Estimation of sex from the human hyoid bone

in a contemporary white European population

By
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A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
In Partial Fulfillment of the Requirements for
the Degree of Honours Bachelor of Science

April 2013, Halifax, Nova Scotia

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Date: April 22, 2013

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Abstract

This project evaluates a method of sex estimation using the human hyoid bone in forensic cases. It evaluates the accuracy of six discriminant functions developed by Kindschuh et al. (2010) on an archaeological skeletal population and then applies the functions to a contemporary white European skeletal population from the McCormick Skeletal Collection at the University of Tennessee, Knoxville. The hyoid body and the left and right greater cornua were measured from 134 individuals (68 male; 66 female). Fifteen measurements were taken from fused hyoids and 12 measurements were taken from unfused hyoid bones. Applying discriminant functions developed from archaeological hyoid bones yielded accuracy rates ranging from 79.1% to 92.3% for contemporary white European hyoid bones. Mean and sex specific accuracy rates indicate that two functions developed on archaeological fused hyoids were not accurate in estimating females in a contemporary white European skeletal population. Discriminant functions developed on the unfused hyoid and the hyoid body of fused and unfused hyoids had accuracy rates ranging from 88.1% to 92.3%, indicating that they were efficient for determining sex for a contemporary white European skeletal population. Two-sample t-tests indicate statistically significant differences between archaeological and contemporary populations in the height of the anterior cornua (CHI) of both fused and unfused males. Significant differences are also observed between the archaeological and contemporary populations' total hyoid length (THL) in both males and females. Four of the six discriminant functions developed by Kindschuh et al. (2010) can be applied to contemporary white European hyoid bones; however significant differences in THL and CHI between archaeological and contemporary skeletal populations indicate that discriminant functions developed solely on archaeological fused hyoids are less accurate when applied to contemporary white European hyoid bones.

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CHAPTER 1: INTRODUCTION

The estimation of sex is an important step in the development of a biological profile within the confines of human osteology, whether studying forensic cases or archaeological populations. Accuracy in the estimation of sex is of the upmost importance, as it can affect evaluation of age and stature, due to the necessity to know sex in order to estimate either of these variables. Together, estimations of biological affinity, sex, age and stature can be used to generate a biological profile of an individual, which can help identify unknown human remains.

Many different methods can be applied to human remains, using different skeletal elements and variations in visual assessments or metric measurements. Many bones have been studied in depth to discern and evaluate levels of sexual dimorphism. Estimation of sex is an important aspect of the biological profile generated during skeletal analysis in forensic cases involving unknown human remains. Estimation of sex is also an important factor in mass disaster identifications, insofar as making certain that all bones are correctly identified as being part of the same individual.

1.1 Objectives

The two main purposes of this research was to examine the relationship between the size of the hyoid bone and biological sex in a modern white European population, and to test the accuracy and reliability of discriminant functions developed on archaeological populations on a modern white European population. Though the methods applied during the research were developed on a skeletal population of black African and white

European individuals (Kindschuh et al., 2010), evidence states that skeletons are becoming larger and more robust (Fogel and Grotte, 2011), and thus methods developed on archaeological populations may not be as accurate when examining contemporary skeletal samples. This study evaluated whether there was a significant difference between the size of the hyoids in the archaeological sample and a contemporary white European skeletal sample. This research was carried out by collecting measurements from 134 hyoid bones (males and females) sampled from the McCormick Collection at the University of Knoxville, Tennessee. Methods developed by Kindschuh et al. (2010) were followed. In addition, the discriminant functions developed by Kindschuh et al. (2010) were evaluated in order to determine the reliability of using the functions on a modern white European population.

1.2 The Hyoid Bone

The hyoid is a u-shaped bone in the anterior neck, approximately level with the fourth cervical vertebra (Figure 1.1). The hyoid can be palpated above the thyroid cartilage, on the surface of the anterior neck. This bone, while not articulating with any other bones directly, functions as an anchor for muscles and ligaments in the neck, connecting the cranium, mandible, sternum and shoulder girdle together, along with the larynx, pharynx and tongue (Mukopadhyay, 2010).

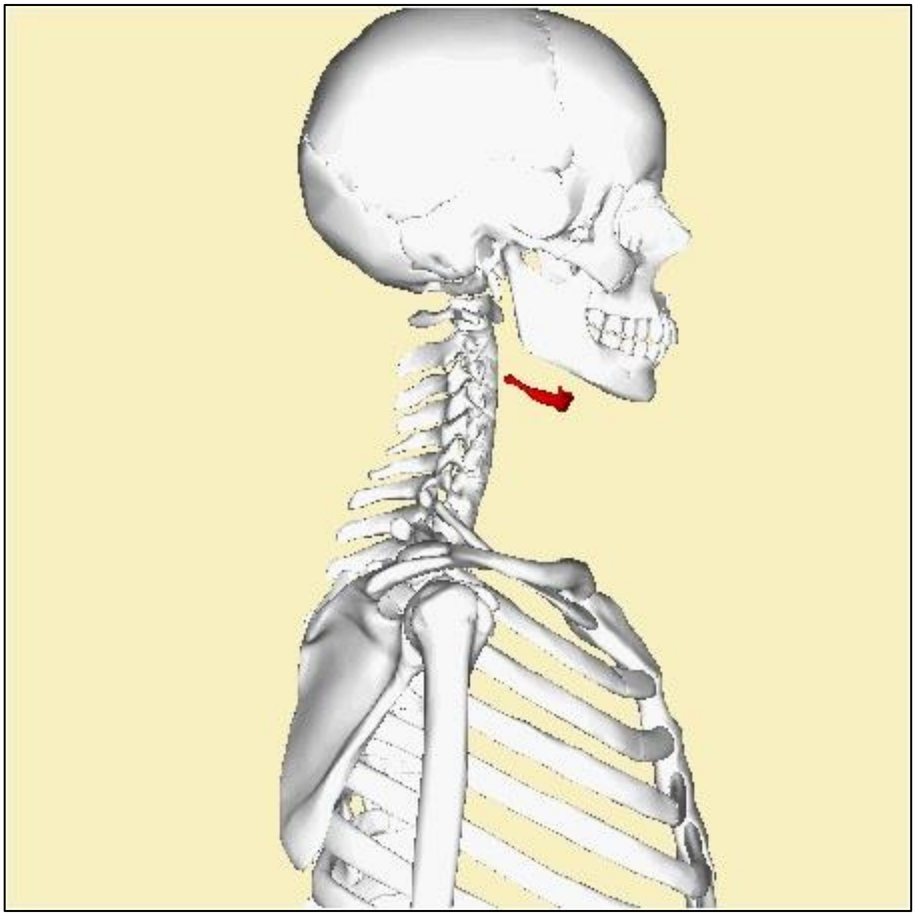

Fig 1.1 Anatomical location of the hyoid bone in the human skeleton (labelled in red) (images taken from the BodyParts3d/Anatomography, under wikimedia creative commons license).

The hyoid consists of three major parts in variable fusion. The body is the middle portion of the bone; it is posterosuperiorly concave, thin and curved. The body of the hyoid is fused or articulated with the horns of the hyoid on the left and right side. The greater horns, or cornua, are thin and long, forming the posterior sides of the hyoid bone by projecting posterolaterally from the body on both the left and right sides (Figure 1.2). The lesser horns are small and conical; these eminences form muscle attachments on the superior surface of the bone where the body and greater cornua articulate and fuse (Koebke, 1978; O'Halloran and Lundy, 1987).

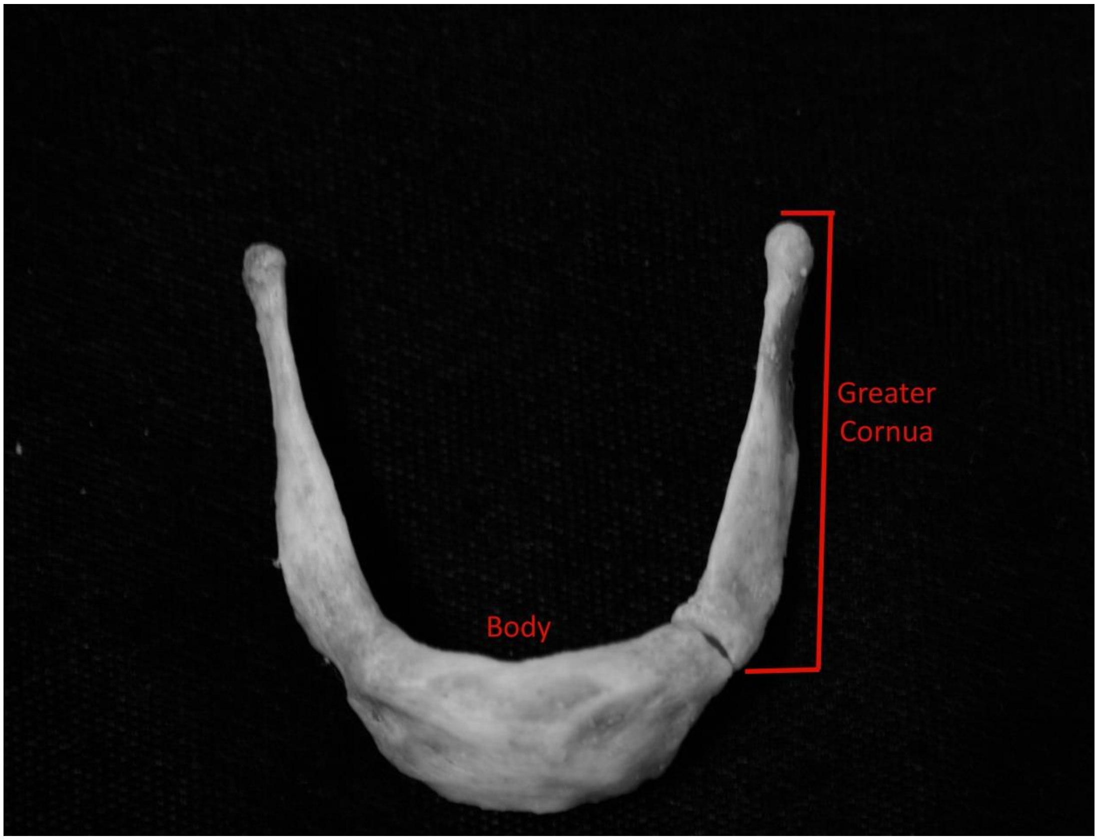

Fig. 1.2 Anatomy of the human hyoid bone (lesser cornua not pictured). (photo by Stephen Walls, permission for photo granted from UTK FAC).

During growth, the hyoid bone develops in a process of endochondral ossification, originating in cartilage of the second and third pharyngeal arches. Ossification begins in six centers; two for the central body, one for the left greater cornua, one for the right greater cornua, one for the left lesser cornua and one for the right lesser cornua. Through development, the first ossification centers to fuse are the ones that form the body of the hyoid. The other fusion centers develop into the cornua independent from the body, attaching by cartilaginous synchondroses, fusing later (if at all) into bony tissue, forming the fully fused hyoid (White et al., 2012).

According to Gupta et al. (2008) the frequency of fusion of the synchondroses into a fused hyoid is correlated with age, though the relationship is not well understood. In general, osseous fusion is not normally found in hyoid bones until the age of 30 years, and even into the third decade remains relatively rare. Incidence of fusion then increases with age, reaching a plateau in the ages of 60-70 years, where the majority of male (70%) and female (60%) hyoids are found to be fully fused. Fusion often does not occur bilaterally, but rather one of the greater cornua's synchondroses will begin to ossify before the other, forming many cases of unilateral fusion in adults (Parsons, 1909). Neither the left nor the right side is more likely to fuse with the body of the hyoid first (Gupta et al., 2008).

1.3 Estimation of sex of the skull

Knowing the sex of human skeletal elements is an incredibly important aspect of forensic cases. Reliability in these methods is required as the estimation of sex is incredibly important to the generation of a biological profile, due to the fact that age, biological affinity and stature estimations depend on the estimation of sex. The skeleton grows in height and ages differently based on sex, and variations in biological affinity can be attributed to sex as well (Stewart, 1979). Many different skeletal elements are used to help determine sex, with varying levels of accuracy. The skull is also used in estimation of sex, with accuracy rates ranging between 80 and 90 % correct-sex identification. Male skulls are generally larger and more robust than female skulls. Aspects of the skull used for estimation of sex include the mastoid process, mental eminence, nuchal crest and the supra-orbital margin and ridge. An issue with estimation of sex from the skull appears to be the influence of biological affinity, as some cranial traits used in estimation of sex are influenced by biological affinity.

1.3.1 Metric methods for estimation of sex from the skull

Metric methods of estimation of sex are comprised of taking measurements of skeletal elements for analyses. These methods are repeatable and more objective than morphological methods, due to the fact that they are more than a visual judgement. Like morphological methods, metric methods for estimation of sex use mainly the pelvis, long bones and skull, along with other skeletal elements.

Norén et al. (2005) measured the lateral angle of the petrous portion of the temporal bone, using angles to determine sex, with accuracies ranging from 77% for estimation of sex for males to 88.3% for estimation of sex for females. Angles less than 45 degrees were identified as males, while those greater than 45 degrees were female. Internal acoustic meatus diameter discriminant function anaylsie generated an accuracy rate of 70% for estimation of sex (Lynnerup et al., 2006), while petrous temporal measurements of 15 landmarks and discriminant function analyses have generated an accuracy rate of 74.19 % for estimation of sex in adult individuals (Akansel et al., 2008).

Gapert et al. (2009) analyzed the foramen magnum and the occipital condyles for identifying sex. Maximum condyle length and width, minimum distance between condyles, maximum interior distance between condyles, maximum bicondylar breadth and external hypoglossal canal were used in discriminant function analyses. The equations developed from these measurements had accuracies ranfing from 59% to 79% for estimation of sex.

Bigoni et al. (2010) studied individuals from Bohemia. Using a contact digitizer, Bigoni et al. (2010) measured 82 ecto-cranial landmarks and 39 other semi-landmarks covering the midsaggital curve of the cranial vault. Sexual dimorphism was observed in the measurements from the midsaggital curve, upper face, nose, orbits and palate. The best indicators of sex from the measurements were found in the upper face and midsaggital curve of the vault, with 99% to 100% of the individuals being correctly identified via this methodology.

Saini et al. (2012) measured the mastioid process of an Indian population. Eight features of the mastoid process were measured, all of which showed sexual dimorphism. Step-wise analysis indicated that mastoid breadth and asterion-mastoidale measurements provided an 87% accuracy rate for sex estimation in an Indian population. Gupta et al. (2012) also measured the sexual dimorphism of the mastoid process in an Indian population, measuring the mastoid length, medio-lateral diameter, anterior-posterior diameter and size of the mastoid process. All features of the mastoid process were indicated to be sexually dimorphic, and discriminant functions developed on each measurement individually indicated that the mastoid length was the best measurement for estimation of sex, with an accuracy rate of 90% (Gupta et al., 2010).

Macaluso Jr. (2010) measured the permanent maxillary molar cusp area for sex estimation in black South African individuals. Cusp areas of both the first and second molars were found to be highly sexually dimorphic, and discriminant function analyses yielded sex estimation accuracy rates between 59.6% and 74.5% for estimation of sex of black South African individuals.

Hassett (2011) measured the buccolingual and mesiodistal cervical diameters of permanent canines in a population from the United Kingdom. Discriminant function analysis using these two measurements indicated a 93.8% accuracy rate for estimation of sex from the measurements of permanent canines in the United Kingdom population group.

Thapar et al. (2011) measured the maximum length and maximum breadth of the cranium along with the maximum mesiodistal and buccolingual diameters of the permanent teeth of right maxillary and mandibular arches in an Indian sample. Logistical regression analysis of both the crania and the teeth together had a sex estimation accuracy of 88.4%.

1.3.2 Morphological methods for estimation of sex from the skull

Morphological methods of estimation of sex use visual observations based on the presence or absence of certain traits. These methods do not require extra tools or calculations and can be made by observation alone. However, due to the relative nature of observations, conclusions drawn can vary greatly between individuals.

Brow prominence, mastoid process size, supra-orbital margin sharpness, nuchal crest development and chin shape have been used as indicators of sex of the skull. Konigsberg and Hens (1998) found that these traits could be used for estimation of sex with 81% accuracy. Walker (2008) applied the same traits, and found that when used in conjunction, accuracy was 89% for estimation of sex, though the mastoid and glabellar were the most successful when used alone.

Cranial indicators tested by Williams and Rogers (2006) reported an accuracy rate of 94% for estimation of sex when the size of the skull was used as an indicator in conjunction with the supraorbital ridge, nasal aperture, zygomatic extension, mastoid size and gonial angle.

1.4 Estimation of sex from postcranial elements

The pelvis is the most accurate skeletal element with which to determine sex; methods range from 90 to 95 % confidence, and thus have become a standard for estimation of sex. Differences in the bone are required for the process of childbirth, making significantly noticeable features in the shape of the pelvis in females when compared to males.

Long bones of the human skeleton have been shown to be useful in the estimation of sex, due to entheses (muscle attachments) present on these bones. These bones have a tendency to be larger and more robust, with more prominently larger muscle attachment in males as opposed to females. Though these methods are quite accurate, these entheses are modified by muscle-building activity, and on an individual basis there can be instances of females developing larger muscle attachments than males, which can lead to misidentification in the estimation of sex.

Other than the pelvis and the long bones, many other skeletal elements have been used for estimation of sex, with varying accuracy based on their methodologies.

1.4.1 Metric methods for estimation of sex from postcranial elements

Metric methods of estimation of sex are comprised of taking measurements of skeletal elements for analyses. These methods are repeatable and more objective than morphological methods, due to the fact that they are more than a visual judgement. Like morphological methods, metric methods for estimation of sex use mainly the pelvis, long bones and skull, along with other skeletal elements.

Cervical vertebrae have been used in the metric estimation of sex. Discriminant function analyses of eight dimensions of the first cervical vertebra have been used on multiple skeletal collections, with sex estimation accuracy ranging from 75% to 85% in the Terry Collection and 60% to 77% in the Hamann Todd collection (Marino, 1995). The second cervical vertebra has also been used for estimation of sex. Discriminant function equations developed from eight measurements of the second cervical vertebra had an accuracy rate of 83% in estimation of sex (Wescott, 2000; Marlow and Pastor, 2011).

Dabbs and Moore-Jansen (2010) developed a five-variable discriminant function for estimation of sex from the scapula, based on previous methods of measuring the maximum height of the scapula (Dwight, 1894). Out of 23 original measurements developed by Dabbs and Moore-Jansen (2010), the five features that displayed the greatest variance in sexual dimorphism were selected to generate the discriminant function on white European individuals with an accuracy rate of 95.7 %. Through testing of this discriminant function, accuracy rates were found to be as low as 84.4 % in a sample that used both black African and white European individuals in the sample to 92.5 % in a sample that only used white European individuals (Dabbs and Moore-Jansen, 2010). Macaluso (2011) measured the photographs left scapulae of 120 black South Africans, collecting measurements of the height, breadth, area and perimeter of the glenoid cavity. Univariate regression analysis yielded sex estimation rates ranging from 85.8% to 88.3% from the measurements of the glenoid cavity (Macaluso Jr., 2010).

Macaluso Jr. et al. (2010) measured the sternum of black South Africans for estimation of sex. Discriminant function analyses indicated that the corpus sterni length and manubrium width were the most sexually dimorphic traits among the black South African population. Resulting discriminant function had an accuracy rate of 86.9% for estimation of sex. Franklin et al. (2012) also used measurements from the sternum in order to estimate sex in a western Australian population. By measuring MSCT scans, Franklin et al. (2010) concluding that using measurements of the lengths of the manubrium and body, manubrium width and corpus sterni width, discriminant functions were developed that had an accuracy range of 72.2% to 84.5% for estimation of sex.

Measurements from the radius and ulna have been useful in estimation of sex. Berrizbeeitia (1989) showed that the maximum and minimum width of the head of the radius were useful for estimating sex, with males having a maximum diameter of 24 mm or greater and females having a measurement of 21 mm or less. Subsequently, discriminant function analyses have been applied to the radius and ulna across various populations. Barrier and L'Abbé (2008) applied discriminant function analyses to a South African population, concluding that using distal breadth, minimum midshaft diameter and maximum head diameter were most accurate in sex estimation in the radius, and the minimum midshaft diameter and olecranon breadth were the most accurate features in sex estimation from the ulna. The accuracies of these features ranged from 76% to 86%.

Purkait (2001) used discriminant function analysis on an Indian population, concluding that the olecranon-cornoid angle and the length of the inferior medial trochlear notch were the most accurate features in estimation of sex of fragmentary ulnae, with an accuracy rate of 90.6%. Cowal and Pastor (2008) applied discriminant function analysis to two archaeological samples, concluding that measurements of the notch length, width of olecranon process, height of olecranon process and radial notch height, four measurements from the proximal ulna were able to discern sex with an accuracy of 85.4%. The use of these discriminant functions was not transferable to contemporary populations, due to the fact that health care improvements and lifestyle change tend to have an effect on bone development (Cowal and Pastor, 2008).

Measurements from the humerus have also been effective indicators of sexual dimorphism. Rogers (1999) suggested that this carrying angle difference results in four different sexually dimorphic traits – trochlear constriction, trochlear symmetry, olecranon fossa shape and angle of the medial epicondyle. Application to archaeological samples has resulted in a 79.1% accuracy rate for estimation of sex. Furthermore, stepwise discriminant function analyses performed on modern Cretan populations resulted in using the maximum humeral length, vertical head diameter, midshaft minimum diameter and epicondylar breadth. Resulting discriminant functions had an accuracy rate of 92.9% for the estimation of sex (Kranioti et al., 2009). Also, modern Japanese populations have been analyzed with stepwise discriminant function analysis, with an accuracy rate of 95 % using the width of distal articular surface of the humerus (Sakaue, 2004).

Measurements of the pelvis have been used to help determine sex of individuals. The greater sciatic notch has been a large part of measurements of the pelvis. Letterman

(1941) concluded that three measurements revealed significant differences between males and females: the maximum height and width of the greater sciatic notch and the distance between the greatest depth of the greater sciatic notch and the posterior inferior iliac spine. Males had a larger greater sciatic notch height, while conversely females had a larger greater sciatic notch width and larger distance between the greater sciatic notch and iliac spine. The greater sciatic notch was also studied by Singh and Potturi (1978), who stated that the posterior angle of the greater sciatic notch was the best parameter for estimation of sex, ranging from 75% to 100% accurate.

Flander (1978) used sacral length and breadth measurements to generate discriminant functions that estimated sex with an accuracy of 80% to 94%. Further study into the sacrum has shown an accuracy rate of 84.2% to 92.1% for estimation of sex using metric analysis of digital photographs of the S1 sacral bone (Bennazi et al., 2009). Gómez-Valdés et al. (2011) found that of 24 measurements of Mexican os coxae and sacral bones, measurements of the transverse acetabular diameter, total pelvic height and total iliac width of the os coxae and maximum transverse diameter of the base, and anterior-posterior diameter of the base of the sacrum were the best estimators of sex for Mexican individuals. Discriminant fucntions based on these measurements yielded sex estimation accuracy rates ranging from 87% to 99% for Mexican individuals. Other measurements of the curvature of the sacrum on North American individuals (Plochoki, 2011) have indicated that sacral curvature is significantly greater in males than in females in the S2-S3 and S3-S4 articualtions. Sex estimation accuracy rates for discriminant functions of the sacral curvature range from 66% to 72% (Plochoki, 2011).

Long bones of the leg have also been useful in estimation of sex. Femoral heads have been studied in Malawian and South African populations, showing significant differences between males and females (Igbigbi and Msamati, 2000). Males in the Malawian population displayed a larger vertical diameter of the femoral head than females (Igbigbi and Msamati, 2000). Males of white European and black African biological affinities in the South African population displayed a significantly larger femoral head than the females, while males of black African biological affinity also showed larger vertical and transverse diameters of the femoral head (Igbigbi and Msamati, 2000). Garcia (2012) measured the nutrient foramen of the tivia for estimation of sex of Portuguese individuals. Discriminant function analysis based on the sexual dimorphism of the nutrient foramen indicated a sex estimation accuracy range of 78% to 90% in two separate Portuguese skeletal collections (Garcia, 2012).

Estimation of sex has been done on both metacarpals and carpals. All metacarpals have been observed for use in estimation of sex. Research by Scheuer and Elkington (1993) indicated a discriminant function developed on measurements from the first metacarpal had an accuracy rate ranging from 74% to 94%. Lazenby (1994) indicated that dominant handedness had a direct effect on the accuracy rates of the discriminant functions developed by Scheuer and Elkington (1993), with accuracy of females in the non-dominant hand dropping as low as 65% accurate. Falsetti (1995) also looked at the metacarpals for estimation of sex, comparing the British samples of Scheuer and Elkington to a North American sample in the Terry Collection using two-way ANOVA, concluding that estimation of sex from this method was 83.3% to 87% accurate. Stojanowski (1999) also tested the metacarpals of a North American population at the

University of New Mexico, concluding the accuracy rate to be 65.2% to 95.7% through linear discriminant function analysis. Barrio et al. (2006) took transverse and longitudinal measurements of the metacarpals for estimation of sex. Males had larger metacarpals than females, with transverse measurements being more different between the sexes than longitudinal.

Case and Ross (2007) found that the carpals and phalanges were more accurate than the metacarpals for estimation of sex, with 80% accuracy using all digits. Further discriminant function analyses indicated the left hand was more relevant for estimation of sex than the right. Sulzmann et al. (2008) researched the carpals in a United Kingdom population; stepwise discriminant function analyses of the measurements of the carpal bones of both hands indicated an accuracy rate of 71.7% to 88.6% in the right hand, while the left hand functions ranged from 73.2% to 87.8%.

Mastrangelo et al. (2011) measured the carpal bones in a contemporary Spanish sample. Using 5 measurements from the lunate, 6 from the scaphoid, 9 from the triquetral, 6 from the capitate, 7 from the hamate, 4 from the pisiform, 7 from the trapezium and 7 from the trapezoid, Mastrangelo et al. (2011) developed a discriminant function that yielded an accuracy rate for sex estimation of 97.8% in a known Spanish skeletal collection.

Introna et al. (1998) measured the patellae of a South Italian population for sex estimation. Using height and width measurements from the articulating facets, along with maximum height, breadth and thickness, Introna et al. (1998) concluded that discriminant functions developed from the patellae in South Italian populations had an accuracy rate of sex estimation of 83.3%. Bidmos et al. (2005) applied the same measurements to a black

South African population, with a resulting sex estimation accuracy of 85%. Using the same measurements, sex estimation accuracy rates have been found to be: 85% in (Kemkes-Grottenhaler), 90.9% in black and white individuals from North America (Mahfouz et al., 2007), 92.9% in an Iranian population (Akhlagi et al., 2010).

The calcaneus and talus of the foot have been used in estimation of sex. Steele (1976) described sexual dimorphism of the calcaneus and talus, developing four discriminant functions for talus and calcaneus measurements with an accuracy range of 79% to 89% for estimation of sex. Furthermore, measurements from the right calcanei in a Southern Italian population had an accuracy rate of 85% in estimation of sex (Introna et al., 1997). Discriminant functions developed on calanei measurements have resulted in accuracy ranges of 73% to 92% in South African white populations (Bidmos and Asala, 2003), and 79% to 86% in South African black populations (Bidmos and Asala, 2004). Gualdi-Russo (2007) found that discriminant functions developed from 18 variables of the left and right talus and calcaneus had accuracies for estimation of sex that ranged from 87.9% to 95.7%, with the talus being more sexually dimorphic than the calcaneus.

Measurements from the talar length, breadth of the trochlea, height of the head and length of the aposterior articular surface have been used for estimation of sex, with accuracies ranging from 80% to 89% in South African white and black populations (Bidmos and Dayal, 2003; 2004). Lee et al. (2012) took nine measurements from the talus, generating three discriminant functions for a Korean population. The first function had an accuracy rate of 86.4% for estimation of sex and used the talar height, trochlear length and length of the posterior articular surface, The second function had an accuracy rate of 72.9% for estimation of sex and used the talar length, talar width and talar height.

The third function had an accuracy rate of 87.9% for estimation of sex and used talar length, talar width, trochlear breadth, trochlear length, head-neck length, head height, length of the posterior articular surface, and breadth of the posterior articular surface.

Harris and Case (2012) measured the length, width and height of all tarsal bones from 160 white European individuals. It was concluded that measurements of the talus, cuboid and cuneiform I were the most sexually dimorphic, and were used to generate discriminant functions with sex estimation accuracies ranging between 88% and 92%.

The metatarsals have also been used in sex estimation. Mountrakis et al. (2010) indicated that measurements of the 1595 metatarsals from 186 adult individuals from a contemporary Greek sample showed significant sexual dimorphism. Discriminant functions developed from the metatarsals in a Greek population has sex estimation accuracies ranging from 80.7% to 90.1%.

1.4.2 Morphological methods for estimation of sex from postcranial elements

Morphological methods of estimation of sex use visual observations based on the presence or absence of certain traits. These methods do not require extra tools or calculations and can be made by observation alone. However, due to the relative nature of observations, conclusions drawn can vary greatly between individuals.

The rhomboid fossa of the clavicle has been shown to be sexually dimorphic. Presence of the fossa was indicated to be indicative that the clavicle is male; the presence of the fossa on a left clavicle was indicative of the specimen being male with 92.2 % accuracy in North American individuals, the presence of the fossa on a right clavicle was indicative of the specimen being male with 81.7 % accuracy in North American individuals (Rogers et al., 2000). Furthermore, in Brazilian individuals, the fossa unilaterally presenting was present in 63.6 % of males and was absent in 97.1 % of females (Prado et al., 2009). Bilateral presentation of the fossa was much less likely, occurring in 29 % of males and only 2.9 % in females (Prado et al., 2009).

Features of the humerus have displayed sexual dimorphism, including trochlear constriction and symmetry, olecranon fossa shape and depth and angle of the medial epicondyle. Accuracy rates have ranged from 91% to 94% for estimation of sex using these features (Rogers, 1999).

The human pelvis is one of the most accurate skeletal elements used in the estimation of sex. Multiple methods were developed for estimation of sex from visual aspects of the pelvis. Early methods developed by Phenice (1969) used the ventral arc, subpubic concavity and ischiopubic ramus. The absence of a trait indicated maleness, while the presence indicated femaleness. Accuracy of this methodology ranges from 83 % (Lovell, 1989) to 96 % (Phenice, 1969).

Işcan and Derrick (1984) concluded that the sacroiliac joint could be used for estimation of sex. Females consistently exhibited a wider postauricular space than males, and iliac tuberisty was shown to be present in all male individuals, but only half the female individuals. Sacroiliac joint bridging has also been studied, with male and female joint bridging being significantly different from one another (Dar and Hershkovits, 2006).

Buikstra and Ubelaker (1994) identified a scoring system for the greater sciatic notch, where classifications of one to five are connected to morphology of the feature. A score of one is an indicator of female morphology, whereas a score of five is strong male morphology.

Bruzek (2002) applied 11 features from the preauricular surface, the greater sciatic notch, the composite arch, the inferior pelvis and the ischiopubic proportions of the pelvis. When all areas were assessed together, accuracy rates ranged from 95% to 98% in estimation of sex. Further testing of this method found accuracies ranging from 90% to 92% in estimation of sex (Listi and Bassett, 2006). An abridged methodology using five features from Bruzek (2002) applied to fragmentary remains in France indicated an accuracy rate of 92.7% (Debono and Mafart, 2006).

Patriquin et al. (2003) used pubic bone shape, subpubic concavity, ischiopubic ramus, ischial tuberosity and the greater sciatic notch to estimate sex. In South African populations, the best indicators for sex were pubic bone shape in females, with accuracies ranging from 88% to 96%, and ischial tuberosity orientation in males, with accuracies ranging from 92% to 96%.

The sacrum has also been used in estimation of sex. Tague (2007) concluded that males exhibited significantly smaller costal processes in S1 than females, and Belcastro et al. (2008) indicated that females show a greater degree of fusion of the sacral body than males.

1.5 Estimation of Sex from the Hyoid Bone

Study of estimation of sex from the hyoid bone has been somewhat limited. The core ideal states that the hyoid displays sexual dimorphism, the basic idea that males and females display differences in size and shape. The hyoid bone has been established to be sexually dimorphic (Jelisiejew et al., 1968), and research has looked into its use as a factor in the estimation of sex. Most research has focused on size and shape differences between sexes, and many have used a variety of statistical methods to attempt to produce standards that can be used to estimate sex in unknown human remains (Papadopoulos et al., 1989; Miller et al., 1998; Reesink et al., 1999; Lekšan et al., 2005, Kim et al., 2006, Kindschuh et al., 2010).

Further investigation of symmetry of the hyoid and the correlation to sex was done by Lekšan et al. (2005). They classified the hyoid into three types, based on morphological variation: symmetrical u-type, symmetrical v-type and asymmetrical type. Types were determined through geometric analysis of an angle derived from the curvature of the overall shape of the fused hyoid. The study stated that female hyoids had higher incidence of the asymmetrical type than males, and a lower incidence of the symmetrical v-type.

Miller et al. (1998) examined hyoids from 188 males and 127 females during autopsy. The hyoid was dissected from the surrounding connective tissue, with care being taken to retain the shape of the hyoid bone. Each hyoid was radiographed at the same distance and exposure, and the resulting radiographs were converted to digital images and 31 measurements (divided into "length" measurements and "width" measurements) were taken from the digital image, along with assessing the overall shape of the hyoid bone. Miller et al. (1998) concluded that hyoid bone dimensions are significantly larger in men than in women, though some dimensions were significantly less dimorphic than others. The hyoid radiograph analyses indicated statistically significant sex differences in 79% of the length measurements, while significant sex differences only presented in 40% of the width measurements. Discriminant function analysis generated an equation that accurately estimated sex in 69.2% of male hyoid bones and 75.2% of female hyoid bones, noting that the most sexually dimorphic measurements came from the distal ends of the greater cornua.

Reesink et al. (1999) examined 33 female hyoids and 26 male hyoids from a cadaveric sample population. Radiographs were taken of the hyoid bones, and 13 measurements were taken directly from the x-ray film. Of the 13 measurements, only three showed statistically significant differences between males and females: the maximum length of the body, the maximum width of the body and the height of the body, with the maximum length of the body being the most significantly discriminating among the differences. A discriminant function based on the three variables was determined to be unreliable, due to an accuracy rate of 72% in estimation of female hyoid bones; the accuracy rate for male hyoid bones was conversely 82%.

Kim et al. (2006) examined hyoid bones from 85 Korean cadavers (52 male and 33 female). Hyoids were dissected from the cadaver and digital photographs were taken of the superior and anterior hyoid bone from a distance of 55 cm. Thirty three measurements were taken from the digital photographs via computer program. Measurements taken from the digital photographs were based on the measurements developed by Miller et al. (1998). A discriminant function developed by Kim et al. (2006) used the distance from the midpoint of the left side of the hyoid body to the midpoint of the right side of the hyoid body, the maximum width of the proximal end of the greater horn, and the length from the narrowest segment of the greater horn to a point equidistant between the distal and proximal ends of the greater horn measured through the central axis of the greater horn on the right greater cornua. Accuracy of this discriminant ranged from 87.9% accurate for females and 88.5% for males.

Kindschuh et al. (2010) selected measurements based on their levels of discrimination between sexes from previous research (Miller et al., 1998, Reesink et al. 1999, Kim et al., 2006). Measurements were taken directly from fused and unfused hyoid bones from the Robert J. Terry anatomical collection, in the Smithsonian Institute's National Museum of Natural History. A total of 398 hyoids were selected, 169 of which were fused and 229 of which were either bilaterally unfused (both the left and right greater cornua were not fused to the body) or unilaterally unfused (either the left or the right greater cornua was fused to the body) (Kindschuh et al., 2010). The study used a uniform sampling population, taking measurements from males and females of both white European and black African biological affinity. The ages of the individuals ranged from 20 years to 79 years, and included 20 individuals from each 10-year increment (20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years, and 70-79 years). Results indicated that there is sexual dimorphism in the hyoid; discriminant functions developed were capable of estimating sex with an accuracy rate ranging from 82% to 85% (Kindschuh et al., 2010).

The discriminant functions developed by Kindschuh et al. (2010) were not developed on contemporary skeletal populations. Humans, as a whole, are becoming larger and more robust over time (Fogel and Grotte, 2011). The discriminant functions developed by Kindschuh et al. (2010) were developed on an archaeological sample, namely individuals born between 1822 and 1943 (Hunt, 2005). With humans becoming more robust, the relationship between the size of the hyoid bone and biological sex in a modern white European population had to be analyzed, and the accuracy of the discriminant functions developed by Kindschuh et al. (2010) had to be tested with regards to a contemporary white European population.

1.6 Legal Significance

A method of estimation of sex, like any aspect of the biological profile, must be tested and accepted by the scientific community before becoming a standard. In the United States in 1993, a ruling based on the *Daubert vs. Merrell Dow Pharmaceuticals* trial introduced strict requirements for introduction of scientific evidence in a courtroom. *Daubert* ruling indicates that in order to be admissible, scientific evidence must be both relevant and reliable. Relevance states that evidence and testimony of that evidence must have bearing to the case it is being given for. Reliability is achieved through scientific means – testing, peer review, known error rates and conclusions that must be met by standard technique (Holobinko, 2012).

Similar to *Daubert*, a ruling in 1994 was established in the Canadian case of *R. v. Mohan*. This ruling established four governing factors of admissibility of evidence in Canada: necessity, relevance, absence of exclusionary rule and proper qualification of the witness. These four factors are an assessment of when expert evidence should be included, which is when the probative value of the evidence outweighs the prejudicial effect it may cause (Holobinko, 2012). Necessity indicates that the evidence presented by an expert witness must be necessary due to the technical nature of the evidence. Relevance is established much the same was as in *Daubert* – testimony must be appropriate for the case it is being given for. The role of the exclusionary rule is relevant to all evidence – it cannot be admissible to court if it has been obtained illegally or inappropriately. This is also true of expert testimony in Canada. Qualification of the witness is the final criteria for expert testimony in Canada, with regards to proper training and certification of experts who give testimony on scientific evidence (Holobinko, 2012).

In both *Daubert* and *Mohan*, peer-reviewed, scientific methodology is important to the admissibility of evidence. This research tested the discriminant functions developed by Kindschuh et al. (2010) on a different population in order to check the validity and reliability of the discriminant function. By testing and validating methodologies established by Kindschuh et al. (2010), the use of the hyoid bone can become more readily available for use in a forensic context.

1.7 Osteological Collection used for this Research

The McCormick Collection is housed at the University of Tennessee, Knoxville, in the Forensic Anthropology Center. The collection contains skeletal elements from more than 900 individuals, with mid- to late-20th century birth years. Bones from this collection are derived from East Tennessee medical examiner cases, consisting of cranial portions with gunshot trauma, clavicles and hyoids. The collection is derived from the post-industrial East Tennessee region, and thus the results may not represent other geographical populations (Fatah et al., 2012). The McCormick Collection is a contemporary skeletal collection containing white European individuals from North America. In being a collection from North America, it can be used to test the limitations of the discriminant functions developed by Kindschuh et al. (2010) on an archaeological North American population.

CHAPTER 2: MATERIALS & METHODS

2.1 Skeletal Materials used for this Research

This research used 136 human hyoid bones, both fused and unfused, from males and females of white European descent. The hyoids were part of the McCormick Collection housed at the University of Tennessee Knoxville Forensic Anthropology Center. The skeletal remains in this collection originated from East Tennessee medical examiner cases from the years 1988 to 1997 which indicate a contemporary skeletal collection. All hyoid bones used in the study were from individuals aged 20 to 49 years old and were separated into three age categories for analyses: 20-19 years, 30-39 years, and 40-49 years. The 136 individuals were distributed evenly between males and females over the age ranges (Table 2.1). The methodology for this project followed the protocol published by Kindschuh et al. (2010).

Table 2.1: Age distribution of male and female hyoid bones examined from the McCormick Collection.

Age Range (years)	**Male Samples (N)**	**Female Samples (N)**
20-29	22	21
30-39	25	23
40-49	23	22
TOTAL	*70*	*66*

The hyoid bones used in this study were selected based on the criteria established by Kindschuh et al. (2010) in order to maintain consistency during the measurement process and to make valid comparisons between the archeological (Kindschuh et al. 2010) and contemporary (current research) skeletal samples. All hyoid bones measured in this study were from white European individuals. Both fused and unfused hyoids were selected for measurement. Eligible hyoids were selected if they displayed no trauma and had both cornua present; hyoid bones were only selected for measurement if both the left and right cornua were present. Furthermore, fused hyoid bones were only selected in samples in which a distinction between the fusion point of the body and the greater cornua was discernible. Discernible fusion points were noted as being faint to clear visible lines of separation between the cornua and the body. These lines had to have been visible on both the left and right side of the fully fused hyoid bone. If there was no discernible line where the cornua fused to the body, the bone was not included in the study. There were a higher number of unfused hyoids in the McCormick Collection, therefore resulting in a larger representation of unfused hyoid bones (Table 2.2).

Table 2.2: Fused and unfused hyoids used for this study.

Sex	**Condition**	**Number (N)**	**Total (N)**
Male	Fused	37	
	Unfused	33	
		Male Total	70
Female	Fused	16	
	Unfused	50	
		Female Total	66
		Cumulative	136

2.2 Methods

This research collected data from both fused and unfused hyoid bones as presented in the protocol of Kindschuh et al. (2010). Fifteen measurements were collected from fused hyoid bones (Figure 2.1a, Figure 2.1b, Table 2.3). Twelve measurements were collected from unfused hyoid bones (Table 2.4, Figure 2.2a, Figure 2.2b).

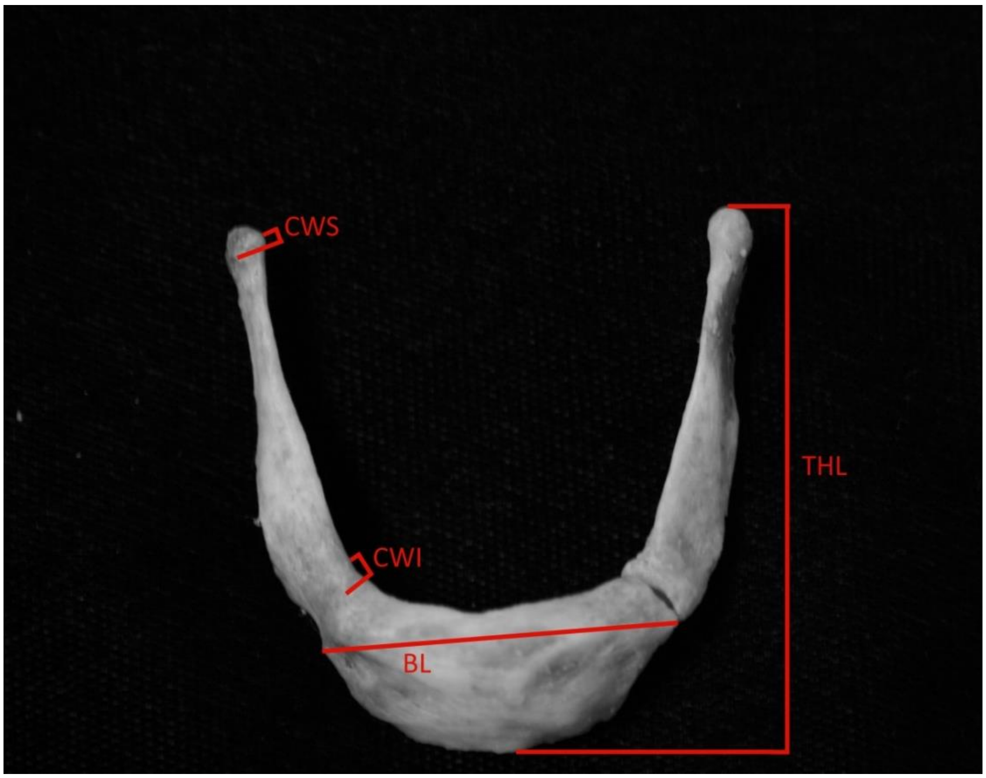

Figure 2.1a: Fused hyoid bone with associated measurements for BL, CWS, CWI, and THL (photo by Stephen Walls, permission for photo granted from UTK FAC).

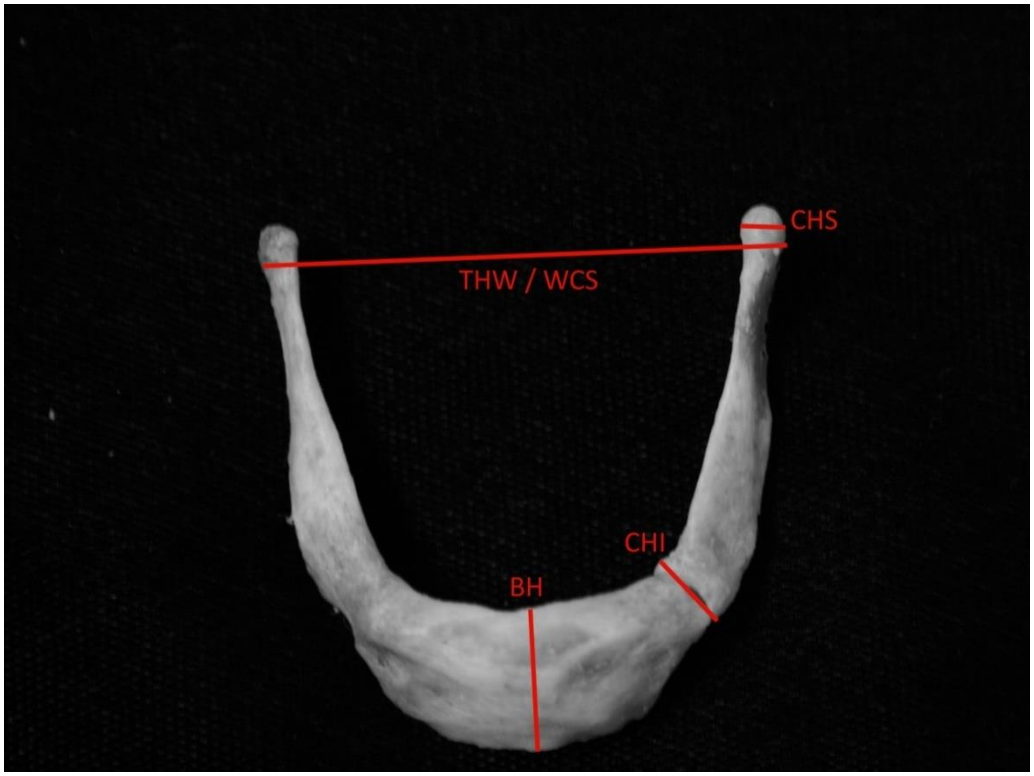

Figure 2.1b: Fused hyoid bone with associated measurements for BH, CHI, CHS, THW and WCS (photo by Stephen Walls, permission for photo granted from UTK FAC).

Table 2.3: Abbreviations used for measurement of fused hyoid bones and their descriptions (modified from Kindschuh et al. 2010).

Abbreviation	Description
BL	The maximum length of the body of the hyoid bone.
BH	The maximum height of the body of the hyoid bone.
CWI	The width of the articulating facet of the hyoid's cornua with the body, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CHI	The height of the articulating facet of the hyoid's cornua with the body, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CL	The maximum length of greater cornua of the hyoid, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CWS	The greatest width of the of the superior end of the cornua, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CHS	The greatest height of the superior end of the cornua, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
THL	Total length of the hyoid from the superior end of the longest cornua to the most anterior protrusion of the hyoid body.
THW	Total width of the hyoid from the two most lateral points on the hyoid bone.
WCS	Total width between superior ends of the right and left greater cornua.

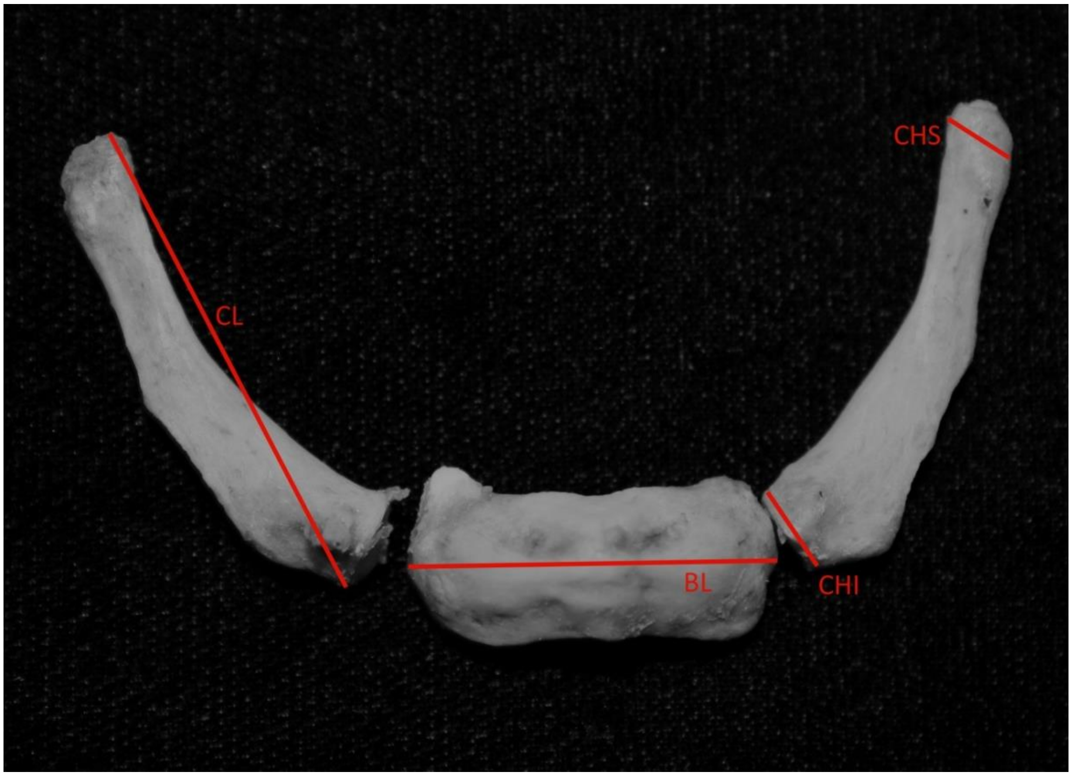

Figure 2.2a: Unfused hyoid bone with associated measurements for CL, BL, CHI and CHS (photo by Stephen Walls, permission for photo granted from UTK FAC).

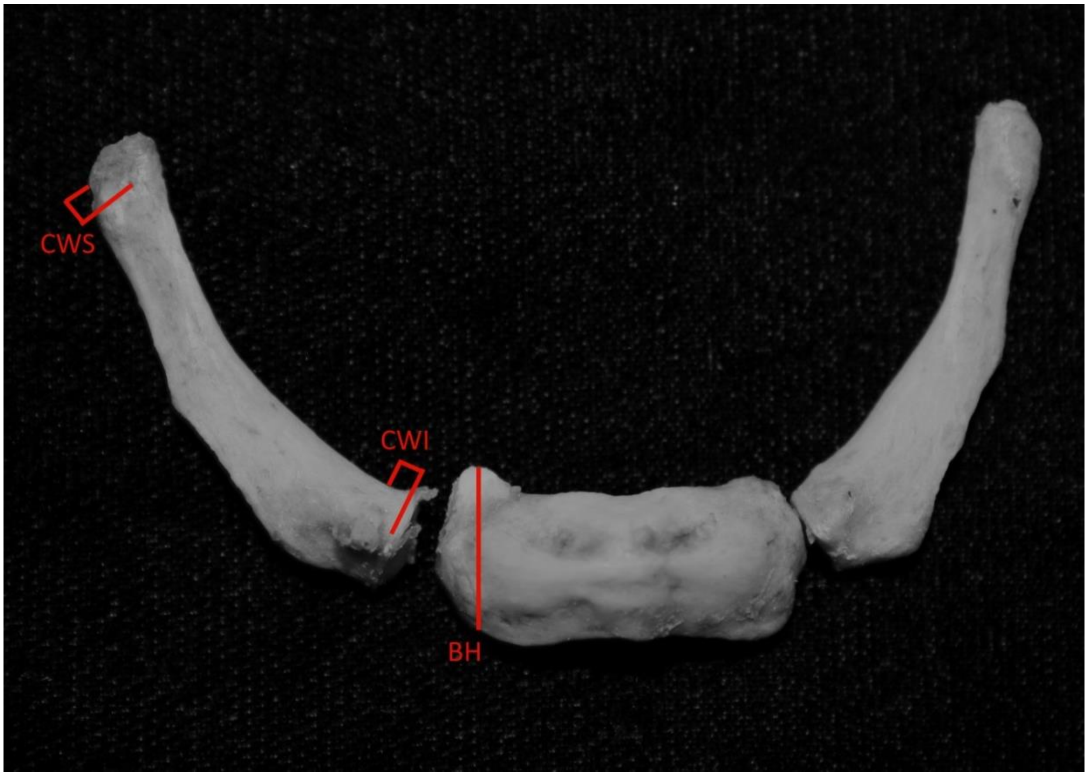

Figure 2.2b: Unfused hyoid bone with associated measurements for BH, CWI and CWS (photo by Stephen Walls, permission for photo granted from UTK FAC).

Table 2.4: Abbreviations used for measurement of unfused hyoid bones and their descriptions (modified from Kindschuh et al. 2010).

Abbreviation	**Description**
BL	The maximum length of the body of the hyoid bone.
BH	The maximum height of the body of the hyoid bone.
CWI	The width of the articulating facet of the hyoid's cornua with the body, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CHI	The height of the articulating facet of the hyoid's cornua with the body, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CL	The maximum length of greater cornua of the hyoid, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CWS	The greatest width of the of the superior end of the cornua, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua).
CHS	The greatest height of the superior end of the cornua, measured from both left and right side (L- prefix denotes left cornua, R- prefix denotes right cornua)

All measurements were determined from each hyoid bone individually by one researcher to control for intra-examiner reliability. Measurements were collected using standard digital calipers measuring to the nearest one hundredth of a millimetre. All data were recorded in an Excel spreadsheet. The information recorded for each individual included: Forensic Anthropology Center reference number, sex, age, year of birth, fused or unfused hyoid condition and all the hyoid measurements collected.

A subsample of ten hyoids was randomly selected and re-measured by the current author to test for intra-observer measurement error rates. This provided data to statistically test that any biological relationships interpreted from this research were not a result of measurement error by the observer.

2.3 Statistical Analyses

2.3.1 Testing for Normality

Normality analyses for BL, BH, THL, THW and WCS were done using standard one-sample Kolmogorov-Smirnov normality testing using Minitab 16 Statistical Software, in order to determine whether parametric (used for normally distributed data) or non-parametric (used for not normally distributed data) tests had to be applied to the data during analyses. Kolmogorov-Smirnov testing was selected for this project as it was the same method of normality testing used by Kindschuh et al. (2010).

In order to test the differences between the individual feature measurements for the left and right cornua, a value was calculated for the difference between each feature measured on both the left and right sides: L-CHI and R-CHI, L-CWI and R-CWI, L-CL and R-CL, L-CWS and R-CWS, and L-CHS and R-CHS. Normality tests were then applied to these differences to ascertain if parametric or non-parametric testing was necessary for comparison between measurements of the sides. The differences between the L-CHI and R-CHI, L-CWI and R-CWI and the L-CHS and R-CHS data were normally distributed, allowing for parametric testing. The differences between the L-CL and R-CL and the L-CWS and R-CWS data were not normal, necessitating non-parametric testing.

2.3.2 Examining differences in size between left and right cornua

Size differences between the left and right sides of all hyoids were examined using several statistical procedures, to examine paired differences between the left and right side of the same hyoid. The purpose of the paired-samples tests is to examine the significances between two related observations – in this research the pairing is between the left- and right-sided features of the same hyoid (Cunningham & Aldrich, 2012, 114). Normally distributed data use paired sample t-testing, while non-normally distributed data use the non-parametric one-sample Wilcoxon test.

Paired sample t-tests were used to examine if there was a significant difference of size between the left and right sides of the normally distributed features, e.g. the L-CHI and R-CHI measurements. The paired sample t-test compares the means of two variables for a single group i.e. in the case of this research, the size in millimetres of a single feature of the hyoid on both the left and right side. The test computes the differences between values of the two variables for each hyoid within the data and tests whether the average calculated from the differences between the two variables differs from zero.

The one-sample Wilcoxon test compares non-parametric matched measurements of samples in order to assess if their population mean ranks are different. Like the paired t-test, the test computes the differences between values of the two variables for each hyoid within the data and tests whether the median differs from zero. The test uses the difference between variables, (i.e. the difference between L-CL and R-CL), and considers information concerning the magnitude of difference between each pair and the sign of the difference (positive or negative) (Cunningham & Aldrich, 2012, 114). Paired sample t-testing was used on the normally distributed CHI, CWI and CHS measurements, and one-sample Wilcoxon testing was used on the non-normal distributed CL and CWS measurements.

2.3.3 Estimation of error rate

Estimating error rates in this research was required in order to ensure that confidence in discriminant functions and comparisons made between contemporary and archaeological samples were due to the actual size of the hyoid features measured in the contemporary population and not due to errors in measurement.

Intra-observer error was assessed by calculating the average error of measurements taken by the same researcher multiple times. Ten hyoids were randomly selected and measured a second time after initial data collection was completed. The differences in measurement for each of the features of the hyoid (BH, BL, average CHI, average CWI, average CL, average CHS, average CWS, THL, THW and WCS) were calculated for each of the 10 hyoid bones selected. The mean difference for each of the features listed was then calculated and recorded as the average error for the feature. All calculations for error rates were completed by Microsoft Excel.

2.3.4 Evaluation of Discriminant Function Accuracy

To test the accuracy of the discriminant functions developed by Kindschuh et al. (2010) on a contemporary population, the overall data set was divided into fused and unfused categories. The fused category contained all hyoids from males and females that were completely fused. The unfused category contained all hyoids from males and females that were unilaterally or bilaterally unfused. This division was due to the fact that the discriminant functions developed by Kindschuh et al. (2010) were dependent on the fusion condition of the hyoid. A total of six discriminant functions were developed for the estimation of sex from the hyoid bone (Kindschuh et al., 2010). Functions 1 to 3 include a variable (A) for biological affinity (Table 2.5). In the cases of the discriminant functions with a variable for biological affinity (A), this research will only apply the coefficient of 2 as indicated by Kindschuh et al. (2010) for samples of white European affinity. Functions 1 and 4 were applied to the measurements taken from fused hyoids. Functions 2 and 5 were applied to the measurements taken from unfused hyoids. Functions 3 and 6 both used only the body length (BL) and body height (BH) measurements, and thus could be applied to both fused and unfused hyoids (Table 2.5). Accuracy rates for each function were calculated by comparing the calculated sex estimation to the biological sex of the hyoid bone used for each function, and then dividing the number of true estimations by the total sample size and multiplying by 100 to achieve %age accuracy. Accuracy rates for each sex were determined by counting the number of true estimations of sex of the male hyoids in the sample and dividing by the total number of males in the sample used for the function, and multiplying by 100 for percent accuracy. For instance, if a function had 26 out of 27 true estimations of sex, the resulting accuracy is therefore ((26/27) x 100 = 96.3%). This method was then repeated for the female hyoids.

Table 2.5: Discriminant functions used for determining sex from hyoid bones (modified from Kindschuh et al. 2010).

Function number	**Hyoid condition**	**Discriminant function**	**Sectioning Point**
1	Fused	(0.133)(THL) + (0.219)(BL) + (0.444)(CHI) + (-0.107)(A) – 12.598	0.0715
2	Unfused	(0.210)(BL) + (0.416)(BH) + (0.498)(CHI) + (0.118)(A) – 13.047	-0.13
3	Body only (fused and unfused)	(0.311)(BL) + (0.498)(BH) + (0.027)(A) – 12.850	-0.092
4	Fused	(0.153)(THL) + (0.220)(BL) + (0.395)(CHI) – 13.123	0.0640
5	Unfused	(0.240)(BL) + (0.413)(BH) + (0.430)(CHI) – 13.006	-0.0765
6	Body only (fused and unfused)	(0.313)(BL) + (0.495)(BH) – 12.827	-0.092

2.3.5 Comparison between Males and Females of the Contemporary Samples

Comparison of the measurements of the hyoid features (Figures 2.1a, 2.1b, 2.2a, 2.2b) of the contemporary males and contemporary females was then completed. Means and standard deviations were calculated for all skeletal features measured in the contemporary skeletal sample. These calculations were done using Minitab 16 software, and were calculated based on criteria set out by Kindschuh et al. (2010). Means and standard deviations were separately calculated for each sex independently for fused and unfused hyoids. This resulted in four separate calculations for each of the skeletal features measured (e.g. separate mean and standard deviation calculations for BL from male, fused hyoids; male, unfused hyoids; female, fused hyoids and female, unfused hyoids). This resulted in a total of 34 calculations being done for means and standard deviations (Table 2.6). Comparison of the mean values of the skeletal features was completed by two-sample t-test. The independent two-sample t-test compares the average difference between the means of two independently sampled groups in order to test if differences between the two groups are significant or occur due to random chance (Cunningham & Aldrich, 2012, 106-107). Minitab 16 was used to compare the skeletal samples by comparing the mean, standard deviation and sample size of each of the skeletal features of the male hyoids to the female hyoids. A total of 17 comparisons were made between contemporary males and contemporary females (Table 2.7). Bonferroni corrections were considered, but deemed irrelevant due to the p-values being below 0.001 in all cases of significant differences. These tests were done to examine whether the features in the contemporary skeletal samples were significantly different between males and females. This was necessary so the results could be interpreted with knowledge of the sexual dimorphism in the contemporary white European sample.

Table 2.6: Breakdown of calculations and recordings made for mean, standard deviation and sample size.

Skeletal Feature	Sex	Fused			Unfused		
		Mean	**S.D.**	**N**	**Mean**	**S.D.**	**N**
BL	M	Calculation 1			Calculation 2		
	F	Calculation 3			Calculation 4		
BH	M	Calculation 5			Calculation 6		
	F	Calculation 7			Calculation 8		
CHI	M	Calculation 9			Calculation 10		
	F	Calculation 11			Calculation 12		
CWI	M	Calculation 13			Calculation 14		
	F	Calculation 15			Calculation 16		
CL	M	Calculation 17			Calculation 18		
	F	Calculation 19			Calculation 20		
CHS	M	Calculation 21			Calculation 22		
	F	Calculation 23			Calculation 24		
CWS	M	Calculation 25			Calculation 26		
	F	Calculation 27			Calculation 28		
THL	M	Calculation 29					
	F	Calculation 30					
THW	M	Calculation 31					
	F	Calculation 32					
WCS	M	Calculation 33					
	F	Calculation 34					

Table 2.7: Breakdown of comparisons made via independent two-sample t-test between contemporary male hyoid measurements and contemporary female hyoid measurements.

Measurement		**Comparison**
BL	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
BH	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
CHI	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
CWI	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
CL	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
CHS	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
CWS	Fused	Contemporary male to contemporary female
	Unfused	Contemporary male to contemporary female
THL	Fused	Contemporary male to contemporary female
THW	Fused	Contemporary male to contemporary female
WCS	Fused	Contemporary male to contemporary female

2.3.6 Comparison between Archaeological and Contemporary Data

Comparison of the skeletal features between the archaeological skeletal sample (Kindschuh et al., 2010) and the contemporary skeletal sample was then completed. Independent two-sample t-tests were used to compare the mean, standard deviation and sample size of each of the skeletal features from the contemporary sample used in the discriminant functions and the analogous mean, standard deviation and sample size in the archaeological sample measured by Kindschuh et al. (2010). A total of 34 comparisons were done between contemporary hyoids and archaeological hyoids (Table 2.8), in order to test to see if there were significant differences in the sizes of skeletal features between males and females in contemporary hyoid bones and archaeological hyoid bones. Bonferroni corrections were considered for the results of the two-sample t-tests, but were deemed to be irrelevant for the differences observed for the variables used in the discriminant functions.

Table 2.8: Breakdown of comparisons made via independent two-sample t-tests between archaeological hyoid measurements (Kindschuh et al., 2010) and contemporary hyoid measurements.

Measurement		**Comparison**	
		Male	**Female**
BL	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
BH	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
CHI	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
CWI	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
CL	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
CHS	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
CWS	Fused	Archaeological to contemporary	Archaeological to contemporary
	Unfused	Archaeological to contemporary	Archaeological to contemporary
THL	Fused	Archaeological to contemporary	Archaeological to contemporary
THW	Fused	Archaeological to contemporary	Archaeological to contemporary
WCS	Fused	Archaeological to contemporary	Archaeological to contemporary

CHAPTER 3: RESULTS

3.1 Statistical Analyses for Normality

Most of the features measured on the hyoid bones from the McCormick Collection had normally distributed data. Kolmogorov-Smirnoff normality testing on all 136 samples (both sexes and all ages combined) identified that distributions for BL, BH, R-CL, R-CWS, L-CHI, L-CWI, L-CL, L-CWS, THL, THW and WCS were all normally distributed. Distributions for R-CHI, R-CWI, T-CHS and L-CHS were not normal (Table 3.1).

Table 3.1: Normality distribution of all features of the hyoid bone in contemporary white European hyoids (136 samples).

Feature	**Normality**
BL	Normally distributed
BH	Normally distributed
R-CHI	Not normally distributed
R-CWI	Not normally distributed
R-CL	Normally distributed
R-CWS	Normally distributed
R-CHS	Not normally distributed
L-CHI	Normally distributed
L-CWI	Normally distributed
L-CL	Normally distributed
L-CWS	Normally distributed
L-CHS	Not normally distributed
THL	Normally distributed
WCS	Normally distributed
THW	Normally distributed

Comparisons were made between the normality plots for all four of the skeletal features that were not normally distributed, and two specimens were identified as outliers among all four normality plots. Both specimens were males from the 30-39 years age subgroup. The remaining 134 samples were tested again for normality (Table 3.2). Two features remained not normally distributed – R-CWI and L-CHS. No significant common outlier was found between the two normality plots, and distributions of these two features were designated to be not normal.

Table 3.2: Normality distribution of all features of the hyoid bone in contemporary white European hyoids (134 samples).

Hyoid Feature	**Distribution**
BL	Normally distributed
BH	Normally distributed
R-CHI	Normally distributed
R-CWI	Not normally distributed
R-CL	Normally distributed
R-CWS	Normally distributed
R-CHS	Normally distributed
L-CHI	Normally distributed
L-CWI	Normally distributed
L-CL	Normally distributed
L-CWS	Normally distributed
L-CHS	Not normally distributed
THL	Normally distributed
WCS	Normally distributed
THW	Normally distributed

In Kindschuh et al. (2010), the mean difference between left and right side measurements of all features were not biologically different from one another. Therefore they averaged the left and right measurements from each feature to obtain one quantity used for the discriminant functions.

Normality of the differences between the left and right side of bilateral features (CHI, CWI, CL, CWS, and CHS) were calculated by Kolmogrov-Smirnoff normality testing (Table 3.3). This was done to assess the capability for averaging measurements take from both left and right sides for discriminant function analyses. The differences between CHI, CWI and CHS measurements were normally distributed, while the differences between CL and CWS measurements were not normally distributed. Paired sample testing was required to see if the measurements from the left and right cornua from the contemporary hyoids were similar enough to be pooled for further comparison analyses.

Table 3.3: Normality distribution the differences between bilaterally measured features of the hyoid bone in contemporary white European hyoids (134 samples).

Name	Calculation	Distribution
Difference of CHI	(R-CHI – L-CHI)	Normally distributed
Difference of CWI	(R-CWI – L-CWI)	Normally distributed
Difference of CL	(R-CL – L-CL)	Not normally distributed
Difference of CWS	(R-CWS – L-CWS)	Not normally distributed
Difference of CHS	(R-CHS – L-CHS)	Normally distributed

The normal distribution of CHI, CWI and CHS allowed for the paired-sample t-test to be utilized (Table 3.4). The comparison of L-CHI to R-CHI resulted in a p-value of 0.511, indicating that the left and right CHI measurements were not significantly different. The comparison of L-CWI to R-CWI resulted in a p-value of 0.038, indicating that the left and right measurements were significantly different from one another. The comparison of L-CHS to R-CHS resulted in a p-value of 0.909, indicating that the left and right CHS measurements were not significantly different from one another.

Table 3.4: Paired-sample t-tests to compare normally distributed left and right measurements of bilaterally measured features of the hyoid bone in contemporary white European hyoids.

Hyoid Feature Comparison	Paired sample t-test		
	t-value	p-value	Sample (N)
L-CHI to R-CHI	-0.66	0.511	134
L-CWI to R-CWI	2.10	0.038*	134
L-CHS to R-CHS	-0.11	0.909	134

*Statistically significant result ($p < 0.05$)

Differences between the left and right sides that were not normally distributed were tested with one-sample Wilcoxon signed rank testing (Table 3.5). For the differences between L-CL and R-CL, the p-value of 0.026 indicated that the measurements for the left and right side were significantly different. For the differences between L-CWS and R-CWS, the p-value of 0.047 indicated that the measurements for the left and right side were significantly different.

Table 3.5: One-sample Wilcoxon signed rank test to compare the not normally distributed left and right measurements of bilaterally measured features of the hyoid bone in contemporary white European hyoids.

Hyoid Feature Comparison	One-sample Wilcoxon test		
	Wilcoxon statistic	p-value	Sample (N)
Difference of CL	5523.5	0.026*	134
Difference of CWS	5264.0	0.047*	134

*Statistically significant result ($p < 0.05$)

Results of the paired-samples tests indicated that L-CHI and R-CHI measurements could be averaged for the discriminant function analyses. L-CHS and R-CHS measurements could also be averaged for discriminant function analyses. There were significant differences between the left and right measurements for CWI, CL and CWS. While statistically significant from one another, these differences were assessed to see if they were biologically relevant (Table 3.6). In the CWI feature, the mean difference in size was 0.08 mm. In the CL feature, the mean difference was 0.33 mm. Finally, the CWS feature displayed a mean difference of 0.06 mm.

Table 3.6: Mean differences of statistically different hyoid features in contemporary white European hyoids.

Hyoid Feature Calculation	**Mean Difference (mm)**
Difference of CWI	0.07
Difference of CL	0.33
Difference of CWS	0.05

Differences between these features were relatively small; they were deemed to be biologically irrelevant in accordance with Kindschuh et al. (2010), who stated that if differences between sides are relatively small, left and right sides can be averaged. Relativity of size was assessed by calculating the mean and standard deviation of each left and right measurement (Table 3.7). If the mean difference was smaller than standard deviation for each of the left and right side of the features independently, the difference was considered to be a biologically irrelevant size difference.

Table 3.7: Independent mean and standard deviations of left and right hyoid features in contemporary white European hyoids.

Hyoid Feature		Descriptive Statistics	
		Mean (mm)	**S.D. (mm)**
CWI	L-CWI	3.98	0.61
	R-CWI	4.06	0.63
CL	L-CL	30.31	3.12
	R-CL	30.65	3.27
CWS	L-CWS	2.97	0.48
	R-CWS	3.02	0.50

Due to mean differences between left and right sides for each feature (Table 3.6) being smaller than the standard deviations for the measurements of each feature (Table 3.7), all of the measurements taken on both the left and right greater cornua were averaged for further analyses.

3.2 Discriminant Function Accuracy

Discriminant functions developed by Kindschuh et al. (2010) from a white European archaeological sample were applied to the contemporary white European sample. The discriminant functions used specific measurements from the hyoid bones as variables in a calculation. The result of the calculation was then compared to a sectioning point, developed specifically for each discriminant function. Results above the sectioning point indicated the sex of the hyoid the measurements were taken from was male; those below the sectioning point were indicated to be female. The estimated sex from the discriminant function was then compared to the biological sex recorded from the McCormick Collection. Correct sex estimations were recorded for each sex individually (Table 3.8).

Table 3.8: Total number of correct sex estimation by discriminant functions developed by Kindschuh et al. (2010) in a contemporary white European population.

Function (See Table 2.5)	**Hyoid condition**	**Sectioning Point**	**Correct estimation of sex of males (correct/total)**	**Correct estimation of sex of females (correct/total)**
1	Fused	0.0715	26/27	8/16
2	Unfused	-0.13	37/41	45/50
3	Body only (fused and unfused)	-0.092	60/68	58/66
4	Fused	0.0640	26/27	5/16
5	Unfused	-0.0765	38/41	46/50
6	Body only (fused and unfused)	-0.092	60/68	58/66

Relevant accuracies of sex estimation were calculated for each discriminant function based on the number of correct sex estimations and the total sample taken. Accuracies for the entire skeletal sample ranged from 72.1% to 92.3% across all discriminant functions. Correct sex estimation for males of all ages ranged between 88.2% and 96.3%, while correct sex estimation for females of all ages ranged between 31.3% and 92.0% (Table 3.9).

Table 3.9 presents the accuracy rate for each of the discriminant functions used in this study, using equations and sectioning points described in previous research (Kindschuh et al., 2010), along with accuracy rates for the hyoid bones used in this study. Function 1 was developed on fused hyoids, using the maximum length of the body (BL), the height of the greater cornua at the fusion point with the body (CHI), and total hyoid length (THL) measurements, along with biological affinity. Function 2 was developed on unfused hyoids, using the maximum length of the body (BL), the maximum height of the body (BH), the height of the greater cornua at the fusion point with the body (CHI), and biological affinity as variables. Function 3 was developed using only the measurements of the body: the maximum length (BL) and maximum height (BH), along with biological affinity. Functions 4-6 utilize the same variables from Functions 1-3, respectively, with the exception of biological affinity.

Table 3.9: Accuracies of Kindschuh et al. (2010) discriminant functions when used on a contemporary white European population.

Function	Hyoid condition	Discriminant function (from Kindschuh et al. [2010])	Mean accuracy	Accuracy of sexes
1	Fused	(0.133)(THL) + (0.219)(BL) + (0.444)(CHI) + (-0.107)(A) – 12.598	79.1 %	M: 96.3 % F: 50.0 %
2	Unfused	(0.210)(BL) + (0.416)(BH) + (0.498)(CHI) + (0.118)(A) – 13.047	90.1 %	M: 90.2 % F: 90.0 %
3	Body only (both fused and unfused)	(0.311)(BL) + (0.498)(BH) + (0.027)(A) – 12.850	88.1 %	M: 88.2 % F: 87.9 %
4	Fused	(0.153)(THL) + (0.220)(BL) + (0.395)(CHI) – 13.123	72.1 %	M: 96.3 % F: 31.3 %
5	Unfused	(0.240)(BL) + (0.413)(BH) + (0.430)(CHI) – 13.006	92.3 %	M: 92.7 % F: 92.0 %
6	Body only (both fused and unfused)	(0.313)(BL) + (0.495)(BH) – 12.827	88.1 %	M: 88.2 % F: 87.9 %

Intra-observer error rates were calculated on repeated measurements of a randomly selected subset of 10 hyoid bones. Differences between the first and second measurement were calculated, and a %age of difference was calculated by dividing the difference in measurement by the original measurement and multiplying the result by 100. Individual error rates for each sample were then averaged for overall average error rate. Average error calculations detailed no error rate above 3.83%, indicating the measurements can be replicated with low levels of error (Table 3.10).

Table 3.10: Average percentage of error of hyoid bone measurements with repeated measurements by one observer.

Hyoid Feature	Average Error
BL	0.54 %
BH	2.35 %
CHI	3.83 %
CWI	3.36 %
CL	2.18 %
CHS	0.1 %
CWS	2.59 %
THL	0.85 %
THW	0.09 %
WCS	0.08 %

3.3 Comparison between Males and Females of the Contemporary Sample

Validation of accuracies of the discriminant functions by Kindschuh et al. (2010) applied to contemporary samples required comparison between males and females of the contemporary sample. This comparison was required in order to make certain that sexual dimorphism of the features measured displayed differences between the sexes. Two-sample t-tests compared the means and standard deviations of skeletal feature measurements of both sexes for fused and unfused hyoids of the contemporary sample (Table 3.11).

Table 3.11 Skeletal feature means (mm) and standard deviations (mm) for the hyoid bones contemporary males and females of white European biological affinity.

Skeletal Feature	Sex	Fused Hyoid			Unfused Hyoid		
		Mean (mm)	S.D. (mm)	Sample (N)	Mean (mm)	S.D. (mm)	Sample (N)
BL	M	25.79	2.08	27	25.31	2.31	41
	F	22.18	1.24	16	21.11	1.85	50
BH	M	11.93	1.28	27	12.39	1.00	41
	F	10.14	0.63	16	10.49	0.87	50
CHI	M	7.37	0.63	27	6.81	0.74	41
	F	6.35	0.59	16	5.52	0.54	50
CWI	M	4.57	0.41	27	4.09	0.60	41
	F	4.053	0.37	16	3.67	0.45	50
CL	M	31.37	2.52	27	32.69	2.71	41
	F	28.51	2.60	16	28.83	2.45	50
CHS	M	4.13	0.79	27	4.38	0.74	41
	F	4.26	0.73	16	4.23	0.53	50
CWS	M	2.94	0.54	27	2.99	0.43	41
	F	3.06	0.37	16	3.02	0.44	50
THL	M	42.91	2.92	27			
	F	38.81	2.59	16			
THW	M	46.53	6.07	27			
	F	39.75	5.57	16			
WCS	M	46.41	6.24	27			
	F	39.10	6.94	16			

Most measurements displayed significant differences between the males and females in both fused and unfused hyoid bones. All measurements used in the discriminant functions (Table 3.11) displayed significant differences between males and females of the contemporary population: this includes the THL (p < 0.001) in fused hyoids, BL in fused hyoids (p < 0.001) and unfused hyoids (p < 0.001), BH in fused hyoids (p <0.001) and unfused hyoids (p < 0.001), and CHI in both fused hyoids (p < 0.001) and unfused hyoids (p < 0.001) (Table 3.12).

Table 3.12: Results of Two-sample Independent T-test showing the differences between male and female hyoids of the contemporary white European sample.

Hyoid Feature	Fused Hyoids			Unfused Hyoids		
	t-value	**D.F.**	**p-value**	**t-value**	**D.F.**	**p-value**
BL	7.13	40	<0.001*	9.42	75	<0.001*
CH	6.12	40	<0.001*	9.56	79	<0.001*
CWI	5.34	33	<0.001*	9.31	71	<0.001*
CHI	4.25	34	<0.001*	3.71	72	<0.001*
CL	3.53	30	0.001*	7.06	81	<0.001*
CWS	-0.55	33	0.588	1.09	70	0.280
CHS	-0.86	39	0.394	-0.33	86	0.744
THL	4.78	34	<0.001*			
THW	3.73	33	0.001*			
WCS	3.46	28	0.002*			

*Statistically significant results (p < 0.05) (D.F. = degrees of freedom)

Only CHS and CWS measurements displayed no significant differences between males and females in the contemporary sample. The lack of significant difference was present in the CHS in both fused (p = 0.588) and unfused (p = 0.280) hyoid bones (Table 3.12). In the CWS measurement, the lack of significant difference was present in both fused (p = 0.394) and unfused hyoid bones (p = 0.744) (Table 3.11). CHS and CWS measurements were not used for any of the discriminant functions and the lack of significant differences for these two features was not explored further.

3.4 Comparison between Archaeological and Contemporary Samples

Comparisons were made between the archaeological and contemporary samples using two-sample independent t-testing. These calculations compared the means, standard deviations and sample sizes of archaeological and contemporary skeletal features that were measured in both Kindschuh et al. (2010) and this research. The means, standard deviations and sample sizes in Kindschuh et al. (2010) were recorded separately for white European and black African individuals, allowing for comparison of archaeological white European individuals to contemporary white European individuals. These means, standard deviations and sample sizes were also recorded separately for fused males, fused females, unfused males and unfused females in Kindschuh et al. (2010), allowing for comparison between archaeological and contemporary skeletal populations for four subgroups per skeletal feature. Two-tailed two-sample t-tests were used to compare the archaeological and contemporary groups, calculating significant differences between the two groups, in order to observe the size difference between archaeological and contemporary hyoid bones.

Significant differences between archaeological and contemporary skeletal features specifically used in the discriminant functions were observed in the CHI measurements for male, fused hyoids ($p = 0.001$), CHI measurements for male, unfused hyoids ($p = 0.022$), THL measurements in male hyoid bones ($p < 0.001$), and THL measurements in female hyoids ($p < 0.001$) (Table 3.13). The mean CHI measurement in the contemporary skeletal sample was smaller in both fused (7.37 mm) and unfused (6.81 mm) hyoid bones (Table 3.13) than in the archaeological sample (8.35 mm for fused and 7.18 mm for unfused). The mean THL measurement for both males (42.91 mm) and females (38.81 mm) of the contemporary population (Table 3.13) were larger than the archaeological population (34.47 mm for archaeological males and 31.86 mm for archaeological females) (Kindschuh et al., 2010).

Outside of the specific measurements used in the discriminant functions, there were several significant differences between archaeological and contemporary males. CWI means in unfused males ($p = 0.001$) were significantly different between archaeological and contemporary skeletal populations, CHS means in fused males ($p = 0.006$) were significantly different between archaeological and contemporary skeletal populations, and CWS means in fused males ($p = 0.007$) were significantly different between archaeological and contemporary skeletal populations (Table 3.13).

Table 3.13: Results of Two-sample Independent T-test showing the differences between archaeological and contemporary male and female hyoid bones.

Hyoid Feature		Fused Hyoids			Unfused Hyoids		
		t-value	**D.F.**	**p-value**	**t-value**	**D.F.**	**p-value**
BL	M	-1.79	41	0.080	-0.45	79	0.655
	F	-0.01	41	0.991	-0.16	76	0.871
BH	M	0.51	47	0.612	-0.64	91	0.522
	F	1.66	42	0.105	-0.12	65	0.903
CWI	M	1.89	27	0.069	3.60	90	0.001*
	F	1.73	41	0.09	-0.19	59	0.852
CHI	M	3.61	32	0.001*	2.33	89	0.022*
	F	1.03	41	0.309	1.44	58	0.156
CL	M	-0.11	27	0.916	-1.97	71	0.053
	F	-1.93	25	0.065	-0.99	25	0.331
CWS	M	2.94	26	0.007*	1.52	56	0.134
	F	-0.52	23	0.606	0.09	31	0.932
CHS	M	2.93	32	0.006*	0.43	69	0.671
	F	-0.82	26	0.421	-0.59	28	0.563
THL	M	-5.57	43	<0.001*			
	F	-7.33	35	<0.001*			
THW	M	-1.58	46	0.121			
	F	0.80	28	0.431			
WCS	M	-1.04	38	0.307			
	F	1.48	30	0.149			

*Statistically significant results ($p < 0.05$) (D.F. = degrees of freedom)

CHAPTER 4: DISCUSSION

4.1 Discriminant Function Accuracy for Estimation of Sex

Discriminant functions in the contemporary white European sample had much more variation in accuracy for estimation of sex than those in the archaeological sample. Functions 1-6 in the contemporary white European sample had accuracies that ranged from 72.1% to 92.3% (Table 3.12), while the archaeological sample mean accuracies ranged from 82.8% to 85.2% (Kindschuh et al., 2010). These differences in accuracy were likely related to differences in skeletal anatomy between the archaeological and contemporary samples. Specifically, secular change in height was well observed in long bones between the years 1800 and 1970 (Trotter and Gleser, 1951; Meadows and Jantz, 1995; Jantz and Jantz, 1999). Allometric changes have been observed in both white European and black African North American individuals between the years 1800 and 1970. Long bones have been observed to be positively allometric with stature, and the total lengths of long bones and stature have both increased significantly between the years 1800 and 1970 (Trotter and Gleser, 1951; Meadows and Jantz, 1995; Jantz and Jantz, 1999). The difference between accuracy rates for the archaeological and contemporary skeletal populations could potentially be due to a similar change in length of the hyoid bone over time.

The overall length of the hyoid bone is represented by the THL measurement (Figure 2.1a). A highly significant difference in THL measurement was seen in both males ($p < 0.001$; Table 3.12) and females ($p < 0.001$; Table 3.12) between the archaeological and contemporary sample. When mean measurements of the THL are compared, male hyoids had a mean THL measurement of 34.47 mm in archaeological white European hyoid bones (Kindschuh et al., 2010) and 42.91 mm in contemporary white European hyoid bones (Table 3.11). Female hyoids had a mean THL measurement of 31.86 mm in the archaeological white European hyoid bones (Kindschuh et al., 2010) and 38.81 mm in the contemporary white European hyoid bones (Table 3.12). Jantz and Jantz (1999) have indicated that secular changes in long bone length are more prominent in males than in females. This is also reflected in the THL measurements between the archaeological and contemporary population. The difference in size between the mean contemporary male hyoid THL measurement (42.91 mm; Table 3.11) and the mean archaeological male THL measurement (34.47 mm; Kindschuh et al., 2010) was 8.44 mm. The difference in size between the mean contemporary female hyoid THL measurement (38.81 mm; Table 3.11) and the mean archaeological male THL measurement (31.86 mm; Kindschuh et al., 2010) was 6.95 mm. Male hyoids showed a greater difference between archaeological and contemporary bones than female hyoids. The difference between accuracy rates for the archaeological and contemporary skeletal populations may be due to changes in the THL measurement between archaeological and contemporary hyoid bones.

THL measurements were used in both Functions 1 and 4 (Table 2.5). The significant increases in the THL measurements for both males and females in the contemporary white European sample resulted in different accuracy rates for estimation of sex due to the difference in size of hyoid bones between the archaeological and contemporary skeletal populations. Larger measurements for THL in contemporary males caused more results of calculations from Functions 1 and 4 to be above the sectioning point, increasing the percentage of males estimated correctly in Functions 1 and 4 in the contemporary white European population when compared to the archaeological population. The accuracy rate for the estimation of sex in males for Function 1 was 96.3% in the contemporary sample (Table 3.9) and 87.3% in the archaeological sample (Kindschuh et al., 2010). The accuracy rate for the estimation of sex in males for Function 4 was 96.3% in the contemporary sample (Table 3.9) and 84.4% in the archaeological sample (Kindschuh et al., 2010). The increases in accuracy rates for estimation of sex of male hyoids in Functions 1 and 4 were connected to larger THL measurement observed in contemporary white European male hyoids. Similarly, the increase in THL measurements for females caused more results of calculations from Functions 1 and 4 to be above the sectioning point, increasing the percentage of females incorrectly estimated as males in Functions 1 and 4 in the contemporary white European population when compared to accuracy rates of the archaeological population. The accuracy rate for the estimation of sex in females for Function 1 was 50% in the contemporary sample (Table 3.9) and 81.9% in the archaeological sample (Kindschuh et al., 2010). The accuracy rate for the estimation of sex in males for Function 4 was 31.3% in the contemporary sample (Table 3.9) and 81.9% in the archaeological sample (Kindschuh et al., 2010). The decreases in accuracy rates for estimation of sex of female hyoids in Functions 1 and 4 were connected to the larger THL measurement observed in contemporary white European female hyoid bones. Much like the significantly larger measurements observed in long bones, the significantly larger THL measurement in contemporary white European hyoids makes Functions 1 and 4 unreliable for the estimation of sex of contemporary white European females. Functions 2, 3, 5 and 6, derived from the archaeological sample, remained reliable for use on contemporary white

European hyoid bones. However, the accuracy rates for estimation of sex in these four discriminant functions were affected by the use of biological affinity as a variable of the discriminant function calculation.

Functions 2 and 5 use the same measurements from the hyoid – BL, BH and CHI (Table 2.5). Functions 3 and 6 also use the same measurements from the hyoid – BL and BH (Table 2.5). However, Functions 2 and 3 used biological affinity as a variable, represented by (A) (Table 2.5), whereas Functions 5 and 6 did not use biological affinity as a variable in the calculation. The mean sex estimation accuracy for Function 2 (90.1%; Table 3.9) is lower than Function 5 (92.3%; Table 3.9), and the mean sex estimation accuracies for Functions 3 and 6 are the same (88.1%; Table 3.9). This indicated that in a contemporary white European sample of hyoids, the use of biological affinity in a calculation has a negative effect on the accuracy of discriminant functions for estimation of sex from the hyoid bone.

The assessment of biological affinity in forensic anthropology can make use of either morphoscopic (visual assessment) or morphometric (mathematical calculations) to assess biological affinity of skeletal elements. There are no systematic methodological approaches to the morphoscopic assessment for biological affinity, nor are there any error rates associated with the visual methods (Hefner, 2009). However, even in morphometric methods, which do contain error rates for estimation of biological affinity, there remain no indications of genetically mediated differences in biological affinity, and that genetic differences between biological affinities are essentially meaningless beneath the 5% to 10% genetic diversity that can be loosely attributed to the concept of ‘race’ (Hunt and Megyesi, 2008). In fact, there is more genetic diversity within a population than between population groups, and that differences between biological affinities that have been seen in the past are disappearing in both genetic variation and skeletal morphology (Hunt and Megyesi, 2008). For example, between 1960 and 1992, the rate of mixed affinity partnership, and therefore the resulting mixed offspring, had risen from 0.4% to 2.2% of all marriages in the United States (Wright et al., 2003); i.e. the archaeological skeletal population is more genetically homogenized than the contemporary population. Therefore, the use of biological affinity within discriminant function analyses may be a reason for the lower accuracy rates of discriminant functions that use biological affinity (e.g. 90.1% in Function 2; Table 3.9) as opposed to discriminant functions that use the same measurements, but do not use biological affinity (e.g. 92.3% in Function 5; Table 3.9).

4.2 Comparison Between Males and Females of the Contemporary Sample

Two-sample t-testing for differences between contemporary white European male and female hyoids revealed some interesting results. The measurements taken from the distal ends of the greater cornua – CWS and CHS (Figures 2.1a, 2.1b, 2.2a and 2.2b) - showed no significant difference between the sexes in the contemporary white European skeletal population (Table 3.12). However this is not in agreement with previous research that has shown sexual dimorphism in the distal ends of the greater cornua (Miller et al., 1998; Kim et al., 2006; Kindschuh et al., 2010). Miller et al. (1998) concluded that there was sexual dimorphism in measurements taken from the maximum diameter of the distal end of the greater cornua ($p = 0.03$). The conclusion of sexual dimorphism from maximum diameter measurements of the distal end of the greater cornua was also found by Kim et al. (2006) in the both the left (p = 0.037) and right cornua (p = 0.01). Comparative measurements to those taken by Miller et al. (1998) and Kim et al. (2006) in this research were the CWS and CHS measurements (Figures 2.1a, 2.1b, 2.2a, 2.2b). Kindschuh et al. (2010) found significant differences between males and females in the CWS measurement of fused hyoids (p = 0.017) and the CHS measurement of both fused (p = 0.004) and unfused (p = 0.037). In the current research, the CWS measurements were not sexually dimorphic in either fused (p = 0.588; Table 3.12) or unfused (p = 0.280; Table 3.12) hyoid bones in the contemporary white European sample. The CHS measurements also showed no difference between the sexes in either fused (p = 0.394; Table 3.12) or unfused (p = 0.744; Table 3.12) hyoid bones in the contemporary white European sample. This indicated that there is no sexual dimorphism in the distal ends of the greater cornua in the contemporary white European sample; which goes against the findings of Miller et al. (1998), Kim et al. (2006) and Kindschuh et al. (2010). However, the indication of no sexual dimorphism between CWS and CHS measurements in the contemporary males and females is likely related to age at death of the individuals included in this research as compared to previous studies.

Sexual dimorphism of the distal ends of the greater cornua may be related to the range of ages at death of the samples of the individuals used in each research population. Shimizu et al. (2005) concluded that the distal ends of the greater cornua of the hyoid bone decrease in size during aging due to fixation of the cornua during fusion. Gupta et al. (2008) indicated that fusion of the hyoid bones generally begins in the third and fourth decade, with mean age of bilateral fusion of female hyoids being 48.50 years and mean age of bilateral fusion of the male hyoids being 53.16 years Shimizu et al. (2005)

indicated that after bilateral fusion of the hyoid bone, the body and greater cornua go through morphological changes, with the distal ends becoming less dense and the body becoming more dense as a result of fixation of the bone. The current research measured a contemporary white European sample with an age range of 20 years to 49 years (Table 2.1), with a mean age of females being 35 years and a mean age of males being 33.8 years. Both the mean ages of males and females in the contemporary sample were well below the mean age of bilateral fusion as stated by Gupta et al. (2008). Also, there was higher representation of unfused hyoid bones than fused hyoid bones in the contemporary white European sample (Table 2.2). Both of these factors indicate that the contemporary white European sample was comparatively too young in age at death to observe the remodeling of the hyoid after bilateral fusion as described by Shimizu et al. (2005), which would have been observed more in the skeletal samples with a wider range of ages at death. Miller et al. (1998) measured males with an average age at death of 41.7 years and females with an average age at death of 53.16 years. Kim et al. (2006) measured the hyoids of individuals with an average age at death of 52 years. Kindschuh et al. (2010) measured an even distribution of individuals between the ages of 20 years to 79 years. All of these samples had an older mean age at death than the contemporary white European sample used in the current study, and likely saw more of the remodeling of the hyoid bone after bilateral fusion and fixation described by Shimizu et al. (2005), which could have led to conclusions of sexual dimorphism that were reliant on both age at death and sex.

4.3 Comparison Between Archaeological and Contemporary Hyoid Samples

Differences between the archaeological and contemporary hyoid bones are quite evident. Laws for immigration and mixed biological affinity relationships in the United States changed radically in the 1950s and 1960s, which lead to more mixing of biological affinity in individuals in the contemporary sample (Wright et al., 2003). However, other differences were observed in the comparisons between archaeological and contemporary hyoid bones. The CWI measurement in unfused males was significantly different between archaeological and contemporary white European samples ($p = 0.001$; Table 3.13), as was the CWS measurement in fused males ($p = 0.007$; Table 3.13). Differences in the CHI measurement between archaeological and contemporary white European individuals were observed only in males in both the fused ($p = 0.001$; Table 3.13) and unfused ($p = 0.022$; Table 3.13) hyoid bones. These differences did not impact the accuracy of the discriminant functions in any negative way, but were worth examining, as they represent differences between populations. Significant differences between the archaeological and contemporary sample may have been due to environmental factors, such as the prevalence of disease in archaeological samples as opposed to contemporary skeletal populations.

Diseases could have been responsible for differences in the hyoid bone between archaeological and contemporary hyoid bones. Some diseases do affect the hyoid bone during their infection. For instance, Ojiri et al. (1998) concluded that infectious abscesses resulting from tonsillitis, odontogenic infection or trauma could extend through the greater cornua of the hyoid bone through the middle layer of the deep cervical fascia. Small abscesses could form around the greater cornua, obliterating paralaryngeal space and changing the shape of the hyoid due to inflammation of the fascial attachment in the infrahyoid muscles, which attach inferiorly in the hyoid body and greater cornua (Ojiri et al., 1998). As untreated abscesses were likely more common in archaeological populations than contemporary, this could account for some of the differences observed in hyoid features measured. CHI and CWI measurements may have been affected by infection of the infrahyoid muscles and abscesses formed around the hyoid.

As stated previously, the age of fusion of the hyoid bone is not a concrete definition, but more of a scale. In the third and fourth decade, bilateral fusion of the hyoid bone becomes more common, increasing with age after this point (Gupta et al., 2008). Shimizu et al. (2005) indicated that after fusion, remodeling occurs in the body of the hyoid and the distal ends of the greater cornua, with the body becoming denser and the distal ends of the cornua becoming less dense after fusion due to fixation of the full hyoid in the neck. These changes continue over the aging process after fusion is complete. Significant differences were observed in the comparison of the archeological and contemporary unfused hyoids in the CWI measurements ($p = 0.001$; Table 3.13) and CHI measurements ($p = 0.022$; Table 3.13). Significant differences observed in the comparison of the archeological and contemporary fused hyoids in the CWS measurements ($p = 0.007$; Table 3.13) and CHI measurements ($p = 0.001$; Table 3.13). These significant differences could be related to the relatively smaller range of ages at death in the contemporary skeletal sample (20-49 years; Table 2.1) than the range of ages at death in the archaeological skeletal sample (20-79 years; Kindschuh et al., 2010). The contemporary sample may not have had much change in the density of the hyoid body and distal ends of the greater cornua, due to the relatively smaller age range, which could result in the significant differences observed in the CWI, CHI and CWS measurements between archaeological and contemporary white European hyoids, due to the proximity of the CWI and CHI to the body and the CWS measurements being taken from the distal ends of the greater cornua.

4.4 Legal Significance

According to the *Daubert* and *Mohan* rulings, scientific evidence must be strongly supported by academic research in order to establish a technique as admissible in court (Holobinko, 2012). Rogers and Allard (2004) have emphasized the need for North American forensic anthropological analyses to conform to standards of *Daubert* in the United States and *Mohan* in Canada. *Daubert* and *Mohan* rulings on expert witness testimony indicate that peer-reviewed scientific methodology in important for the admissibility of expert witness evidence, such as estimation of sex from the hyoid bone. Validation studies and error rates are a major focus of the reliability of forensic anthropological techniques. This research aimed to validate the discriminant functions developed by Kindschuh et al. (2010), from an archaeological population, on a contemporary white European population.

One of the major issues with validation of methodologies in forensic anthropology is the tendency of researchers to adapt techniques rather than testing methods as originally presented (Christensen et al., 2009). In the United States, Federal Rules of Evidence Rule 702 - which deals with the admissibility of evidence - was appended in 2001 to emphasize a connection between the data, methods and conclusions rather than the credentials of the expert witness (Dirkmaat et al., 2008). The focus on forensic anthropological methods from this point has been on replicable and quantitative methods, with a focus on testability and reliability of conclusions.

This study was designed to test the measurement technique and discriminant functions developed by Kindschuh et al. (2010). Measurement techniques were not modified from those described by Kindschuh et al. (2010) and discriminant functions were not changed in any way when being used with the contemporary white European skeletal sample. This study did not aim to modify the discriminant functions used for estimation of sex from the hyoid bone, but to evaluate their reliability when used on a separate population. Four of the six discriminant functions were accurate in estimating both contemporary males and contemporary females reliably. Two of the discriminant functions (Functions 1 and 4; Table 2.5) were unreliable for estimation of sex of females (50% accuracy in Function 1, 31.3% accuracy in Function 4; Table 3.9) in the contemporary white European sample. These unreliable accuracies indicate that each of the discriminant functions developed by Kindschuh et al. (2010) must go through more rigorous testing to be valid for use as expert testimony in forensic cases.

Another major issue with forensic anthropology methods and *Daubert* and *Mohan* rulings are the measurement and report of error rates. *Daubert* and *Mohan* are concerned largely with methodological error (Christensen et al., 2009), which can be established with validation studies such as in this research. Error rates were calculated by measuring 10 of the 136 hyoids a second time after initial data collection was completed. Measurements were repeated for each of the 10 hyoids, and percentage error rates were calculated by subtracting the second measurement from the original measurement, dividing that result by the first measurement and multiplying by 100. This provided separate error rates for each of the hyoid features, which were then averaged to find mean error rate for each of the hyoid features. This provided a methodological error for the measurement of the hyoid bone.

Error rates were relatively low in both the archaeological and contemporary hyoid bone measurements. Intra-observer error was below 5% in all features for the archaeological sample (Kindschuh et al., 2010), and intra-observer error was below 4% in all features for the contemporary white European sample (Table 3.10). There are currently no evaluations of inter-observer error for this methodology, which would be required for better validation of the methods by *Daubert* and *Mohan* rulings (Christensen et al., 2009). Future focus on estimation of sex from the hyoid bone should evaluate inter-observer error in order to further meet the criteria for *Daubert* and *Mohan*.

4.5 Further Investigation

The current research has contributed to the understanding of using the hyoid bone as an estimator of sex in a contemporary white European population. This provided some comparison for the effectiveness of the discriminant functions developed by Kindschuh et al. (2010) when applied to contemporary cases involving white European individuals. However, discriminant functions developed by Kindschuh et al. (2010) focused on both black African and white European individuals from an archaeological skeletal population. The current research only focused on white European individuals from a contemporary skeletal population. Some differences were found in both Functions 1 and 4 (Table 3.9), due to their low accuracy rates for estimation of sex in females of the contemporary white European population (50.0% for Function 1, 31.3% for Function 4; Table 3.9). Further investigation should evaluate the accuracy of the discriminant functions on a contemporary black African population. Continued focus into use of the hyoid as an estimator of sex should focus on measurements directly from the bone, and must look into inter-observer error for this methodology.

CHAPTER 5: CONCLUSION

The current research indicated a sex estimation accuracy rate of 88.1% to 92.3% using four valid discriminant functions (Functions 2, 3, 5 and 6; Table 3.9) on contemporary white European fused and unfused hyoid bones. A total of six discriminant functions, developed previously by Kindschuh et al. (2010) on an archaeological population, were tested. Four of the six discriminant functions were reliable for use on contemporary white European individuals (Functions 2, 3, 5 and 6; Table 3.12); however two discriminant functions that used measurements from the total hyoid length (THL) were highly unreliable for estimation of sex of female hyoids (Functions 1 and 4; Table 3.12).

This research has indicated that measurements from THL are too different between archaeological and contemporary hyoid bones (Table 3.12) to be used reliably in discriminant functions for white European contemporary individuals. Biological affinity did have a negative impact on sex estimation accuracy rates from discriminant functions that included measurements from the greater cornua, as seen in comparisons between Functions 2 and 5 (Table 3.12). The use of biological affinity as a variable in discriminant function calculations had no impact on discriminant functions that took measurements from the body of the hyoid alone, as noted by comparisons between Functions 3 and 6 (Table 3.12). The larger total hyoid length (as seen in contemporary white European individuals), mean age at death of the skeletal sample and prevalence of disease within a population are factors that must be considered when measuring the hyoid bone for discriminant function analyses for estimation of sex. Larger hyoid length in contemporary individuals generated unreliable accuracy rates for estimation of sex in contemporary white European females when using discriminant functions developed on archaeological populations. Also, sexual dimorphism of the body and greater cornua of the hyoid bone is reliant on age, and disease can affect the morphology of the hyoid bone.

The use of the hyoid bone for estimation of sex from discriminant function calculations remains promising, with accuracy as high as 92.3% (Table 3.9). Further investigation must look into the accuracy rates for estimation of sex of black African individuals, and inter-observer error rates for measurements of the hyoid bone in order to be established in forensic cases in the face of *Daubert* and *Mohan* rulings.

ACKNOWLEDGEMENTS

The author would like to thank the Forensic Anthropology Center at the University of Tennessee, Knoxville for allowing access to the McCormick Collection for research. Special thanks also to the supervisor of the project, Dr. Tanya Peckmann and the internal supervisor within the biology department at Saint Mary’s University, Dr. Doug Strongman, for helping to make this a stronger thesis. Finally, thanks also to Dr. Susan Meek of the biology department at Saint Mary’s University for helping with statistical analyses.

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