexts. Research focused on the pelvis most commonly investigates changes in age, and few projects attempt to metrically determine sex (8,9). It is especially important today to develop accurate metric analyses due to the need for reliable and objective results in the court room (10). Improving the specificity of sex determination in the pelvis has the potential to improve both modern forensic anthropology as well as analyses within bioarchaeology.

The goal of this dissertation project will be to use a 3D Microscribe digitizer to assess sex from the pubic bone, as well as to compile a list of standard landmarks that can be used in future geometric morphometric studies of the pubic bone and pelvis. This project will also assess patterns in morphological change related to age as an aid in determining whether or not the differences between males and females change as individuals age. If available, ancestry will also be recorded and tested to determine whether or not ancestry plays a significant role in shape differences. The information gained from this research should be applicable to modern forensic cases as well as other various scenarios, such as mass or commingled graves. The initial hypothesis of this project, based on a review of the existing literature and experience is that this 3D geometric morphometric analysis will accurately determine the sex of unknown skeletons based on the shape of the pubic bone.

The Human Pelvis

The primary function of the human pelvis is to transfer the weight of the upper body into the legs during standing, walking, and running (11). The pelvis consists of two large bones, known as the hip bones, or os coxae. These two bones connect anteriorly at the pubic symphysis, and posteriorly with the sacrum at the sacroiliac joints (11). These bones form a very stable structure, known as the pelvic ring, which allows close to no mobility, transfers weight into the legs, and helps to protect and hold the abdominal organs (11). Each os coxae is actually formed from the fusing of three different bones; the ilium, the ischium, and the pubis. These three bones eventually fuse together at the acetabulum to form the large and complex structure of the pelvis. The three bones of the os coxae grow throughout the juvenile years of each individual, and sometimes do not fully fuse into one bone until the age of 25 (11). Such a long growth period through childhood, puberty, and adulthood is part of what contributes to making the pelvis the absolute best indicator of adult sex in the human skeleton; and speaks as to why sub-adult sex estimation can be so difficult.

Although the pelvis has a unique morphology when compared to the rest of the human skeleton, it develops much the same way as the other bones throughout the body, beginning with an intra-uterine mesenchymal template which transforms into cartilage, and later ossifies to form bone (11). The first indication of pelvic development in-utero occurs on day 28 when the lower limb buds begin to develop (11). Following this, between days 34 and 36, the obturator, femoral, and sciatic nerves rapidly extend into the developing limb bud, establishing their location before any larger structures are formed (11). Chondrification, the process of transforming the mesenchymal templates into cartilage, begins to occur between intra-uterine weeks six and seven (11). Chondrification centers are well established in the ilium, ischium, and pubis by eight weeks, and by the third intra-uterine month, the cartilaginous pelvis is well developed (11).

Ossification of the pelvis occurs through the process of endochondral ossification, during which, chondrocyte cells begin the stimulation of mineralization and blood vessel growth, both of which allow bone cells to be laid down within the cartilaginous template (11,12). Each of the three bones in the os coxae begin ossifying at primary ossification centers which follow the timing and location of the prior chondrification process (11). An ossified ilium is recognizable at around four to five intra-uterine months, the ischium is recognizable after six months, and the pubis begins ossification last, and is recognizable around the seventh month (11,13). All three bones are easily identifiable by birth, and experience rapid growth during the first three months of life, which then slows until approximately three years of age, after which, the growth slows even more (11,13). During these first few years of postnatal life, multiple secondary ossification centers form in each of the three bones (11). The secondary ossification centers help to expand bone, much like the epiphyseal plates in long bones (11,13). They also help to form the epiphyses of the ilium, the ischium, and the pubis, which will fuse later in life. A slow rate of growth is maintained until puberty, during which a growth spurt causes rapid size and shape changes. The ilium epiphyses are generally fully fused by about 20 years of age, the ischium is generally completely fused by about 20-23 years of age, and the pubis is completely fused by 25 years of age (11,13). It should be noted however that shape changes continue to occur in the pelvis over an individual's lifespan. These changes can be due to age, giving birth, trauma, disease and more.

Since the nineteenth century, osteologists have followed a simple rule, coined Wolff's Law after the man who developed the concept, that bone will adapt to the load placed upon it (14). This adaptation affects the morphology of bone throughout the body and can be easily seen in various contexts, one classic example being that of a professional weight lifter's skeleton exhibiting much larger muscle attachment sites on their bones than any non-weight lifter counterpart. A lot of research has been done using this principle in an attempt to determine the handedness of a skeletal individual due to the simple concept that one using their right hand more often to complete physical tasks would develop larger muscles, and therefore larger attachments throughout the bones of their right hand and arm (15–17). Unfortunately, no concrete method was ever validated to determine the handedness of an unknown individual; however a look into the symmetry and asymmetry of the human skeleton can offer some useful insights (16).

Although asymmetry between the upper limbs cannot reliably predict handedness, it is observed quite often (16). This observation occurs most frequently in archaeological samples however, and does not seem to exhibit anything close to a pattern in modern populations, likely due to the shift away from all-day physical labor in contemporary developed nations (16,18). Asymmetry between the lower limbs is usually much smaller than asymmetry between the upper limbs, and sometimes manifests as crossed symmetry (16). Crossed symmetry is when the bones of the right arm are larger as well as the bones of the left leg, or vice versa (16). This seems to occur due to the natural act of bracing oneself bilaterally, such as when a ball is thrown with the right hand, and the individual steps forward with their left to compensate (16). However, crossed symmetry is also not often seen in modern populations and likely does not affect the pelvis to much degree. Locomotor requirements tend to produce much more symmetrical limb sets, which explains why significant asymmetry between the lower limbs of humans is relatively rare (16). It is unlikely that any significant asymmetry exists between the right and left pubic bones of individuals seeing as the pubic bones are not directly affected by any common one-sided movement of the body. Any asymmetry that does exist would likely be due to shape changes related to age and may be easier to observe in older individuals.

This research focuses specifically on the adult pubic bone, which is one of the more sexually dimorphic and age-revealing bones of the pelvis (12,19). The pubic bone is located on the anterior portion of the os coxae and forms an articulation at the pubic symphysis. This joint consists of a fibrocartilaginous joint which sits between the articular surfaces of the pubic bones (20). This joint allows for approximately 2mm of movement in adults, and otherwise resists tearing, rotating, shearing, and any further movement (20). The shape of the adult pubic bone offers many clues as to the biological sex of the individual and can be used to quite accurately assign a designation of male or female (12,21). The articular surfaces of the pubic bone continually change shape and appearance as an individual ages (12,20,21). These changes can be categorized into stages and thus used to estimate the age-at-death for an unknown individual (12,21). The ability to accurately estimate sex and age from the pubic bone make it one of the most important bones to recover when an unknown skeletonized individual is found, and therefore accurate estimation methods are also necessary.

Traditional Sex Estimation

It is well known that the pelvis is the absolute best indicator of sex within the human skeleton, although the cranium and other post-cranial elements can also be used (7,12,21). The pelves of males and females are functionally different due to the basic fact that females give birth (22). The pelvis of a female must be shorter and wider to offer space for childbirth while the pelvis of a male tends to be taller and narrower (22). Other morphological traits have developed between the two biological sexes as well due to the difference in functionality required in the pelvis. These traits can only be used to determine the sex of an adult skeleton though, because children do not develop these distinct traits until after puberty has occurred and their bones are fully fused (12,21). Traditional sex estimation uses a visual analysis of these traits to determine the sex of an unknown individual. In contrast, metric methods, such as geometric morphometrics, use measurements and statistics to calculate a sex determination.

There are morphological indicators of sex on all three bones of each os coxa, and when used in conjunction, a visual analysis performed by an experienced forensic anthropologist can yield a 98% accuracy rate (7). The greater sciatic notch and the preauricular sulcus on the ilium; the ischiopubic ramus ridge on the ischium; and the ventral arch, subpubic angle, and the body shape on the pubic bone are some of the most reliable traits visible on the pelvis (12,21). These traits are generally visually scored on a 1-5 scale, 1 being very female and 5 being very male, to aid in the overall determination of sex (12,21). Each trait can be used to estimate sex alone, for example, if the pelvis is fractured and only fragmentary pieces are recovered. It is possible for female individuals to exhibit male traits however, and vice versa, meaning that a sex estimation determined from just one trait may not be accurate (22). This is also true when utilizing traits of the cranium to determine sex, and simply means that whenever possible, multiple traits should be used in conjunction in order to determine the most accurate sex estimation possible.

There are surprisingly few metric methods to determine sex using the pelvis. A popular method which uses both morphoscopic methods and metric analysis was published in 2008 (23). This method requires the user to conduct a morphoscopic analysis of the subpubic concavity, the ischiopubic ramus ridge, and the ventral arch. The user then enters these scores into a spreadsheet which uses a logistic regression calculation to determine the overall probability of the specimen being male or female (23). This method is popular because it offers a percentage of likeliness, however it is still based on subjective morphoscopic analysis. A study in 2010 used geometric morphometrics and a Microscribe digitizer to locate landmarks on the entire pelvis (19). The research was conducted in much the same way as the current project, the largest difference being that in 2010, the authors focused on the entire pelvis, rather than just the pubic bone(19). This study resulted in a 100% correct classification rate for males and 98% for females, an incredibly promising result for future metric analysis of the pelvis (19). This geometric morphometric analysis is better than past morphoscopic analyses because it offers just as high a rate of correct classification as an experienced anthropologist and it is metrically based, which will inevitably stand up better in court if necessary (10,19). The main concern with this particular method is that the entire pelvis is needed, which often is not available. To improve upon this method, more specific research should be done using geometric morphometrics on smaller portions of the pelvis, such as the pubic bone.

Age Estimation

The age of an unknown human skeleton can be estimated based on numerous parts of the body, including the teeth, epiphyseal closure sites, the cranium, sternal rib ends, the pubic symphysis and the auricular surface (21,22). Aging methods for sub adults are based on bone growth and development, while aging methods for adults are based on bone degeneration (22). Teeth are best used to determine the age of sub adults because human teeth erupt at very regular intervals which can offer an accurate age estimation within a few months (24). Epiphyseal closures are also best used for sub adults because the bones tend to develop at regular rates, and are mostly to completely fused by ages 25-30 (25). The sutures on the cranium can also be used to estimate age based on how open or closed they are, however, this method has been shown to be inaccurate and is no longer widely used among forensic anthropologists unless it is the only method available (26). The sternal rib ends, however, have proven to be quite useful in age estimation. By analyzing the morphology and condition of the sternal end of the fourth rib, an experienced forensic anthropologist can estimate a range for age at death of the individual (27).

The auricular surface and the pubic symphysis are the best age indicators in the body (although the sternal rib ends have been shown to be just as accurate as the pubic symphysis) (22,27). The auricular surface is the portion of ilium which articulates with the sacrum, together, forming a tight joint which offers stability and little to no movement in the pelvis as a whole. This surface has been shown to degenerate at a relatively regular rate, which can offer an age range for unknown individuals (28). Similarly, the articulating surface of the pubic symphysis can also be used to estimate the age of an unknown set of remains (29). Both methods are based on descriptions and photographs of the bone surfaces at different ages. As with sex estimation, the most accurate age estimations are determined when multiple analyses are used together.

Effects of Parturition

Forensic scientists have studied the effects of parturition on the human skeleton for many years. Knowing the parturition status of an unidentified female skeleton could aid greatly in the investigation to identify her, and is of great interest to forensic anthropologists (30,31). While it has been shown that parturition and related events can affect the pelvis and leave changes and marks, it is not the only event which can create these same marks and alterations (30,31). The alterations found on the pelves of parous women can also be found on nulliparous women and men (30). The amount of alterations or intensity of them also do not seem to correlate with multiple pregnancies, and are sometimes entirely absent on parous and multiparous women (30). This means that it is known that pregnancy can affect the shape and appearance of the pelvis, but it cannot be concluded that pregnancy occurred when these alterations are viewed on the pelvis of an unknown individual.

The main alterations which have been noted over time as possibly indicating a past pregnancy include various alterations and manifestations of the preauricular sulcus, pubic pitting, trabecular bone loss, and an extension of the pubic tubercle (30). Most of these alterations are associated with changes in hormones and physical forces which are a product of the ligaments and muscles stretching and relaxing around the pelvis in anticipation of childbirth (30). It has also been shown that pregnant women can experience a decrease in bone density during their pregnancy due to the transfer of calcium to the fetus and their breast milk (32–34). Unfortunately, these alterations have also been found to be caused by general age changes, urinary tract infections, minor and/or major traumas, surgery, joint and pelvic instability, anomalies in the lumbosacral bones and joints, occupation-related activity, congenital anomalies, degenerative conditions, as well as general pelvis and body size (30). The decrease in bone density can also be caused by some of these other factors and is completely reversible in women once the pregnancy has ended (32). Most of these other occurrences can happen to both men and women, regardless of their parity history, making a forensic determination of pregnancy impossible.

Considering how closely this research is looking at the shape of the pubic bone, and that pregnancy can create alterations in the shape of the pelvis, it is important to work with a sample that consists of females with available pregnancy records. A 2016 study showed that parturition could affect the accuracy of age estimation when using the Suchey-Brooks pubic symphysis method (35). The researchers showed that when their sample was separated into groups of men, nulliparous women, and parous women, the parous women were inaccurately aged most often (35). When they grouped all the females together however, there was no significant error in the age estimations, meaning that the Suchey-Brooks method (created using a mixed parous and multiparous sample) already accounts for the changes observed in some parous female samples (35). This research shows, however, that parous females could present pubic bodies which are quite different from nulliparous females, and which, could potentially bias this geometric morphometric research. To control for this possibility, the method will be created using a sample of both known parous and nulliparous females in an effort to account for these alterations. It will also be noted though whether or not there is a difference in the results of parous and nulliparous women when grouped separately in the statistics of this research.

Geometric Morphometrics

As stated earlier, geometric morphometrics is most simply a 3D analysis of shape (4). The method has been used in biological fields for many years, although has only recently become widespread in anthropology. Research using geometric morphometrics varies in many ways, from focusing on age or sex estimation, to which elements of the skeleton are used. One of the largest variances between studies, however, is the method of data collection. There are two primary methods for gaining digitized data on shape variation. The first is to use a camera to take photos of the element or bone surface, and to then use a computer to measure and record landmark measurements and sliding semi-landmark measurements (2,36,37). While these studies offer great potential and demonstrate the usefulness of 3D research, they are limited by the fact that they use a 2D technique to analyze 3D shapes (36,37). A study on the use of sliding semi-landmarks showed that when morphological variation is small, such as that between modern humans, the differences between a landmark and a sliding semi-landmark study using the same sample can be altered, thus raising questions about any results obtained by using sliding semi-landmarks (36). The second method is to use a digitizer arm or 3D scanner, like a computed tomography (CT) machine, to record 3D measurements in space. These methods tend to be more precise, however they require more expensive tools as well as more experience to use them effectively (1,38,39).

A study by San-Millan et al. used a camera to take images of the acetabulum of 327 males and 355 females of known sex and age from various collections in Europe to study shape changes within the acetabulum as individuals age (37). Using the digitized images, the researchers placed one landmark at the apex of the anterior acetabular horn of the lunate surface and one on the apex of the posterior horn, as well as at 32 sliding semi-landmarks along the outline of the lunate surface (37). The authors analyzed these points using various statistical tests to determine shape changes related to sex and age of the individuals (37). The results found significant shape differences in both sex and age between individuals; however, as females aged, their acetabular shape began to look much more similar to males and it became harder to distinguish between sexes of older ages (37). This age confluence could introduce errors when attempting to assess the sex of individuals based on joint morphology. For this reason, this dissertation project will also look for patterns in morphology changes related to age in the body of the pubic bone. The authors of this study also point out that digitizing a 3D shape (the acetabulum) using a 2D technology (camera photography) is limited and that further research using 3D methods should be employed to expand on their results concerning acetabular morphology between the sexes (37).

Within the second main area of data collection, using 3D digitizers or CT machines, there are also various methods to collect data. The first method is collecting 3D points to measure traditional interlandmark distances, such as maximum length of a bone (1,2). The second method consists of recording multiple fixed landmarks to conduct a shape analysis of the element in question (38,40,41). This method would record landmarks such as the medial intercondylar tubercle and the maximum anterior point on the medial condyle of the proximal tibia (41). The third method uses a mixture of fixed landmarks, similar to the second method, while adding points evenly spaced across the bone surface (3). For example, Franklin et al. used fixed landmarks on mandibles as well as three evenly-spaced points along the posterior ramus and another three evenly-spaced points along the mandibular body in an attempt to gain a better representation of the shape of the mandible (3). A project which needs traditional distance measurements should use the first method, while projects researching the shape morphology of skeletal elements should use either the second or third method. Currently, the research does not show many advantages or disadvantages to adding evenly-spaced points to existing landmarks. One study on intraobserver error in geometric morphometric methods, however, did show that the largest amount of error comes from locating landmarks (39). Due to this error, using evenly-spaced points might be more easily replicated, but future research is needed to substantiate this.

A study by Katherine Spradley and Richard Jantz focused on estimating ancestry from the cranium used interlandmark distances measured by digitizing landmarks using a Microscribe digitizer on craniums in the Forensic Anthropology Data Bank (FDB) collection housed at the University of Tennessee (1). The authors used the digitizer to capture 31 landmarks from each skull in their study, and were then able to calculate all possible interlandmark distances between the 31 points, which resulted in 465 interlandmark distances per skull (1). Using these landmarks, the authors also tested geometric morphometric shape analysis to compare to the use of interlandmark distance measurements. The results of their study showed that the interlandmark measurements considered nonstandard (or measurements that have not been described before in research protocol books like Standards (42)), were more accurate in ancestry estimation than were the geometric morphometric analyses (1). While geometric morphometrics was not the most accurate method to determine ancestry in this study, the use of a Microscribe digitizer proved accurate and useful in data collection (1).

The authors determined that the shape information provided by geometric morphometric analysis is still important and that further research should be conducted in this area (1). The authors also pointed out that using the digitizer prompts the observer to focus on one landmark at a time, which reduces error, and then the landmark is automatically recorded through a computer program, like Excel, which reduces recording error and immediately saves data (1). An earlier project using geometric morphometric analysis of the cranium set out to develop a database similar to FORDISC which could categorize unknown skulls' ancestry and sex (43). The authors collected 75 craniofacial landmarks on over 1,000 skulls of European, African, and Hispanic ancestry using a Microscribe digitizer (43). While this study did not use interlandmark distances, it did find significant shape differences between ancestries and the authors' called for future research in geometric morphometrics concerning both ancestry and sex determination (43). The database created from this research is an ongoing project as more research contributes to the field of geometric morphometrics. Although this dissertation will not be focusing on cranial measurements, these studies provide support for continued research in the field of geometric morphometrics and emphasizes the advantages of using a Microscribe digitizer (1,43).

Most studies using a digitizer focus on analyzing the shape of a bone or bone surface, rather than measuring interlandmark distances. One of the earliest studies using a Microscribe digitizer was a dissertation written by Frederick John Snow at the University of Tennessee in 2004. Snow's goal was to assess sex from the scapulae of a sample that consisted of 241 individuals from the William M. Bass Donated Skeletal Collection, all of either White or Black ancestry (44). The author collected ten landmarks on both right and left scapulae (when possible) of each individual (44). The main analysis of this study showed that the mean centroid size could significantly discriminate between males and females, but could not discriminate between White and Black ancestries (44). The author observed that the canonical discriminant function and principal component analysis were also able to significantly distinguish males from females, and were able to observe shape differences between distinct ancestral backgrounds (44). Snow concluded that this was the first study to use geometric morphometrics to exhibit sexual dimorphism between male and female scapulae, and reported that his analysis indicates that shape differences in ancestry are present (44). This early research clearly displayed the potential of digitized geometric morphometrics and indicated the need for further study of the scapula.

A thesis project from the Boston University School of Medicine set out to test sex assessment through metric, non-metric, and geometric morphometric analysis of the distal humerus (38). This study used a Microscribe digitizer to collect 28 points on the distal ends of

227 humeri from the William M. Bass Donated Skeletal Collection at the University of Tennessee (38). The analysis of this study was particularly interesting because the author ran statistical analyses on all 28 landmarks together, only the posterior landmarks, and only the anterior landmarks (38). This type of analysis could help in future work when presented with fragmentary remains. The results of these analyses were not much different from each other; males and females were classified correctly anywhere from 50-70% of the time, which are relatively low classification rates (38). Overall, the author determined that the geometric morphometric analysis was not more accurate than the metric and non-metric methods; although it did show some shape differences and has potential for future research (38). The author stated that her inexperience in using geometric morphometric techniques most likely contributed to the overall low results of the geometric morphometric analysis and declared that more research was needed to determine which landmarks are most useful in shape analysis of the distal humerus (38). This is a significant preliminary study in the use of geometric morphometrics and emphasizes the need for further research and experience in the data collection process.

A study by Bytheway & Ross in 2010 looked to determine how well the pelvis, when digitized, could determine sex (19). The authors used 200 left os coxae from the Terry Collection housed at the Smithsonian, and collected 36 landmark points per os coxa (19). The authors found that the landmarks they wished to collect did not fit into the traditional classifications of type I, II, or III (an explanation of these landmark types can be found in the Materials and Methods section); so they revised classifications and descriptions to create categories such as "constructed" or "traditional fuzzy" landmarks (19). The "fuzzy" landmarks are considered to be areas of the bone, rather than one point, and are found and collected by following the author's description of the point (19). Overall, the authors found that by using geometric morphometric analyses on the pelvis, they could determine sex 98-100% of the time (19). This is an incredibly high rate of accuracy and is consistent or better than traditional methods (generally 90-95% accuracy (45)) to determine sex using the pelvis. The authors concluded that the pelvis is, as expected, a highly accurate element to use to determine sex; they also determined that geometric morphometrics is a reliable and accurate method to apply (19). The geometric morphometric analysis was able to display the direction of shape change between males and females in different areas of the bone, information that is not available via traditional analytical methods, and has great implications for identification in fragmentary contexts (19). The authors also point out that the greatest amount of shape variation was found in the pubic and ischium bones, as well as the connecting areas of the illium (19). This study did not perform an evaluation of intraobserver error on data collection, however, so it is unknown how the "fuzzy" landmark points would perform in further use of this method and the authors state their intention of further research concerning this subject (19).

Most other studies using the pelvis to determine sex focus on morphoscopic and visual index methods. One of the earliest metric analyses of the pubic bone was a thesis project by Tiffany Burch in 2003. Burch measured the pubic width on the dorsal side of the pubic bone, measuring the width between the medial edge of the pubic symphysis to the margin of the obturator foramen (46). The author's samples consisted of 110 males and 50 females which had been collected at autopsy from the Department of Coroner, Los Angeles (46). This collection was originally curated by Dr. J. Suchey and used to develop the widely used Suchey-Brooks method to age skeletal individuals based on the pubic symphysis. Using samples collected from autopsy offers the advantage of using a completely modern sample of known age, sex, and ancestry. The author only used individuals 17-30 years in age, and later further narrowed this field to 21-30 years old because younger females tended to classify incorrectly (46). The author averaged the width of both right and left pubic bones for each individual, and then found sectioning points to distinguish between male and female (46). The highest correct percentage of classified males and females, aged 21-30 was 91.4%(46).

This is a high classification rate and is at least on par with traditional morphoscopic methods. While this project did not complete a 3D analysis, a shape analysis, or use more than one measurement or landmark, it is one of the only metric analyses of the pubic bone in an attempt to determine sex. Burch is able to successfully show that the pubic bone is metrically sexually dimorphic and that its size and shape should be further studied (46). The author also utilized a sample collected from autopsy, although she utilized this sample almost 30 years after it was originally collected (46). This gap in time may influence the results applicability because the sample is not completely contemporary, although this would need to be tested. To improve on this method, this dissertation research will utilize a contemporary sample taken from autopsy to improve on the forensic applicability of the results.

The third method of data collection discussed above consists of using landmark points in conjunction with points that are placed after measuring evenly-spaced distances across a bone. The study by Franklin et al. set out to use geometric morphometrics to determine sex of subadults based on the mandible (3). The study consisted of 96 subadult mandibles of known age, sex and population, pulled from the Hamann-Todd Osteological Collection, the Raymond A. Dart Collection of Human Skeletons, and the Natural History Museum of London (3). The authors collected 38 bilateral points with a Microscribe digitizer (3). Most of the landmark points collected were traditional, however the authors did add evenly-spaced points along the posterior ramus and the mandibular body in an effort to more accurately capture the shape of the entire mandible (3). While the authors were not able to significantly determine differences between males and females, they did observe some shape differences which are promising for future research to expand on. They also reported finding more significant differences between populations, which indicate that subadult mandibles might be able to be classified by ancestry, which could be quite useful in forensic contexts (3). This study also emphasizes the usefulness of a digitizer and the resulting shape analysis it can provide (3).

Another method to utilize landmark data is to study landmark measurements through 3D Computed Tomography (CT) scans. One project used 100 randomly selected CT scans of *in situ* pelves to test the reliability of landmark distance measurements and the traditional Phenice traits based on CT scans (47). The advantages of using modern CT scans are that the sample consists of modern, living people, the researcher does not need access to the actual bone, and a computer program is used to identify and complete all measurements meaning there is less subjective error. The disadvantages of using CT scans usually involve obtaining access to the scans themselves. It can be very difficult to obtain access to current medical records of living people due to the many privacy laws currently in use. A researcher must also have access to the computer software necessary to manipulate CT scans and gain accurate results. The authors of this study however were able to reach results of 100% accuracy in male and female determination (47). This is astonishingly high and further supports the fact that using the pelvis is the best way to determine sex and supports a 3D metric analysis of the pelvis. While not all future researchers will have access to modern medical records, this research clearly indicates need for further research into 3D shape analysis, specifically of the pelvis because of the incredibly accurate results achieved (47).

Many research projects focused on the pubic bone study how to improve age estimations of skeletal remains (8,9). The pubic symphysis is one of the best age indicators on the human skeleton, so understandably, most research focuses here. An example of this which uses a somewhat different method of 3D shape analysis is a thesis by Gray (2011). Here, the author uses 3D laser scanning which is similar to using a CT scan to perform a shape analysis, however different measurements are obtained from a laser scan (48). The author attempted to determine age from the symphyseal face of the pubic symphysis on a sample of 40 male pubic bones (48). Gray used 3D laser scans of the symphyseal face to quantify and measure seven morphoscopic traits from the Suchey-Brooks method of aging the pubic symphysis (48). The results showed that rim completeness, billowing height and area, and depth of symphyseal face depression were most closely correlated with correct age (48). While projects like this are continually improving the precision of aging human skeletons based on a small portion of the pelvis, there is a gap created which ignores how to determine sex from the same small portion of bone. Continued 3D studies show that this is an accurate and reliable field to further study, and there is a clear need to improve aspects of sex determination within this field.

Conclusion

The human pelvis is a widely studied element within forensic anthropology and has been used as the best sex indicator for many decades (7,21,22). It is also known that the pelvis can predict age as well as sex, although it is always important to use other methods in tandem when possible (29,49). Although the current literature can conclude no true correlation between pregnancy and alterations of the pelvis, it is known that some pregnancies can cause changes, and future research may be able to fully establish what a pelvis could reveal about parturition (30,35). Most traditional methods used on the pelvis to conduct these analyses consist of visual observation and analysis however, and it has become more necessary over time to create potentially more accurate metric methods to estimate this information for unidentified skeletal remains (10). Due to this need, geometric morphometrics has become increasingly more popular in the field of forensic anthropology, but there is still a gap visible in the existing metric studies of the pelvis.

It is clear based on these previous studies that more research in the field of geometric morphometrics is necessary. Researchers are still exploring the best methods to use within this field and many of these studies indicate that geometric morphometrics has great potential (50). It is also clear, however, that most of the larger projects have focused on sex and ancestry based on the cranium (1,43). The projects which do focus on the pubic bone mainly investigate changes in morphology due to age (48,51,52). This means that more research on the postcranial skeleton is definitely needed, and that more research and literature on shape differences in the pelves of males and females is needed (7,50). This dissertation will draw from aspects of past research, including data collection methods, certain landmarks, and statistical analyses in an effort to improve upon existing research, as well as to improve standardization throughout geometric morphometric studies.

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Chapter Three: Materials and Methods

The methods of this research derive from those within the field of geometric morphometrics. At its most basic definition, geometric morphometrics is the analysis of shape (1). The basic process of this consists of recording multiple homologous shapes (multiple pubic bones), rotating them all onto the same plane and removing size as a variable (Generalized Procrustes Analysis), and finally distinguishing differences between group clusters (Principal Components Analysis, etc.) (1). This research will use a Microscribe Digitizer to collect 3D points on multiple collections of pubic bones. This data will then be analyzed using the computer software MorphoJ to identify differences in shape between male and female specimens.

Hypothesis: by using 3D geometric morphometric analysis, differences in the shape of female and male pubic bones can be identified and used to classify unknown individuals as either male or female.

A pilot project was conducted on a small test sample of pubic bones and preliminary statistics were run on the data gathered. The initial results are promising for this research overall and indicate that the method and hypothesis will be successful when tested on larger sample sizes.

It is well known within anthropology that when developing methods which are to be applied to modern humans, the samples used should also consist of modern humans. In order for this research to be forensically relevant, it must be based on a modern skeletal collection (2,3). There are a series of modern human skeletal collections in the United States, the most popular being the William M. Bass Donated Skeletal Collection at the University of Tennessee, the

Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History, and the Terry Collection at the Smithsonian. These collections consist of modern humans of known sex, ancestry, age-at-death, and sometimes profession. Most studies within the existing literature use samples from one or more of these collections (4–9). This research will use the University of New Mexico Maxwell Museum Documented Collection, as well as the University of Montana Skeletal Pubic Symphysis Collection, both of which consist of modern, documented individuals.

Materials

The Samples: The University of New Mexico Maxwell Documented Skeletal Collection

The primary sample will consist of individuals from the University of New Mexico Maxwell Museum's Documented Skeletal Collection. This collection was established in 1984 and now has over 300 individuals of both sexes, varying ages, and many population groups (10). The known information for most individuals within this collection include sex, age-at-death, population affinity, and cause of death (10). All donations after 1995 have also been asked to provide past health information as well as occupation information so that more research using the collection can be conducted (10). This is one of the best modern collections in the western United States and is particularly important to forensic anthropology because it consists entirely of individuals who have passed away within the last 50 years (10). The sample as a whole is made up of 60% males, most of the males and females are aged 51-75, and 80% of the total sample identified as White (10).

The Maxwell Documented Skeletal Collection has been chosen as the primary sample for this research for multiple reasons. First, it is one of the most modern skeletal collections in the United States. It is important to develop new methods using a contemporary collection so that the method(s) is more likely to be accurate when used on modern forensic cases (11). This helps to avoid biases that may be introduced by secular change in a population. Along with this, it is also important to develop new methods using a collection that derives from the ancestry affiliation of which it will be used on in the future (11). This is not always possible, especially when modern admixture and self-identification are taken into account, but it is always important to record ancestry when available and to notice any trends or correlations that may arise. The Maxwell Documented Collection is identified as 80% White, which will correlate well with any sample recovered in Montana.

"White" can mean many things, and a White sample from New Mexico will likely be quite different from a White sample in Montana, but it is at least a similar overall characteristic that can be used to combine and contrast the two samples. It is important to remember that the ancestry classifications are self-reported, which means they represent which group of people each individual identified with, and not necessarily where their genetic ancestors came from. In the U.S., as with many other places, "White" has become an umbrella term for people with light skin, who usually reside in a middle to high socioeconomic class, and who might recognize having ancestors from Europe. In reality, white is a color and not an ancestry, meaning that the genetic ancestry of people who identify as "White" could vary drastically. It has also been shown that those who are multiracial, or identify with multiple ancestries or ethnicities, often change their identification over time, and usually choose to list a single ancestry rather than multiple ones (12). Self-reported information on ancestry may not be the most reliable, however it does create a group basing that can still offer important information when compared and contrasted.

Lastly, the Maxwell Collection offers a good sample for this research because most of the individuals within the collection are 51-75 years old (10). Only individuals 21 and older will be considered for this research to avoid morphologically indeterminate juveniles. Determining the sex of a subadult human skeleton is nearly impossible, and it is generally considered bad practice to do so within forensic anthropology. An overall older sample will provide more individuals and hence more data for the current research.

The Samples: The University of Montana Skeletal Pubic Symphysis Collection

The second sample for this research will be curated throughout the duration of the project. The Medical Examiners at the State Crime Lab in Missoula, Montana have agreed to procure the pubic bodies from recently deceased individuals who receive autopsies. Families of the deceased are asked to fill out a consent form to allow for the donation of the pubic bone portions to the University of Montana. Through the use of this form, the family has the chance to deny or allow donation, offer demographic information about the deceased, and offer access to the deceased's medical records. The collection process is simple; during autopsy, the medical examiner makes four cuts using the bone saw, one on each ramus of the right and left pubic bone, to easily remove the two pubic bodies around the pubic symphysis. The bones are then soaked in hot water, bleach, and soap, and left to dry. Once dry, the procured samples are transported to the University of Montana Anthropology Department, where they are curated into the existing skeletal collection. Samples will be collected from all possible individuals aged 21 and older. Younger individuals will not be included in this sample to avoid the ambiguities between male and female juvenile skeletons.

Samples will ideally be collected for two years, after which, sample collection will stop so that analyses can proceed. The author hopes to collect data points from the samples throughout this time frame, so that analyses can occur immediately after the collection process ends. The overall size of this sample depends on the availability of remains, which in turn depends on the autopsy rate in Missoula, as well as the willingness of families to donate tissue of the deceased. In a perfect world, after two years, there would be at minimum 200 individuals included in this sample; whether or not that number will be reached remains to be seen. Whatever the size of the sample at the end of this project, the individuals curated will still contribute to this research by determining whether or not they fit within the new method based on the Maxwell Museum sample. The author will also consider this collection process as an exercise in learning how to curate a new collection and how to work with multiple agencies in the process of sample procurement. It is the hope of the author that regardless of the size of the collection at the end of this project, the curation can continue through other individuals at the University of Montana in order to continue building an irreplaceable collection which will result in further research.

Equipment

The most important piece of equipment needed for this research is the Microscribe Digitizer. This consists of a stylus at the end of a moveable arm, which is attached to a heavy base which keeps the digitizer in place. The digitizer also comes equipped with a foot pedal to ease the recording of data, and all necessary cords to connect to the research computer. The use of the digitizer is simple, one must simply place the point of the stylus at the point which is to be recorded, and simultaneously click the foot pedal to record the data point. This data point is automatically entered into an Excel Spreadsheet. Once data collection is finished, the data can be imported into other software, such as Past or MorphoJ. This software is necessary to perform further statistical tests, such as a Generalized Procrustes Analysis, a Principal Components Analysis, and a Canonical Variates Analysis.

Other necessary equipment includes a vice to hold each individual sample in place while data recording occurs. In order to achieve the necessary stillness, a small, rubber clamp was set into a larger, steel vice. The steel vice holds the clamp quite still, while still allowing the clamp to be easily opened and closed to hold each bone. A crockpot, bleach, soap, and water are also needed to clean each bone sample once it has been removed during autopsy. These materials are readily available in the Medical Examiner's office, and once the samples are clean and dry, they are placed into individual bags and boxes to be transported to the University of Montana. Once at the university, each individual is assigned a number to ensure anonymity during data collection and analysis.

Methods

Background: Geometric Morphometrics

Geometric morphometrics has been used in a variety of fields for some time now and is a relatively new method within anthropology. Biology, on the other hand, has utilized geometric morphometrics for many years and quite a few interesting studies have been published using variations of the methods behind geometric morphometrics. As in anthropology, biological studies have also used photography, outlines and semi-sliding landmarks, as well as Microscribe Digitizers to collect 3D data (13–17). Geometric morphometric data can also be used in simple or complex ways, depending on how many traits are being observed and what kind of phylogenetic or environmental traits they are being compared to (17). This section will outline a sample of the various types of research within biology that have used the methods of geometric morphometrics to inform on larger evolutionary questions.

Neosauropod dinosaurs lived during the Jurassic period, were herbivorous, and exhibited the extreme end of gigantism (18). A 2007 study on these giant dinosaurs used geometric morphometrics as one of their methods to research long bone scaling patterns (18). The authors set out to test the hypothesis that neosauropod long bones scaled isometrically as well as to investigate any paleobiological implications of these trends (18). Previous studies have indicated that as neosauropods age, their long bones grow isometrically, meaning that no significant shape change occurs through growth (18). This is somewhat surprising, considering the enormous weight and size gain each individual experienced aging from juvenile to adult (18). Supporting previous studies with more accurate geometric morphometric data would indicate that adult neosauropods were quite limited in their locomotive movements and speed (18). The authors also looked for any indication of allometric growth, which would mean that different body parts grow at different rates. The samples used consisted of femurs and humeri and represented six neosauropod taxa; both geometric morphometric and distance-measured data were collected on all samples (18). The geometric morphometric data was collected through photography and computer software which identified landmarks on each fossil (18).

After many multivariate statistical tests, the authors found that their data did support the hypothesis that neosauropod long bones grew isometrically and did not exhibit a pattern of allometry (18). Based on this data, as well as previous studies, and other observations (such as the fact that neosauropod dinosaur long bones did not have a medullary cavity), the authors hypothesize that with increasing mass, it might have been necessary for neosauropods to shift more weight support to their limb bones and away from their limb muscles (18). This, along with possible behavioral adaptations which could have included avoiding strenuous physical activity, could have resulted in the lack of muscle "sculpting" exhibited in the long bones and the observed isometric growth (18). It is likely that neosauropods were simply highly efficient walkers, and that they did not change their locomotive patterns as they grew from juvenile to adult (18). This research is a great example of how geometric morphometric analysis can help to increase accuracy and further inform on existing research.

A later study on theropod dinosaurs, in 2012, used geometric morphometrics to help answer remaining questions concerning macroevolutionary patterns of their cranial morphology and how these may or may not relate to their feeding patterns (13). Theropod dinosaurs existed for over 160 million years, and included the famed *Tyrannosaurus* rex. A long-standing question in all evolutionary fields is whether or not phylogenetic constraint or functional adaptation is more important in shaping species morphology. The authors used landmark-based two-dimensional geometric morphometrics to complete their analysis (13). The authors attempted to identify 24 type one and type two landmarks on each of the 51 specimens (one specimen per species) available for study, although, because of incomplete fossils and missing data, they were forced to create two data sets (13). The first included 26 species on which all landmarks could be recorded, and the second included 36 species for which a reduced set of 13 landmarks could be recorded (13). The second set maximized the number of landmarks while still including representatives from all major subclades of nonavian theropod dinosaurs (13). The landmarks were recorded through two-dimensional photography and computer software, including MorphoJ (13). The authors then ran a series of multivariate statistics, including a Generalized Procrustes Analysis, and multiple Principal Components Analyses, to compare the landmark data to bite force and speed data, as well as phylogenetic data (13).

Through their analyses, the authors found that theropod crania differ primarily in relative antero-posterior length and snout depth, and that "oviraptorosaurs deviate most strongly from the 'typical' and ancestral theropod morphologies" (13). They also found that large-bodied carnivores independently converged on the same region of morphospace, while noncarnivorous taxa generally fell out in distinct regions of morphospace and exhibited greater overall disparity (13). Morphospace is considered the region in which landmarks from the crania are plotted into, along with information about diet and environment, and which can then offer information about broad patterns based on crania shape (13). Overall, the distribution of taxa in morphospace was strongly correlated with phylogeny but only weakly correlated with biting and feeding behavior (13). The authors conclude that phylogeny and not adaptive feeding behavior was the major determinant of broad patterns in the skull shape of theropod dinosaurs (13). This research is a good example of how geometric morphometrics can inform on larger evolutionary trends in morphology and help to unravel remaining mysteries. The authors indicate that more research will continue as more data, including possibly 3D techniques, become available (13).

A 2007 study on East African cichlids also set out to answer the question of whether or not phylogeny or adaption held a stronger sway on shape change over time (14). East African cichlids are famous for their intense variation and explosive speciation and offer great opportunities for studies on evolution, diversification, and adaptative radiation (14). The authors used over 1,000 specimens, which represented 45 species and 14 of the 17 major fish tribes in Lake Tanganyika (14). Digital pictures were taken of the profile of each fish, and 17 landmarks were then digitally recorded from each photo (14). The authors also coded each fish species for its preferences of habitat, such as preferred water column depths, preferred substrate (mud, sand, rock, etc.), feeding preferences and type of prey, the type of parental care given within the species, the mating system (monogamy, polygamy, etc.), and it's breeding type (mouthbreeders or substrate guarders) (14). The phylogenetic analysis of each fish species consisted of mitochondrial DNA sequencing and comparison (14). All of these types of data were then compared to each other and run through statistical analyses to determine whether the adaptive characteristics or the phylogenetic information was more influential to the shape changes among the cichlids (14).

The authors found that the influence of phylogeny on the evolution of shape in the Lake Tanganyika cichlids is small and that shape is much more influenced by ecology and habitat adaptation (14). The authors were able to conclude that ecology plays a very large role in generating morphological diversity (14). These results are different from the study on Theropod dinosaurs which concluded that phylogeny was the more important factor (13). These varying results are probably due to the fact that the research on Therapods consisted of animals which spanned millions of years, while the research on cichlids consisted of fish that have relatively recently speciated (13,14). Both studies display the informative power of geometric morphometrics and how the method can help to further understandings of evolution, shape change, phylogeny, and habitat adaptation.

Attempting to determine sex through the use of geometric morphometrics is not unique to research on *Homo sapiens*; a recent study on neon flying squid (*Ommastrephes bartramii*) also set out to find shape differences between males and females, as well as to attempt to differentiate between stocks of the squid within the northwestern Pacific Ocean (15). This species of squid is fished annually as a good source of protein for human consumption, and management of the species and the two stocks is important to ensure the continued survival of the species for the ocean ecosystem as well as a source of food for people (15). The researchers investigated the shape of the squids' upper and lower beaks, as well as the pigmentation levels of the beaks to see if these characteristics could be used to determine sex and/or which stock (eastern or western) they originated from (15). The upper and lower beaks of 216 random *O. bartramii* were digitally photographed, and then 8 landmarks on the upper beak and 10 landmarks on the lower beak were digitized (15). Semi-sliding landmarks were then added to both upper and lower beaks of all specimens (15). The authors used a series a multivariate statistics to analyze the shape change among the beaks, including a Generalized Procrustes Analysis, a Principal Components Analysis, a MANOVA test, and thin-plate spline grids (15).

The results showed that the upper and lower beak shapes differed significantly between the western and eastern stocks of squid, and could be used to differentiate between the two (15). The authors also found that the degree of pigmentation of the upper beak changed between the stocks, adding a second indicator to which regional group they originated from (15). The only shape differences between sexes the authors could identify occurred in the western stock of squid, and could not be used to differentiate males and females on random squid from both stocks (15). The authors hypothesize that these results occur due to the variation of feeding habits between the two stocks of squid but the sharing of a similar habitat by both sexes (15). Shape differences between the sexes could not be identified in this study, however, the authors indicate that further research will be done in an effort to determine the best way to differentiate between the sexes using other parts of the squid (15). This research is a great example of how geometric morphometrics can be used across multiple species in an effort to learn more about shape differences in males and females as well as different geographical groups.

A very early study (2000) used a Microscribe Digitizer to determine how high in taxonomic orders geometric morphometrics could effectively be used (16). The authors explain that most geometric morphometric research focuses on one species, or perhaps one genus or family (16). To test how much variability could be effectively studied through geometric morphometrics, the authors digitized 23 skulls all representing different ordinal mammalian groups (16). The authors chose 35 landmarks to collect on each skull, the identification of which proved to be quite difficult considering the vast variability among mammalian crania (16). Rather than using the word “homologous” to describe all of the landmarks between each species, the authors defined their landmarks as “equivalent” (16). TPSSMALL and Morpheus computer software was then used to analyze the Procrustes distances and the tangent space distances of the data (16).

The authors found that the tangent space analyses were decent representations of the Procrustes distances, however when the data was used to create a phylogeny, it displayed almost no consensus with current (2000) morphological phylogenies (16). The authors conclude that this study included too much variation for geometric morphometrics to be completely useful (16). The essential shape analysis was correct; however, the data was unable to create any useful comparison of phylogeny (16). The authors also explain that part of this is likely due to the difficulty of choosing and identifying landmarks on such variable crania (16). This study is a good example of researchers testing the boundaries of geometric morphometrics in biology as a whole to show that the method is valid but must be applied to the correct type of specimens with less variation among them.

It is also quite common for geometric morphometrics to be used in tandem with other methods, such as earlier when pigmentation in squid was analyzed alongside their beak shapes (15). Another recent study used geometric morphometrics to study the evolutionary patterns and trait characteristics of ground squirrels (*Marmotini*) (17). The authors compared data sets of body size, and cranial, mandibular, and molariform tooth shape to analyze variation, covariation, and disparity patterns in a phylogenetic framework (17). A mitochondrial DNA dataset was used for the phylogenetic analysis and comparison, while geometric morphometric data was used for the rest of the analyses (17). The authors used a Microscribe Digitizer to collect 50 3D landmarks from 136 specimens, representing 65 marmotine species (17). After these landmarks were digitized, computer software was used to add semi-sliding landmarks along the midline of each cranium (17). The authors also used a previously collected dataset of 2D landmarks to gather information on molariform tooth shape and mandibular shape (17). This dataset consisted of 58 marmotine species and also included regular landmarks and semi-sliding landmarks (17). Multivariate statistics, such as GPA, PCA and MANOVA were used to compare and analyze all of this data together (17).

The authors found strong correlations between body size and cranial traits, and that evolutionary modes were concordant across traits. They also found "divergent dynamics on the macroevolutionary landscape, with phenotypic disparity being differentially shaped by convergence and conservatism" (17). These findings reiterate the mosaic nature of morphological evolution and indicate that the evolution of ground squirrels is poorly captured by single process descriptors (17). This research shows that when researching evolutionary patterns, morphological traits should be studied in groups, against multiple phylogenetic and environmental factors, rather than a single-trait approach. It also demonstrates how different types of geometric morphometric data can be used together to help inform on many evolutionary patterns and can be used for many types of research, no matter how simple or complex. The success of these research projects using geometric morphometrics, and the ability to show shape change within and between species, indicates that this type of analysis should yield useful information when applied to male and female human pelves.

Landmarks

For this research, landmarks are defined as a specific point on bone that can be located on each sample and subsequently recorded in 3D space (19). A preliminary list of landmarks was used in the pilot project stage of this research and can be found in Appendix A. These preliminary landmarks were chosen based on previous research, traditional landmark location, and some new landmarks were chosen by the author (6,20,21). New landmarks were chosen in an effort to improve existing landmarks and overall shape analysis. When collecting data on landmark points, whether it is collected with a digitizer or through the use of a camera, it is important to distinguish which type of landmark it is. Landmarks can be categorized as type I, II, or III (1,2). Type I landmarks are considered the easiest to find and consist of one single point on the bone where tissue transitions, such as an intersection of sutures (2,6,22). Type II landmarks are considered the points of maximum curvature or greatest muscle attachment, an example of this would be the euryon on the cranium (2,22). Type III landmarks are the most extreme points of a structure overall, sometimes labelled as the "posteriormost" or "anteriormost" points (2,6). A category that has since been added to this list by subsequent studies is constructed landmarks, which is defined as "points corresponding to locations that are defined using a combination of traditional landmarks and geometric information" (4). For example, calculating and using the midpoint along a line of maximum width as a landmark. Most studies using geometric morphometrics distinguish which types of landmarks are used to help determine which types of points are most useful or which points introduce the most error (2,4,22). Sliding semi-landmarks were not used in this study because when morphological variation is small, such as that between modern humans, the differences between a landmark-based study and a sliding semi-landmark based study using the same sample can be altered or skewed, based on differences in initial alignment; thus raising questions about any results obtained by using sliding semi-landmarks (23). Throughout the pilot project, the author tested the preliminary landmarks to determine if they were easily located and if they accurately captured the shape of the pubic bone (see Appendix A for landmark locations). These preliminary tests also helped the author determine which type of landmark is most effective, and if types II, III, and constructed landmarks could be easily replicated. The results of these tests, and the explanation of the final set of landmarks can be found in a later section.

Statistics

Once all data is recorded, a series of statistical analyses will be run in order to identify what, if any, differences exist between the shape of male and female pubic bones. For the purpose of geometric morphometrics, shape has been define as "all the geometrical information that remains when location, scale and rotational effects are filtered out from an object" (19,24). The first statistical step of every geometric morphometric project is a Generalized Procrustes Analysis, or GPA. Before any analysis is completed, the landmark coordinates include information on size, shape, position and orientation (25,26). In order to analyze just shape however, all of this other information must be excluded (26). A GPA eliminates the non-shape variation, and rescales and rotates each sample so that they are relative to each other in the same plane (1,26). By doing this, the GPA translates all landmark configurations to the same centroid, scales all configurations to the same centroid size, and iteratively rotates all configurations until the summed squared distances between the landmarks and their corresponding sample average is a minimum (26). After the GPA is run, all configurations are super-imposed on each other, and the resulting coordinates are called Procrustes shape coordinates and only include information about the shape of the configurations (26). This statistical step alone does not offer a lot of useful information, but rather, it prepares the data to be analyzed further in ways that will offer more information about the shapes that are present.

The next step is to run a Principal Components Analysis, or PCA. A PCA is a way to represent the variation that is present within the sample, and the goal of a PCA is to determine which variable introduces the most amount of variation (19,27). When the PCA is run, it creates a covariance matrix which shows the principal components of the data, and from this, the eigen vectors of the present variation (19). The principal components provide a means of comparing the relative importance of each dimension of the data (19). If the first two eigen vectors represent 50% of the variance, or more, then the test can be considered successful. The PCA also offers a scatter plot, which shows groupings or clusters and outliers (28). The PCA scatter plot is very useful in determining whether or not the variation in the data is being explained by the variable in question. To do this, the researcher can see on the plot which specimens are grouping together and can then go to those specimens to see what they have in common. Vice versa, the researcher can identify the specimens on the plot which graph far apart and can then identify the differences between the physical specimens.

Further statistical tests will be explored once data collection at the Maxwell Museum is completed. These future tests include Discriminant Function, MANOVA, and independent group t-tests of centroid sizes. At this point, it will be determined which statistical analyses will yield the most useful information concerning the differences between male and female pelves.

The Pilot Project

The goals of the pilot project stage of this research were to both familiarize the author with geometric morphometric data and the Microscibe Digitizer equipment, as well as to indicate whether or not this specific approach to sexing a skeleton would work. It was also used to refine the methods and landmarks used in order to lessen error when the larger datasets are collected. The project used specimens from the University of Montana Forensic Anthropology Teaching Collection and consisted of 14 individuals, 9 males and 5 females, with a total of 24 pubic bones available. There is no known provenience information for these individuals. 13 data points were collected on each pubic bone using the Microscribe Digitizer. These points in space were recorded in an Excel Spreadsheet, and then transferred to MorphoJ, a software program designed to analyze 3D data.

Limited analysis was conducted on this data because of the nature of a pilot project, in that it should only be used as an early indicator and test for what should follow when larger data sets are analyzed. Generalized Procrustes Analysis and Principal Components Analysis were the main statistical tests run on this preliminary data and showed promising results. The PCA Eigen values clearly showed more than 50% of the variance within the first two principal components, which indicates a successful test (see Appendix B for graph). The scatter plot of the PCA displays a tight cluster in the middle, which consists of all male specimens, except for one female. The rest of the female specimens are scattered throughout the rest of the graph (see Appendix B for graph).

These results are promising because the male specimens are clearly clustering together, which indicates that sex is explaining most of the variance within the sample. The largest question is why the female specimens did not form a separate cluster. This may be answered by the fact that females tend to undergo an intense form of trauma which alters the pelvis in unknown ways, pregnancy and child birth (29). The lasting changes and effects of pregnancy on the female pelvis is still poorly understood and it seems as though pregnancy affects different women in different ways (29,30). There is no way to know if any of the female individuals included in this preliminary research had ever been pregnant or given birth, but if some of them had while others hadn't, it may explain why they do not cluster together. It is also possible that age may be explaining some of the variance in the sample. It is known that age affects shape change in the pelvis, and it is hard to tease that out of this preliminary data, considering how small the sample size is (21,31,32).

Intraobserver error was also tested for this data to determine how consistently the author was recording landmarks. Unfortunately, the average standard error was 0.79, and overall error ranged from 0.5-1.1, all of which are relatively high error rates. Some error can be explained by inexperience of the author, which should improve with time. To help lower error further however, the author determined which landmarks had the lowest and highest error rates. Landmarks 1, 2, 4, and 13 (see Appendix A for landmark locations) displayed the lowest error rates. Landmark 1 is the pubic tubercle, and 2, 4, and 13 are all landmarks which are found by identifying maximum lengths, which means these are all quite easy to find. The landmarks with the highest error rates (landmarks 5, 6, 7 and 8) were constructed landmarks, and proved quite difficult to locate. In order to lessen overall error rates, the landmarks which proved to be the most difficult to locate have been removed and the definitions of the easier, remaining landmarks have been refined (see Appendix C for final list of landmarks).

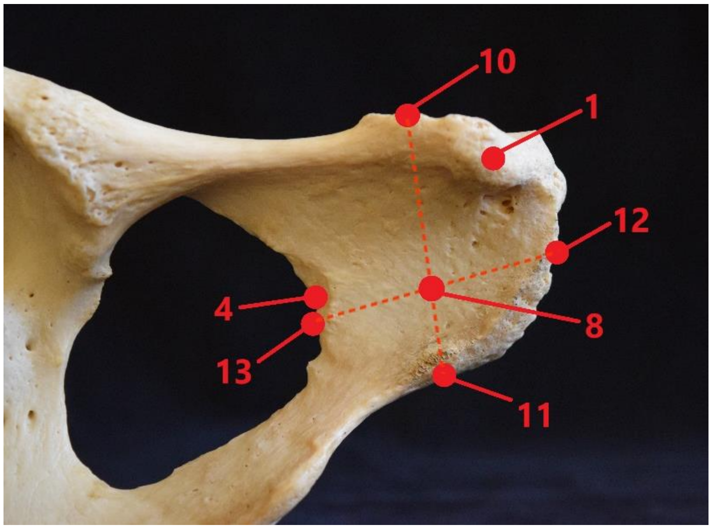
It is also important to discuss the sample biases present in this pilot project and address how they may be controlled for in future research. The largest bias is the small sample size, which will easily be remedied by simply gaining access to larger collections, such as the

Maxwell Museum. This sample also included almost twice as many males as females. While this is hard to control considering researchers generally want to use as many specimens as is possible, it may be prudent to exclude some in some analyses to determine if evening out males and females affects the data in any way. More than likely though, once the sample size reaches a respectable number, the number of males and females will be quite close to each other and should not present further issues. The individuals within this pilot research are also likely to have varying ancestries, which can introduce variation in unknown ways. Knowing the ancestry of individuals is one of the large advantages when using a documented collection. The question of ancestry will be easily addressed through the curation of known individuals within Montana and the use of the documented collection at the University of New Mexico. Once the ancestries are known, individuals can be grouped according to ancestry to determine whether or not ancestry affects the overall sex determination process. It will also be known whether or not the female specimens were ever pregnant, which may help to explain the wide variation that is already being observed between female individuals. Using samples that include known provenience and health backgrounds will vastly improve the information offered by future research based on this preliminary study.

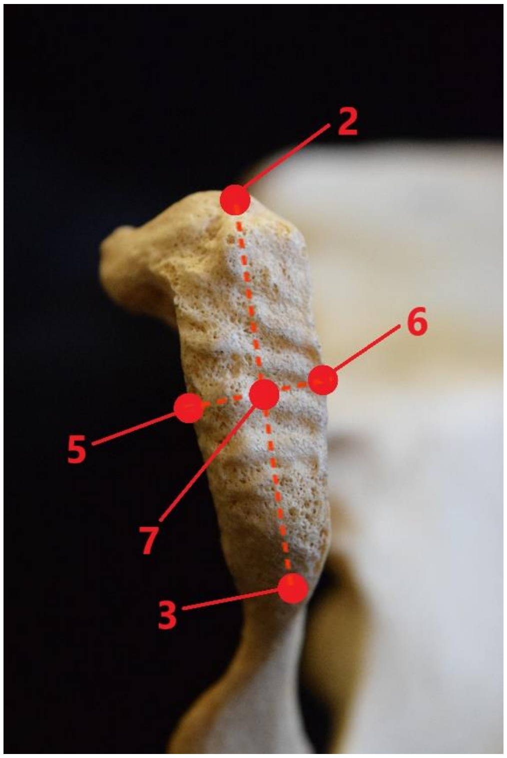
Overall, the pilot project met the original goals of the researcher. Experience and familiarity using the equipment and software was gained and will hopefully be evident in lower error rates as future research occurs. The author was also able to refine the process of gathering data and narrow down the landmarks which should be most useful in attempting to determine sex of unknown individuals. Most importantly, the preliminary data offered promising results that at least male specimens may cluster together and lead to a sex differentiation among larger sample sizes. The pilot project acted as a very important learning experience and test for the larger dissertation research that will follow and should prove to have been one of the most important steps in creating a new method to metrically sex unknown human pelves.

Appendix A: Preliminary Landmark Descriptions and Types

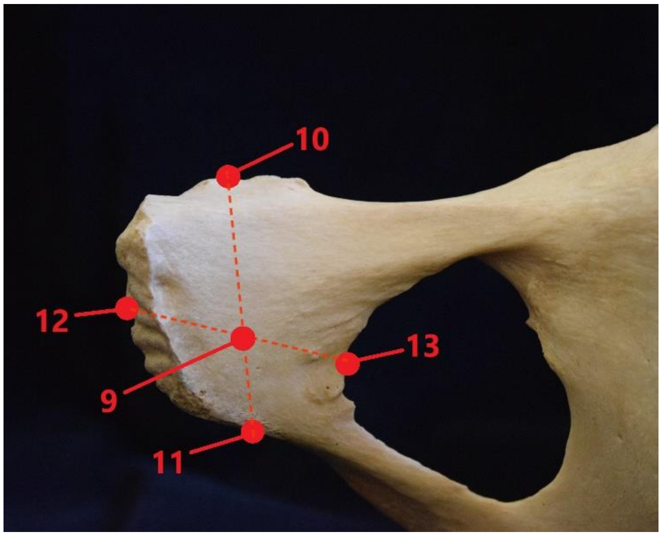
Number	Landmark	Description	Type
1	Pubic Tubercle	Most prominent point of the pubic tubercle	Traditional (II)
2	Superior Pubic Symphysis	The most superior point of the pubic symphysis	Extremal (III)
3	Inferior Pubic Symphysis	The most inferior point of the pubic symphysis	Extremal (III)
4	Lateral Border	Point on the lateral border of the pubic body which would create the maximum breadth of the obturator foramen	Extremal (III)
5, 6	Symphysis Width	The anterior (5) and posterior (6) points which create the maximum width of the pubic symphyseal surface	Constructed
7	Pubic Symphysis	Midpoint of the pubic symphysis; found by determining both the maximum length and width, point is at their intersection	Constructed
8	Anterior Surface	Midpoint of the anterior surface; found by determining both the maximum length and width, point is at their intersection	Constructed
9	Posterior Surface	Midpoint of the posterior surface; found by determining both the maximum length and width, point is at their intersection	Constructed
10, 11	Pubic Body Height	The superior (10) and inferior (11) points which create the maximum height of the pubic body	Constructed
12, 13	Pubic Body Width	The medial (12) and lateral (13) points which create the maximum width of the pubic body	Constructed



Anterior Surface,
Right Pubic Bone

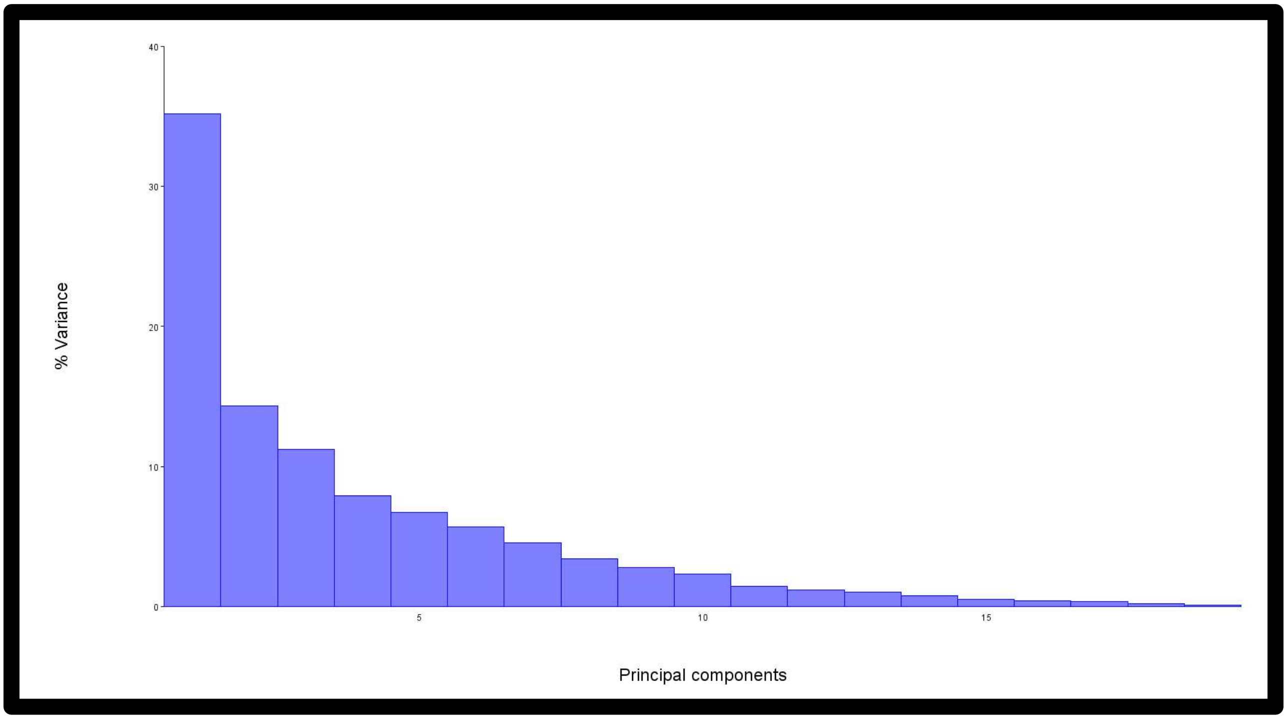


Sympheseal Surface,
Right Pubic Bone

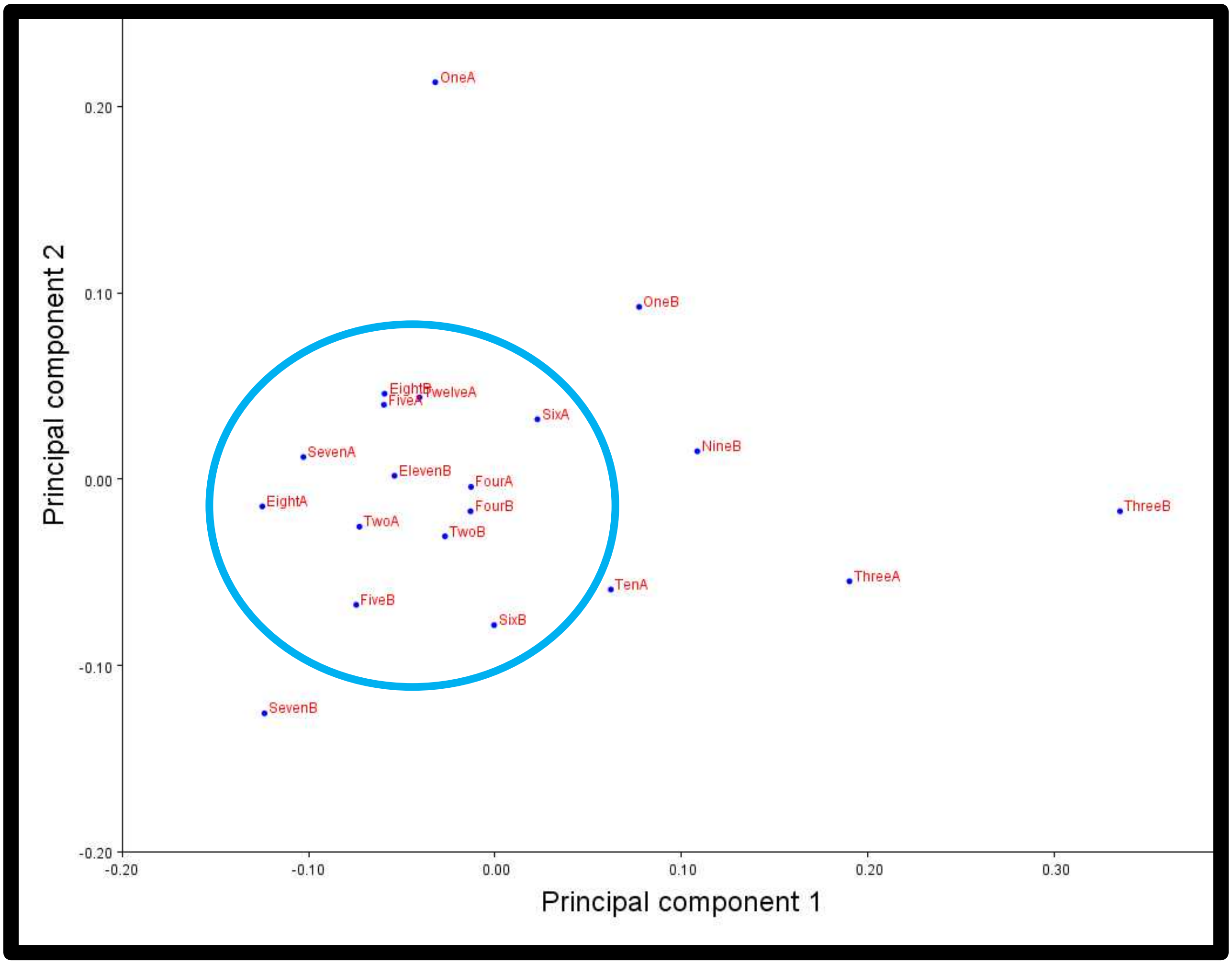


Posterior Surface,
Right Pubic Bone

Appendix B: Graphed Results of Pilot Project (Principal Components Analysis)



PCA Eigen Vectors



PCA Scatter Plot (arbitrary circle placed to show general clustering)

Appendix C: Final List of Landmarks, Descriptions and Types

Number	Landmark	Description	Type
1	Pubic Tubercle	Most prominent point of the pubic tubercle	Traditional (II)
2	Superior Pubic Symphysis	The most superior point of the pubic symphysis	Extremal (III)
3	Inferior Pubic Symphysis	The most inferior point of the pubic symphysis	Extremal (III)
4	Lateral Border	Point on the lateral border of the pubic body which would create the maximum breadth of the obturator foramen	Extremal (III)
5, 6	Pubic Body Height	The superior (5) and inferior (6) points which create the maximum height of the pubic body	Extremal (III)
7, 8	Pubic Body Width	The medial (7) and lateral (8) points which create the maximum width of the pubic body	Extremal (III)

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Chapter Four: Implications and Relevance

The available research and literature in geometric morphometrics has been growing, although, there are still gaps that need to be filled (1). It is also important to repeat existing studies in order to replicate results, determine usefulness, and propose revisions to improve upon past research. This project will draw on preexisting research and integrate new methods to study the shape of the pubic bone in an effort to bolster the available information and further the applicability of geometric morphometrics in forensic and bioarchaeological contexts. This method has the potential to aid in the sex determination of both whole and fragmentary skeletal remains in modern forensic anthropological casework and bioarchaelogical sites, mass graves, and commingled remains. 3D methods are also becoming more and more popular in the research setting, meaning it is important to study their reliability and test the boundaries of their uses in each field. There is still a gap in the field of forensic anthropology when it comes to 3D techniques, although research similar to this is quickly starting to bridge existing knowledge. This research is also important because it discusses the difficulties and technical aspects of curating a new and modern collection of human skeletal remains, a goal which is often unattainable for many university programs. All of these aspects contribute to the importance and contemporary relevance of the current research and indicate that this new method has the potential to contribute greatly to the field of forensic anthropology.

It is rare in forensic and bioarchaeological contexts to recover entire skeletons (2–4). Oftentimes, long periods of time will pass before a body is discovered, which means that in a clandestine burial, there are plenty of chances for weather events, like rain, and outside forces, like grazing cattle, to disturb, break, and move bones. In a forensic case, the body may have been deliberately separated from itself, in order to more effectively hide it, and only parts may be found. It is also very difficult to ensure that all elements for each individual are paired and sorted correctly when in the context of a mass grave or commingled burial. For these reasons, it is important to continue improving and revising more accurate ways to determine sex from fragmentary remains of all parts of the body (2–4). This research focuses on the body of the pubic bone, which is a very dense piece of bone that may stand better chances of surviving through time to later be recovered than, other, less-dense parts of the skeleton. This type of technique could not only improve modern forensic casework, but also help in the identification of victims of mass or commingled graves.

A forensic anthropologist would count themself lucky if given an entire human skeleton to analyze for a case. More often than not, case work consists of fragmentary remains, which may or may not represent most elements of the body. Sometimes, only a cranium is recovered, while other times, broken pieces of many bones are recovered. The anthropologist is still expected to offer their expert opinion on as many aspects of the biological profile as possible. This includes sex, age, stature, ancestry, and an analysis of trauma (5). Multiple methods exist to estimate all of these characteristics; however, it is generally agreed that multiple methods should be used in conjunction to make determinations. This can be quite difficult to do when presented with an incomplete skeleton. Adding into the complicated nature of fragmentary remains is the knowledge that metric analyses are more trusted by court-systems and juries than are subjective morphoscopic analyses (6). There are few widely accepted metric assessments of sex and age based on fragmentary pieces of the skeleton in the existing anthropological literature (7). The current research will create a starting point for a new method which could greatly aid in the metric sex assessment of fragmentary remains.

Research in metric sex assessment is growing and quickly being added to, however, assessments in fragmentary portions are still lacking. A recent study looked to determine how well the pelvis, when digitized, could determine sex (7). The authors used 200 left os coxae and collected 36 landmark points per os coxa (7). Overall, the authors found that by using geometric morphometric analyses on the pelvis, the could determine sex 98-100% of the time (7). This is an incredibly high rate of accuracy and is consistent or better than traditional methods (generally 90-95% accuracy) to determine sex using the pelvis (8). The authors concluded that the pelvis is, as expected, a highly accurate element to use to determine sex; they also determined that geometric morphometrics is a reliable and accurate method to apply (7). One issue, making the applicability of this research an issue, is that an entire os coxa is needed to make a sex estimation. The current research will improve on this study further by focusing the method onto one small portion of the pelvis, which could potentially be applied when the pelvis is recovered in a fragmentary state.

Commingled remains and mass graves present unique problems in forensic and bioarchaeological casework and sites. Usually, determining a minimum number of individuals (MNI) is the first main goal, after which, assigning elements to individuals is attempted. Pair matching elements, for example, both of these humeri belonged to the same individual, or, this pelvis belongs with this cranium, can be very difficult (9). Many times, using a sex estimation of different elements can aid in the individualization of bones. It is likely that in a commingled setting, not all elements would be complete, meaning that accurate metric methods on small portions of bone would offer an advantage to the anthropologist. Confidently sexing pubic bones could potentially aid in the overall analysis of a mass and/or commingled grave, at the very least, offering information about the male to female ratio of the recovered individuals. In some situations, it may also help to associate a pelvis with a certain cranium or other elements in order to attempt to complete more individuals. In a forensic mass grave context, it could also help to identify unknown individuals by confidently assigning them to one sex or the other, especially if the rest of the pelvis and/or cranium are missing or too fragmented to use.

3D technologies are becoming more and more common in many areas of research and science (1,10). As 3D techniques become more common in physical anthropology laboratories, it is vital to determine what type of data collection is most useful and to create standard methods of collection that can be used in various scenarios. The ultimate goal of forensic anthropology is to help identify victims and unknown individuals, so it is important that as a science, the results stand up to other disciplines and the court systems in which we, as forensic anthropologists, work. It is also important to continue working to determine which type of techniques can accomplish which types of analysis. 3D technologies are becoming more common, unfortunately though, many are still very expensive and not available to all researchers. The goal of this project is to use a less expensive piece of equipment (Microscribe Digitizer) to create an accurate metric method. The results of this project will determine whether or not the Digitizer should be used for data collection similar to this and whether or not the proposed method is accurate.

It is also important in a modern forensic context to improve quantitative proof of results because a subjective morphoscopic review is less likely to be accepted in a court of law. Metric results are much harder to contest than a morphoscopic assessment and are more useful to the expert witness and the victim because of the subjectivity of a morphoscopic assessment. Since the 1993 *Daubert* decision, scientific standards for court cases have been tested and questioned for all fields of inquiry, however, it is particularly important in forensic anthropology that we be able to display the validity of our determinations considering how much of the field is based on experience (6,11,12). Most of the standard methods in forensic anthropology consist of morphoscopic analysis, such as the classification of a phase on the auricular surface to determine age, and these methods are inherently subjective. It is still imperative to ensure consistency and reliability in the application of forensic anthropological methods; because of this, many researchers are moving towards more metric and 3D technologies which are not subjective and can still offer accurate and reliable results and determinations (11). As with all new technologies, it is important to standardize the way in which they are used to obtain results (11). It has been shown that a digitized pelvis can accurately determine sex (7), so testing and refining this method is one step closer to being able to standardize the use of a Microscribe Digitizer in the determination of sex in the human pelvis.

Most literature concerning the pelvis focuses on how to improve age-at-death estimations (13,14). Based on a review of related literature, metric sex determination of the pelvis falls in a clear gap. This dissertation will begin to fill this gap by providing a full 3D shape analysis of the pubic body. The existing literature which does focus on sex determination uses morphoscopic techniques on the pelvis as a whole. While these techniques have proven trustworthy by an experienced observer, they are subjective and individuals new to the technique will often make mistakes (15,16). It is important to have methods that are capable of determining sex on fragmentary remains when the entire pelvis is not available, and to have methods that are easily replicable. A review of the existing literature on applications of geometric morphometrics to the human pelvis is included in the background chapter of this dissertation.

It is well known and accepted within anthropology that when developing a method which is intended to be used on modern humans, it must also be developed and based on modern humans (17,18). A part of this project consists of attempting to curate a new sample of modern pubic symphyses from autopsy. This process, while rewarding, has been quite slow and has presented many problems and learning opportunities for the author. The process included gaining approval from the Medical Examiner's Office as well as the University's Internal Review Board to begin a new collection consisting of those deceased individuals who have received an autopsy. It was also necessary to gain the support and cooperation of the local Sheriff's Office and Coroners; because Montana does not employ Medicolegal Death Investigators, the Coroners are in charge of determining whether or not an individual needs an autopsy. Ideally, any time an individual was sent for an autopsy, the involved Coroner would email the autopsy request form to the author of this project, as well as the State Crime Lab, to allow the author quick access to the demographic information of the deceased individual. The author would then determine if the individual seemed like a good donor based on the age of the individual, and whether or not their driver's license denoted them as an organ donor. If the author was also able to obtain the next of kin information, she could then contact them to ask for permission for the donation. The next of kin would need to sign a consent form and return it to the author before the autopsy was completed so that the Medical Examiners could view consent and subsequently remove the sample from the body.

Communication and timing proved to be the most problematic aspects of this process. As it was not their main duty, Coroners would often forget to email the request to the author as well as the Crime Lab, resulting in many individuals not being considered for donation. When the author was aware of a new individual for consideration, it was often impossible to obtain next of kin consent before the autopsy was completed and the deceased had been moved to a funeral home. The first issue of communication is something that could be improved on through continuation of a large project such as this. The longer the Coroners are asked to send on information, the more often it would hopefully occur. The second issue is more difficult to improve on considering how difficult it is to ask a family for consent of a donation. If the next of kin simply does not want to discuss it, or move too slowly, there is nothing more to be done about it. These occurrences are to be expected however, not all who are asked will consent to a donation of part of their loved one. It is the hope of the author that graduate students at the University of Montana will continue this curation in an attempt to create a much larger and more useful contemporary sample of pubic symphyses, and it is the hope that with time, the process will become more regular and faster in order to improve on the number of samples obtained.

The importance of developing new methods based on contemporary, modern, human skeletal collections is to ensure that new methods can be applicable to modern forensic cases (19,20). Contemporary collections can offer an abundance of information and research material to forensic anthropologists and bioarchaeologists. A contemporary collection can be used to study sex and sexual dimorphism, age, growth and development, morphological variation, nutrition, disease, and can support the creation of new and improved methods concerning skeletal analysis (20–23). Anatomically modern *Homo sapiens* have existed for approximately 200,000 years and the modern human skeleton has changed throughout this time, a concept referred to as secular change (24,25). Due to this morphological change over time, it is important to develop new methods and research on contemporary collections of skeletal remains (20).

A recent study showed that secular change can happen relatively quickly, and can affect traits on the pelvis that are regularly used in forensic anthropology (24). Samples from the Hamaan-Todd Osteological Collection and the William M. Bass Donated Skeletal Collection were compared to denote any changes in the ischiopubic ramus, the subpubic angle, and the ventral arc of the pelvis (24). The Hamaan-Todd Collection includes individuals that were born between the mid-nineteenth century and the early twentieth century, while the William M. Bass Collection includes individuals born since 1940 (24). The results showed significant shape differences in all three traits for females and the ventral arc for males (24). The sex determination methods which utilize these traits still offer high accuracy determinations for both populations, but with such significant shape changes occurring over time, it is unknown how long those changes will not have an effect on these methods (24). This study demonstrates the need for continued contemporary skeletal collections.

It is also important to develop new collections world-wide because regional morphological variations can also affect the accuracy of new and established methods (20). Cross-comparison of collections throughout the world could help further understandings of how humans develop and age, what kinds of sexual dimorphism is present, and how different disease processes and cultural differences may or may not be expressed in the human skeleton (20). Developing methods that are population-specific can help to increase accuracy for that specific region, and may aid further in forensic investigations of unknown identity and missing persons. By developing a new method based on the University of New Mexico Maxwell Documented Skeletal Collection (consisting of only individuals who have passed away in the last 50 years), this research will develop a new method to assess the sex of a modern human skeleton based on the pubic bone (26). This method will then be tested on the new contemporary sample of pubic symphyses at the University of Montana (consisting of individuals who have passed away in the last year) in order to determine if the method seems to be regionally specific, or, if like most sex determination methods, it is accurate across regions. This method will be usable for modern forensic cases because it was developed based on very recent contemporary populations, rather than an older collection, such as the Hamaan-Todd Collection.

This dissertation project is a small, but necessary step towards a better understanding of the bones which make up the human pelvis, specifically, the shape of the pubic bone. Based on the existing knowledge of morphoscopic sex differences in the pubic bone, it is expected that a thorough metric analysis will yield significant and useful results. Creating a method which will allow future forensic anthropologists to metrically determine the sex of an unknown pubic bone fragment can potentially aid both future research in forensics and bioarchaeology, as well as expert witnesses in court. As 3D technology becomes more common, affordable, and understood, it will become the norm for research and analysis and so it is important to continue filling in the existing gaps with new hypotheses and methods. Ultimately, the goal of forensic anthropology is to recreate the life history of unknown people based on their skeletal remains. Improving the methods in which we do this is necessary and vitally important to ensure our accurate portrayal of those individuals we aim to help. This project also explores the successes and failures of attempting the curation of a new collection and may hopefully lead to better methods of communication and curation in Montana in the future.

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Chapter Five: Three-dimensional geometric morphometric sex determination of the human pubic bone

3-D sex determination of the pubic bone

Katherine Baca[1], Brandon Bridge[2], Meradeth Snow[3]

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[1]Department of Anthropology, University of Montana, Missoula, Montana, United States of America

[2]Department of Economics, University of Montana, Missoula, Montana, United States of America

[3]Department of Anthropology, University of Montana, Missoula, Montana, United States of America

Abstract

Geometric morphometrics has become a more popular method in anthropology as three-dimensional data and research become more widely recognized and accessible. This research provides a refined method utilizing 3-D geometric morphometric analysis to determine sex from the human pubic bone. The study used a sample of N=378 individual pubic bones from the University of New Mexico Maxwell Documented Collection. Eight landmarks were digitized on each individual bone using a Microscribe Digitizer. Results from the Principal Components Analysis provide promising clustering between male and female groups, as well as indications that the method may be ancestry-specific, and that parity may have an effect on the shape of female pubic bones. The Discriminant Function analysis of the training data set resulted in 96.2% accuracy in predicting the correct sex, and the testing data set resulted in 95.5% accuracy, P<0.0001. To test the ability to replicate this method, the author collected data a second time on a random set of 50 individuals, N=100 pubic bones and reran the GPA, PCA, and discriminant function analyses. This second test resulted in 96.5% accuracy of the training data set, and 93.8% accuracy of the testing data set. To test interobserver error, the author collected all eight landmarks from the same bone once a day, six days in a row. The PCA scatter plot of this test is presented to exhibit the extremely low variance between each instance of measurement.

Introduction

The use of geometric morphometrics has become increasingly popular in forensic anthropological research [1–3]. Geometric morphometrics is, at its most basic level, the metric analysis of shape [4]. This method has been used in many biological studies for at least the past three decades, but is not commonly used in the field of Forensic Anthropology [4]. Geometric morphometrics differ from traditional methods because morphoscopic analysis does not metrically assess bone, and interlandmark distance measurements do not fully capture the shape of a bone. In contrast, geometric morphometrics metrically analyzes the shape of a bone in two or three dimensions [4,5]. Metric analyses are more objective and generally require less training and experience than visual analysis techniques [6]. Thus, it is important to continue improving metric analyses to aid in the forensic anthropologists' biological profile determinations.

Much of the existing geometric morphometric work focuses on sex and ancestry assessment of the cranium [1,5,6]. It is accepted, however, that the pelvis is the best indicator of sex in human skeletal remains [6,9,10]. Research has also shown that metric analysis of the post-cranial skeleton can be just as accurate, if not more accurate, in sex estimation than the cranium [10]. Further research using 3D techniques on the post-cranial skeleton is needed to help increase accuracy of sex estimation in forensic and archaeological contexts. Research focused on the pelvis most commonly investigates changes in age, and few projects attempt to metrically determine sex [11,12]. The DSP method is one of the few large-scale metric methods of sex determination using the pelvis and has been shown to be quite accurate [13]. This method utilizes interlandmark distance measurements however, and is based on archaeological samples; both of which are not ideal for use in modern forensic casework [13]. It is especially important today to develop accurate metric analyses due to the need for reliable and objective results for instances in which the remains might be evidence in a court room [14]. Improving the specificity of sex determination in the pelvis has the potential to improve both modern forensic anthropology as well as analyses within bioarchaeology.

It is important to develop new methods using a contemporary collection in order to ensure the method's accuracy when applied to modern forensic cases [15]. This helps to avoid biases that may be introduced by secular change in a population. Anatomically modern *Homo sapiens* have existed for approximately 200,000 years and the modern human skeleton has changed throughout this time due to the environment and random genetic drift, a concept referred to as secular change [16,17]. Due to this morphological change over time, it is important to develop new methods and research on contemporary collections of skeletal remains [18]. A recent study showed that secular change can happen relatively quickly, and can affect traits on the pelvis that are regularly used in forensic anthropology [16]. Samples from the Hamaan-Todd Osteological Collection and the William M. Bass Donated Skeletal Collection were compared to denote any changes in the ischiopubic ramus, the subpubic angle, and the ventral arc of the pelvis [16]. The Hamaan-Todd Collection includes individuals that were born between the mid-nineteenth century and the early twentieth century, while the William M. Bass Collection includes individuals born since 1940 [16]. The results showed significant shape differences in all three traits for females, and the ventral arc for males [16]. The sex determination methods which utilize these traits still offer high accuracy determinations for both populations, but with such significant shape changes occurring over time, it is unknown how long changes will continue to not affect the methods used [16]. This study demonstrates the need for continued contemporary skeletal collections. Due to the locomotor demands of the lower limbs, it is unlikely that any significant asymmetry exists between the right and left pubic bones; however, crossed symmetry has been observed in the past, and it will be important to note whether any significant asymmetrical patterns indicate that a right or left pubic bone would be more accurate in determining male or female [17].

Along with this, it is also important to develop new methods using a collection that derives from the ancestry affiliation for which it will be used in the future [15]. This is not always possible, especially when modern admixture and self-identification are considered, but it is always important to record ancestry when available and to notice any trends or correlations that may arise in order to ensure that data derived from these methods is used most appropriately and accurately in the future.

The goal of the present research is to provide a refined method using a 3D Microscribe digitizer to assess sex from the pubic bone, as well as to compile a list of eight standard landmarks that can be used in future geometric morphometric studies of the pubic bone and pelvis. This research refines similar methods used in two previous studies which utilized a 3D Microscribe Digitizer to estimate sex from landmarks located across the entire os coxa [6,20]. This research also noted patterns in morphological change related to age, ancestry, and parity in females, all of which likely play a role in pelvis morphology, but to what extent is unclear. The information gained from this research is applicable to modern forensic cases as well as other various scenarios, such as mass or commingled graves, where it would be advantageous to understand the demographics of the individuals involved, even if identifications may not be possible.

The initial hypothesis of this research was that this method of 3D geometric morphometric analysis will formulate a statistically accurate method of sex determination based on the shape of the human pubic bone.

Materials and Methods

The main method of data collection for this research was done utilizing a Microscribe Digitizer G2X. The sample bone is held in a small, rubber clamp, which is held by a heavy steel vice to ensure the bone does not move during data collection. Due to the nature of recording points in three-dimensional space, if the bone moves during data collection, all points must be re-

recorded. It is important to ensure the digitizer is calibrated and working correctly by pressing the “home” button on the digitizer and testing the origin point by measuring a known distance in millimeters or centimeters (setting determined by researcher) [21]. This data point is automatically entered into an Excel Spreadsheet open on the computer. Once data collection is finished, the data can be imported into other software, such as Past, Python, or MorphoJ [22, 23]. The subsequent analyses were performed using Python.

The University of New Mexico Maxwell Documented Skeletal Collection

The larger sample for this research consists of individuals from the University of New Mexico Maxwell Museum’s Documented Skeletal Collection. This collection was established in 1984 and now has over 300 individuals of both sexes, varying ages, and many population groups. The known information for most individuals within this collection includes sex, age-at-death, population affinity, and cause of death. All donations after 1995 have been asked to provide past health information as well as occupation information so that more research using the collection can be conducted. This is one of the best modern collections in the western United States and is particularly important to forensic anthropology because it consists entirely of individuals who have passed away within the last 50 years. The sample as a whole is made up of 60% males, most of the males and females are aged 51-75, and 80% of the total sample identified as White [24].

The Maxwell Documented Skeletal Collection has been chosen as the primary sample for this research for multiple reasons. First, it is one of the most modern skeletal collections in the United States. As discussed earlier, it is vital that methods which are to be applied to modern human remains are developed from known modern collections [15]. Second, the Maxwell Documented Collection is identified as 80% White, which allows for a large collection of similar ancestry. This research utilized 213 of the 307 available individuals, 133 males and 80 females; each individual had to be older than 18 years at time of death to avoid the indeterminate morphology of juveniles; the oldest individual in the sample was 101. Each individual had to offer at least one intact pubic bone. A total of 378 pubic bones were recorded, as not all individuals offered intact right and left bones. Both the right and left bones were collected to ensure the method was viable for use on both pubic bones, considering an anthropologist may not have the luxury of choosing between the two when presented with a fragmented skeleton. The right and left bones were not compared to each other (other than a brief check for significant asymmetry) because orientation is one of the variables of information removed before a geometric morphometric analysis can begin, so they can all be analyzed together.

Table 1. Demographic information

	Number of Individuals	Parous	Non-Parous	Age Group 1: 18-35	Age Group 2: 36-50	Age Group 3: 51-60	Age Group 4: 60+	White Ancestry	Hispanic Ancestry	Black Ancestry
Male	133	N/A	N/A	17	16	31	69	112	5	4
Female	80	34	10	2	6	8	64	78	4	1
Total Number of Individuals	213	34	10	19	22	39	133	190	9	5

Demographic information including the number of individuals, parous and non-parous females, age groups, and self-reported ancestry of the 213 individuals included in the sample, note that parity and ancestry information was not available for all individuals.

It is important to recognize that “White” is an ambiguous term when it comes to determining ancestry or regional belonging; “White” can mean many things, and a White sample from New Mexico will likely be quite different from a White sample elsewhere. Although, it is at least a similar, overall characteristic that can be used to combine and contrast future population data. Ancestry classifications are self-reported, which means they represent which group each individual identified with, and not necessarily where their genetic ancestors came from. In reality, white is a color and not an ancestry, meaning that the genetic ancestry of people who identify as "White" could vary drastically. It has also been shown that those who are multiracial, or identify with multiple ancestries or ethnicities, often change their identification over time, and usually choose to list a single ancestry rather than multiple ones [18]. Self-reported information on ancestry may not be the most reliable, however it does create a group basing that can still offer important information when compared and contrasted, so long as the general issues with the classification are taken into account.

Landmarks

For this research, landmarks are defined as a specific point on bone that can be located on each sample and subsequently recorded in 3-D space [19]. The landmarks used in this study were chosen based on previous research, traditional landmark location, and some new landmarks were chosen by the author [27–29]. New landmarks were chosen in an effort to improve or expound on existing landmarks and overall shape analysis.

When collecting data on landmark points, it is important to distinguish which type of landmark it is. Landmarks can be categorized as type I, II, or III [4,30]. Type I landmarks are considered the easiest to find and consist of one single point on the bone where tissue transitions, such as an intersection of sutures [20,24,25]. Type II landmarks are considered the points of maximum curvature or greatest muscle attachment, an example would be the euryon on the cranium [24,25]. Type III landmarks are the most extreme points of a structure overall, sometimes labelled as the “posteriormost” or “anteriormost” points [20,24]. A category that has since been added to this list by subsequent studies is constructed landmarks, which is defined as “points corresponding to locations that are defined using a combination of traditional landmarks and geometric information” [26]. For example, calculating and using the midpoint along a line of maximum width as a landmark. Constructed landmarks tend to be more difficult to find by inexperienced users due to the need for a measurement of some kind to locate the landmark. Landmark types II and III, which use extremes to locate, tend to be consistently easier to find. Most studies using geometric morphometrics distinguish which types of landmarks are used to help determine which types of points are most useful or which points introduce the most error [24–26]. Sliding semi-landmarks were not used in this study because when morphological variation is small, such as that between modern humans, the differences between a landmark-based study and a sliding semi-landmark based study using the same sample can be altered or skewed based on differences in initial alignment, thus raising questions about any results obtained [32]. There are no visible suture intersections on an adult pubic bone, so only landmark types II and III were utilized in this research. Type III were found to be reliably consistent when they represented both extremes of a width or height. Table 2 lists the landmarks used and the type of landmark they represent, figure 1 shows the approximate physical location of each landmark.

Table 2. Written landmark descriptions and locations.

Number	Landmark	Description	Type
1	Pubic Tubercle	Most prominent point of the pubic tubercle	Traditional (II)
2	Superior Pubic Symphysis	The most superior point of the pubic symphysis	Extremal (III)
3	Inferior Pubic Symphysis	The most inferior point of the pubic symphysis	Extremal (III)
4	Lateral Border	Point on the lateral border of the pubic body which would create the maximum breadth of the obturator foramen	Extremal (III)
5, 6	Pubic Body Height	The superior (5) and inferior (6) points which create the maximum height of the pubic body	Extremal (III)
7, 8	Pubic Body Width	The medial (7) and lateral (8) points which create the maximum width of the pubic body	Extremal (III)

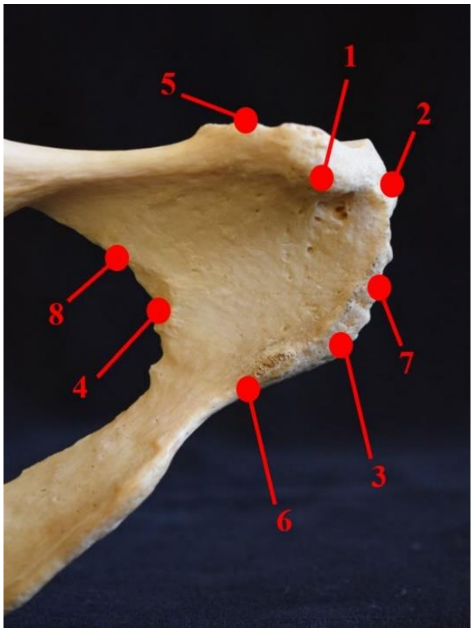

Fig 1. Location of landmarks. Anterior surface of the right pubic bone displaying approximate locations of the eight landmarks recorded on each bone.

For the purpose of geometric morphometrics, shape has been defined as the geometrical information that is left when location, scale and rotational effects are removed from an object [19,28]. The first statistical step of this research was to perform a Generalized Procrustes Analysis, or GPA. Before any analysis is completed, the landmark coordinates include information on size, shape, position and orientation [34,35]. In order to analyze just shape, however, all of this other information must be excluded [35]. A GPA eliminates the non-shape variation and rescales and rotates each sample so that they are relative to each other in the same plane [4,35]. By doing this, the GPA translates all landmark configurations to the same centroid, scales all configurations to the same centroid size, and iteratively rotates all configurations until the summed squared distances between the landmarks and their corresponding sample average is at a minimum [35]. After the GPA is run, all configurations are superimposed on each other and the resulting coordinates are called Procrustes shape coordinates and only include information about the shape of the configurations [35]. This statistical step alone does not offer a lot of useful information, but rather it prepares the data to be analyzed further in ways that will offer more information about the shapes that are present.

The next step was a Principal Components Analysis, or PCA. A PCA is a way to represent the variation that is present within the sample, and the goal of a PCA is to determine which variable introduces the most amount of variation [19,31]. The Principal components provide a means of comparing the relative importance of each dimension of the data [19]. If the first two eigen vectors represent 50% or more of the variance then the test can be considered competent at finding a linear classifier that effectively separates the classes (sex).

Lastly, a Discriminant Function analysis was run through Python to determine the predictive power of the sample based on the two groupings of males and females. The first part of a Discriminant Function uses multivariate F tests to determine whether or not there are any significant differences between both groups (male and female) with regard to all variables [37]. This results in finding the statistically significant means across the groups, which can then be used to classify all samples into which of the two groups they most likely to belong to [37]. The analysis randomly breaks the data set into two sets, placing 70% of the data in the first and the remaining 30% of the data into the second. The first set is used as a training set to teach the software the difference between the two classifying groups (male and female). The second set is used as a test to predict how well the machine learned to distinguish between the two groups. Essentially, this will result in the predictive power of the method's ability to determine an unknown bone as either male or female.

In order to determine ease of reproducibility, the author resampled 50 individuals from the previously sampled 213. The author then collected data for a second time on each of the 50 individuals, N=100 pubic bones. One of the advantages of geometric morphometrics is that the data can be collected on any plane and rescaled to perform analysis; because of this, one of the clearest ways to asses replicability was to simply run the entire statistical process again using only the resampled data and compare these results to the results of the larger sample. This analysis was performed exactly as described above, using Python software.

To test the presence of interobserver error, the author collected all eight landmarks from a single bone, once a day, six days in a row, to determine whether or not the landmarks were consistently recorded in the same location. This data collection occurred approximately one year after the initial data collection, and the individual bone came from the University of Montana Forensic Anthropology Laboratory Collection, which is not a collection of known provenience. Due to this, the data collected from this individual was not added to the previous, large sample from the University of New Mexico Maxwell Museum. The six instances of data collection were treated as their own data set, and a GPA and PCA were run using MorphoJ. The resulting PCA scatter plot, presented below, displays how much variance was present between each collection instance.

Results

The first two eigenvectors of the PCA test represented 43% of the total variance, which was close to, but not exceeding the ideal 50% for class separability. The resulting PCA scatter plot displaying principal components one and two can be seen in Figure 2. The third principal component represented 11.4% of the total variance, but was not mapped because it did not add to the visualization of the data. The scatter plot was also coded to show males and females, age groupings, parity of females, and ancestry groups (see Figures 2-4). While it does not in itself offer definitive class separability, it is clear from Figure 2 that the males and females do show some difference in how they cluster, and the male specimens cluster much more tightly than the females. It should be noted that the only African American female individual in the sample is represented by the two uppermost outliers of the scatter plot (right and left pubic bones). This individual was not removed from the subsequent analysis because she provided two complete pubic bones with valid measurements and was in line with the higher variance of females. While a sample size of one is not nearly enough to confirm the necessity of an ancestry-specific method, this does indicate that future research should use larger and more diverse samples to determine whether or not ancestry is a defining variable.

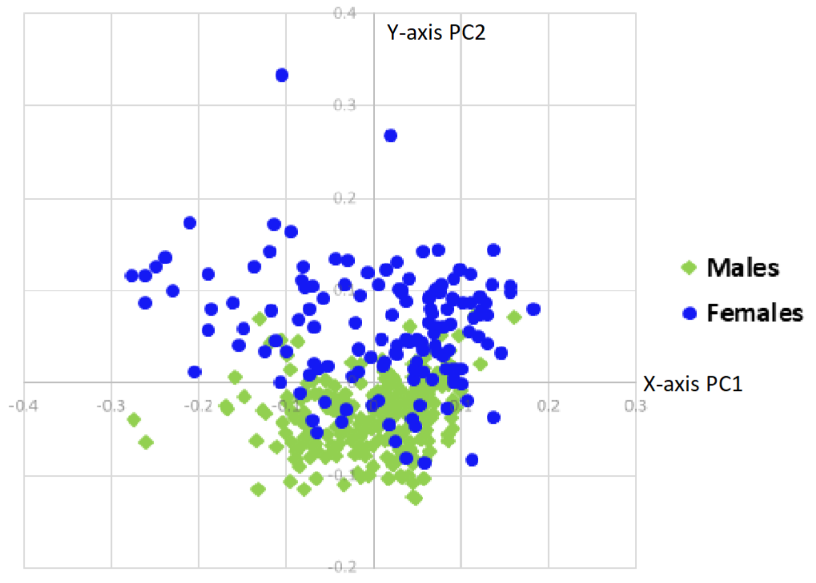

Fig 2. PCA scatter plot. Principal Components Analysis scatter plot displaying male and female groups.

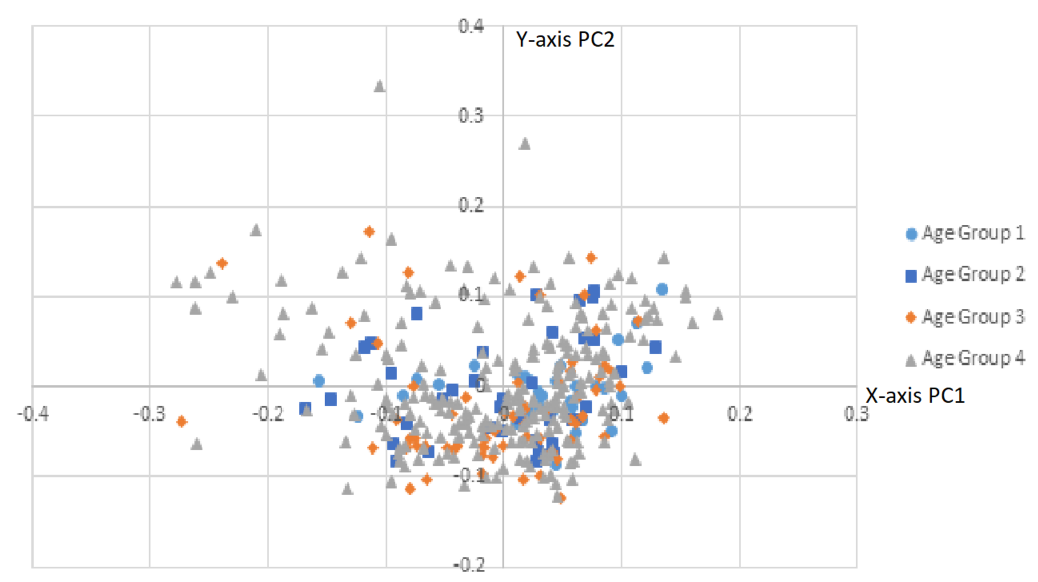

Fig 3. PCA age scatter plot. Principal Components Analysis scatter plot displaying age groups (males and females grouped together); group 1 (18-35), group 2 (36-50), group 3 (51-60), group 4 (61+).

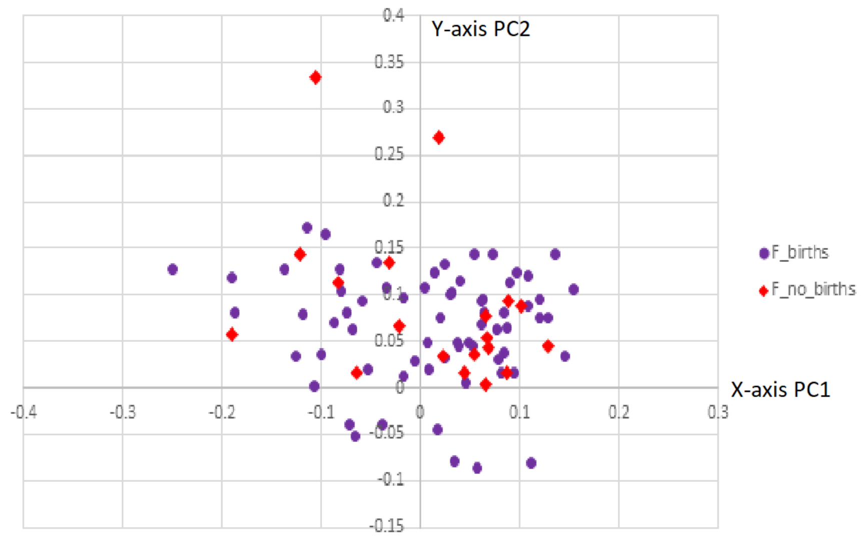

Fig 4. PCA parity scatter plot. Principal Components Analysis scatter plot displaying available data on parity of females (parous vs. non-parous).

By coloring the scatter plot based on age groupings, it is clear that as the sample groups get older, the variance becomes greater (Figure 3). The younger age groups (groups 1 and 2) cluster much tighter than groups 3 and 4. By coloring only the females with available data concerning their parity, it can be seen that parous females exhibit a slightly greater variance than non-parous females (Figure 4). Unfortunately, this data was not available for every female individual; however, this data indicates the need for future research, especially considering how little is known about the shape changes a female pelvis experiences after giving birth.

The Discriminant function analysis was run through Python software. The training discriminant function analysis resulted in 96.2% accuracy between male and female predicted group classifications. The testing set discriminate function analysis resulted in 95.5% accuracy between male and female groups with a P-value of <0.0001. Figure 5 shows the bar graph of the testing set discriminant function analysis.

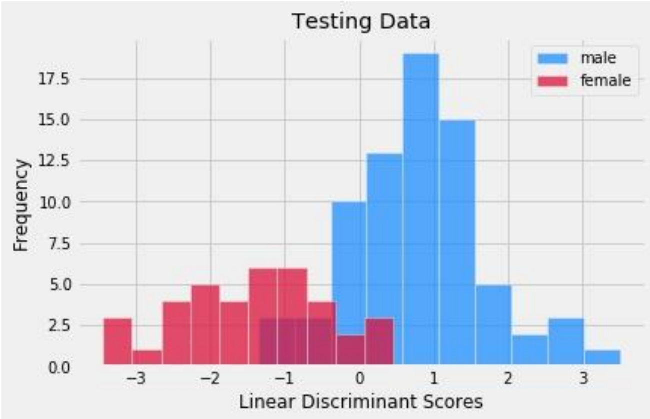

Fig 5. Discriminant function bar graph. Results of the discriminant function test set. The x-axis displays the degree of maximum separability, the y-axis displays the frequency.

This Discriminant Function analysis was validated through a second software program, MorphoJ, by running the same tests (GPA, PCA, DFA). The testing set resulted in 96.3% accuracy, and the test set resulted in 95.5% accuracy (P<0.0001).

The last analysis performed on this large data set, which consisted of running the GPA, PCA, and discriminant function analysis on the second set of resampled data, N=100, resulted in a training score of 96.5% accuracy, and a cross-validation score of 93.8% accuracy, P<0.0001.

The interobserver error test ran a GPA and PCA on a smaller data set which consisted of six instances of landmark collection from the same bone. This resulted in a PCA scatter plot, figure 6, which shows incredibly small amounts of variation between each instance of data collection. All six instances of landmark collection graph on top of each other, clearly representing that each data collection instance occurred on the same individual. The first two principal components contained 78% of the variation, this indicates a successful PCA and does not necessitate graphing of the remaining principal components due to their small amount of represented variation.

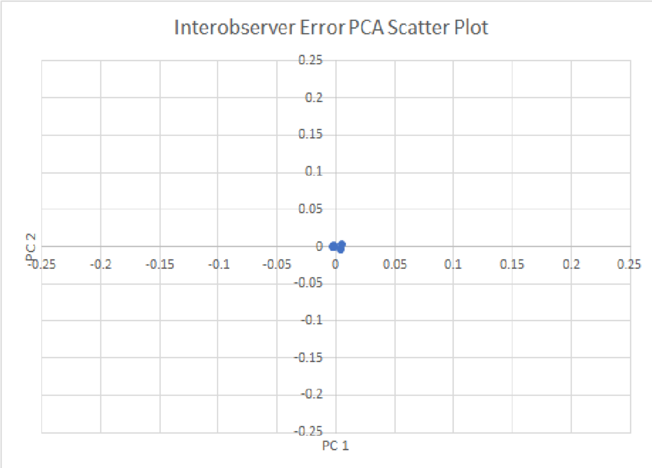

Fig. 6 Results of the interobserver error PCA analysis. This chart shows the PC scores (X axis displays PC 1; Y axis displays PC 2) of the six instances of data collection from the same bone, by the same author, once a day, six days in a row.

No significant asymmetry was observed between the right and left pubic bones of individuals. Most individuals' right and left bones were relatively mirrored when displayed on the PCA scatter plot. The mirroring is not exact; however, asymmetrical shape differences are likely due to shape changes related to aging rather than sex differences. No further analysis was conducted concerning asymmetry because it did not seem to affect the results of the research in any significant way; this indicates that this method can be just as effective when either the right or left pubic bone is used. The PCA scatter plot with each individual labeled can be viewed in Appendix A.

Discussion

The main goal of this research was to test whether or not the method of digitizing landmarks on the human pubic bone could be used to accurately distinguish between male and female individuals. With a result of 95.5% accuracy and a p-value of <0.0001 from the discriminant function, this method has been successful on this sample. Experienced forensic anthropologists using morphoscopic methods can accurately determine sex approximately 90-95% of the time, depending on the state of the bones and the experience of the user [6,20]. Similar studies, which collected landmarks across the entire os coxa reported similarly high results, so it is promising to see that the method can be narrowed down to a smaller portion of bone and still retain high accuracy [6,20]. These results indicate that this method could potentially be used in forensic casework to aid in the identification of unknown human remains. It could also be useful in instances of mass graves, commingled graves, or fragmentary remains when portions of the pubic bones are recovered. This study helps to fill the existing gap of metrically assessed sex determination methods in forensic anthropology and introduces an applicable method of geometric morphometrics to the field.

Supporting forensic anthropological determinations in the court room with metric analysis is becoming more important as more and more scientific expertise is used to support criminal cases. With a 95.5% accuracy rate, the present method has great potential as an aid in supporting a sex determination of an unknown individual. The validation of the discriminant function analysis results using MorphoJ software indicates that this type of analysis is consistently accurate when using various brands of software. This demonstrates that the data is robust enough to exhibit the same pattern when analyzed in distinct software programs, which also means it could be available to more future researchers. It is also quite promising that the resampled data resulted in significant accuracy rates quite close to the larger sample percentages. The resampled data pool was much smaller than the overall data set, which may explain some of the difference. It is clear though that with experience using a Microscribe Digitizer, this method should be replicable. The interobserver error test also shows that data collection can be incredibly consistent and that using type II and type III landmarks did not create replication issues. Future research should determine how long it takes for a user to become proficient at collecting data using a digitizer. A forensic anthropologist should use multiple methods to support their determinations and there are very few metric options available for sex determination. This method offers a very accurate, metric determination which could be added to the anthropologist's traditional analysis. It is also vital that this method was developed using a sample which only contains individuals that have died in the last 50 years because it is more applicable on modern populations and there is less concern of secular changes biasing the results when used on contemporary individuals.

Limitations of this research demographic sample size and applicability issues. While large, the sample for this research includes many more males than females, which could be improved in future research. The sample also consists almost entirely of White ancestry individuals, so it is unknown whether ancestry differences introduce enough shape variation to alter the effectiveness of this method. Within this sample, it is clear that the single African American individual is an outlier, however, it is not known if this is due to her ancestry or some unrelated reason. The same can be said for the effects of parity because this sample had relatively few known instances of parity information. This method also requires that the pubic bone be nearly complete for analysis, which is an unrealistic expectation in modern forensic casework. It is suggested that future research test this method on larger samples of varying ancestry, and on fragmented bones which may be missing landmarks, to improve the accuracy and usefulness of this technique.

The data and code that support the findings of this study are available within the public repository GitHub, and can be found at https://github.com/brandonbridge/GeoMorpho [36].

Acknowledgments

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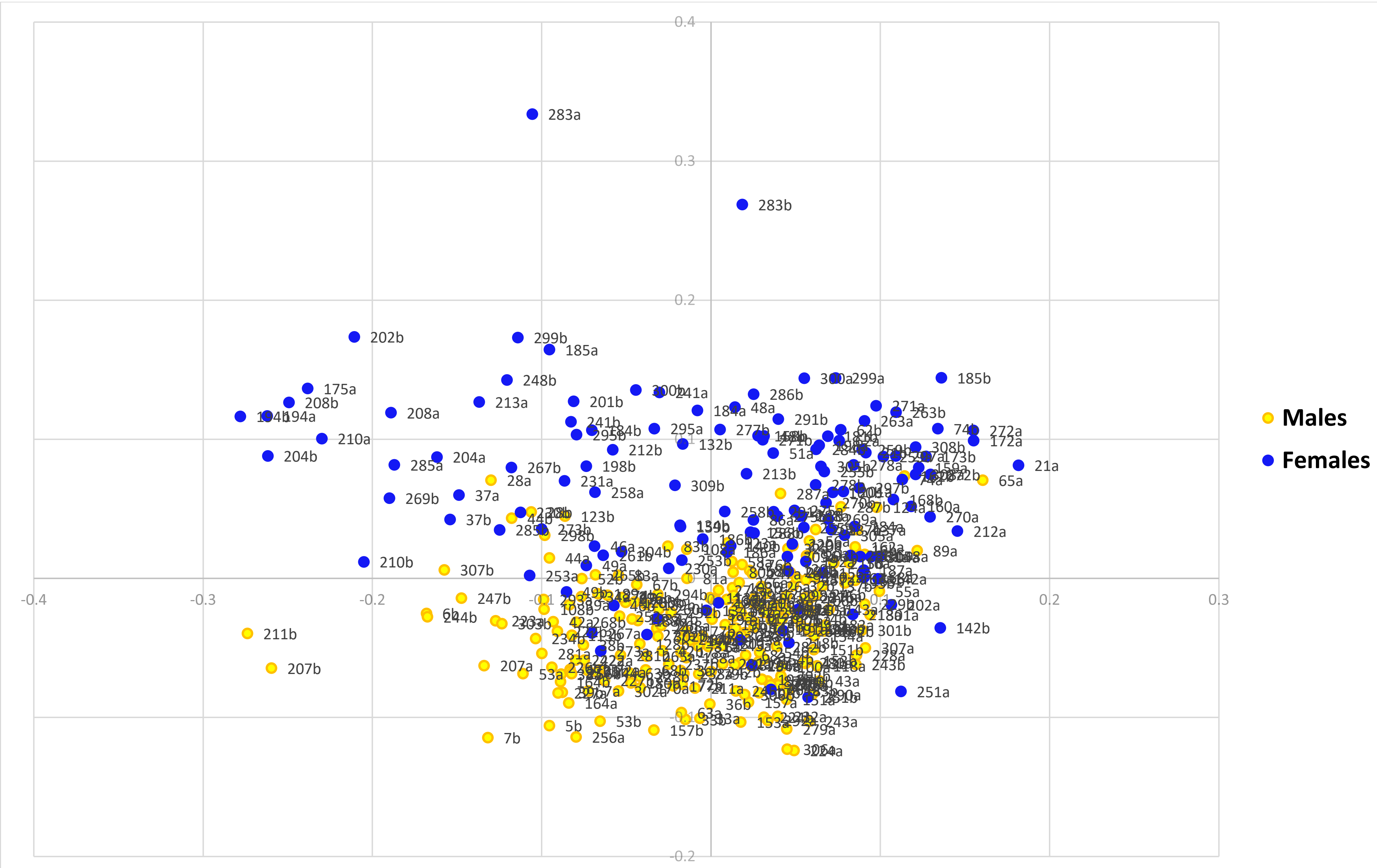
Appendix A: PCA scatter plot displaying labeled individuals (a indicates the right bone, b indicates the left bone)



Chapter Six: Modeled Fragmentary Analysis using a Geometric Morphometric Sex Determination Method of the Human Pubic Bone

Submitted to the Journal of Forensic Sciences

Katherine Baca, MA
University of Montana

Brandon Bridge, PhD
University of Montana

Meradeth Snow, PhD
University of Montana

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ABSTRACT

Sex determination of the human pelvis has traditionally been done through visual analyses of morphoscopic traits and there are limited metric methods available to forensic anthropologists to add metric credibility to these analyses. The goal of this research was to create a new metric method using three dimensional geometric morphometrics to determine sex from a fragmented pubic bone. The sample consisted of n=378 pubic bones from the University of New Mexico's Maxwell Museum Documented Skeletal Collection and eight landmarks were collected from each bone. Statistical analyses and machine learning algorithms were used to mimic fragmented remains that included tests run on each possible landmark combination of three or more landmarks to simulate fragmented bones (218 combinations). The results of the modeled fragmentary analysis consisted of 133 combinations which exhibit a 90% or higher accuracy in sex prediction; and nine combinations which exhibit 95.5% accuracy in sex prediction. In particular, three landmarks clustered around the ventral arc of the pubic bone performed the best, indicating this is the most sexually dimorphic portion of the bone. These results indicate that three-dimensional geometric morphometrics is a valid method to be applied to sex determination in forensic anthropology.

KEYWORDS: Forensic Anthropology, Sex Determination, Geometric Morphometrics, Pubic Bone, Fragmentary, Geomorph.

Sex determination using the pelvis has traditionally been done using a series of morphoscopic traits, including the overall shape of the pelvic inlet, the ventral arc, the ischiopubic ramus, subpubic concavity, and the greater sciatic notch (1). It is well accepted that the pelvis is the best element to use to determine sex when available (2,3). An experienced biological anthropologist can generally estimate sex from a visual analysis of the pelvis correctly approximately 90-95% of the time (1–5). The pelvis exhibits a series of sexually dimorphic differences as an individual matures, including a wider pelvic inlet and sub-pubic arch for females, and a narrower greater sciatic notch and pubic bone for males, most of which are due to the physiological ability of females to give birth (1,2,4–6). Very few accepted metric analyses of these shape differences in the pelvis exist, but metric analysis is becoming more important as it becomes more common for forensic anthropologists to testify as experts within the court system (2,7).

Geometric morphometrics is an analysis of shape and can be performed in both two-dimensional and three-dimensional planes (8). This research applies 3-D geometric morphometrics to the problem of metrically determining sex from the human pelvis. The basic process of this specific method consists of recording multiple homologous shapes (multiple pubic bones), rotating them all onto the same plane, removing size as a variable (Generalized Procrustes Analysis), distinguishing differences between group clusters (Principal Components Analysis), and then performing a discriminant function analysis to determine the predictive power of the method (8). This method is advantageous because it offers more metric information about the specimen than traditional visual or interlandmark distance measurements do. Traditional visual analyses rely on the expertise of the forensic anthropologist to judge the size and shape of the bone by simply examining it. Whereas completing a statistical analysis on multiple points in space (landmarks on bone) offers measurements between the x, y, and z coordinates of each landmark and a much more complete and exact analysis of the bone as a whole. Geometric morphometrics is commonly utilized in biological studies, however, is fairly new to the field of anthropology (9–11). Existing research in biological anthropology mainly utilizes the method for cranial ancestry estimation and age-at-death estimation; there are few metric methods available for sex determination from the pelvis (12,13).

It is rare to recover complete sets of skeletal remains in most forensic and archaeological scenarios (2). Often, remains have been buried or exposed to the elements for some time before recovery, which results in broken, partially disintegrated, and/or incomplete bones available for analysis. Due to this commonality, it is necessary to develop methods of sex estimation that can be used on fragmentary remains. The hypothesis of this research is that a geometric morphometric shape analysis of the pubic bone will result in highly statistically accurate sex determinations on both whole and fragmented human pubic bones and that certain landmarks will be more effective than others in establishing a sex determination.

Adding to the complicated nature of fragmentary remains is the knowledge that metric analyses are more trusted by court-systems and juries than are subjective morphoscopic analyses (7). An expert should use multiple methods to validate their determinations, and a precise metric method to validate a sex determination could aid in establishing the credibility of the testimony. This method could also be useful in other contexts, such as mass graves, commingled and fragmentary remains, and bioarchaeological studies. In archaeological contexts when multiple individuals are identified, one of the first demographics determined is how many males and females are present; this method could easily offer a metric answer to this question.

Materials/Methods

In order to perform a three-dimensional geometric morphometric analysis of the pubic bone, a Microscribe digitizer was utilized to collect a set of landmarks from each specimen. The bone must be held stationary during data collection; if it were to move while collecting data points, the collection for that specimen would have to be deleted and started again. The author used a rubber clamp, bolted to the table, to hold the bone in place. The rubber clamp was able to hold the bone still without damaging the bone. The bone could be placed into the clamp in any manner as long as the landmarks were accessible to reach with the digitizer. Before data collection, the digitizer was calibrated by pressing the "home" button and checking a known measurement in millimeters (14). The stylus of the digitizer was placed at the point of the landmark and a foot pedal was pressed to record that location; the x, y, z coordinate was then entered into a spreadsheet which can be uploaded to any statistical software. Eight landmarks were collected on each specimen when available, only specimens that were missing three or fewer landmarks were utilized (missing landmarks were recorded as -9999 to differentiate them from existing landmarks). See Table 1 for the description of the landmark locations; it was necessary to always record landmarks in the same order (one through eight) so the computer knew which landmark it was recording. The landmarks were chosen by the author based on previous research and a pilot project which aided in narrowing down landmarks that were easily identified and replicable (2). 213 individuals were utilized from the University of New Mexico's Maxwell Documented Skeletal Collection, which resulted in 378 total pubic bones. The Maxwell Documented Collection consists only of donated individuals who have passed away in the past 50 years, making it one of the largest modern collections in the US. The collection houses over 300 individuals, 60% of which are male, the majority of adults are aged 51-75, and the sample as a whole is self-identified as 80% White (15). All 213 individuals utilized were 18 or older to avoid the indeterminate morphology present in juvenile pelves, and when available, both right and left pubic bones were recorded.

Number	Landmark	Description
1	Pubic Tubercle	Most prominent point of the pubic tubercle
2	Superior Pubic Symphysis	The most superior point of the pubic symphysis
3	Inferior Pubic Symphysis	The most inferior point of the pubic symphysis
4	Lateral Border	Point on the lateral border of the pubic body which would create the maximum breadth of the obturator foramen
5, 6	Pubic Body Height	The superior (5) and inferior (6) points which create the maximum height of the pubic body
7, 8	Pubic Body Width	The medial (7) and lateral (8) points which create the maximum width of the pubic body

Table 1: *Description of the location and landmark type of the eight landmarks recorded on each bone.*

The statistical analysis began with transferring the raw data from the spreadsheets into Python 3 (16). Before the data is manipulated in any way, it contains information on size, position, and orientation; all of which must be removed in order to analyze shape alone (17,18). A Generalized Procrustes Analysis was run to transform the data so that it is scaled and rotated to the same plane, leaving only the shape information (8). Next, a Principal Components Analysis was run to isolate which variables were providing the most variation within the sample (19). The analysis first performs an eigen-decomposition on the covariance matrix of the Procrustes shape coordinates and then sorts the eigenvalues and eigenvectors. The top two eigenvectors exhibit the greatest variance in the data and are known as the first two principal components (19). The component scores of each observation were then plotted on the first two principal component axes to form a scatter plot to visualize the variation present within the data. This visualization displays groupings, clusters, and outliers. This scatter plot indicated that it was possible to distinguish between males and female groups based on all data points being present.

The bulk of the statistical analysis consisted of a series of discriminant function analyses to determine the predictive power of the sample when classifying into two groups; males and females. A discriminant function analysis in Python 3 is performed as a supervised machine learning algorithm. “Supervised” in this case means the input data (the Procrustes shape coordinates) are labeled male or female, and the program learns to predict the sex classification from this input data. First, the data is randomly split into two sets, the training set and the testing set. The training set in this study was 70 percent of the sample, with the remaining 30 percent reserved as the testing set. The training set is used to train the prediction model while the testing set is left out. The trained prediction model is then applied to the testing set to validate its predictive accuracy. The training score reported in the model results indicates how well the model predicts the classes of the samples in the training set, while the testing score reports the model’s predictive accuracy on the data samples previously left out.

The discriminant function analysis is a dimensionality reduction technique similar to the principal component analysis. The primary difference between the two techniques is that a principal component analysis is an “unsupervised” attempt to project the samples onto a subspace whose axes maximize the variance in the data, while the “supervised” discriminant function analysis attempts to project the samples onto a subspace whose axes maximize the separability between classes (20). The end result is the predictive power of the method’s ability to determine whether an unknown bone originated from a male or a female individual. The first discriminant function was run on the entire sample to establish a baseline for how well the method worked when presented with whole, non-fragmented bones. To begin the simulated fragmentary analyses, a discriminant function analysis was run without landmarks 1, 2, and 5, which were the landmarks most commonly missing from the UNM sample. Next, in order to determine which combinations of landmarks offer the best predictive power, every possible combination of three landmarks or more was then run through a discriminant function test.

After the initial data collection, the author used a random number generator to create a list of 50 individuals from the original data set, all of which provided both right and left pubic bones. This resulted in a second sample of N=100 pubic bones, which the author collected data from a second time, in order to test the replicability of this method. A GPA, PCA, and discriminant function analysis was run on this second data set to determine whether or not the method was replicated accurately by the researcher. It was also important to test interobserver error to ensure the author could consistently identify the correct landmark locations. Approximately one year after the initial data collection, the author collected the same eight landmarks on one bone from the University of Montana Forensic Anthropological Laboratory once a day, six days in a row. This resulted in a small data set of six instances of data collections all representing the same bone. A GPA and PCA was run on this data set to determine how consistent the author was when collecting landmarks on the same bone.

Results

The first discriminant function test, which included all available landmarks for all 378 specimens resulted in 96.2% accuracy based on the training data, and 95.5% accuracy based on the testing data, $P<.0001$. These results are on par with other sex determination methods on the pelvis, which as mentioned earlier range from approximately 90-95% (1–5). The second discriminant function test removed landmarks 1, 2, and 5 from the samples and resulted in a training set accuracy of 93.5% and a testing data set accuracy of 91.7%, $P<.0001$.

The larger discriminant function loop which tested all possible combinations of three landmarks or more offered results for 218 different combinations. The least effective combination consisted of landmarks 1, 4, and 8 and resulted in a training score of 86.9% and a testing score of 77.6% accuracy. Nine different combinations all resulted in the highest testing score of 95.5% accuracy, see table 2 for which landmarks were included in each of these fragments. It should also be noted that 133 of the 218 (61%) possible combinations resulted in testing scores of 90% or higher. Results for all 218 landmark combinations can be viewed in the supplemental information.

Combination Number	**Training Score**	**Testing Score**	**Landmarks Included**
24	95%	95.5%	2, 3, 6
44	95.7%	95.5%	3, 6, 7
93	96.1%	95.5%	2, 3, 4, 6
96	95.4%	95.5%	2, 3, 5, 6
115	96.9%	95.5%	3, 4, 6, 7
121	95.7%	95.5%	3, 6, 7, 8
172	94.6%	95.5%	2, 4, 5, 6
177	96.5%	95.5%	3, 4, 5, 6, 7
181	95.4%	95.5%	3, 5, 6, 7, 8

Table 2: The nine landmark combinations which resulted in the highest testing scores, showing their corresponding training scores and which landmarks were included in each combination.

The second data set which consisted of 50 individuals, n=100 pubic bones, to test replicability of the method, resulted in a training set accuracy of 96.5%, and a testing set accuracy of 93.8% accuracy. The last analysis based on the data set which consisted of six instances of data collection from the same bone resulted in very low variance between instances of landmark collection. Figure 1 displays a scatter plot graphing the PC1 and PC2 scores of each of the six data collection instances and shows how close each instance of data collection is to each other. This demonstrates a successful second use of the method on a separate, albeit smaller, data set, as well as low intra-observer error when repeatedly tested on the same bone.

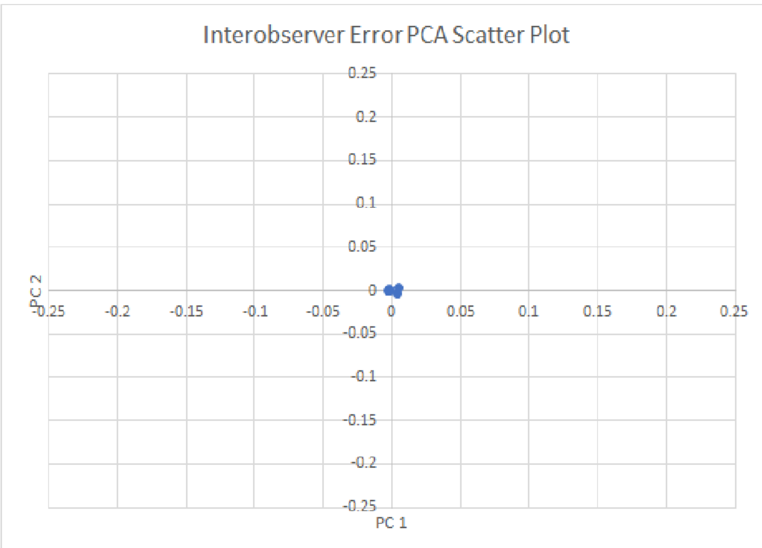

Figure 1: Results of the interobserver error PCA analysis. This chart shows the PC scores (X axis displays PC 1; Y axis displays PC 2) of the six instances of data collection from the same bone, by the same author, once a day, six days in a row.

Discussion

The results reported here clearly indicate that this three-dimensional geometric morphometric method of sex determination has the potential to increase the accuracy and credibility of sex estimations on both whole and fragmented human pubic bones. More than half (61%) of all possible combinations of these landmarks result in an accuracy rate of 90% or higher, which is in the same range as previously used visual analysis methods (1–5). This result indicates that this method could be used on many differently sized and broken fragments of pubic bone to gain an accurate estimation of sex. A single discriminant function was run without landmarks one, two, and five, because those were the landmarks most commonly missing from the UNM samples and easily replicated a real fragmentary context. A result of 91.7% is a promising indication that this method worked well in an actual fragmentary context. Finally, nine simulated fragments resulted in an accuracy result of 95.5%, which is just as high as the overall accuracy of the method when all eight landmarks are included in the discriminant function analysis.

When considering the nine combinations of landmarks that all resulted in 95.5% accuracy, there are two specific results within that appear quite significant to the applicability of this method. First, combination 44 utilized only landmarks three, six, and seven, which are the inferior pubic symphysis, the inferior point which creates the maximum height of the pubic body, and the medial point which creates the maximum width of the pubic body, relatively (see Figure 2). All three landmarks are clustered around the general area of the ventral arc. This indicates that the ventral arc, which is already used in visual analysis sexing methods, is the most sexually dimorphic area of the pubic bone (1,4). Secondly, landmarks three and six are utilized in eight of the nine combinations with the highest accuracy results; the one combination that does not include both three and six, does include six (combination 172). Figure 3 displays a heat map of the landmarks' predictive powers. To create this map, the average predictive power of each combination containing landmarks one and two was calculated; the same for each combination containing landmark one and three, one and four, and so on with every two-landmark duo. The darker the color, the higher the average predictive power for combinations including that landmark duo. It is clear that combinations including landmarks three and six have the highest average predictive powers. The variance in the average of the predictive powers is not large simply due to the high accuracy of the data as a whole. Landmarks three and six appear to be the landmarks that offer the most shape information in relation to male or female individuals and are most effective in predicting sex. This is quite promising to the real-world applicability of this method because it indicates that very high results could still be obtained even if the forensic anthropologist is only presented with a small piece of the inferior pubic bone. At this point, the expert could perform both a visual analysis on the ventral arc, as well as a metric analysis using this method to make their overall sex determination.

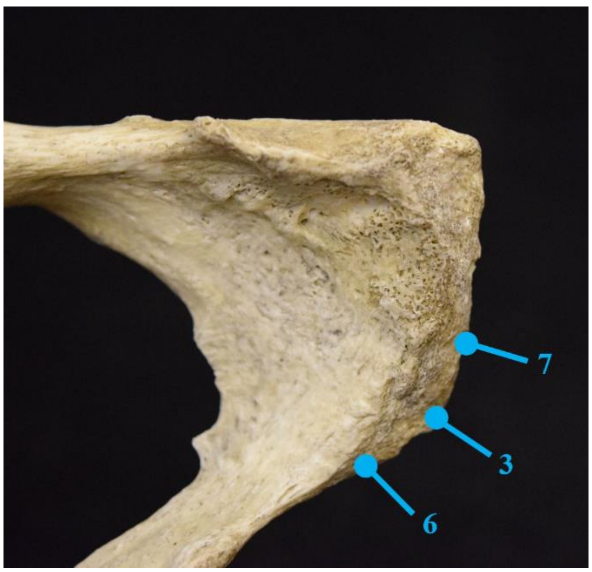

Figure 2: The locations of landmarks three, six, and seven; notice that they are clustered around the ventral arc area of the pubic bone.

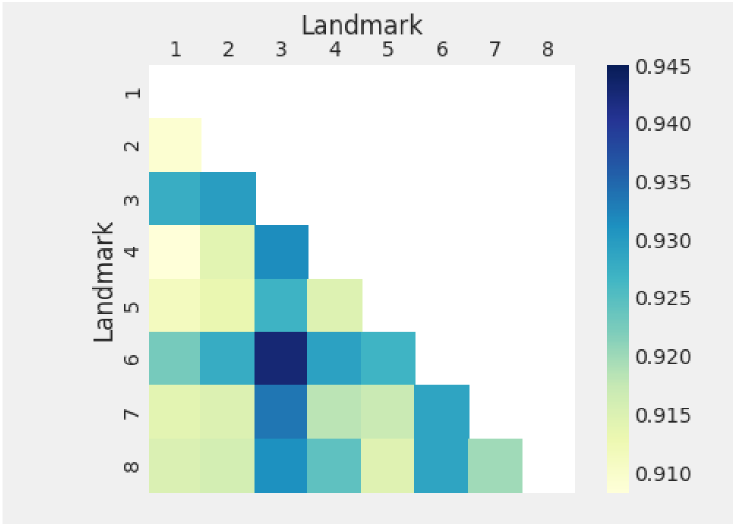

Figure 3: Heatmap displaying the average predictive powers of combinations including each landmark due; note that landmarks three and six make consistently more powerful combinations than other landmarks.

A limitation of this simulated fragmentary research is that not all of the high scoring landmark combinations reflect real possible fragmented bones. For example, combination 77 which includes landmarks three, four, five, six, and seven, requires that essentially all sides of the bone are present, which is highly unlikely if the bone is fragmented. This is the case with most of the combinations which require more than four landmarks. Multiple combinations which only require three or four landmarks to result in accuracy levels just as high as using all eight landmarks are therefore an important finding. Combinations 24, 44, and 115 are particularly applicable when it comes to simulating actual broken bone because each combination uses landmarks that are relatively close together. A second limitation is that this research was conducted using a sample of primarily white individuals, further research into ancestry specific biases is needed. An early Principal Components Analysis exhibited the only African American individual in the sample as an outlier; this could be due to her ancestry or an unknown variable, more research is needed to determine the cause.

It is imperative to develop methods which can be used on fragmentary remains, considering how often incomplete remains are recovered. This is true not only in forensic contexts, but mass graves, commingled remains, and bioarchaeological contexts as well. It is likely that in these scenarios not all elements would be complete, meaning that an accurate metric method on a small portion of bone would offer an advantage to the anthropologist (2). This method has been narrowed from the entire os coxa, to just the pubic bone, and then further to fragmentary scenarios, ensuring that it is applicable and useful to actual recovered remains (2). The method also appears to be easily replicable based on the second, repeated, data set, which exhibits significant results very close to the accuracy exhibited by the larger sample and the higher scoring simulated fragmentary samples. It is also clear that interobserver error is low for this author. Future research should investigate how long it takes a new user to become comfortable and adequate at locating and collecting landmark data.

Based on the promising results here, future research into this method is needed. The method should be applied to larger samples to continue validating it as an established method so that it can be used in legal contexts in the future. This research should continue to develop the fragmentary application of the method as well; replication of this research is vital if it is to ever be applied to actual forensic casework. Any ancestry specific biases should also be further explored on larger samples to ensure the method can be used across populations. This method may also have great potential in exploring the shape changes in female pelves related to pregnancy and birth. Early analyses showed that the female specimens exhibited more variation overall than did male specimens—this may be due to the traumatic event of giving birth, however more research is needed to determine whether or not this is the case.

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Supplemental Information: All possible landmark combinations and their accuracy scores; columns 0 – 20 show which landmarks were included in each combination as x, y, z coordinates.

Combination Number	Training Score	Testing Score	col0	col1	col2	col3	col4	col5	col6	col7	col8	col9	col10	col11	col12	col13	col14	col15	col16	col17	col18	col19	col20
combo_15	0.869731801	0.776785714	x1	y1	z1	x4	y4	z4	x8	y8	z8												
combo_2	0.869731801	0.794642857	x1	y1	z1	x2	y2	z2	x4	y4	z4												
combo_12	0.881226054	0.794642857	x1	y1	z1	x4	y4	z4	x5	y5	z5												
combo_29	0.869731801	0.803571429	x2	y2	z2	x4	y4	z4	x7	y7	z7												
combo_64	0.896551724	0.8125	x1	y1	z1	x2	y2	z2	x4	y4	z4	x7	y7	z7									
combo_6	0.869731801	0.821428571	x1	y1	z1	x2	y2	z2	x8	y8	z8												
combo_14	0.896551724	0.821428571	x1	y1	z1	x4	y4	z4	x7	y7	z7												
combo_27	0.858237548	0.821428571	x2	y2	z2	x4	y4	z4	x5	y5	z5												
combo_36	0.888888889	0.821428571	x2	y2	z2	x7	y7	z7	x8	y8	z8												
combo_62	0.885057471	0.821428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5									
combo_83	0.900383142	0.821428571	x1	y1	z1	x4	y4	z4	x5	y5	z5	x7	y7	z7									
combo_5	0.842911877	0.830357143	x1	y1	z1	x2	y2	z2	x7	y7	z7												
combo_33	0.892720307	0.830357143	x2	y2	z2	x5	y5	z5	x8	y8	z8												
combo_65	0.881226054	0.830357143	x1	y1	z1	x2	y2	z2	x4	y4	z4	x8	y8	z8									
combo_13	0.900383142	0.839285714	x1	y1	z1	x4	y4	z4	x6	y6	z6												
combo_30	0.865900383	0.839285714	x2	y2	z2	x4	y4	z4	x8	y8	z8												
combo_49	0.892720307	0.839285714	x4	y4	z4	x5	y5	z5	x8	y8	z8												
combo_3	0.835249042	0.848214286	x1	y1	z1	x2	y2	z2	x5	y5	z5												
combo_32	0.896551724	0.848214286	x2	y2	z2	x5	y5	z5	x7	y7	z7												
combo_34	0.865900383	0.848214286	x2	y2	z2	x6	y6	z6	x7	y7	z7												
combo_48	0.888888889	0.848214286	x4	y4	z4	x5	y5	z5	x7	y7	z7												
combo_55	0.904214559	0.848214286	x5	y5	z5	x7	y7	z7	x8	y8	z8												
combo_82	0.927203065	0.848214286	x1	y1	z1	x4	y4	z4	x5	y5	z5	x6	y6	z6									
combo_90	0.904214559	0.848214286	x1	y1	z1	x5	y5	z5	x7	y7	z7	x8	y8	z8									
combo_104	0.904214559	0.848214286	x2	y2	z2	x4	y4	z4	x5	y5	z5	x8	y8	z8									
combo_110	0.911877395	0.848214286	x2	y2	z2	x5	y5	z5	x7	y7	z7	x8	y8	z8									
combo_8	0.911877395	0.857142857	x1	y1	z1	x3	y3	z3	x5	y5	z5												
combo_18	0.911877395	0.857142857	x1	y1	z1	x5	y5	z5	x8	y8	z8												

combo_19	0.858237548	0.857142857	x1	y1	z1	x6	y6	z6	x7	y7	z7												
combo_54	0.911877395	0.857142857	x5	y5	z5	x6	y6	z6	x8	y8	z8												
combo_63	0.938697318	0.857142857	x1	y1	z1	x2	y2	z2	x4	y4	z4	x6	y6	z6									
combo_67	0.896551724	0.857142857	x1	y1	z1	x2	y2	z2	x5	y5	z5	x7	y7	z7									
combo_68	0.91954023	0.857142857	x1	y1	z1	x2	y2	z2	x5	y5	z5	x8	y8	z8									
combo_71	0.91954023	0.857142857	x1	y1	z1	x2	y2	z2	x7	y7	z7	x8	y8	z8									
combo_84	0.911877395	0.857142857	x1	y1	z1	x4	y4	z4	x5	y5	z5	x8	y8	z8									
combo_103	0.911877395	0.857142857	x2	y2	z2	x4	y4	z4	x5	y5	z5	x7	y7	z7									
combo_124	0.923371648	0.857142857	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8									
combo_4	0.842911877	0.866071429	x1	y1	z1	x2	y2	z2	x6	y6	z6												
combo_21	0.91954023	0.866071429	x1	y1	z1	x7	y7	z7	x8	y8	z8												
combo_31	0.858237548	0.866071429	x2	y2	z2	x5	y5	z5	x6	y6	z6												
combo_42	0.896551724	0.866071429	x3	y3	z3	x5	y5	z5	x7	y7	z7												
combo_43	0.911877395	0.866071429	x3	y3	z3	x5	y5	z5	x8	y8	z8												
combo_50	0.904214559	0.866071429	x4	y4	z4	x6	y6	z6	x7	y7	z7												
combo_70	0.877394636	0.866071429	x1	y1	z1	x2	y2	z2	x6	y6	z6	x8	y8	z8									
combo_72	0.938697318	0.866071429	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5									
combo_1	0.91954023	0.875	x1	y1	z1	x2	y2	z2	x3	y3	z3												
combo_7	0.931034483	0.875	x1	y1	z1	x3	y3	z3	x4	y4	z4												
combo_16	0.850574713	0.875	x1	y1	z1	x5	y5	z5	x6	y6	z6												
combo_17	0.865900383	0.875	x1	y1	z1	x5	y5	z5	x7	y7	z7												
combo_23	0.915708812	0.875	x2	y2	z2	x3	y3	z3	x5	y5	z5												
combo_28	0.892720307	0.875	x2	y2	z2	x4	y4	z4	x6	y6	z6												
combo_35	0.881226054	0.875	x2	y2	z2	x6	y6	z6	x8	y8	z8												
combo_57	0.938697318	0.875	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4									
combo_58	0.927203065	0.875	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5									
combo_85	0.927203065	0.875	x1	y1	z1	x4	y4	z4	x6	y6	z6	x7	y7	z7									
combo_87	0.931034483	0.875	x1	y1	z1	x4	y4	z4	x7	y7	z7	x8	y8	z8									
combo_109	0.908045977	0.875	x2	y2	z2	x5	y5	z5	x6	y6	z6	x8	y8	z8									
combo_120	0.91954023	0.875	x3	y3	z3	x5	y5	z5	x7	y7	z7	x8	y8	z8									
combo_145	0.91954023	0.875	x1	y1	z1	x2	y2	z2	x5	y5	z5	x7	y7	z7	x8	y8	z8						

combo_11	0.91954023	0.883928571	x1	y1	z1	x3	y3	z3	x8	y8	z8												
combo_20	0.865900383	0.883928571	x1	y1	z1	x6	y6	z6	x8	y8	z8												
combo_22	0.9348659	0.883928571	x2	y2	z2	x3	y3	z3	x4	y4	z4												
combo_52	0.885057471	0.883928571	x4	y4	z4	x7	y7	z7	x8	y8	z8												
combo_53	0.888888889	0.883928571	x5	y5	z5	x6	y6	z6	x7	y7	z7												
combo_66	0.888888889	0.883928571	x1	y1	z1	x2	y2	z2	x5	y5	z5	x6	y6	z6									
combo_69	0.873563218	0.883928571	x1	y1	z1	x2	y2	z2	x6	y6	z6	x7	y7	z7									
combo_98	0.9348659	0.883928571	x2	y2	z2	x3	y3	z3	x5	y5	z5	x8	y8	z8									
combo_101	0.9348659	0.883928571	x2	y2	z2	x3	y3	z3	x7	y7	z7	x8	y8	z8									
combo_107	0.892720307	0.883928571	x2	y2	z2	x4	y4	z4	x7	y7	z7	x8	y8	z8									
combo_126	0.9348659	0.883928571	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8									
combo_127	0.938697318	0.883928571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5						
combo_138	0.908045977	0.883928571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x7	y7	z7						
combo_161	0.931034483	0.883928571	x1	y1	z1	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_10	0.904214559	0.892857143	x1	y1	z1	x3	y3	z3	x7	y7	z7												
combo_26	0.915708812	0.892857143	x2	y2	z2	x3	y3	z3	x8	y8	z8												
combo_39	0.923371648	0.892857143	x3	y3	z3	x4	y4	z4	x7	y7	z7												
combo_46	0.927203065	0.892857143	x3	y3	z3	x7	y7	z7	x8	y8	z8												
combo_47	0.911877395	0.892857143	x4	y4	z4	x5	y5	z5	x6	y6	z6												
combo_61	0.938697318	0.892857143	x1	y1	z1	x2	y2	z2	x3	y3	z3	x8	y8	z8									
combo_78	0.9348659	0.892857143	x1	y1	z1	x3	y3	z3	x5	y5	z5	x8	y8	z8									
combo_89	0.9348659	0.892857143	x1	y1	z1	x5	y5	z5	x6	y6	z6	x8	y8	z8									
combo_91	0.9348659	0.892857143	x1	y1	z1	x6	y6	z6	x7	y7	z7	x8	y8	z8									
combo_102	0.931034483	0.892857143	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6									
combo_105	0.908045977	0.892857143	x2	y2	z2	x4	y4	z4	x6	y6	z6	x7	y7	z7									
combo_113	0.923371648	0.892857143	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7									
combo_25	0.9348659	0.901785714	x2	y2	z2	x3	y3	z3	x7	y7	z7												
combo_56	0.931034483	0.901785714	x6	y6	z6	x7	y7	z7	x8	y8	z8												
combo_92	0.927203065	0.901785714	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5									
combo_94	0.946360153	0.901785714	x2	y2	z2	x3	y3	z3	x4	y4	z4	x7	y7	z7									
combo_129	0.965517241	0.901785714	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x7	y7	z7						

combo_133	0.942528736	0.901785714	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x8	y8	z8						
combo_140	0.91954023	0.901785714	x1	y1	z1	x2	y2	z2	x4	y4	z4	x6	y6	z6	x7	y7	z7						
combo_157	0.9348659	0.901785714	x1	y1	z1	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_37	0.91954023	0.910714286	x3	y3	z3	x4	y4	z4	x5	y5	z5												
combo_51	0.927203065	0.910714286	x4	y4	z4	x6	y6	z6	x8	y8	z8												
combo_74	0.954022989	0.910714286	x1	y1	z1	x3	y3	z3	x4	y4	z4	x7	y7	z7									
combo_81	0.927203065	0.910714286	x1	y1	z1	x3	y3	z3	x7	y7	z7	x8	y8	z8									
combo_88	0.888888889	0.910714286	x1	y1	z1	x5	y5	z5	x6	y6	z6	x7	y7	z7									
combo_97	0.946360153	0.910714286	x2	y2	z2	x3	y3	z3	x5	y5	z5	x7	y7	z7									
combo_108	0.923371648	0.910714286	x2	y2	z2	x5	y5	z5	x6	y6	z6	x7	y7	z7									
combo_114	0.950191571	0.910714286	x3	y3	z3	x4	y4	z4	x5	y5	z5	x8	y8	z8									
combo_146	0.931034483	0.910714286	x1	y1	z1	x2	y2	z2	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_163	0.954022989	0.910714286	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7						
combo_176	0.950191571	0.910714286	x2	y2	z2	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_45	0.946360153	0.919642857	x3	y3	z3	x6	y6	z6	x8	y8	z8												
combo_60	0.9348659	0.919642857	x1	y1	z1	x2	y2	z2	x3	y3	z3	x7	y7	z7									
combo_80	0.942528736	0.919642857	x1	y1	z1	x3	y3	z3	x6	y6	z6	x8	y8	z8									
combo_111	0.923371648	0.919642857	x2	y2	z2	x6	y6	z6	x7	y7	z7	x8	y8	z8									
combo_125	0.954022989	0.919642857	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8									
combo_136	0.954022989	0.919642857	x1	y1	z1	x2	y2	z2	x3	y3	z3	x7	y7	z7	x8	y8	z8						
combo_137	0.942528736	0.919642857	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6						
combo_143	0.91954023	0.919642857	x1	y1	z1	x2	y2	z2	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_144	0.938697318	0.919642857	x1	y1	z1	x2	y2	z2	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_151	0.957854406	0.919642857	x1	y1	z1	x3	y3	z3	x4	y4	z4	x6	y6	z6	x8	y8	z8						
combo_159	0.9348659	0.919642857	x1	y1	z1	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8						
combo_160	0.961685824	0.919642857	x1	y1	z1	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_164	0.957854406	0.919642857	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x8	y8	z8						
combo_178	0.961685824	0.919642857	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_40	0.950191571	0.928571429	x3	y3	z3	x4	y4	z4	x8	y8	z8												
combo_41	0.946360153	0.928571429	x3	y3	z3	x5	y5	z5	x6	y6	z6												
combo_75	0.9348659	0.928571429	x1	y1	z1	x3	y3	z3	x4	y4	z4	x8	y8	z8									

combo_76	0.961685824	0.928571429	x1	y1	z1	x3	y3	z3	x5	y5	z5	x6	y6	z6									
combo_77	0.931034483	0.928571429	x1	y1	z1	x3	y3	z3	x5	y5	z5	x7	y7	z7									
combo_106	0.942528736	0.928571429	x2	y2	z2	x4	y4	z4	x6	y6	z6	x8	y8	z8									
combo_112	0.954022989	0.928571429	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6									
combo_119	0.954022989	0.928571429	x3	y3	z3	x5	y5	z5	x6	y6	z6	x8	y8	z8									
combo_122	0.927203065	0.928571429	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7									
combo_123	0.942528736	0.928571429	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8									
combo_142	0.938697318	0.928571429	x1	y1	z1	x2	y2	z2	x4	y4	z4	x7	y7	z7	x8	y8	z8						
combo_147	0.957854406	0.928571429	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6						
combo_170	0.961685824	0.928571429	x2	y2	z2	x3	y3	z3	x5	y5	z5	x7	y7	z7	x8	y8	z8						
combo_9	0.950191571	0.9375	x1	y1	z1	x3	y3	z3	x6	y6	z6												
combo_38	0.954022989	0.9375	x3	y3	z3	x4	y4	z4	x6	y6	z6												
combo_73	0.957854406	0.9375	x1	y1	z1	x3	y3	z3	x4	y4	z4	x6	y6	z6									
combo_79	0.957854406	0.9375	x1	y1	z1	x3	y3	z3	x6	y6	z6	x7	y7	z7									
combo_86	0.957854406	0.9375	x1	y1	z1	x4	y4	z4	x6	y6	z6	x8	y8	z8									
combo_100	0.957854406	0.9375	x2	y2	z2	x3	y3	z3	x6	y6	z6	x8	y8	z8									
combo_116	0.957854406	0.9375	x3	y3	z3	x4	y4	z4	x6	y6	z6	x8	y8	z8									
combo_118	0.954022989	0.9375	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7									
combo_130	0.942528736	0.9375	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x8	y8	z8						
combo_141	0.961685824	0.9375	x1	y1	z1	x2	y2	z2	x4	y4	z4	x6	y6	z6	x8	y8	z8						
combo_148	0.946360153	0.9375	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7						
combo_149	0.961685824	0.9375	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x8	y8	z8						
combo_150	0.965517241	0.9375	x1	y1	z1	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7						
combo_154	0.961685824	0.9375	x1	y1	z1	x3	y3	z3	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_156	0.961685824	0.9375	x1	y1	z1	x3	y3	z3	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_169	0.961685824	0.9375	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_173	0.965517241	0.9375	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_174	0.954022989	0.9375	x2	y2	z2	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8						
combo_182	0.957854406	0.9375	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_59	0.957854406	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x6	y6	z6									
combo_95	0.954022989	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x8	y8	z8									

combo_99	0.957854406	0.946428571	x2	y2	z2	x3	y3	z3	x6	y6	z6	x7	y7	z7									
combo_117	0.954022989	0.946428571	x3	y3	z3	x4	y4	z4	x7	y7	z7	x8	y8	z8									
combo_128	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6						
combo_131	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6						
combo_132	0.965517241	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x7	y7	z7						
combo_134	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x6	y6	z6	x7	y7	z7						
combo_135	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x6	y6	z6	x8	y8	z8						
combo_139	0.957854406	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x8	y8	z8						
combo_152	0.954022989	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x7	y7	z7	x8	y8	z8						
combo_153	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_155	0.9348659	0.946428571	x1	y1	z1	x3	y3	z3	x5	y5	z5	x7	y7	z7	x8	y8	z8						
combo_158	0.954022989	0.946428571	x1	y1	z1	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8						
combo_162	0.957854406	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6						
combo_165	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7						
combo_166	0.957854406	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x8	y8	z8						
combo_167	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x7	y7	z7	x8	y8	z8						
combo_168	0.965517241	0.946428571	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_171	0.957854406	0.946428571	x2	y2	z2	x3	y3	z3	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_175	0.954022989	0.946428571	x2	y2	z2	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_179	0.965517241	0.946428571	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8						
combo_180	0.954022989	0.946428571	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8						
combo_183	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6			
combo_184	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7			
combo_185	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x8	y8	z8			
combo_186	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7			
combo_187	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x8	y8	z8			
combo_188	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x7	y7	z7	x8	y8	z8			
combo_189	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7			
combo_190	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x8	y8	z8			
combo_191	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x7	y7	z7	x8	y8	z8			
combo_192	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x6	y6	z6	x7	y7	z7	x8	y8	z8			

combo_193	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7			
combo_194	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8			
combo_195	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8			
combo_196	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_197	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_198	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7			
combo_199	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8			
combo_200	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8			
combo_201	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_202	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_203	0.961685824	0.946428571	x1	y1	z1	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_204	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7			
combo_205	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8			
combo_206	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8			
combo_207	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_208	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_209	0.961685824	0.946428571	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_210	0.961685824	0.946428571	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8			
combo_211	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7
combo_212	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x8	y8	z8
combo_213	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x7	y7	z7	x8	y8	z8
combo_214	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7	x8	y8	z8
combo_215	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8
combo_216	0.961685824	0.946428571	x1	y1	z1	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8
combo_217	0.961685824	0.946428571	x1	y1	z1	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8
combo_218	0.961685824	0.946428571	x2	y2	z2	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8
combo_24	0.950191571	0.955357143	x2	y2	z2	x3	y3	z3	x6	y6	z6												
combo_44	0.957854406	0.955357143	x3	y3	z3	x6	y6	z6	x7	y7	z7												
combo_93	0.961685824	0.955357143	x2	y2	z2	x3	y3	z3	x4	y4	z4	x6	y6	z6									
combo_96	0.954022989	0.955357143	x2	y2	z2	x3	y3	z3	x5	y5	z5	x6	y6	z6									
combo_115	0.969348659	0.955357143	x3	y3	z3	x4	y4	z4	x6	y6	z6	x7	y7	z7									

combo_121	0.957854406	0.955357143	x3	y3	z3	x6	y6	z6	x7	y7	z7	x8	y8	z8									
combo_172	0.946360153	0.955357143	x2	y2	z2	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_177	0.965517241	0.955357143	x3	y3	z3	x4	y4	z4	x5	y5	z5	x6	y6	z6	x7	y7	z7						
combo_181	0.954022989	0.955357143	x3	y3	z3	x5	y5	z5	x6	y6	z6	x7	y7	z7	x8	y8	z8						

Chapter Seven: The Curious Case of Trophy Skulls in Montana: A Case Study of Six Crania Profiled by the University of Montana Forensic Anthropology Lab (UMFAL)

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Kirsten Green, PhD

University of Montana

Katherine Baca, MA

University of Montana

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ABSTRACT

Cranial remains have been processed and kept postmortem throughout human history. Whether it be for ancestor veneration, social memory, or as a trophy, the idea of keeping a skeletal element, especially the cranium, has a lot to do with our social acceptance and understanding during a specific time. It is not unusual to encounter these crania in archaeological or historical context and they can be used to speak to a specific cultural, social, or temporal practices. That being said, how do we, as forensic anthropologists, handle those crania that are brought to us as forensic cases through a Medical Examiner's office usually under questionable or unknown circumstances. This paper reviews the cases of six trophy skulls received by the University of Montana Forensic Anthropology Lab (UMFAL) within the time span of three years. The authors attempt complete biological profiles, to reconcile police reports and oral histories with the biological findings, and reconstruction of the individual's life history in order to properly repatriate the individuals. The authors also present a change to our terminology of trophy skulls to include souvenir skulls in order to better describe the likely circumstances of their creation.

KEYWORDS: Trophy Skull, Souvenir Skull, Forensic Anthropology, Crania, Montana, Forensic Archaeology.

The term “trophy skull” harkens to the idea of crania being absconded with during times of war. The taking of body parts, specifically crania, has been well researched in the archaeological and forensic record (1–4). From the archaeological context the concept of trophy skulls, along with other body parts, are typical of most societies that took part in some type of warfare and/or those who participated in ancestor veneration. This concept is not new or uncommon, for example there are shrunken heads from South America, the Cheyenne would take body parts as trophies from battle, and the Aztec’s are famous for keeping and displaying the crania of their sacrifices. While it is no longer common to actively create and collect trophy skulls; they still exist and are found quite often (5–7). The question remains, what should be done with trophy skulls in a forensic context?

The intriguing thing about the trophy skulls in Montana is the sheer number, six, that have come though the University of Montana Forensic Anthropology Lab (UMFAL) since 2016. In a state that has a small population and relatively low violent crime rate (compared nationally), skeletal forensic cases are few and far between. Any skeletal material found in the state is sent to the State Crime Lab and then to the UMFAL where forensic analysis is completed. Based on Montana’s geographical location and cultural history, it is usually assumed that any trophy skull found is of Native American ancestry and likely archaeological. It turns out that these assumptions are likely unfounded. They are not trophies of overseas war or from a conflict on American soil. Instead, the majority seem to be archaeological finds that have been altered more recently to serve as a souvenir that can be used as décor or for some other personal purpose.

This paper focuses on the contextual evidence associated with each of the six crania and the biological profile created based on metric and non-metric traits, taphonomy, and postmortem alterations. The goal of this paper is to bring to light the unusual amount of souvenir crania in

Montana, combat the assumptions of bioarchaeologists and forensic anthropologist concerning these types of remains, highlight the methods used to ascertain the biological profile, and discuss the future of the crania as they leave the lab setting. The combination of multiple lines of evidence lead the authors to discuss the various scenarios of post-analysis deposition. For example, do they fall under NAGPRA, are they seized and then sit on a shelf at a law enforcement agency, or are they accessioned into a university collection. Finally, this paper tackles the concept of trophy skulls and suggests a change in the nomenclature to reflect the variety of contexts in which they are found.

Methods

Due to the nature of the UMFAL being an educational facility, cases are assigned to graduate students with a professor supervising all activities in the lab including report writing. That being said, the methods used on each case vary and are described below.

Documentation Methods

Each cranium was photographed using a Nikon D5300 camera, with both a wide angle and a macro lens. Profiles of the crania were photographed, as well as any trauma, pathology, and taphonomic alterations deemed important to the case. In the case of the Wheatland Skull, photos were taken both before and after the cranium was removed from the frame. A Dinolite Edge Digital Microscope with 20x magnification was used to analyze bone anomalies which could not be clearly viewed with a human eye. Standard documentation forms were used to collect data as the analysis of each cranium proceeded. The forensic forms include individual pages for different types of analyses, such as skeletal inventory, sex, age, stature, ancestry, osteometry, pathology, and taphonomy. All data and observations were first recorded on these forms, and were then transferred into a professional report for local law enforcement and the State Crime Lab-Missoula.

Skeletal Analysis Methods

The skeletal analysis of each individual consisted of a biological profile, which includes an estimation of sex, age, ancestry, trauma, pathology, and taphonomy. Stature was not estimated for any of the individuals because of the lack of postcranial remains. The sex of each individual was determined using morphoscopic trait analysis, logistic regression, and metric analysis (8–10). The morphoscopic traits include the nuchal crest, glabella, mastoid process, supraorbital margin, and mental eminence (8). Each of these traits was scored on a 1-5 scale, 1 being very female, and 5 being very male (8). These scores offered an overall view of how male or female a cranium appeared to be and allowed a visual assessment of sex to be determined. The scores of each trait were next entered into Walker's Logistic Regression Excel Spreadsheet to determine male or female. Once each scored trait is entered, the regression offers a male or female determination based on each trait, or combination with a percentage of likelihood (9). All possible cranial measurements were recorded for each individual cranium and were analyzed using FORDISC 3.1 discriminant function analysis which aided in the determination of sex (10) and provided a probability and typicality score.

Estimating the age of unknown skeletal remains can be very difficult without postcranial remains, however, age ranges were estimated for each individual in this sample. A dental analysis was useful in determining adult versus subadult, which offered a starting point in creating an age range. Cranial suture closure was the primary method used for more specific age ranges (8). It is known that cranial suture closure can be quite variable, but without any other skeletal elements, it was agreed that this method might offer useful information (11).

The ancestry of each individual was estimated through use of morphoscopic and metric methods. Morphoscopic traits of the crania were observed and used to place each individual into a likely ancestral group (12). These traits include, but are not limited to, nasal sill, prognathism, shovel shaped incisors, the shape of the zygomatics, and orbital shape (12). Next, cranial measurements were recorded, according to the methods outlined in Standards (8). These measurements were run through FORDISC 3.1 for a metric determination of ancestry (10). The observed traits were also entered into Kales et.al (13) spread sheet to gain another estimation of ancestry. All of these methods were used as multiple lines of evidence to determine the most likely ancestral background for each individual.

Each analysis also consisted of an assessment of pathology, if present. This analysis consists of the initial observation of any pathological anomalies; and the subsequent analysis and comparison to reference material in order to determine the significance, and potential causes. The most common pathology noted within these cases is dental pathologies.

The analysis of taphonomy for each individual included a distinction between normal and expected weather-related taphonomic changes versus changes due to excavation and handling. Weather-related taphonomy includes soil staining, cortical flaking, drying, cracking, root etching, and warping due to exposure to moisture, among other things. Taphonomic changes due to excavation and handling include things like shovel cuts or fractures, crushing, worn edges, or smooth and shiny surfaces. All observable taphonomic changes were recorded for each individual within this sample.

Postmortem alterations were recorded for each cranium. These alterations are what marks these crania as being definitive trophy skulls, and include things such as drawing or markings on the crania, drilled holes, presence of wax and/or glue, etc. The specific alterations may offer information on how each cranium was displayed or kept and factor into the discussion of why these crania are so common and where they may be coming from.

Case	Sex Results	Age Results	Ancestry Results
1	Male	18+	Asian
2	Male	18+	Vietnamese/Asian
3	Male	18+	Native American/Asian
4	Male	30+	Mixed; Asian/African/European
5	Male	14-50	Native American
6	Male	18+	Mixed: Native American/European

Table 1: Results of the biological profile assessment of each cranium.

Case 1

Case 1 came from the town of Sheridan, MT and was received by the UMFAL on November 16th, 2016. The remains consisted of a nearly complete cranial vault and part of the facial skeleton (Image 1).

Postmortem Alterations, Taphonomy, Trauma

The cranium exhibits some postmortem color change including green staining on the right parietal and temporal, likely due to contact with copper, a small area of silver discoloration on the posterior right parietal of unknown origin, and some white staining on the superior aspects of the cranium are potential bird excrement.

The skull exhibits both perimortem fracturing and sharp force trauma. The perimortem fracturing comprises two fractures on the right pterygoid process and one on the left pterygoid process of the sphenoid, as well as one fracture through the right zygomaticomaxially suture. The postmortem fracturing effectively hollowed out the inside of the cranium and created a rectangular hole on the basicranium. The sharp force trauma included a cut through the basilar portion of the occipital, cut marks through the right and left petrous portions of both temporals, multiple cut marks bilaterally along the lambdoidal suture, and two cut marks in the left superior eye orbit with associated bilateral orbital fractures. The postmortem modifications to the base of the skull may represent an attempt at mounting, which supports the conclusion that this cranium represents a souvenir skull.

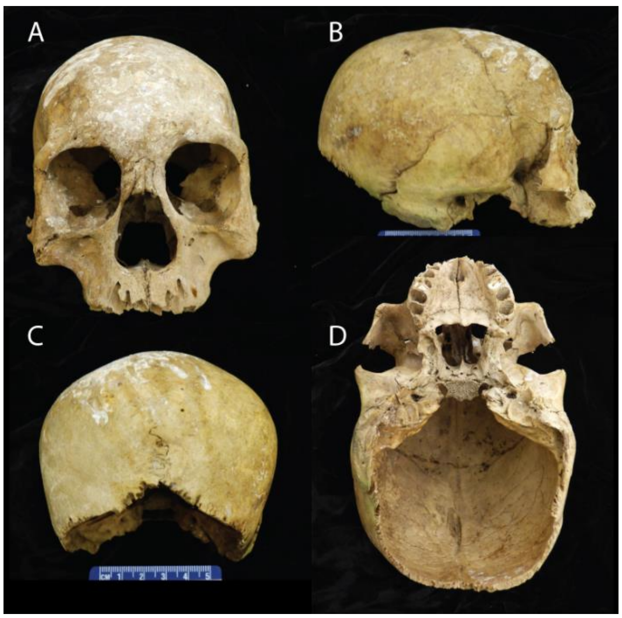

Image 1: Case 1 from Sheridan, MT.

Case 2

Case 2 came from the Billings Police Department and was recovered during a traffic stop in 2015. The UMFAL received the remains on September 25th, 2017. The remains consisted of a complete articulated cranium and mandible (Image 2). All teeth in the mandibular portion of the crania are missing with possible resorption of both third mandibular molars. On the maxillary portion of the crania teeth 5, 3, 13 and 14 are present.

Postmortem Alterations, Taphonomy, Trauma

There is the presence of a glue residue on the condyles of the mandible and in the temporo-mandibular joint of the crania. The glue present on the cranium is consistent with ornamental display of the crania and mandible. According to Yucha et al. (7) the two main sources of trophy specimens in the United States are from WWII and the Vietnam conflict. We have concluded that this individual may have been a trophy skull or souvenir taken during WWII or the Vietnam conflict based on the ancestry assessment (Table 1). This coincides with the statement given by the driver of the vehicle that the cranium was found in a "Vietnam-era" foot locker that was purchased by the father.

Image 2: Case 2 from Billings, MT.

Case 3

Case 3 came from Wheatland County, MT, and the UMFAL received it on October 20th, 2017. The remains consisted of a single, mostly complete cranium and mandible.

Postmortem Alterations, Taphonomy, Trauma

The mandible had been glued and wired to the cranium, and both had subsequently been wired a wooden board which was oval in shape, painted black, framed, and covered with a clear convex piece of plastic (Image 3). Also contained within this frame was a large stone which had at one point been wired to the wooden board as well, but had come lose before our analysis began, likely causing some of the postmortem damage to the cranium (Image 3). Most of the cranial sutures were separated, and some had been glued back together, previous to our analysis. Most of the cranial vault was covered in a clear lacquer or glue. An orange-colored glue was used to attach the mandible to the cranium, and it was present on the left mandibular dentition, as well as a few small spots spattered around the cranium. There was also extensive coffin wear on the left side of the cranium, due to rubbing against the wooden board the cranium was mounted to. There were also small flecks of black paint on the cranium, likely due to the black paint used on the wood mounting board. The only pathology present on these remains was extreme tooth wear.

Image 3: Case 3 from Wheatland County, MT; note the extensive measures taken to display this cranium.

Case 4

Case 4 came from Lewis and Clark County, MT and was received by the UMFAL on October 24th, 2018. The remains consisted of a complete cranium (Image 4).

Postmortem Alterations, Taphonomy, Trauma

Postmortem alterations include a red star drawn on the frontal bone, burn marks right laterally of opisthion, and burn marks on the left occipital condyle. There are also postmortem alterations consisting of red paint located laterally on the occipital condyle and pink paint located posteriorly of the mastoid process, inferiorly of the temporal bone, superior to the right sagittal suture and parietal bone, inferiorly on the left parietal bone, and inferior on the parietal bone, and pencil markings on the sphenoid. Shelf rot is present on the posterior region of the cranium, likely due to sitting in the same location for an extended period of time.

Image 4: Case 4 from Lewis and Clark County, MT; note the red star drawn on the frontal bone of the cranium.

Case 5

This case came from Missoula, MT and was received by the UMFAL on November 13th, 2018. The remains consisted of two large portions of one cranium that can be refitted (Image 5).

Postmortem Alterations, Taphonomy, Trauma

Postmortem damage includes breakage of facial and vault bones where the internal structure of the bone is lighter in color. This is evidence of the break occurring after the cranium was removed from the soil. There are scratch marks on the endocranial surface indicative of an individual trying to clean out the inside of the cranium. There is postmortem tooth loss along with breakage of the alveolar bone above the left C^1, P^1, and P^2. There is glue on the occipital and sphenoid bones, which is likely from a previous parties' attempt at reconstruction. Finally, there is a clear wax on the inside and outside portion of the face, particularly the right orbit and endocranial portion of the right and left petrous. No trauma is present, however, several dental pathologies are present including calculus, abscesses, and resorption.

Image 5: Case 5 from Missoula, MT; notice the wax covering parts of the facial cranium.

Case 6

Case 6 came from Lewis and Clark County, MT and the UMFAL received it on October 30th, 2018. The remains consisted of a mostly complete cranium and mandible (Image 6).

Postmortem Alterations, Taphonomy, Trauma

Postmortem alterations and damage include a large hole cut into the frontal and parietals, approximately at bregma. The hole measures 70.59mm medial-lateral by 76.58mm anterior-posterior. There are fracture lines radiating from the hole down the frontal and right parietal. The edge of the hole is smooth and rounded with beveling on the endocranial surface likely done with a sharp object such as an electric saw or knife. The smooth edge could indicate wear or filing. There is also residual gum substance on the inferior portion of the sphenoid, glue on the right and left temporomandibular joint (TMJ) surfaces, a pink hued wax covering a large part of the inferior endocranium and exuding out the left jugular fossa and the right hypoglossal canal. There is thin wire wrapped through the foramen magnum and the posterior palate.

There is postmortem damage to both right and left squamosal sutures as well as the naso-maxillary suture. There was an attempt to glue most of the sutures including the squamosal sutures, nasal suture, zygotemporal sutures, zygofrontal sutures, and the lambdoidal suture. A small portion of the occipital is missing that runs along the lambdoidal crack. There is a reddish-brown discoloration/stain on the left parietal. The mandible has two drilled holes located on each superior portion of the ascending ramus. There is residual glue on both mandibular condyles. The right M_1 was broken postmortem. Some dental pathology is present and the left TMJ shows evidence of misalignment.

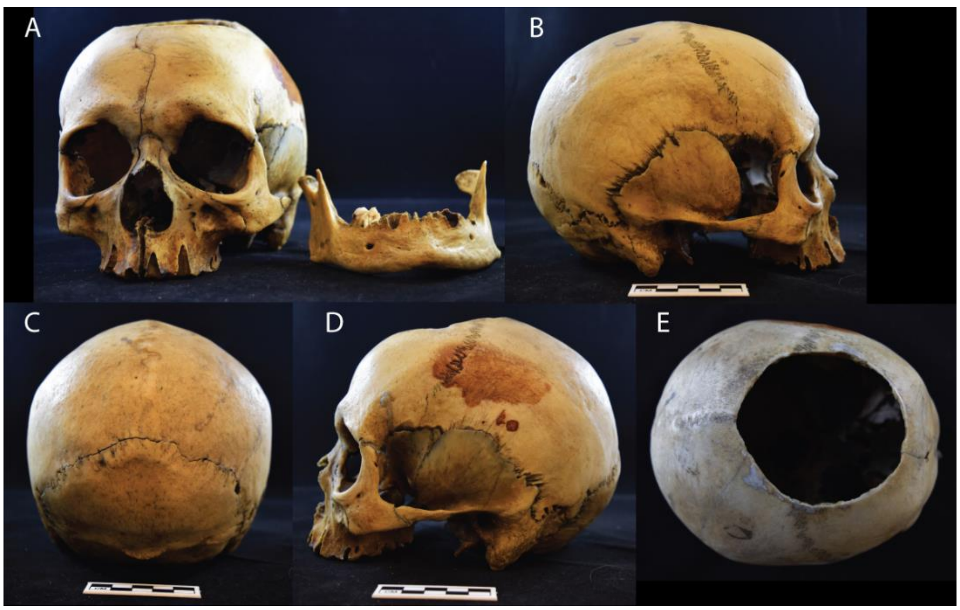

Image 6: Case 6 from Lewis and Clark County, MT; notice the large hole purposefully cut from the top of the cranium.

Discussion

Each case presents its own unique set of obstacles, whether it be a lack of provenience or contestation of disposition that need to be accounted for when considering the future of the skeletal material. While one case may be clearly Native American and should fall under either the federal or states human remains law, another case may not be so cut and dry. Ambiguous cases tend to lead to crania sitting on shelves and falling through the proverbial cracks of bureaucracy.

As a lab we tend to err on the more conservative side and any case that could potentially be Native American, no matter if it's found on private property or not, it is suggested that consultation with and repatriation to the closest Federally Recognized tribe take place. If there is a confident finding that the individual is not of Native ancestry the Montana State Crime Lab has reached out to state and federal agencies to provide guidance on deposition of the crania. If no guidance is given it is suggested that the UMFAL accession the cranium into the permanent collection at the University of Montana. This would provide a safe environment in perpetuity for the remains as well as allow for the crania to be used in teaching the next generation of Forensic Anthropologists.

Where we run into trouble is once the crania leave our possession and return to the crime lab (via the chain of custody), there is no guarantee that the law enforcement agency that takes charge of the material will follow our suggestion. Several ways we have combated this is by creating relationships with our medical examiners, autopsy technicians, and law enforcement agents. This allows for open dialogues even after cases are closed. We have also attempted to implement consistent education of law enforcement by attending coroner trainings and workshops. Information on NAGPRA, repatriation, basic forensic anthropological analyses, and case studies are used to educate those individuals that are the first to come into contact with these craniums and usually the final agency to retain these remains.

Future case studies should aid in demystifying the idea of trophy skulls. These crania are not always from war or conflicts, they are not always Native American, and they are not always of forensic significance. We need to change the nomenclature to reflect the true nature of these crania as souvenir skulls. This suggested terminology encompasses those crania found with no provenience, those found that cannot be assessed an ancestry, and those with alterations that suggest being displayed. We also suggest creating open research plans and online databases to create a sense of transparency that will eventually lead to a collection of legacy data to be used for future research. This will enable research even after the cranium is returned, repatriated, or otherwise retained.

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Chapter Eight: Conclusion

This research demonstrates that the main hypothesis of the project was accepted, indicating that three-dimensional geometric morphometric methods can be accurate and useful in a forensic anthropological context. The results that analyzed the predictive power of all eight landmarks exhibited a 95.5% accuracy rate. This is a quite promising refinement of the method used by Bytheway et. al and indicates that three-dimensional geometric morphometric methods should continue to be studied and tested (1). The second set of results analyzed modeled fragmentary scenarios, which represented fragmented bones in order to determine the predictive power of various combinations of landmarks. These results are of particular importance to the field of forensics because they indicate that this method will be useful in actual fragmentary contexts, something a forensic anthropologist is presented with commonly. All possible landmark combinations of three or more (combinations of two landmarks were not analyzed because that is simply an inter-landmark distance measurement and not a 3-D shape analysis) resulted in 218 possibilities, each of which represented a simulated fragmentary bone. More than half of the 218 possible combinations resulted in accuracies of 90% or higher, and nine specific combinations resulted in an accuracy of 95.5% (just as high as using all eight landmarks).

The implications of the modeled fragmentary results are extremely important because they display how applicable this method could be in forensic analysis. Adding to the credibility of expert forensic anthropologists' testimony in the court of law is especially important as forensic findings are used more commonly in court cases. Improving metric analyses of sex determination is a necessary way to improve accuracy and credibility in an expert's report. There are relatively few metric sex determinations of the pelvis, and metric analyses are more likely to be taken seriously by a jury than is a subjective visual analysis based on the experience of the observer. This research begins to fill this developing need and clearly indicates that this method and similar techniques could soon be used as validated methods in forensic case reports.

This research also indicates that this method will be useful in other contexts and applied to other research areas. Determining sex from only the pubic bone will be useful in a commingled and/or mass grave in which gaining demographic data is important. It could also be applied to bioarchaeological scenarios in which the remaining bone is quite fragmented and other sex determination techniques may not be applicable. The results of this research also indicate that this method could be applied to aging and parity research as well. The PCA scatter plot which is colored to show age groups indicates that the variance increases as individuals age. This is a good indication that this method could be refined to apply to estimating an accurate age range for unknown individuals. When the PCA scatter plot is colored to show parous females versus non-parous females, the parous females display a larger variance than the non-parous females. This data was limited, but it does show that this method has potential to aid in better understanding the shape changes a female individual's pelvis undergoes during the process of pregnancy and/or birth.

The overview of souvenir skulls in Montana opens an important discussion as to how these skulls should be handled in a modern forensic context. Most of the crania analyzed appear to be recently manipulated and simply found, rather than actual trophies from warfare. This suggests that most souvenir crania are not actually relics of war and are more likely to be archaeological in nature and contemporarily modified. This indicates that the general associations and discussions concerning souvenir crania need to shift to a more realistic description of where they may have originated and how they should be repatriated to Native American or other native populations. This includes referring to them as souvenir skulls, rather than trophies. It is suggested that more open research plans and online databases be created to help disseminate knowledge of these crania and to demystify the idea of trophy skulls. This will also allow for larger studies of these crania, even after they have been repatriated or otherwise retained.

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