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Ancestry assessment in subadult skeletons

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ANCESTRY ASSESSMENT IN SUBADULT SKELETONS

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ANCESTRY ASSESSMENT IN SUBADULT SKELETONS

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ABSTRACT

The identification of individuals is the primary goal in any forensic investigation. To facilitate an identification, a biological profile (age, sex, ancestry, stature) for the unknown individual is created by a forensic anthropologist. For adult individuals, the aspects of the biological profile are largely straightforward. For subadult individuals, the only aspect of the profile that can be reliably estimated is age. However, an important but difficult aspect of the biological profile is ancestry. When working toward an identification of a set of subadult remains, it can only be said that the remains are consistent with the demographic profile of a missing child. Little research exists that examines the use of nonmetric traits for ancestry assessment in subadult individuals, and little is known about how the traits are expressed in different age groups. This study examines ancestry assessment in subadult skeletons using the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History, the Terry Osteological Collection at the Smithsonian National Museum of Natural History, and the Johns Hopkins Fetal Skull Collection at the Cleveland Museum of Natural History. To assess ancestry, the skull and mandible of 307 subadult individuals, aged 0-20 years with known demographics are examined using the 15 nonmetric traits examined by Hefner (2009) normally used with adult individuals. Despite difficulties in scoring all 15 traits, there were differences found with each trait when compared to ancestral groups. Even among the youngest individuals in the sample, the traits could be identified and scored.

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LIST OF ABBREVIATIONS

CMNH...Cleveland Museum of Natural History

HTH...Hamann Todd Human Osteological Collection

LDFA...Linear Discriminant Function Analysis

NAGPRA...................................Native American Graves Protection and Repatriation Act

NMNH...National Museum of Natural History

OCME..Office of the Chief Medical Examiner

OSSA...Optimized Summed Scored Attributes

QDFA...Quadratic Discriminant Function Analysis

CHAPTER 1: INTRODUCTION

When skeletal remains are discovered, a forensic anthropologist is often called to aid in the identification of the individual. They must determine if the remains are human and provide a biological profile, which includes estimating the sex, age, stature, and ancestry of the individual in addition to noting any pathologies and past trauma evident on the bone that may aid in identification and circumstances of death. When the remains of a subadult are found, the biological profile becomes more complicated. As children are always growing, it has been a common belief that evidence of sex and ancestry evident in the skeleton, as examined by the forensic anthropologist after death, are obscured by skeletal growth indicators.

Most of the skeletal material examined by forensic anthropologists are those of adults as there are more adults than children in the world and adults are more frequently the victims of crimes (Kerley 1976). However, crimes involving children are increasingly in the news, yet methods to identify them, particularly when the recovered remains are badly decomposed or fragmentary, are falling behind studies involving the identification of adults. Often, the only reliable estimation the forensic anthropologist can make from a set of subadult skeletal remains is that of age, as minimal research has been conducted on forming reliable methodologies to estimate sex and ancestry.

This study seeks to examine ancestry assessment of subadult skeletal remains by establishing frequencies for 16 cranial traits used in adult ancestry estimation. Using the Terry Collection at the Smithsonian National Museum of Natural History (NMNH) in addition to the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History (CMNH) and the Johns Hopkins Fetal Skull Collection at the CMNH, this study aims to answer three questions: Can the ancestry of African American and European American subadult individuals be assessed with significant accuracy? Which cranial traits are useful in assessing the ancestry of subadult skeletons of these two ancestral groups? At what age do nonmetric traits manifest in the subadult skeleton for these individuals?

Note on Terminology

In the United Kingdom and North America, the terms “immature,” “subadult,” and “nonadult” are used to describe any individual that is not an adult, meaning the bones in the skeleton are still in the stages of fusing (Scheuer and Black 2004). However, the term “juvenile” is growing to replace these terms. In this thesis, the term subadult is used, except in cases where the author of each study uses alternate terms in their individual studies. The same can be said for ancestry terms. In this thesis, the terms “African American” and “European American” are used except in cases where the authors of referenced studies used alternate terms or the collections themselves have different terminology (i.e., Black and White).

Race, Ancestry, and Physical Anthropology

Why do anthropologists, particularly biological anthropologists study ancestry given the racist background to the research? Anthropology is the study of humankind and anthropologists are interested in studying and understanding all aspects of humans. From studying what makes us different and where we are the same, to attempting to unravel our origins and predicting our future selves, nothing is not of interest to anthropologists, and ancestry falls well within these interests.

Current research on ancestry is focused on two main goals: understanding the range of human variation and how to use that variation for human identification purposes in forensic contexts (DiGangi and Hefner 2013). Moving past the belief that race exists at the biological level to the understanding that it exists solely in the sociocultural realm, it is nevertheless understood that individuals belonging to one of four major ancestral groups (African, Asian, European, Indigenous/Native Americans) share morphological characteristics due to evolutionary and adaptational differences that can distinguish them from the other groups, differences that are by and large continental in scope

A Brief History of Race

Humanity has been grouping people based on physical characteristics since they began encountering the "other" through exploration in the fifteenth century (DiGangi and Hefner 2013). Carolus Linnaeus, known for his creation of the binomial nomenclature taxonomic system still used today, wrote in *Systems Naturae* (1759) the manner in which these well-defined (to those of the time period) races were related. He argued that humans represented one species, called *Homo sapiens*, but the species could be further separated into different subspecies according to geography, physical characteristics, and personality characteristics. These subspecies were called *africanus, americanus, asiaticus,* and *europaenus*, and they formed the basis of classification for the next two centuries (Linnaeus 1759).

To address the question of race, anatomists and physicians of the 1800s and earlier studied the craniofacial form of various human population groups to determine the number of varieties. For example, Johann Blumenbach, a German physician and anatomist, determined that there were five distinct races which diverged and degraded from a single original form (Brace 2010). These five races were organized in a hierarchy with Europeans at the top. Samuel George Morton, in his comparisons of the cranial form of Native Americans and Egyptians to Blumenbach's observations on human variety, came to similar conclusions; however, he believed that the five races of humans each represented distinct lineages that were fixed and unchanging.

Through the ordering and ranking of races, attempts emerged to explain why there were differences and how they came to exist. This led to two competing schools of thought, monogenism and polygenism, which both ordered the different races into hierarchical systems (DiGangi and Hefner 2013). Monogenists believed in *The Great Chain of Being* developed by ancient Greek philosophers, placing the Christian God at the top and human beings arranged directly below. It was after the coupling of Adam and Eve that environments changed and populations shifted, creating races other than

Caucasoid. This system provided religious support for the ranking of system in use by monogenists, placing Caucasians above all other races.

Popular in the nineteenth century, particularly in the U.S., was the opposing viewpoint of polygenism. Polygenists held the belief that each race had its own origin, not shared by any other race. Caucasians, naturally, were members of the oldest race and were said to be the most evolved. In support of slavery, polygenists believed that the Negroid race was the youngest and least evolved of the races of the world. As this view was prominent in the U.S., and formed the basis for much anthropological thought, European anthropologists dubbed it "The American School of Anthropology" (Brace 2010).

A notable polygenist was Samuel George Morton, an American anatomist with an interest in craniometry (Brace 2005; DiGangi and Hefner 2016). He collected crania from around the world to measure cranial capacity, and he believed that there was a hierarchical order of the races. He concluded that Caucasoids had the largest cranial capacity, though his work has been reanalyzed and proven to have been possibly manipulated to support his beliefs (Brace 2005; DiGangi and Hefner 2016).

Morton's work on cranial capacity demonstrated that white males were superior to white females and whites were superior to all other races (Morton 1840). This was not only used by other researchers as a way of justifying their hierarchical classification methods but also by the people of his time to justify social policies like slavery in the United States (Brace 2009; DiGangi and Hefner 2013). In the mid-nineteenth century in Paris, Paul Broca showed similar interests with Morton. He examined brain weights as a way of relating race with both brain size and intelligence. Contrary to Morton's biased and manipulated conclusions, Broca's work was inconclusive.

Franz Boas, Earnest Hooton, Aleš Hrdlička: American Physical Anthropology

Three individuals – Franz Boas, Earnest Hooton, and Aleš Hrdlička – are acknowledged to be the most important historical figures in the development of American Physical Anthropology. Each of these three men had differing views on race that continue to impact the field today. On one hand, Hrdlička and Hooton believed that human variation was the result of different evolutionary pathways that created the different races that are seen today. On the other hand, was Boas who instead believed that human variation was influenced by environmental variables like climate, nutrition, and culture. The typological nature of Hrdlička's and Hooton's view on human variation created categories based on physical characteristics and linked cultural characteristics as well, leading to the belief that biology not only dictates physical traits but sociocultural ones as well. This idea came to be known as biological determinism (DiGangi and Hefner 2016).

Aleš Hrdlička

Originally from Eastern Europe, Aleš Hrdlička emigrated to the U.S. in the late nineteenth century as a child (Spencer 1979). He received his first training in anthropology in France after first training as a medical doctor. His training in France, and his inspiration by Paul Broca, likely influenced his views on race. Hrdlička was inspired by Broca's creation of the *Anthropology Society of Paris* and upon his return as a field anthropologist at the American Museum of Natural History in New York then the

Smithsonian Institution in Washington, D.C., he attempted to set up a similar organization for the United States. Hrdlička ultimately succeeded in creating both the America Journal of Physical Anthropology in 1918 and the American Association of Physical Anthropologists in 1929 (Brace 2005; Spencer 1981). With his position at the museum and with the organization, Hrdlička was able to control how the field of physical anthropology could inform public discourse on race, even testifying before Congress in 1922 on his views regarding biological determinism and the hierarchical arrangement of the races (Caspari 2009; Oppenheim 2010).

Hrdlička's main questions regarding race focused on the number of races that existed in the world, as he already believed similarly to the polygenists that the different races had different evolutionary histories. For Hrdlička, physical anthropology was the study of comparative racial anatomy and particularly focused research on studying the three racial groups that he argued existed (white, black, yellow/brown) as a way of more fully understanding the one he and his contemporaries felt was the important one (whites). Again, like the polygenists, he believed that the social differences between the races were evolutionary in origin and that the white race was superior over the others.

Earnest Hooton

Similar to Hrdlička, Earnest Hooton's views on race were deterministic and polygenic, though his views were also typological (Brace 1982; Caspari 2009). Hooton was originally trained in Classics, but as a Rhodes Scholar to Oxford his interest in anthropology was peaked. After receiving his Ph.D. from the University of Wisconsin in 1911, Hooton joined the faculty at Harvard in 1913 where he became influential in the field of anthropology. As there did not yet exist training programs for physical anthropology, Hooton's program at Harvard was the first program to produce Ph.Ds. in physical anthropology, resulting in 28 Ph.D. recipients under his tutelage. His students went on to teach at other universities and established programs of their own.

Hooton's typological views on race led to his interest in using cranial nonmetric traits as a way of classifying individuals. He created the Harvard Blanks as a way of standardizing the recording of nonmetric traits, general cranial observations, cranial measurements, and anything that he felt was useful for answering the questions he had about body form and race (Brues 1990). His students shared his research interests, many went on to publish books and articles on the deterministic and typological views of race. However, despite his beliefs and his research that supported a hierarchical arrangement of the races, Hooton was antiracist and even attempted to create an antiracist group in anthropology (Caspari 2003).

Franz Boas

While Hrdlička and Hooton primarily held sway in the emerging field of physical anthropology, Franz Boas advocated for the importance of the four-field approach in American Anthropology, stressing the importance of a holistic viewpoint in anthropology. Despite receiving his Ph.D. in physics from a German university, Franz Boas took a position at the American Museum of Natural History for several years before joining the faculty at Columbia University in New York in 1905 (Caspari 2009; Spencer 1981). His views on race came from his early ethnographic research among the Eskimo (Inuit) in the Canadian Arctic. It was there that he came to the understanding that culture plays a crucial role in impacting behavior and biology (Erikson 2008). This was in opposition to the views of most other researchers of the time, including Hrdlička and Hooton.

Boas believed that rather than basing research on typological ideas and biological determinism, research should instead be focused on investigating links between environment, culture, and the biological variation that results from the effect of the two (Caspari 2009; Erikson 2008). Boas himself was an example of his views on race; he was white, but through his ethnographic research he sought to learn the languages of the people he studied as well as participate in their cultural activities (Erikson 2008). In 1910, his views on race were further supported in his publication *Changes in Bodily Form of Descendants of Immigrants*. Based on his research on the cranial sizes of immigrants and their children, Boas proposed that the cranial size of immigrant children born in the U.S. was different than that of their siblings who had been born overseas (Boas 1910). Boas determined that this change was due to differing environments, possibly resulting in better nutrition and better access to medical care than their parents had been able to receive in their home country. Boas' research demonstrated the importance of testing hypotheses to provide a scientific background to research, an element lacking in the research of the biological determinists.

Boas' research was not only important in the twentieth century, but through his twenty students who went to teach at other universities, passing his views on to an expanding number of new students, his research continues to be important today. His views on the holistic nature of anthropological research continue to provide a foundation for many research questions and guide the discipline of anthropology. Boas' research into secular change, how each generation of individuals is different from previous generations due to their environment, is still continued today.

Scientific Racism and Social Darwinism

Early research and views used phenotypic traits to provide a hierarchy of the races beginning with Carolus Linneaus' classification system. When Charles Darwin published his *On the Origin of Species* in 1859, scholars latched onto his ideas about evolution and natural selection to classify races by level of development and cultural achievements, despite Darwin's near omission of natural selection and humans (Blakey 1999; Darwin 1859; Graves 2001; Nash 1962).

This movement of Darwin's ideas on the biological nature of natural selection into the social arena to address topics including culture became known as Social Darwinism and was used to justify their racist leanings. The term "survival of the fittest," coined by a contemporary of Charles Darwin, Herbert Spencer, was also beginning to be applied to humans as a way to define an individual's "fitness" in terms of culturally bound categories, including intelligence and attractiveness (DiGangi and Hefner 2013). This was used to justify the favoring of one group of people over another as in capitalism and colonialism (Marks 2008). Proponents of Social Darwinism argued that evolution created differences between people; it was not the fault of any person or institution that some groups were less evolved than other groups.

Eugenics

Social Darwinism allowed for the belief that, like physical traits, social and cultural traits could be inherited, unlike learned behaviors. This was a view shared by Charles Darwin's cousin, Francis Galton, who coined the term "eugenics" in 1883 to categorizing his idea for the artificial selection of human beings (Shipman 1994). Galton believed that it was society's responsibility to prevent the lower classes from spreading their undesirable traits to future generations through selective breeding programs that allowed only those considered desirable to breed (Gould 1981; Graves 2001; Paul 2008). The effects of environment on the development of populations was completely removed and ignored.

Galton's ideas were further expanded by a German biologist named Ernst Haeckel. His argument was for the Aryan ideal, advocating that evolution was goal-directed and progressive and that the environment did not influence social and cultural traits (Graves 2001). Haeckel believed firmly in a social hierarchy, even going so far as to suggest that those races he deemed to be the lowest were "psychologically nearer to the mammals (apes or dogs) than to civilized Europeans; we must therefore assign a totally different value to their lives" (Haeckel 1904:406).

Eugenics in the U.S. can be traced to the twentieth century American biologist Charles Davenport (Marks 2008; Shipman 1994). In 1911, Davenport, feeding on the fears held by upper class Americans about the incoming immigrants, published his book *Heredity in Relation to Eugenics* (Davenport 1911). In the book, Davenport claimed that biology was responsible for the downfall or development of civilization. In 1916, Madison Grant used these ideas to propose the sterilization of those who were determined to be unfit and undesirable in society (Grant 1916).

It was these books and ideas that led to the creation of eugenics laws in 30 states in the United States and the involuntary sterilization of people over the next two decades, people who were deemed to have undesirable traits (Marks 2008; Suzuki and Knudson 1989). These laws helped to form the basis of genocidal practice in Nazi Germany, making the leap from involuntary sterilization to the complete removal of undesirable groups through extermination (Suzuki and Knudson 1989). It was only with the Great Depression that shifted the focus to domestic economic problems and the horrors witnessed with the Holocaust that eugenics ended in the United States and slowly removed the eugenics laws (Blakey 1999; Marks 2008). However, starting with the State of North Carolina's 2012 decision to financially compensate the approximately 7,500 living victims of the policies in the state, restitution for those forcibly sterilized finally began (Severson 2012).

Ancestry and Anthropology Today

Current anthropological research on ancestry is focused on two main goals: understanding the range of human variation and how to use that variation for human identification purposes in forensic contexts. These goals can be attributed to the publications and research interests of Franz Boas (Caspari 2009). The overall shift in research focus can be traced to Sherwood Washburn's New Physical Anthropology in which he described a new direction for the discipline; a movement towards evolutionary change, population genetics, and human variation studies, and away from the typological research of the past (Washburn 1951). Additional researchers have argued that race cannot exist as it cannot explain human variation, is imbued with social meaning, ignores evolutionary forces, and does not explain the role environment plays in the expression of traits (Comas 1961; Livingstone 1962; Montagu 1964; Washburn 1964).

CHAPTER 2: PREVIOUS RESEARCH ON ANCESTRY ASSESSMENT

Ancestry Estimation in Adult Skeletal Material

The skull is the only area of the skeleton where a relatively accurate estimation of ancestry can be achieved (Bass 2005). Typically, facial nonmetric traits have been used, showing the extremes of each racial type (Burns 2013; Gill 1998; Rhine 1990). Historically and typologically, Africans were recognized by the prominence of the mouth, Europeans by the prominence of the nose, and Asians by the prominence of the cheeks, all in relation to the rest of the face.

Rhine (1990) utilized 81 skulls from the Maxwell Museum of Anthropology with known identity and evaluated the mid-facial region for ancestry estimation. His sample included individuals who were residents of New Mexico at the time of death, and nine precontact skulls that were included to round out the sample and provide a baseline for the interpretation of Hispanic morphology. He utilized 18 traits from the neurocranium, 13 from the face, seven dental, and seven mandibular. He concluded that the Hispanic group showed the most variation, some appearing more “Anglo” and some appearing more “Indian.” However, Rhine (1990) remarked that race is not a matter to be decided by the reference to a single index or an isolated nonmetric trait. Instead, one must examine a large number of traits and attain a sense (gestalt) from the cluster of variables that expresses the morphology of the individual. Rhine’s study relied purely on observer experience and lacked statistics to reinforce his findings, leading to a “method” based on a small reference sample that is inappropriate to use for ancestry assessment.

In order to advance the methodologies used by forensic anthropologists to estimate ancestry and update them to reflect differences seen in modern humans, Hefner (2009) compiled eleven traits from a number of sources - largely from Hooton's "Harvard List" - that have been used to determine ancestry in forensic anthropology. These traits include the anterior nasal spine, inferior nasal spine, interorbital breadth, malar tubercle, nasal aperture width, nasal bone contour, nasal overgrowth, postbregmatic depression, transverse palatine suture, supranasal suture, and the zygomaticomaxillary suture. Hefner (2009) developed trait frequencies for four ancestral groups (African, Native American, Asian and European) and found that no single individual had all eleven expected trait frequencies, suggesting that the compiled trait lists for ancestry ignore a substantial amount of variation within and between groups.

Hefner (2009) concluded that there has historically been an emphasis on experience, simplified trait lists, and extreme trait values in the traditional forensic anthropological analysis. This unscientific approach led to ambiguous or discordant traits becoming viewed as "admixture" without the consideration of the actual distribution of traits in a given population. It was determined that labelling a person of "mixed" ancestry only works if groups had been mixed for some time. However, groups are comprised of individuals that show traits from different ancestral groups, and every decedent would be labelled of mixed ancestry if one were to use the traditional published trait lists, such as Rhine's (1990). When combined with standard (nonmetric) ancestry estimation, statistical analyses such as those below can show the probability that an individual will belong to each ancestral group and allow for a more accurate prediction of the individual's actual ancestry (Hefner 2009).

Klales and Kenyhercz (2015) tested the utility of using the traits from Hefner (2009) for the estimation of ancestry using the historic Hamann-Todd Collection. The authors examined 208 crania from American Whites and Blacks. The traits were scored by both authors, both of whom were familiar with and experienced in using the traits outlined by Hefner (2009) and the Osteoware (2016) software. Another observer with basic skeletal knowledge but no significant experience using the methods was also included.

Klales and Kenyhercz (2015) found that their results were consistent with that of Hefner (2009); the traits show a wide range of trait expression within and between groups. This study also corroborated Hefner's (2009) notion that phenotypic variation is present between the groups in trait expression. Klales and Kenyhercz (2015) suggest that this phenotypic variation is a likely contributor to the continued utility and application of morphoscopic traits for ancestry estimation. The authors argue that adequate use of the traits is dependent on trait selection and the multivariate classification techniques employed by the researcher due to the overlap of trait frequencies. It is also important to note that if a trait is significantly different from the available ordinal scores, it should remain unrecorded rather than attempting to make it fit with the closest available option. That is, the current descriptions of trait expressions may not sufficiently capture human variation.

Cranial nonmetric ancestry assessment is typically viewed as less precise than craniometrics due to its subjective nature (Byers 2008). This subjective nature also makes this method of assessing ancestry more difficult than metric methods. Statistical classification methods, like those below are helpful in standardizing the assessment of ancestry, as do the improved illustrations and definitions that are provided in Osteoware (2016). These new aids in assessing nonmetric cranial ancestry allow investigators to move past the experience-based determinations and towards a standardized method of practice.

Optimized Summed Scoring Attributes

Developed by Hefner and Ousley (2014) using six of the traits scored by Hefner (2009), the Optimized Summed Scoring Attributes method (OSSA) dichotomizes each trait to maximize the between-group differences between American Black and White individuals. This method uses the scores from the nonmetric cranial traits anterior nasal spine, inferior nasal aperture, nasal aperture width, nasal bone shape, interorbital breadth, and postbregmatic depression outlined in Hefner (2009), dichotomizes the scores to a 1 or a 0, and these scores are summed to indicate an ancestry assessment. For the purposes of this method, a summed score of 3 or below indicates American Black ancestry and a score of 4 or above indicates American Whites.

The OSSA method is only applicable for American Black and White individuals, and it does not account for Hispanic, Asian, or Native American individuals. This is due to the sample that was collected for the creation and testing of the method, which was selected to represent the range of casework seen in most forensic anthropology laboratories across the U.S.

Kenyhercz *et al.* (2017) evaluated the OSSA method developed by Hefner and Ousley (2014) for classification accuracies using the historic Hamann-Todd Osteological (HTH) Collection, in addition to positively identified forensic cases from Mercyhurst University (DAFS) and the New York City Office of the Chief Medical Examiner (OCME). As stated by Kenyhercz *et al.* (2017), the OSSA method is used in current forensic casework due to the standardized illustrations and descriptions provided by the Osteoware (2016) software that avoids the previously used typological approach of assigning individuals to an ancestral group based on the presence or absence of certain traits. In addition, the method is easy to use as it relies on only a few traits to make an assessment of ancestry.

Kenyhercz *et al.* (2017) evaluated the OSSA method on a historic skeletal sample, in addition to positively identified forensic cases from U.S. Black and White individuals, resulting in a sample of 274 crania. A portion of the sample that came from the OCME were scored from detailed photographs taken of the skull as the remains were no longer in the custody of the OCME. The scoring of these individuals was only included when all six traits for OSSA could be confidently scored. For each case, the authors tested the classification accuracies again with a shifted sectioning point to reduce bias in the samples.

Using the methods outlined in Hefner and Ousley (2014), Kenyhercz *et al.* (2017) obtained a classification accuracy of 68.3% for the Hamann-Todd Collection (Black =

50.5%, White = 85.0%). The authors found that by shifting the sectioning point from ≤3 to ≤4 improved the classification accuracy for the entire sample to 77.9% (Black = 80.2%, White = 69.2%). The two forensic samples were pooled for the analysis, resulting in a classification accuracy of 87.9% (Black = 53.3%, White = 98.0%). For this sample, the authors again shifted the sectioning point to ≤ 4, resulting in an improved classification accuracy of 93.9% (Black = 50.9%, White = 89.2%). With each sample pooled together, the classification accuracies were 73.0% (Black = 80.2%, White = 78.5%) for the sectioning point of ≤ 3, and 79.2% (Black = 80.2%, White = 78.5%).

Statistical Classification Methods

Hefner and Ousley (2014) propose several methods for assessing ancestry based on the nonmetric traits defined in Hefner (2009). The difficulty with most multivariate statistical classification methods is the requirement that the data have a multivariate normal distribution (Hefner and Ousley 2014). Nonmetric trait scores are not normally distributed, though methods like linear discriminant function analysis (LDFA) and quadratic discriminant function analysis (QDFA) can still be used with caution in interpreting the posterior probabilities. Logistic regression is the best method to use with the nonmetric trait scores when differentiating two groups as it does not require that the data be normally distributed (Hefner and Ousley 2014; Hefner 2009).

Hefner and Ousley (2014) also tested the trait scores that they had collected using statistical classification methods that use individual similarities instead of group similarities to classify unknown individuals. These two tests are k-nearest neighbor analysis and Kernel probability density. These classification methods work best with this type of data because they compare the unknown individual to known reference samples and classify an unknown individual to the group they have the highest probability of belonging to; for example, an unknown individual may be classified as African American as the individual is most similar to that ancestral group.

"Machine Learning"

"Machine learning" methods are the newest classification methods and rely on the speed and power of computers to pool individuals into appropriate groups (Hefner and Ousley 2014). Machine learning has not been used for ancestry classification but has been used successfully in other areas of biological anthropology, including sex estimation (McBride *et al.* 2001; Konigsberg *et al.* 2001). This method of classification based on trait scores has the ability of testing the unknown against thousands of classification cutoff points to find the most accurate way of pooling the unknown individual into a group. However, the classification accuracies can be affected by differing data transformations.

Osteoware

Osteoware (2016) is a database program available from the Smithsonian National Museum of Natural History designed to make collecting data, including cranial nonmetrics, easier. Osteoware was created as a result of the need to catalogue the massive

Native American collection at the Smithsonian Natural Museum of Natural History due to the passage of the National Museum of the American Indian Act in 1989 and the Native American Graves Protection and Repatriation Act (NAGPRA) a year later. It uses the definitions provided by Buikstra and Ubelaker (1994) for the collection of data that including trauma and pathology. The newest module to this software is Macromorphoscopics, the module most relevant to nonmetric cranial ancestry assessment studies. This module provides detailed descriptions and illustrations to provide assistance on scoring the traits outlined in Hefner (2009).

Ancestry in Subadult Skeletal Material

The number of research articles examining ancestry assessment of adult skeletal material number in the hundreds and there are many different methods that can differentiate groups using both metric and nonmetric variables (see: Gill 1984, Hanihara *et al.* 2003, Işcan 1988, Spradley *et al.* 2015 for example). However, when one looks to the literature for subadult ancestry assessment methods, there exists a significant deficit.

With adult skeletal material, ancestry can be difficult to assign due to the fact that there are no "pure" races (Buck and Vidarsdottir 2004); however, subadult material is even more difficult to classify due to the ontogenetic changes occurring in bone. As a result of these changes, the discriminant functions obtained from ancestry estimation studies involving adult material cannot be used to classify subadults. Buck and Vidarsdottir (2004) examined the mandible for its utility and reliability in estimating the ancestry of both adult and subadult material. They examined 174 individuals of African

American, Native American (Arikara), Caucasian, Inuit, and Pacific Islander ancestry and assigned a biological age to subadults for graphical purposes based on standards set by Ubelaker (1989).

In Buck and Vidarsdottir's (2004) study, sex was not considered a variable and was not assigned to unknown individuals; if the sex was known, the individuals were sampled to avoid bias. Seventeen nonmetric landmarks were chosen for the study that allowed a good representation of mandibular morphology, and the landmarks were identified on both adult and subadult material. Using discriminant function analyses with cross-validation, the authors found that individuals were placed in their correct sample groups with an average accuracy of 70% for all five groups. If the group number was decreased, the accuracies increased, rising to an average of 88%. When the authors used the mandibular ramus and corpus separately (to simulate fragmentary remains), average accuracies were determined to be 67% for the corpus and 73% for the ramus. The authors concluded that the mandible can be used reliably to assign an ancestry to both adult and subadult individuals, whether the sample was whole or fragmentary.

In a Master's thesis, Szen (2018) examined ancestry in subadult skeletons using metric analysis. Using the Hamann-Todd, Terry and American Museum of Natural History Osteological Collections, Szen (2018) examined if ancestrally related craniofacial variations could be metrically assessed in subadult skeletons and at what point in development the variations occur. Her sample totaled 169 individuals ranging in age from birth to 21 years of age and the individuals were examined using a 3D digitizer. Szen's (2018) analysis yielded no statistically significant results. However, this does not necessarily mean that ancestry of the individuals in her sample could not be determined. It is possible that the ancestrally related craniofacial differences might not be metrically visible until after the completion of puberty. Alternately, the variations related to ancestry may be too minute for quantification with metric methods such as the 3D digitization she used in her study.

CHAPTER 3: DEVELOPMENT OF THE SKULL

Introduction

Ancestry, even among adult individuals is difficult to estimate. Reasons for this difficulty include the differences in craniofacial growth and development, population variation, methodological approaches, lack of appropriate samples, etc. The craniofacial skeleton adult individuals is the result of the rapid growth-related alterations that occurred during childhood. These alterations make interpretations and measurements from this area particularly difficult. While overall growth and development is understood, details regarding the specific elements of craniofacial growth and development are not, particularly the ways in which environment can affect growth.

What is Growth?

Development of the cranium falls under a mixture of two types of growth during the subadult period. The subadult skull undergoes a combination of static growth and periodic spurts of growth. While some areas will undergo more static growth with intermittent growth spurts, other areas experience slow static growth throughout the entire period of development. Once an individual has reached the adolescent stage, the growth spurts slow until the individual has reached late adolescence, at which point these growth spurts fall to their slowest rate.

Human growth has been defined by Scheuer and Black (2004) as alterations in size and morphology during the development of an individual. Differences in growth do exist both between the sexes and population groups as well as within both sex and population groups. Additionally, growth is affected by the interaction between an individual and their environment at a genetic level, which allows for the suppression or expression of genes that are related to growth (Scheuer and Black 2004). This affect can be seen in all body systems, including the skeleton. One of the most studied interactions between environment and growth has been the affect that illness has on growth and development. Stress, caused by illness or other environmental factors, during any period of growth and development can cause a cessation in growth as the body must divert energy to the more important task of sustaining vital systems. As soon as the individual recovers from a stressor, or the stress is removed, growth can resume as the body diverts the energy back to its original task. Bones and teeth however, can record marks of these stress periods through the appearance of linear enamel hypoplasia, Harris lines, and similar pathologies that can be visible for years after the event (White *et al.* 2012).

The Effect of the Brain on Cranial Development

It has been established through decades of research by skeletal biologists that the shape of the adult brain is established early during embryonic development. The size and shape of the brain is set by the enclosure of the fetal brain by the endomenix and ectomenix during embryonic development and the endocranial bones must be set by this time as they directly reflect the shape of the adult brain (Neubauer *et al.* 2009). Additional research into embryonic development has proposed that the shape of the craniofacial region may develop, at least partially, by the final prenatal trimester. At this time, the skull also experiences an increase in the size of the anterior cranial base, palate, and basicranium (White *et al.* 2012).

It is also possible that craniofacial traits begin their formation in the embryonic period. Development of the cranial and facial skull during the embryonic period involves expansion anteroposteriorly and superoinferiorly and takes place in four regions on the skull: the chondrocranium, desmocranium, neurocranium, and viscerocranium (Zumpano and Richtsmeier 2003).

Cellular Processes

It is important to first understand the processes that generate bone growth to fully understand how overall craniofacial development occurs. At the cellular level, there are two types of cells that are responsible for the generation and removal of bone. These cells are known as osteoblasts, which are responsible for the synthesizing and deposition of osteological material that has not yet ossified, and osteoclasts, who are responsible for the breakdown of that material (Dixon *et al.* 1997; White *et al.* 2012). Working together, osteoblasts and osteoclasts build and shape bone to generate either woven or lamellar bone. Woven bone is deposited rapidly and is typically seen in response to injury or in regions that must grow at a rapid pace, while lamellar bone is deposited relatively slowly and with more structure (Dixon *et al.* 1997; Liberman 2011; White *et al.* 2012). This process conducted by these cells is known as ossification or osteogenesis.

Methods of Bone Formation

Bone in every region of the body ossifies in one of two ways. Endochondral ossification involves the use of a cartilaginous model around which bone is deposited (White *et al.* 2012). Bone cells enter this model and begin to form bone, while chondroclasts (transformed white blood cells), break down the cartilaginous model. Within the cartilaginous model, chondrocytes change into osteoblasts and begin to generate bone inside the internal cartilaginous structure (Dixon *et al.* 1997; White *et al.* 2012). Due to the manner of ossification, the resulting bone shape is highly influenced by the cartilaginous model. Endochondral growth is most commonly seen in the postcranial skeleton, though the cranial base also ossifies in this manner.

The other form of ossification, intramembranous ossification, forms through the use of a mucus tissue known as the mesenchyme that surrounds organs and related material (White *et al.* 2012). Whereas in endochondral ossification, the resulting shape of the bone is influenced by the cartilaginous model, in intramembranous ossification, the bone shape is influenced by the surrounding organs. In this ossification process, the cells of the mesenchyme change into osteoblasts which build the framework and initial network of bone. The outer layer of this initial structure becomes what is known as the periosteum. Inside of this structure, cells generate and mineralize collagen fibers known as osteoid. Intramembranous ossification is the main ossification method of the cranial vault and facial skeleton (Dixon *et al.* 1997; Liberman 2011; White *et al.* 2012).

The Movement of Bone

Bone formation inevitably causes bone movement which affects the position and the shape of bone. Five processes are responsible for this movement and each of which often work in tandem with the others.

The first of these processes, and the longest occurring of them, is bone remodeling (Dixon *et al.* 1997). With bone remodeling, bone is broken down by the osteoclasts and replaced by the osteoblasts. This process is not only seen in bone growth but is also seen during bone repair in the case of trauma. With the increase in size of the bone undergoing remodeling, it moves away from the other bones, thereby creating additional space for continued expansion. This movement can bring the articulating ends of the bones into contact, creating joint contacts, in a process known as displacement (Enlow and Hans 1996; Liberman 2011; White *et al.* 2012).

Within cranial growth, two processes are most commonly found: drift and rotation (Dixon *et al.* 1997). Drift occurs when osteoblasts deposit new bone on one end of the bone while osteoclasts break down the other end. During this process, bone drifts towards different bones and away from the bones it was near before. With rotation, resorptive and depository sides of the bone are positioned opposite to another resorptive and depository field (Liberman 2011; Liberman *et al.* 2000). The final process that is responsible for the movement of bone is known as relocation and refers to the movement of the bone as it is increased in size (Enlow and Hans 1996).

Growth of the Cranial Base

Basicranium

Growth of the cranial base begins in the fourth week of fetal development when a mass of mesenchymal cells cluster in the occipital area (Cunningham *et al.* 2016). This mass of mesenchymal cells is known as the chondrocranium and is a cartilaginous outline of what will become the basicranium (Cunningham *et al.* 2016; Liberman *et al.* 2000). In the second month of development, this mass spreads anteriorly from about forty-one ossification centers and will surround the pituitary region and create the basicranium through the formation of a cartilaginous mass (Cunningham *et al.* 2016; Lieberman 2011, Liberman *et al.* 2000). The fetal brain will grow on this platform and become surrounded by the dual layer of mesenchymal material known as the endomeninx and the ectomenix. The endocranial bones will continue to grow through the intramembranous process described above (Neubauer *et al.* 2009). The fetal basicranium will continue to grow throughout this period, though the major changes it experiences relate primarily to the width of the bone (Zumpano and Richtsmeier 2003).

During the first two years of life, the endocranium increases in overall size while the basicranium flexes, altering the angle at which it sits (Neubauer *et al.* 2009). The basicranium continues to grow rapidly until approximately seven years of age, at which point growth slows. There is an elongation of the bone via bone drift along the anterior and posterior borders of the basicranium and deposition along the midline. The elongation also occurs from the deposition and drift of the occipital portion of the bone (Liberman *et al.* 2000). The basicranium is viewed as a platform for the growth of the brain due to its early maturation and the tapering of growth coincides with the cessation of brain growth.

After seven years of age, the rapid growth of the basicranium slows and it has reached about 95% of its adult size, corresponding with the size of the brain. The basicranium does not cease its growth; it continues to grow from internal to external structures much more slowly than before through adolescence and mid-pubescence (Bastir *et al.* 2006).

The endocranium also grows in conjunction with the brain, but its growth is comparatively slower than the basicranium. It appears to grow as more of an accommodating force in relation to the brain rather than directly relating to brain growth. The endocranium reaches mature size and shape by approximately eleven to twelve years of age, once brain growth has ceased (Neubauer *et al.* 2009).

Neurocranium

The neurocranium, also known as the braincase, is the combination of the superior portion of the cranial vault in addition to the remaining elements at the base of the skull and constitutes most of the protective covering of the brain (Scheuer and Black 2004). Included in this portion of the cranium are the parietals, temporals, frontal, occipital, and sphenoid. As this portion is directly surrounding the brain, the brain itself is responsible for much of the growth in this area.

Similar to the basicranium, the neurocranium forms from an embryonic cartilaginous precursor and appears not long after the chondrocranium, approximately four to eight weeks in utero (Dixon *et al.* 1997). This embryonic precursor is referred to as the desmocranium. The desmocranium develops via intramembranous ossification in an area known as the ectomeninx, a mesoderm (cellular/tissue layer present in early embryological development) and moves along with the growth of the developing brain (Lieberman 2011).

The neurocranium experiences its most rapid growth during the first two years after birth through the expansion of the temporal and parietal lobes of the brain, elongation of the neurocranium, and the downward movement of the cranial base (Cunningham *et al.* 2016; Dixon *et al.* 1997). This rapid growth allows the neurocranium to achieve much of its adult size by the age of eight, with continued growth into late childhood (Liberman 2011; Neubauer *et al.* 2009). As the brain achieves its adult size by the age of ten, the neurocranium follows closely and achieves its adult size at about the same time (Liberman 2011). This early maturation of the brain and neurocranium allows for the setting of an individual's head shape and sets the platform upon which the craniofacial skeleton will grow (Enlow and Hans 1996).

Viscerocranium

The viscerocranium, more commonly known as the facial skeleton, is made up of the bones of the orbits, nose, maxilla, and mandible (Scheuer and Black 2004). The facial skeleton is referred to as the splanchnocranium during fetal development and is later separated into both the viscerocranium (skeletal) and splanchnocranium (cartilaginous) portions. It initially appears around four weeks in utero and the morphology completes at around ten weeks (Cunningham *et al*. 2016; Scheuer and Black 2004). In about the sixth week in utero, the bones of the face begin to undergo intramembranous ossification from a multitude of ossification sites (Liberman 2011).

The development of the facial skeleton is fairly slow except for the upper face which undergoes quite rapid growth until about the age of twelve. This divide in the speed of growth of the facial skeleton can be attributed to the effect of the neurocranium on the upper face. The growth of the remainder of the facial skeleton is characterized by an overall enlargement in relation to the growth of the basicranium and the neurocranium.

Until about seven years of age, the growth of the facial skeleton occurs mainly at suture sites, while after this point, growth occurs from the periosteum (Bulygina *et al.* 2006; Dixon *et al.* 1997; Liberman 2011). The growth of the facial skeleton is largely characterized by periods of rapid growth with longer periods of rest. These periods of rapid growth occur in conjunction with the development and eruption of the dentition. Major growth of the facial skeleton slows or ceases around fourteen to twenty years of age, though there is no agreement on the exact time at which growth ceases (Bastir et al 2006, Liberman 2011, Libermann et al 2000).

CHAPTER 4: MATERIALS AND METHODS

This study examines subadult skeletal material using three collections located in the U.S. to examine the frequencies of trait expression for those used to assess ancestry of adult African American and European American individuals. This research uses subadult skeletal remains ranging in age from birth to 20 years from the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History and the Terry Collection at the Smithsonian National Museum of Natural History. Additionally, fetal and infant remains were examined from the Johns Hopkins Fetal Skull Collection at the Cleveland Museum of Natural History and the DS Lamb/FP Mall Collection at the Smithsonian National Museum of Natural History.

The Hamann-Todd Osteological Collection

Originally located at Western Reserve Medical School, the Hamann-Todd Osteological Collection was initialized by anatomy professor, Dr. Carl August Hamann in 1893 (Jones-Kern and Latimer 1996). He was succeeded by Dr. T. Wingate Todd in 1912, who is responsible for amassing the majority of the complete human skeletal remains in the collection. In 1951, the collection began the transition to its current resting place and is now housed in the Cleveland Museum of Natural History in Cleveland, Ohio.

Thus far, the collection consists of approximately 3,100 individuals with birth years ranging from 1825 to 1910, as well as nearly 900 non-human primate specimens. All of the human specimens are donated, most becoming a part of the collection as a result of a law stipulating local, unclaimed cadavers be donated to the collection with the expectations of being utilized for investigative and educational purposes. This process allowed for the documentation of age at death, sex, group affiliation, cause of death, anthropomorphic measurements, and when possible, living stature and donation source. In most cases, Todd examined the cadavers before and after autopsies, at which point he identified any pathological lesions and abnormalities associated with the specimens, including, but not limited to, evidence of healed fractures, degenerative changes, dietary deficiencies and disease. Cadaver weight and height were also obtained through a process of suspending the specimens from the auditory canal by ice tongs, where height was measured with a graduated wooden staff.

As is the nature of anatomical collections, the Hamann-Todd osteological collection possesses inherent limitations primarily due to the manner of acquisition and selection criteria utilized in choosing donated specimens. Consequently, the collection is not representative of the general population. Dominated by unclaimed cadavers, the remains predominantly represent a common socioeconomic background of poor working-class Americans of the late 1800s to the early 1900s (Arney 2011). Each of the specimens is designated as either black or white and are individuals who have died in or around Cleveland, Ohio, but were not necessarily born in the region. The collection contains specimens from a variety of states. In most cases, the individuals are first generation Americans, where white individuals are natives of the northern United States and black individuals of southern states. The inclusion of southern blacks is attributed to the migration of migrants following their emancipation.

The collection is also comprised of all age groups, from neonate to elderly. While it is beneficial to researchers for the collection to be comprised of all age groups, this poorly represents the population of the time, suggesting that age at death was evenly distributed when, in fact, life expectancy of the time was between 20 and 40 years of age. Remains were often selected for pathological lesions present on the skeletal elements. In these cases, the individuals are considered the most robust or healthiest of the populations due to their survival of a particular disease long enough to develop lesions.

Robert J. Terry Anatomical Collection

One of the most widely studied skeletal collections in the world, the Robert J. Terry Anatomical Collection was collected by Robert Terry beginning in 1910 for research and educational purposes (Hunt and Albanese 2005). Terry was interested in variations of the human skeleton, particularly normal and pathological variations and was aware of the lack of documented anatomical or osteological collections in which could be studied for skeletal biology, anatomy, and pathology. This awareness began during his medical training under George S. Huntington in 1893 at the College of Physicians and Surgeons in New York. Huntington was a strong proponent for saving the skeletal material that remained from the documented human cadavers that came into the school for dissection purposes. During his tenure at the school, Huntington acquired and kept over 3,800 human skeletons from his dissection classes, however his collection continues to be overlooked in the course of research in biological anthropology utilizing skeletal remains.

The current Terry collection that is used today is actually the third attempt for Terry at beginning a documented skeletal collection. His first was begin after his return from the United Kingdom where he had trained under Sir William Turner. This collection was begun when he obtained the position of demonstrator of anatomy at the Missouri Medical College beginning in 1898 and expanding in 1900 when he was appointed assistant professor. Unfortunately, this first collection was destroyed by a fire. Terry began collecting skeletal material after the fire, fully recognizing the need to have a documented skeletal collection, but this collection also did not survive to become the collection used today. This second collection was mismanaged when Terry went to teach at Harvard in 1906-1907 as an Austin Teaching Fellow. The disasters of the first two collections helped Terry to create a protocol for the collection and documentation of his third and final attempt.

The cadavers that joined the Terry Anatomical Collection beginning with Terry's appointment as Chair of the Anatomy Department in 1910 came primarily from the medical school's anatomy classes. They were collected from local St. Louis hospitals and institutional morgues when they were not claimed by relatives. Some of the cadavers came from other institutions throughout Missouri as well. All of the cadavers were from the lower socioeconomic classes whose families could not afford to pay for a burial and therefore they went unclaimed at the morgues. The cadavers were made available to medical schools to save the taxpayers the expense of a burial.

By 1910, Terry had developed a well-established uniform protocol for the collecting, cataloguing, maceration, and storage of the skeletons that were collected.

Understanding the need to be able to show a complete range of human variation, Terry did not confine himself to collecting only human skeletons that had pathological conditions evident on the bones. He made sure to collect “normal” skeletons as well as the pathological specimens. Maceration was conducted by stripping the bones of as much soft tissue as possible before soaking the skeleton in hot water for 72 hours, brushing, and drying the bones. Hands and feet were individually confined in cotton gloves to avoid the loss of any element. Each element, aside from the tiniest, were labelled with a catalogue number, many were labelled in different inks to correspond to the side the element was from. Inventory checklists were done for each step in the process to ensure no loss of elements.

Ultimately, due to their extensive handling, some individuals needed to be replaced in the collection. Each new specimen was given a retired specimen number, but to prevent confusion, the new specimen number would also contain the letter “R” at the end to show that it replaced the previous specimen with that number. Not all individuals remained with the main Terry Collection, some were sent to other museums and universities to be added to their own collections. Recipient collections include the Harvard Peabody Museum and the Raymond Dart Collection in South Africa.

Upon Terry’s retirement in 1941, Mildred Trotter took over the position and continued collecting skeletons until she retired in 1967. It was she who changed the name of the collection from the Washington University Collection, as it was then known, to the Terry Collection, as it is known today. As nearly 80% of the collection had already been amassed by the time Trotter took over, her major contribution to the collection was to attempt to balance the collections demographic composition. She focused her collections on females and younger individuals in addition to reinstating 90 individuals to the collection to further increase the number of female and younger individuals in the collection. By the 1950s and 1960s, the Anatomy Department at Washington University changed its research focus to brain morphology and function and interest in maintaining the Terry Collection decreased. Trotter began corresponding with T. Dale Stewart at the Smithsonian Institution concerning the transferring of the collection for permanent curation. The transfer of the Terry Collection to the Smithsonian Institution was conducted in 1967.

Smithsonian Fetal Collection – D.S. Lamb/F.P. Mall

The D.S. Lamb/F.P. Mall Collection, also referred to as the Smithsonian Fetal Collection was collected by Aleš Hrdlička upon his arrival to the U.S. National Museum (which became the National Museum of Natural History in 1910) in 1903 (Freilich and Hunt 2016). He corresponded with anatomists and scholars in medical research and training institutions throughout the country but most actively in the Washington D.C. area. The medical scholars he corresponded with sent donations or conducted exchanges for the sharing of the osteological collections under their curation.

Regarding the fetal collections at the Smithsonian Institution, the two most notable donors were Daniel S. Lamb and Franklin P. Mall, both from the Washington D.C. area. Mall is considered by most to be the father of the modern practice of anatomical training. He co-founded the American Journal of Anatomy with Charles S.

Minot and George S. Huntington in 1901. His research at John's Hopkins University included the function and physiology of both non-human and human internal organs in addition to contributing to neo-natal growth embryology and developmental growth research. Lamb was the head of the Anatomy Department at the Army Medical Museum where he single-handedly ran the collection from 1865-1917. He not only curated and researched the skeletal collections at that museum, but he also collected specimens to add to the collection as well. He is also known as the anatomist who autopsied William Garfield after his assassination in addition to examining the assassin, Charles Giteau, as well.

The collection today numbers 320 individuals with known demographics (Freilich and Hunt 2016). Infant mortality rates were high in the beginning of the twentieth century among all groups in the United States (Gindhart 1988) and the individuals from this collection are representative of that. Many of the individuals were noted to be the result of miscarriages by the mother, though some of those indicated as such were suspected by the curators to have actually been abortions. A number of the individuals in the collection were also from stillbirths in the area as well as children who had succumbed to childhood diseases (Hunt, personal communication, 2018).

METHODS

The demographics of the sample used in this study is shown in Table 4.1. The sample composition based on collection used is shown in Table 4.2.

Table 4.1: Sample Demographics

Age Range	African American	European American	Mixed Ancestry	Total
Fetal	39	39	17	95
Birth -5 years	62	36	18	116
6-10 years	11	1	0	12
11-15 years	8	3	0	11
16-20 years	59	14	0	73
		Total		307

Table 4.2: Sample Composition by Osteological Collections

Collection	Total
Hamann-Todd Osteological Collection (CMNH)	86
Terry Collection (NMNH)	31
Johns Hopkins Fetal Skull Collection (CMNH)	108
DS Lamb/FP Mall Collection (NMNH)	82
Total Sample	307

Features Examined

For ancestry estimation, the traits examined include those presented in Hefner (2009) and include the traits added by Klales and Kenyhercz (2015) all of which are included in Osteoware (2016). These traits and their scoring systems are presented in Table 4.3.

Table 4.3: Cranial Traits, Scoring System, and Associated References Assessed in This Study.

Trait	Scoring System	Associated References
Anterior Nasal Spine	1 = slight, 2 = moderate, 3 = marked	Gill 1998; Hefner 2009, 2012; Rhine 1990
Inferior Nasal Aperture	1 = smooth transition, 2 = sloping, 3 = right angle, 4 = weak vertical ridge, 5 = pronounced sill	Gill 1998; Hefner 2009, 2012; Rhine 1990
Interorbital Breadth	1 = narrow, 2 = intermediate, 3 = broad	Bass 1995; Hefner 2009, 2012; Rhine 1990
Malar Tubercle	0 = no projection, 1 = trace, 2 = medium, 3 = pronounced, 4 = double	Hauser and De Stefano 1989; Hefner 2009, 2012; Rhine 1990
Nasal Aperture Shape	1 = teardrop, 2 = bell, 3 = bowed	Hefner 2012; Rhine 1990
Nasal Aperture Width	1 = narrow, 2 = medium, 3 = broad	Bass 1995; Hefner 2009, 2012; Rhine 1990
Nasal Bone Contour	0 = low, rounded, 1 = oval, high walls, 2 = steep walls, broad plateau, 3 = steep walls, narrow plateau, 4 = triangular cross section, 5 = flat	Brues 1990; Hefner 2009, 2012; Rhine 1990
Nasal Bone Shape	1 = no pinch, 2 = superior pinch, minimal lateral bulging, 3 = superior pinch, pronounced bulging, 4 = triangular	Hefner 2012; Rhine 1990
Nasal Overgrowth	0 = absent, 1 = pronounced	Hefner 2009, 2012; Rhine 1990
Nasofrontal Suture	1 = rounded, 2 = square, 3 = triangular, 4 = irregular	Hefner 2012; Rhine 1990
Orbital Shape	1 = rounded, 2 = rectangular, 3 = rhombic	Hefner 2012; Rhine 1990
Postbregmatic Depression	0 = absent, 1 = present	Bass 1995; Hefner 2009, 2012; Rhine 1990
Posterior Zygomatic Tubercle	0 = absent, 1 = weak, 2 = moderate, 3 = marked	Hefner 2012; Rhine 1990
Supranasal Suture	0 = obliterated, 1 = open, 2 = closed, visible	Hauser and De Stefano 1989; Hefner 2009, 2012; Rhine 1990
Transverse Palatine Suture	1 = straight, 2 = anterior bulging, 3 = M-shaped, 4 = posterior bulging	Gill 1998; Hauser and De Stefano 1989; Hefner 2009, 2012; Rhine 1990
Zygomaticomaxillary Suture	0 = greatest lateral projection inferior, smooth, 1 = greatest lateral projection midline, one angle, 2 = visible lateral projection, two or more angles, 3 = obliterated	Gill 1998; Hauser and De Stafano 1989; Hefner 2009; Rhine 1990

STATISTICAL METHODS

The statistical tests used to analyze the data include chi-square analysis, binary logistic regression, Spearman's rank order correlation coefficients, and the Cohen's kappa statistic. Chi-square analyses were used to determine if there are differences between the ancestral groups in the expression of nonmetric traits. Binary logistic regression was used to determine which nonmetric traits are better at differentiating African and European American subadults. Spearman's rank order correlation coefficients were used to determine if there is a correlation between age and trait scores. Lastly, the Cohen's kappa statistic was used to test for intraobserver error using 10% of the examined sample.

All of the data collected in the course of this research were collected using Microsoft Excel (2016) with the comparative assistance of Osteoware (2016) and were analyzed using IBM SPSS Statistics Version 24 (IBM 2016). Assistance in interpreting the results came through the use of Field (2018).

CHAPTER 5: RESULTS

General Observations

Due to the nature of the collections, the preservational quality of the remains was good for all individuals in the sample. However, due to the nature of preservation for the fetal and infant remains, the nonmetric traits examined in this study were difficult or impossible to score and many traits were not scored as a result. For example, the bones of the face of the majority of the fetal and infant individuals, which was the focus of this study, were for the most part unfused to each other and were no longer held together through the use of glue or with any dried, adhering soft tissue. This made it difficult to score traits such as orbital shape, where the frontal, zygomatic, and maxilla need to be articulated. A few individuals in the study group had matured to the point where many of the bones had become articulated, which made this study easier.

An additional problem affecting many of the individuals in the sample was the curling that was affecting the edges of the flat bones of the skull. This was seen in both of the fetal collections and affected individuals of all ages. For some individuals, the curling did not affect the areas where traits were scored; however, those that they did affect were removed from the study. Additionally, it was noted that with the fetal and infant remains, the trait of postbregmatic depression could not be scored. Individuals in these age groups have a soft spot in that area that is filled with cartilage. Had any individual in the sample had that trait scored, it would have received a score of “0” as the soft spot does cause a depression located immediately posterior to bregma.

Statistical Analyses

Chi-Square

Chi-square analyses were conducted to determine if there are differences between the ancestral groups in the expression of the nonmetric traits between two groups; a summary of the results is given in Table 5.1. The analysis showed that there was a difference with each trait when compared to ancestral group. The traits with the highest degree of diagnostic difference are the anterior nasal spine (29.849), inferior nasal aperture (35.134), malar tubercle (21.068), nasal aperture width (27.097), and orbital shape (20.409).

The trait with the lowest chi-square value and the lowest degree of diagnostic difference was nasal overgrowth (1.908). However, the low value for this trait could be traced to the preservation and handling of the collections. The nasals are easily broken and any nasal overgrowth that an individual may have had, could have easily broken off by accident by previous researchers or in the initial cleaning of the bones.

Table 5.1: Summary of Chi-square Analyses.

Trait	Value	Asymptotic Significance	Likelihood Value	Asymptotic Significance	N of Valid Cases
Anterior Nasal Spine	28.849	0.000	33.074	0.000	307
Inferior Nasal Aperture	35.134	0.000	36.468	0.000	307
Interorbital Breadth	16.124	0.041	17.750	0.023	267
Malar Tubercle	21.068	0.007	24.362	0.002	283
Nasal Aperture Shape	14.742	0.005	14.360	0.006	307
Nasal Aperture Width	27.097	0.000	29.702	0.000	307
Nasal Bone Contour	16.894	0.154	23.173	0.026	78
Nasal Bone Shape	10.386	0.407	11.384	0.328	231
Nasal Overgrowth	1.908	0.984	2.867	0.942	216
Nasofrontal Suture	8.801	0.359	10.563	0.228	76
Orbital Shape	20.409	0.002	17.861	0.007	116
Postbregmatic Depression	15.239	0.004	16.354	0.003	105
Posterior Zygomatic Tubercle	5.564	0.474	5.533	0.478	306
Supranasal Suture	9.508	0.147	10.561	0.103	81
Transverse Palatine Suture	15.755	0.107	15.324	0.121	206
Zygomaticomaxillary Suture	12.521	0.014	12.603	0.013	306

Binary Logistic Regression

Binary logistic regression was used to determine which nonmetric traits are the best at differentiating between African and European American subadults. The results are summarized in Table 5.2. The traits that show the highest correlation with ancestry are indicated in bold in Table 5.2 and include inferior nasal aperture, nasal bone contour, nasal aperture width, supranasal suture, and the zygomaticomaxillary suture.

Table 5.2 Binary Logistic Regression Classification Table.

Variable	B	S.E.	Wald	df	Sig.	Exp(B)
Anterior Nasal Spine	-0.085	0.510	0.028	1	0.868	0.919
Inferior Nasal Aperture	1.351	0.384	12.378	1	0.000	3.860
Interorbital Breadth	22.200	4661.777	0.000	1	0.996	4377581428
Malar Tubercle	19.299	10511.952	0.000	1	0.999	224310287.4
Nasal Aperture Shape	-0.644	0.650	0.981	1	0.322	0.525
Nasal Aperture Width	0.967	0.640	2.286	1	0.131	2.630
Nasal Bone Contour	4.906	2.289	4.595	1	0.032	135.058
Nasal Bone Shape	1.105	20274	.236	1	0.627	3.018
Nasal Overgrowth	-12.103	41518.001	0.00	1	1.000	0.000
Nasofrontal Suture	-.0565	2.154	0.069	1	0.793	0.569
Orbital Shape	2.440	2.652	0.847	1	0.357	11.473
Postbregmatic Depression	22.881	10404.907	0.000	1	0.998	8654808998
Posterior Zygomatic Tubercle	0.066	0.392	0.028	1	0.867	1.068
Supranasal Suture	-3.324	1.747	3.620	1	0.057	0.036
Transverse Palatine Suture	-1.180	2.500	0.223	1	0.637	0.307
Zygomaticomaxillary Suture	-0.603	0.545	1.224	1	0.269	0.547
Constant	-58.756	41805.696	0.000	1	0.999	0.000

Spearman's Rank Order Correlation Coefficients

Spearman's rank order correlation coefficients were used to determine if there are correlations between age and trait scores. The results are summarized in Table 5.3. Those traits that had a statistically significant positive correlation with age were nasal aperture width (0.347), posterior zygomatic tubercle (0.257), and zygomaticomaxillary suture (0.243).

Those traits with a statistically significant negative correlation with age was anterior nasal spine (-0.268). The remainder of the traits were either found to not be correlated with age or were not statistically significant with age.

Those traits that showed no correlation with age were interorbital breadth, malar tubercle, nasal overgrowth, and postbregmatic depression. For these traits the lack of correlation may also be explained by the collections themselves. The fetal and infant remains were unarticulated and disassembled so scoring traits like interorbital breadth was difficult in many cases and impossible in most. Nasal overgrowth is in an area of the facial skeleton that is so fragile that the trait could have been broken and therefore could not be observed. The malar tubercle, in the fetal and infant remains, was difficult to see though in most cases a slight bump where one would be visible in a larger specimen could be felt. Due to the difficulties in scoring these traits, it is possible that they are not the best at diagnosing ancestry in subadult individuals.

Table 5.3: Summary of Spearman's Rank Order Correlation Coefficients.

Trait	Correlation Coefficient	Significance	N
Anterior Nasal Spine	-0.268	0.000	307
Inferior Nasal Aperture	0.060	0.292	307
Nasal Aperture Shape	-0.104	0.069	307
Nasal Aperture Width	0.347	0.000	307
Posterior Zygomatic Tubercle	0.257	0.000	306
Zygomaticomaxillary Suture	0.243	0.000	306

Cohen Kappa Statistic

The Cohen Kappa Statistic was used to test intraobserver error using 10% of the examined sample of 307 individuals. The results of this analysis are presented in Table 5.4 with their associated level of repeatability following Landis and Koch (1977). With this statistic, it was determined that the accuracy for scoring the traits consistently between trials was highest with anterior nasal spine (0.971), inferior nasal spine (0.971), interorbital breadth (0.941), malar tubercle (1.00), nasal aperture shape (1.00), nasal aperture width (0.971), posterior zygomatic tubercle (1.00) and zygomaticomaxillary suture (1.00).

The traits with the lowest accuracy between the trials were nasal bone contour (0.314), nasal bone shape (0.743), nasal overgrowth (0.714), nasofrontal suture (0.314), orbital shape (0.543), postbregmatic depression (0.486), supranasal suture (0.514), and transverse palatine suture (0.600). However, upon review of the data and the analysis, these traits are the ones that had the highest number of non-observations due to the nature of the collections and the preservation encountered. This could have some effect on the analysis.

Table 5.4. Summary of Cohen Kappa Statistic results.

Trait	Value	Landis and Koch (1977) Agreement
Anterior Nasal Spine	0.971	Almost Perfect
Inferior Nasal Aperture	0.971	Almost Perfect
Interorbital Breadth	0.914	Almost Perfect
Malar Tubercle	1.00	Almost Perfect
Nasal Aperture Shape	1.00	Almost Perfect
Nasal Aperture Width	0.971	Almost Perfect
Nasal Bone Contour	0.314	Fair
Nasal Bone Shape	0.743	Substantial
Nasal Overgrowth	0.714	Substantial
Nasofrontal Suture	0.314	Fair
Orbital Shape	0.543	Moderate
Postbregmatic Depression	0.486	Moderate
Posterior Zygomatic Tubercle	1.00	Almost Perfect
Supranasal Suture	0.514	Moderate
Transverse Palatine Suture	0.600	Moderate
Zygomaticomaxillary Suture	1.00	Almost Perfect

CHAPTER 6: DISCUSSION

Many traits were difficult to score, particularly with the fragile, and paper-thin, fetal and infant remains. Many of these sets of remains were incomplete and fragmentary, particularly among the youngest individuals where the elements containing the traits to be examined were no larger than a fingernail. It was also noted on many of the individuals that the degree of curling on the flat bones of the skull was so severe that they affected the expression of the traits, therefore eliminating the impacted traits from that individual or the individual entirely from the study.

For the older individuals, preservation was better; however, many still had issues that may have affected the analysis of the traits. Many of the older individuals in the study had been sectioned through the midline to expose the sinuses for an earlier research study utilizing these remains. For these individuals, they needed to be reassembled using dental wax to hold the halves of the skull together for trait observation. Often the halves would not line up exactly due to the way they were cut, so the affected traits could not be scored. In addition, for those who were able to be reassembled accurately, often the sectioning would damage one or both of the fragile nasal bones, rendering that trait unobservable.

Can the Ancestry of African American and European American Subadult Individuals be Assessed with Significant Accuracy?

Chi-square analysis has shown that those nonmetric traits examined in this study, commonly used to assess adult ancestry, can be used to assess ancestry in subadult individuals. Identifying the traits can be difficult in the youngest individuals, but the majority of the traits can be identified and scored for all age groups.

Which Cranial Traits are Useful in Assessing the Ancestry of Subadult Skeletons?

As was shown above, the majority of the cranial nonmetric traits examined are useful in assessing the ancestry of subadult skeletal remains, depending on the age of the individual. Those of the most utility are anterior nasal spine, inferior nasal aperture, malar tubercle, nasal aperture width, and orbital shape. The trait with the least utility in assessing the ancestry of subadult individuals is postbregmatic depression as in the youngest individuals, those in the fetal and infant age groups, do not have this feature on their skulls due to the presence of the soft spot. However, postbregmatic depression is not often diagnostic in adult individuals and may even be affected by the sex of the individual (Hefner 2003).

At What Age do Nonmetric Traits Manifest in the Subadult Skeleton for African and European Americans?

As was shown above, all of the traits, except for postbregmatic depression could be seen even in those individuals in the fetal age group, with an age as young as three months in utero. The viewing of the traits can be complicated by the state of the remains for the younger individuals. For example, in this study, the majority of the fetal and infant remains were stored in the collections separated into individual bones; the bones of the skull were all separated along the suture lines as all of the soft tissue had been removed. Additionally, many of the flat bones of the skull exhibited curling of the edges which prevented the scoring of any traits they obscured and affected the reconstruction of the skulls.

Comparison with Previous Studies

As this study has shown, those traits examined by Hefner (2009) can also be seen in the facial skeleton of individuals even as young as three months in utero with the exception of only a few traits. In general, the frequencies at which the traits are seen in each ancestral group are similar between this study and Hefner's (2009). These similarities show that the traits examined by Hefner (2009), and expanded to show a greater range of human variation in that same study, are able to capture variation among all age groups.

As was seen with the geometric morphometric study conducted by Buck and Vidarsdottir (2004), it is possible to assess ancestry in subadult age groups. This study did not examine the mandible as a way of assessing ancestry in subadult individuals as, for much of the sample the mandible was not available for examination, and therefore cannot directly compare results with Buck and Vidarsdottir's (2004) study. However, the results are similar; in each study, it has been shown that it is possible to assess the ancestry even of subadult individuals despite the long held belief by many forensic anthropologists and skeletal biologists that features commonly used to assess ancestry in adults are obscured by ontogenetic changes in the subadult skeleton.

In comparison with Szen (2018), this study has shown that, nonmetrically, ancestry can be examined in subadult skeletons. This may validate one of the possible reasons why the study by Szen (2018) was unable to assess ancestry in her study. Her study used a 3D digitizer to examine the subadult skull for ancestry assessment using metrical analysis. In her study, Szen (2018) came to the conclusion that the differences in the ancestral groups may be too small to discriminate metrically, and as this current study was able to locate differences between the two ancestral groups examined, this assumption may be confirmed. Nonmetric analysis may be able to separate out ancestral groups when the individual studied is a subadult.

CHAPTER 7: CONCLUSIONS

The goal of this study was to determine the frequencies of nonmetric traits commonly used to assess ancestry in adult African American and European American individuals for subadult individuals, and to determine which traits are better to use for each age group. This research shows whether or not the traits that are commonly used to assess ancestry for adult African American and European American individuals can be used to assess ancestry in subadult individuals as is commonly believed.

Despite the difficulties described in the preceding pages, this thesis can be said to have accomplished its goals. For those individuals whose remains were not affected by preservation issues or damage to the trait areas, the nonmetric traits that are commonly used to diagnose ancestry in adult individuals were able to be scored for subadult individuals. However, due to the nature of the remains of the youngest individuals, the fetal and infant remains, a note of caution must always be taken into account. As the bones are fragile and paper-thin, and are not yet fused together with most of the age range, many traits that difficult to diagnose. In a fetal or infant skull that is still largely held together through soft tissue and cartilage, the traits may be easier to score. However, postbregmatic depression cannot be scored until the fontanelle creating the soft spot surrounding bregma is ossified.

This study has shown through Chi-square that the nonmetric traits examined in this study, commonly used to assess adult ancestry, can be used to assess ancestry in subadult individuals. Identifying the traits can be difficult in the youngest individuals, but the majority of the traits can be identified and scored for all age groups.

As was shown above, the majority of the cranial nonmetric traits examined are useful in assessing the ancestry of subadult skeletal remains, depending on the age of the individual. Those of the most utility are anterior nasal spine, inferior nasal aperture, malar tubercle, nasal aperture width, and orbital shape. The trait with the least utility in assessing the ancestry of subadult individuals is postbregmatic depression as in the youngest individuals, those in the fetal and infant age groups, do not have this feature on their skulls due to the presence of the soft spot. However, postbregmatic depression is not often diagnostic in adult individuals and may even be affected by the sex of the individual (Hefner 2003).

As was shown above, all of the traits, except for postbregmatic depression could be seen even in those individuals in the fetal age group, with an age as young as three months in utero. The viewing of the traits can be complicated by the state of the remains for the younger individuals. For example, in this study, the majority of the fetal and infant remains were stored in the collections separated into individual bones; the bones of the skull were all separated along the suture lines as all of the soft tissue had been removed. Additionally, many of the flat bones of the skull exhibited curling of the edges which prevented the scoring of any traits they obscured and affected the reconstruction of the skulls.

The Future of Ancestry Analysis?

DNA Analysis

It is possible that the future of the diagnosis of ancestry in skeletal material, subadult or adult, will fall into the realm of DNA analysis, particularly as it becomes easier and more accessible for use. The popularity of these tests has the benefit of making them more widely accessible to people everywhere, and can make it easier for law enforcement to gain access to tests. However, due to the popularity of these tests, the definitions that are used for race (in the social realm) are changing. People are able to find out their entire ancestral background and that can make it difficult to determine how to classify an individual who has gone missing. Additional research needs to be done, both in biological and cultural anthropology to see the effects these tests can have on the future of creating the biological profile in forensic investigations.

Future Research

Additional research into the assessment of ancestry in subadult skeletal remains should be focused on applying these traits to one of the statistical classification methods listed above before this study can be applied to cases that may cross the desk of the forensic anthropologist. This thesis simply scored the traits to determine if it was possible to score them on even fetal and infant remains. It has been proven that with time and careful examination of the skeletal elements, it is possible to score these nonmetric traits on subadult skeletons. However, it is not known just how well these scores will work for actually providing a diagnosis of ancestry and therefore should not be used in forensic anthropology casework without further analysis. It has been proven by other researchers that the metric analysis to diagnose ancestry does not work for subadult individuals, possibly due to the size of the remains. In situations like this, nonmetrics can excel as they are not as affected by the size of remains. Nonmetric traits are scored relative to the overall size of the remains and can be used even when the remains available are tiny.

APPENDIX: TRAIT FREQUENCY TABLES

Table A.1. Anterior nasal spine frequencies in two ancestral groups.

	African American (n = 214)		European American (n = 93)	
Anterior Nasal Spine	N	%	N	%
1	69	32	7	7.5
2	89	42	38	41
3	56	26	48	52

Table A.2. Inferior nasal aperture frequencies in two ancestral groups.

	African American (n = 214)		European American (n = 93)	
Inferior Nasal Aperture	N	%	N	%
1	68	32	8	8.6
2	51	24	17	18
3	45	21	19	20
4	40	19	37	40
5	10	4.7	12	13

Table A.3. Interorbital breadth frequencies in two ancestral groups.

	African American (n = 193)		European American (n = 74)	
Interorbital Breadth	N	%	N	%
1	16	8.3	11	15
2	142	74	59	80
3	35	18	4	5.4

Table A.4. Malar tubercle frequencies in two ancestral groups.

	African American (n = 191)		European American (n = 92)	
Malar Tubercle	N	%	N	%
0	40	21	8	8.7
1	89	47	56	61
2	55	29	27	29
3	7	3.7	1	1.1

Table A.5. Nasal aperture shape frequencies in two ancestral groups.

	African American (n = 214)		European American (n = 93)	
Nasal Aperture Shape	N	%	N	%
1	38	18	12	13
2	140	65	76	82
3	36	17	5	5.4

Table A.6. Nasal aperture width frequencies in two ancestral groups.

	African American (n = 214)		European American (n = 93)	
Nasal Aperture Width	N	%	N	%
1	43	20	42	45
2	137	64	49	53
3	34	16	2	2.1

Table A.7. Nasal bone contour frequencies in two ancestral groups.

	African American (n = 67)		European American (n = 11)	
Nasal Bone Contour	N	%	N	%
0	15	22	0	0
1	35	52	6	55
2	2	3.0	0	0
3	2	3.0	0	0
4	13	19	5	45

Table A.8. Nasal bone shape frequencies in two ancestral groups.

	African American (n = 164)		European American (n = 67)	
Nasal Bone Shape	N	%	N	%
1	57	35	12	18
2	71	43	35	52
3	9	5.5	7	10
4	27	16	13	19

Table A.9. Nasal overgrowth frequencies in two ancestral groups.

	African American (n = 151)		European American (n = 66)	
Nasal Overgrowth	N	%	N	%
0	106	70	47	71
1	45	30	19	29

Table A.10. Nasofrontal suture frequencies in two ancestral groups.

	African American (n = 61)		European American (n = 15)	
Nasofrontal Suture	N	%	N	%
1	18	30	6	40
2	19	31	6	40
3	1	1.6	1	6.7
4	23	37	2	13

Table A.11. Orbital shape frequencies in two ancestral groups.

	African American (n = 94)		European American (n = 21)	
Orbital Shape	N	%	N	%
1	41	44	10	48
2	29	31	6	29
3	24	25	5	24

Table A.12. Postbregmatic depression frequencies in two ancestral groups.

	African American (n = 87)		European American (n = 18)	
Postbregmatic Depression	N	%	N	%
0	78	90	17	94
1	9	10	1	6.0

Table A.13. Posterior zygomatic tubercle frequencies in two ancestral groups.

	African American (n = 214)		European American (n = 92)	
Posterior Zygomatic Tubercle	N	%	N	%
0	55	26	16	17
1	91	43	46	50
2	56	26	25	27
3	12	5.6	5	5.4

Table A.14. Supranasal suture frequencies in two ancestral groups.

	African American (n = 65)		European American (n = 16)	
Supranasal Suture	N	%	N	%
0	39	60	7	44
1	7	11	5	31
2	19	29	4	25

Table A.15. Zygomaticomaxillary suture frequencies in two ancestral groups.

	African American (n = 213)		European American (n = 93)	
Zygomaticomaxillary Suture	N	%	N	%
0	102	48	46	49
1	53	25	35	38
2	58	27	12	13

Table A.16. Transverse palatine suture frequencies in two ancestral groups.

	African American (n = 135)		European American (n = 71)	
Transverse Palatine Suture	N	%	N	%
0	0	0	1	1.4
1	40	30	31	44
2	61	45	24	34
3	31	23	14	20
4	3	2.2	1	1.4

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