QUANTIFYING CRANIAL SHAPE CHANGE WITH AGE IN ADULT MODERN HUMANS: THROUGH A GEOMETRIC MORPHOMETRIC APPROACH

by

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Skeletal ontogenetic changes in shape have been closely examined and researched in modern humans during the prenatal to subadult stages of development. However cranial shape changes during adulthood are less notable and studied. This thesis used 35 three-dimensional landmarks from 13 cranial specimens of known age to estimate shape changes associated with age. Since research on this topic is less well known I hypothesized that the human cranium does undergo shape change during adulthood and that these changes will provide more information on cranial ontogeny.

Three-dimensional surface models of the superior and inferior portions of crania were created by photogrammetry using *Agisoft Photoscan*. *Geomagic Control* was used to unite these parts into a single 3D model of each specimen. The 35 craniometric landmarks were digitized using *Landmark Editor*. The landmarks were superimposed through a Generalized Procrustes Analysis in *MorphoJ*. Variations due to size, position, and orientation were removed from the data leaving the variable of shape for each specimen. The resulting 13 configurations of Procrustes coordinates were regressed against chronological age.

Results of the regression analysis demonstrated a correlation between cranial shape with age. Age has a subtle effect on cranial shape that accounts for approximately 5.7% of shape variance. Though minimal, as the cranium ages the position of the zygomaxillare anterior narrows resulting in a sunken or hollowed look of the craniofacial region of the skull.

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Introduction

Present research on cranial aging techniques for use in forensic anthropology and bioarchaeology along with cranial growth and development has primarily focused on changes from the prenatal stages to the subadult stages of life (White and Folkens, 2005). In comparison, research examining cranial change after attaining adulthood is limited. These changes in shape with age are commonly researched in the post-crania, however, the cranium does offer indicators of age: tooth eruption and sutural fusions or closures (Christensen et al., 2014).

The use of geometric morphometrics (GM) has enabled anthropologists to recognize these shape changes and correlate them to variations within and between species to identify patterns of variability (Zollikofer & Ponce de León, 2005). Previous GM research has primarily focused on taxonomic variation (Zollikofer & Ponce de León, 2005), however, my research used GM to distinguish cranial shape changes in adults due to age to provide a more accurate age estimation and additional information on skeletal change during ontogeny or growth and development (Baab et al., 2012).

To achieve this, photogrammetry, geometric morphometrics, and cranial landmarking were used to create 3D models of 13 cranial specimens with recorded ages at death from the Terry Collection. The Terry Collection was chosen because it houses cranial specimen of known ages. For this thesis, I addressed the following questions: Are there quantifiable patterns of shape change correlated to age in adults? If so how does the research assist in cranial age estimation techniques? What do these changes tell us about human ontogeny?

Acknowledgement of Collection Sample: The Terry Collection

The Robert J. Terry Anatomical Skeletal Collection (Terry Collection) was named after Robert J. Terry a professor of anatomy and head of the Anatomy Department at Washington University's Medical School in St. Louis, Missouri (Hunt and Albanese, 2005). The collection, assembled between 1898 - 1967, is made of a total of 1,728 human skeletons housed in the Smithsonian Institution, Department of Anthropology, National Museum of Natural History, in Washington D.C. It is important to acknowledge that the individuals within the collection were non-consenting. The majority of Dr. Terry's collection consists of remains from Washington University's institutional morgue and hospitals in the local St. Louis area who were not claimed by their next of kin. Since the remains were not claimed, they were awarded to the state, and in turn, the state of Missouri decided to donate the collection to science for medical research (Hunt and Albanese, 2005). Since they were wards of the state these individuals did not consent during their life to be donated to the collection.

The Terry Collection contains real human remains and their bodies deserved to be treated with the utmost respect through any type of medical or anthropological research including my own. The Terry Collection was chosen because it included records of known age, sex, and ancestry. The remains within the collection have been utilized for considerable amounts of anthropological research in attempts to create new methods for biological profiles and now a more accurate aging method and human growth and development studies (Hunt and Albanese, 2005).

I agree with the statement provided by Dr. Grover Krantz, who is one of the consenting individuals found within Dr. Terry's collection, "I've been a teacher all my life, and I think I might as well be a teacher after I'm dead (Dr. Grover Krantz)" Dr. Krantz consented or chose to donate his body to science after his death in 2002. I would like to emphasize that the Terry Collection is not just a set of remains for scientists to use for research, it is a collection of actual people who continue to help educate us even passed their deaths. While the individuals in the Terry Collection are referred to as "specimens" throughout this thesis, I would like to acknowledge they were and are still human beings and were treated with care throughout my research.

Background

Current Aging Techniques for Adult Modern Humans

As humans age into adulthood, there is a lack of identifiable features that represent age-related shape changes of the skeleton (Tanner, 1998). It is important to look at age-related changes in adults because once adults reach this developmental stage, selective structures are no longer acting to manage the skeleton's biological maintenance thus the rates of degenerative changes are highly variable (Christensen et al., 2014). The range in variability is one focus of this research to obtain knowledge of adult aging techniques.

Anthropologists use a variety of postcranial bones to estimate adult age, including the pubic symphyses, auricular surfaces, and sternal rib ends (Christensen et al., 2014). Aside from postcranial bones, the cranium also has a set of features anthropologists use for age estimation. The general features that are primarily used to age the adult cranium are tooth wear and sutural fusion/closure (White and Folkens, 2005). To estimate age from suture fusions, anthropologists examine the visibility of the cranial sutures. Based on the degree of visibility, the sutures are scored as (1) Open, (2) Minimal, (3) Significant, (4) Obliteration. Each score has a corresponding age range with which it is associated (Christensen et al., 2014).

Adult age estimates tend to be correlated with broad ranges of uncertainty (White and Folkens, 2005). When anthropologists estimate age, a range of equally possible ages based on examined features is produced (i.e. pubic symphysis 15-24, 19-40 years, etc.). The steps used for quantifying skeletal morphology introduces this range of error as the data is transformed into a chronological age or the length of time an individual has been alive (Christensen et al., 2014). The wide intervals of age can be seen as accurate, but not particularly precise. However, there is no current method that provides ample amounts of accuracy and precision to age estimation. The research presented in this thesis examines the question of "Are there additional features and methodologies that will be used to assess cranial age?"

<u>This research looked to add to existing forensic aging knowledge as it introduces a method of improved accuracy and precision to cranial age estimation within adults along with providing information on growth patterns examined in human cranial ontogeny.</u>

Human Ontogeny

The term "ontogeny" refers to the growth and development of an individual from prenatal stages until death (Tanner, 1998). In this paper, the terms "ontogeny" and "growth and development" will be used interchangeably. Growth is the increase in size or number of a structure whereas development is the change in shape, pattern, proportion, or a history throughout an individual's life. Phases of an individual's life history reflect in morphological shape changes as humans transition through developmental stages. Examining life history allows anthropologists to view evolutionary and developmental changes (Leigh & Blomquist, 2007). The human skeletal system, just like the rest of the body is changing within and between developmental stages. The growth and development of an adult skeleton is considered complete post cranially when all epiphyses have been fused, and cranially when all permanent teeth have erupted (Christensen et al., 2014).

This research will enhance anthropologists' ability to examine developmental changes of the adult cranium. The skeleton has undergone modeling and remodeling leading up to and during this point in an individual's life history. There are constant changes through the use of the skeleton, lack of nutrition, and an individual's response to biomechanical needs (Christensen et al., 2014). However, as Christensen et al. (2014) mention, forces are no longer active in controlling the skeleton's biological maintenance; this is why examining human skeletal ontogeny is important. Since the skeleton is no longer responding to biological and biomechanical needs it is pertinent to see how the skeleton changes once a human reaches adulthood. Doing this allows for the introduction of new knowledge regarding the process of human skeletal growth and development that is lacking in modern literature.

The data analyzed in this research will provide a foundation for forensic anthropologists to study and build a new cranial aging method, paleoanthropologists to more appropriately compare crania of different ages, and will supply insight on modern human and hominid ontogeny. With the existing literature of the focused topics outlined it is important to introduce and address the validity of the methods used for the research.

Reliability and Validity of Photogrammetry

Photogrammetry is a rising technique used by anthropologists and archaeologists for surface data acquisition. Photogrammetry is a method that is accessible and flexible for collecting digital measurements. This method utilizes computerized software that constructs multiple photographs from different angles into a complete 3D digital image (Waltenberger et al., 2021). Photogrammetry is a non-invasive technique that preserves the structural integrity of specimens. This technique is convenient for documenting specimens as only a camera is required while working in a museum or other collections, but it is labor intensive to produce the full models (see methods below). These photos are easily transmitted into digitized files that make them easier to share among the scientific community. Wong et al. (2007) have shown that the use of photogrammetry is a technique that produces reliable digital measurements.

Laser scanning and the use of micro-scribes are also techniques anthropologists use for collective digital measurements. As opposed to laser scanning, photogrammetry overcomes the limitations of space and time (Wong et al. 2007, Jurda & Urbanová, 2016). In this instance, space would mean the area needed to transport equipment when analyzing specimens since digital cameras and cameras found on phones can be used for photogrammetry. This makes it easier for anthropologists to carry equipment across long distances. Photogrammetry overcomes the limitation of time by the fact that image capture from a camera is faster than the image capture of a laser scanner. The use of a micro-scribe allows anthropologists to obtain more cranial landmarks and examine a large number of specimens over a short period. However, with a micro-scribe, if a cranial landmark is placed incorrectly, all the points that have been previously collected must be replaced making this technique more tedious and time consuming. In comparison, the use of photogrammetry allows for the creation of a relatively complete surface model so that landmarks are viewed from all perspectives once placed in the computerized software and edited or refined as needed.

Unlike the other techniques, photogrammetry helps retain the surface features and textures of the crania photographed (Fourie et al., 2011). The retainment of these features assists anthropologists in identifying shape changes.

This research looked to provide validation for the use of photogrammetry and geometric morphometrics as opposed to other forms of data collection that are used within the field. Photogrammetry methods are best used when partnered with geometric morphometrics as these methods allow for the introduction of visual representations of quantified data.

Geometric Morphometrics

Geometric morphometrics (GM) is a coordinate-based set of tools that enable the identification and interpretation of complicated patterns of morphological variation, between and within groups of specimens, represented by configurations of homologous landmarks (Zollikofer & Ponce de León, 2005). Furthermore, GM presents the ability to visualize the shape changes examined in this research. Geometric morphometrics is commonly used to view variation and covariation within and among species (Baab et al., 2012). Studies such as Ackerman and Krovitz (2002), Mitteroecker et al. (2004), Cobb and O'Higgins (2004), and McNulty et al. (2006) have used geometric morphometrics to examine juvenile ontogeny and differences in shape with the goal of characterizing taxonomic variation among or between species of primates or hominids. This research, however, utilized GM to analyze age-related changes within modern humans to draw inferences on adult human ontogeny.

Geomorphic morphometrics has proven to be a useful technique in identifying age-related developmental changes in the human skeleton. Gayzik et al. (2008) used GM to quantify shape changes within the rib cage. There have been numerous studies with primate models that have studied the morphology of their crania; Frost et al., 2003, Turley and Frost, 2013, and Jorganic and Hueze, 2019. For instance, Jorganic and

Hueze (2019) provide context on how the aging of the craniofacial region structurally changes the cranial shape of female baboons, where the central facial region is narrowed. However, only a handful of studies have been conducted using geometric morphometrics to identify age-related changes (Bastir et al., 2006).

This established the framework of this research to explore age-related shape changes in the human cranium. The software tools utilized by GM provide an advantage when compared to linear morphometrics in its capability to produce visualized coordinates of shape changes found on the cranium (Baab et al., 2012). Geometric morphometrics combined with photogrammetry allows this research to implement a new aging methodology used in cranial aging techniques for modern humans.

The research presented looked to expand on the use of geometric morphometrics in the field of forensic anthropology and how it is used to assess cranial age estimation.

Cranial Landmarking

A landmark is "a precisely defined point on a specimen" (Baab et al., 2012) and is represented by a set of x, y, and z coordinates. For this research, I collected cranial landmarks and analyzed them with geometric morphometrics to describe patterns of shape change associated with adult age. Like age identification, using the method of landmarking holds its own measurement of error. Barbeito-Andrés et al. (2012) examined the measurements of error between the three types of landmarks. Addressing the range of error for this method is necessary to ensure that all variables are accounted for. This research utilized landmarks Type (I) which are isolated in the cranial structure of each specimen that are easily identified (Barbeito-Andrés, 2012). Harvati & Weaver (2006) and Mitteroecker et al. (2004) provide great examples of a studies that implemented the method of cranial landmarking. Their studies provided cranial landmarks that set the framework for the research outlined in this thesis.

Thirty-five landmarks outlined in Langley et al (2016) were exclusively used during the process of this thesis. It is important to note that the landmarks selected are identifiable on all of the specimens examined in the data collection process. This research provided additional information on the measurement of error calculated in cranial landmarking. When combined with geometric morphometrics, the two methods yield results of geometric shape change.

Objective and Hypotheses

Objective: Identify cranial shape change correlated with age.

To achieve the primary objective, I addressed the following hypotheses:

Hypothesis 1: The human cranium undergoes shape change due to aging during adulthood.

Null Hypothesis: There is no shape change.

Hypothesis 2: Shape changes will provide information on cranial growth and development.

Hettena (2004) provides support that age can be assessed through craniofacial features meaning the study focused primarily on the facial bones. This in turn provides supplemental support that the proposed approach will produce viable results of shape change explained by age as it focuses on markers of shape change to the whole cranium. Modern literature has also shown that the human skeleton undergoes maturational changes (Bastir et al., 2006) supporting that markers of cranial shape change can be found throughout an individual's life history.

Materials and Methods

Sample

Originally 50 specimens were photographed, however, only 14 complete 3D models were created due to laboratory restrictions caused by COVID-19. Specimen 1572 was excluded from the study due to damage to the top of the skull which left a hole causing a missing landmark leaving only 13 viable specimens. The photographs of the viable 13 human cranial specimens, 8 male and 5 females were digitized and used for this research. The specimens range in age from 34 – 71. There were two instances of duplicate age where two specimens shared the age of 38 and two other specimens shared the age of 41 (See Table 1). The ancestry of these specimen was not recorded in the initial collection of specimens, however, the specimen number (placed by the Terry Collection), notes of missing features, and preliminary caliper landmark measurements were all reviewed, used, or referenced during the research process.

While 13 specimens are not an ideal sample size for accuracy, the sample still provided a glimpse into the possible degrees of shape change. In future stages of this research, the sample size will be increased to include the remaining specimen of the original 50 to showcase the amount of variation among a larger sample.

Table 1. Sample Composition

Identification	Sex	Age
745R	Female	34
1482R	Female	35
62RR	Male	38
14R	Male	38
41R	Female	41
1599	Female	41
34R	Male	44
1572	Female	45*
517	Male	46
605R	Male	52
548	Male	54
413	Male	55
59R	Male	59
925	Female	71

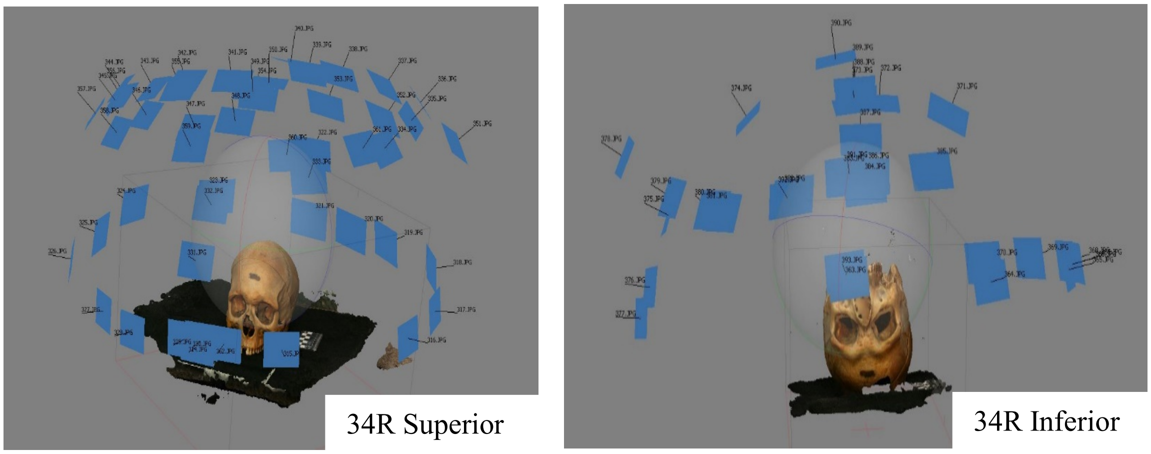
*The asterisk (*) and highlighted area indicates the specimen that was excluded due to damage.

Data Collection Protocol

Part 1: Photogrammetry

Three-dimensional surface models of the 13 cranial specimens from the Terry Collection were generated through photogrammetry. In this process, each specimen was photographed from different elevations and angles, approximately every 15 degrees in a circle around the specimen, using a digital camera. In order to capture the entire surface, two sets of photographs of each specimen were taken from superior (top) and inferior (bottom) views. Figure 1 depicts the photogrammetric analysis of Specimen 34R, a 44-year-old male. Specimen 34R will be used as a visual throughout the methodology section for consistency and to show the progression of the methods through my research. The blue squares in Figure 1 indicate the different angles photographs of the specimen were taken from. Two surface models were generated for each individual for a total of 100 surface scans, one collection of superior photos and one collection of inferior photos as seen in Figure 1. Taking photos from different angles ensures that all perspectives of the specimens are accounted for during the remaining process of data collection.

Once all of the photos were captured, they were placed into *Agisoft Photoscan*, a photogrammetry pipeline software, that digitized each specimen creating a 3D model. Photogrammetry is an ideal method as it allows for the surface textures and features of each specimen to be retained which helps when creating accurate 3D models (Waltenberger et al., 2021). Retaining the surface textures and features is important, especially when placing landmarks on a 3D model, since it is beneficial to have the model be as close to the physical features of the specimen as possible to ensure accuracy in measurements and possible changes in shape.


34R Superior

34R Inferior

Figure 1: Photogrammetry perspectives of Specimen 34R superior and inferior views.

Part 2: Creating Whole 3D Models

The digitized files of these photos were then uploaded into *Geomagic Control*, a professional engineering software, to construct complete 3D models of the specimens. The digitized files were then separated where one cranial specimen has two positions; superior and inferior (i.e. 34R top, 34R bottom see Figure 2). The superior position provides a view of the parietals and temporal bones, while the inferior position provides a view of the occipital bone, foramen magnum, mastoid processes, and maxillary teeth.

When the models in Figure 2 were uploaded into *Geomagic Control*, the surface the original specimen was placed on (i.e. a table) was included as part of the surface model. These areas such as the area underneath 34R superior needed to be removed. In this instance, the unwanted area was highlighted and deleted from the model leaving just the 3D model of the cranial specimen.

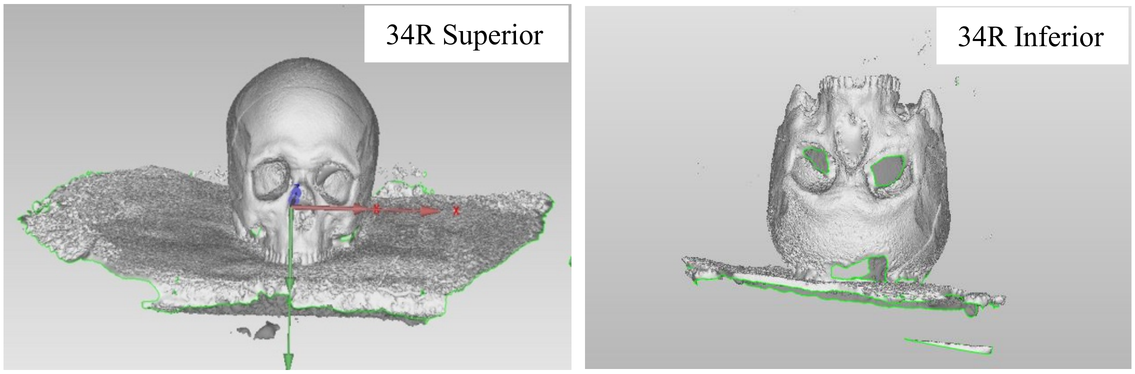


Figure 2: 3D representations of the superior and inferior views in *Geomagic Control*.

Part 2.1: Scaling

Geomagic Control programming allows for both positions of the cranium to be adjusted and measured. When placed in the software, one position of the cranium (superior or inferior) was metrically scaled larger than the other. The distance between cranial landmarks (i.e. Maximum cranial length, bizygomatic width, etc.) found on both positions was calculated. Using the Forensic Anthropology Training Manual, I chose a set of base landmarks that were shared by all specimens within the sample. Table 2 contains the list of chosen landmarks that were used for scaling the positions of each specimen to size. The average of all the distances for each position was measured and recorded. The averaged number was used to scale the models to equal size, and they were placed into an alignment program to ensure they fit together. Figure 3 illustrates the alignment process.

Table 2. Scaling Landmarks

Abbreviation	Measurement Name	From This Point	To This Point
GOL	Maximum cranial length	Glabella (g)	Opisthocranion (op)
ZYB	Bizygomatic width	Zygion (zy)	Zygion (zy)
UFHT	Upper facial height	Naison (n)	Prosthion (pr)
MFB	Minimum frontal width	Frontomolare (ft)	Frontomolare (ft)
NLH	Nasal height	Naison (n)	Nasospinale (ns)
NLB	Nasal width	Alare (al)	Alare (al)
BIOB	Bi-orbital width	Ectoconchion (ec)	Ectoconchion (ec)
MAST	Mastoidal distance	Mastoidale (mas)	Mastoidale (mas)
EAM	External auditory meatus distance	External Auditory Meatus	External Auditory Meatus

(Source: Forensic Anthropology Training Manual, by Karen Ramey Burns)

In Figure 3, the inferior, or bottom, position (left panel) is colored red while the superior, or top position (right panel) is colored green. The numbers found on each skull represent the landmarks outlined above. The bottom panel of Figure 3 shows the top placed into the bottom position. Here was where I ensured the two positions "fit" or were scaled to the same size. Once the positions have been scaled to equal size, they are saved and used to create the complete 3D model of each specimen.

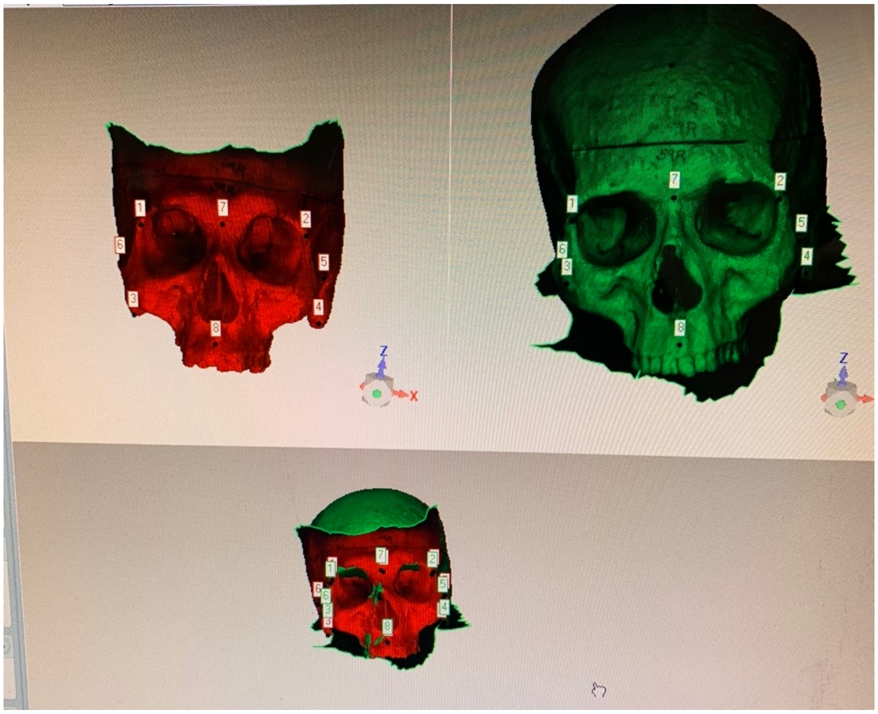
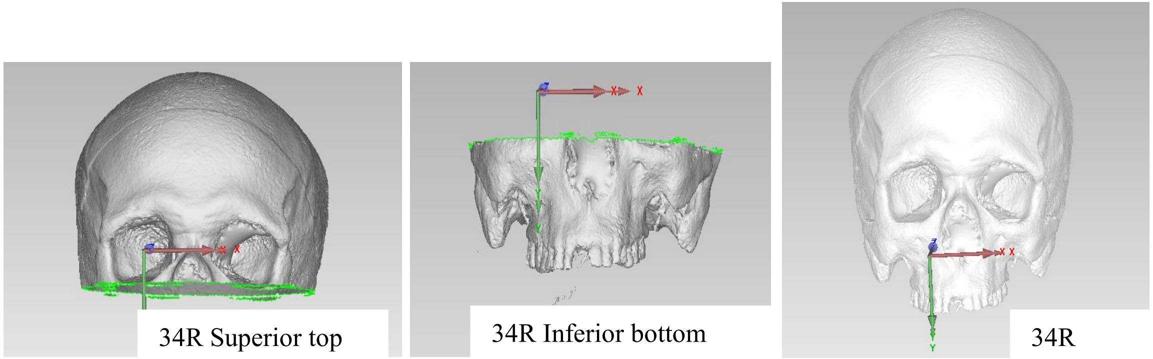


Figure 3: Scaling and alignment process.

Part 2.2: Completing the 3D Models

After the superior and inferior positions of each specimen have been scaled to fit, the bottom of the superior position and the top of the inferior position is cut and deleted leaving the remaining halves of each position (top half of superior and bottom half of inferior). The two halves are then both selected and merged creating a complete 3D model of the cranium (See Figure 4). The scaling process was imperative for a seamless merger of the two halves. If one position is too big, the two halves would not merge correctly causing a need for a recalculation. If a cranium is merged incorrectly, it risks losing the structural/feature integrity of the specimen. Digitized data is significant to this research in that the 3D models and photographs taken are easy to share with other researchers for repeatability and further research purposes (Algee-Hewitt & Wheat 2016).


34R Superior top

34R Inferior bottom

34R

Figure 4: Cut positions and merged (complete) model of Specimen 34R.

Part 3: Cranial Landmarking

After all of the 3D models of each specimen are adjusted, cut, and merged into complete models, the ply (ply.) or object (obj.) file of the merged 3D model was imported into the landmarking software, *Landmark Editor* (IDAV, 2002-2005). *Landmark Editor* was used to examine shape change and variation across all models created using the sample collection. Thirty-five landmarks, as outlined by Langley et al. (2016), were chosen and placed around the complete cranial scans. The chosen set of landmarks are found on the majority of the specimens in the sample except for one specimen, Specimen 1572, that is missing one landmark (#5 - Bregma). The list and landmark order of all 35 landmarks can be found in Table 3.

This set of landmarks excludes landmarks found on the mandible because the mandible itself was not photographed during the photogrammetry stage of this research. Each landmark was placed in the same order found in Table 3 on each specimen. If a landmark has two sides, a right and a left, the specimen left (viewer right) was landmarked first followed by the specimen right (viewer left). This essentially means that the left landmark was placed first followed by the right landmark.

Table 3. Landmark List and Order*

Abbreviation	Point	No.	Position
alv	Alveolon	1	Midline
ast	Asterion	2/3	Bilateral (right/left)
ba	Basion	4	Midline
b	Bregma	5	Midline
d	Dacryon	6/7	Bilateral (right/left)
ec	Ectoconchion	8/9	Bilateral (right/left)
ecm	Ectomolare	10/11	Bilateral (right/left)
eu	Euryon	12/13	Bilateral (right/left)
fmt	Frontomalare temporale	14/15	Bilateral (right/left)
ft	Frontotemporale	16/17	Bilateral (right/left)
g	Glabella	18	Midline
l	Lambda	19	Midline
ms	Mastoidale	20/21	Bilateral (right/left)
n	Nasion	22	Midline
op	Opisthocranion	23	Midline
o	Opisthion	24	Midline
pr	Prosthion	25	Midline
po	Porion	26/27	Bilateral (right/left)
ra	Radiculare	28/29	Bilateral (right/left)
zy	Zygion	30/31	Bilateral (right/left)
zma	Zygomaxillare anterior	32/33	Bilateral (right/left)
zo	Zygoorbitale	34/35	Bilateral (right/left)

*The list of chosen landmarks from Langley et al. 2016 and their order. Single numbers represent landmarks that are found along the midline of the skeleton. Numbers separated by a slash (/) are bilateral landmarks separated by the midline producing landmarks found on both the right and left sides of the skull.

Figure 5 illustrates the landmarks from Table 3 placed on Specimen 34R. This figure shows the placement of the 35 landmarks from the anterior, posterior, inferior, and side positions. Cranial landmarks are coordinates (i.e. x,y,z) for anatomical features of importance, for the cranium those include the Biorbitalis, Glabella, Bregma, etc. In Figure 5, the blue dots on the cranium indicate each placed landmark. If a specimen were to be missing a landmark, the landmark would be placed in the region where the landmark would normally be found and labeled “Missing” in *Landmark Editor* with coordinates recorded as “9999” for all three dimensions.

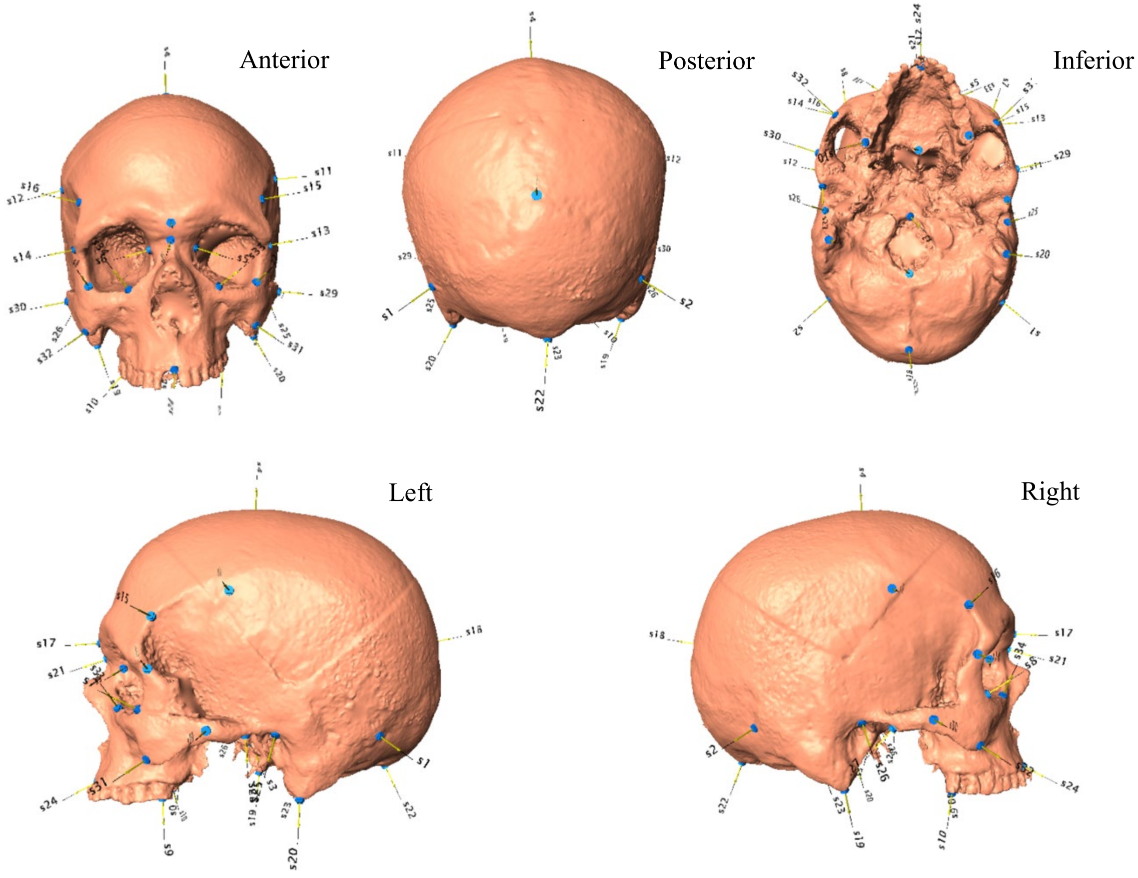


Figure 5: Multiple views of landmark placement on Specimen 34R.

Analytical Methods

Part 1: Replication Study

Before further analyzing the data, a replication study was performed to ensure the accuracy of landmark placement across the 13 3D models. The 13 models were placed into a single *Landmark Editor* project and the 35 landmarks were placed on each specimen. After the landmarks of the initial round were placed, I repeated this process four more times, each on a new *Landmark Editor* project file for a total of five replicates for each of the 13 models. I landmarked each cranium in the same order to ensure consistency within each project. (See Table 3). The titles of each project are as follows: Unmarked Specimens, Marked Specimens 1, Marked Specimens 2, Marked Specimens 3, Marked Specimen 4, and Marked Specimen 5. Placing each landmark was very tedious as it is important to place the landmark on the exact point of the feature.

A Principal Component Analysis (PCA) was also performed to assess the measurement error in relation to the amount of variation among different specimens. A PCA is a data reduction technique that takes the original set of variables that are likely correlated with one another and summarizes them as a new set of uncorrelated variables. These uncorrelated variables are then ranked to summarize as much variance as possible in as few variables as possible (Frost et al, 2003).

Part 2: Generalized Procrustes Analysis

One hundred five coordinates of the 35 landmarks for each specimen were used as the data set for this analysis. The landmark configurations of each specimen were superimposed using a Generalized Procrustes Analysis (GPA) (Rohlf and Slice, 1990) in *MorphoJ* (C. P. Klingenberg. 2011). Specimen centroid sizes were calculated by finding the square root of the sum of squared distances of the set of landmarks from their centroid during superimposition (Frost et al. 2003). A centroid is a point with the average coordinates of all the landmarks.

Superimposition essentially evaluated whether the repeated measurements and landmark placement fell within specific degrees of variations (Barbeito-Andres et al, 2012). During superimposition nuisance variables (size, position, and specimen orientation) were removed so that only shape remains (Rohlf and Slice, 1990).

Part 3: Regression Analysis

I conducted a series of multivariate regressions to assess the effect of age on cranial shape. For each specimen, I found the averages for each raw coordinates from the five replicates and used these averages for the regression. To run the regression, I uploaded the averaged coordinate dataset and age as a covariate into *MorphoJ*. I ran the regression once both files were uploaded into the *MorphoJ* new project. For the regression analysis, the dependent variable was shape (as represented by the superimposed coordinates) and the independent variable was the age of each specimen.

The finished regression for the 13 models can be seen in Figure 6 which is the regression of shape change explained by age.

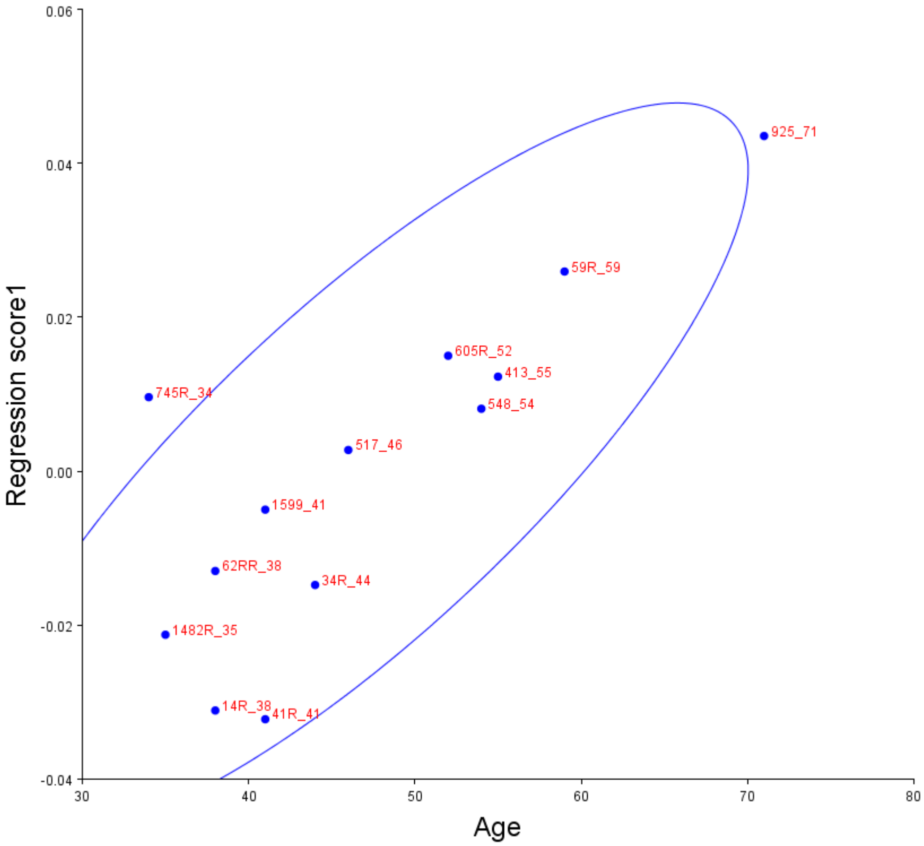


Figure 6: Linear Regression.

As you can see, the regression graph is formatted with age on the x-axis and shape associated with age on the y-axis. The blue circle or confidence ellipse around the specimens summarizes the variance of the data. For the purpose of visualization, the regression coefficients were magnified by 37 which is the difference between the oldest individual, Specimen 925 (71), and the youngest individual, Specimen 745 (34).

Results

Principle Component Analysis

Table 4 represents the reported list of eigenvalues from the PCA of the replication study. An eigenvalue is a total amount of variance in a sample correlated with the PC. The total variance is calculated by summing the variance of the 105 variables from the replication study.

Table 4. Eigenvalue Report

PC	Eigenvalues	% Variance	Cumulative %
1.	0.00102179	17.340	17.340
2.	0.00076499	12.982	30.322
3.	0.00067180	11.400	41.722
4.	0.00061299	10.402	52.125
5.	0.00057699	9.791	61.916
6.	0.00048320	8.200	70.116
7.	0.00044315	7.520	77.636
8.	0.00038489	6.532	84.168
9.	0.00032631	5.538	89.706
10.	0.00026964	4.576	94.281
11.	0.00020850	3.538	97.820
12.	0.00012848	2.180	100.000
Total Variance 0.00589272		**Predicted Percentage**: 5.6544%	

Regression

The results of the regression and description of eigenvalues show that cranial shape change in adults due to age is minimal accounting for 5.6544% of the total variance. These findings support *Hypothesis 1* that the human cranium does undergo shape change during adulthood. Figures 7 and 8 are lollipop diagrams that depict the amount and pattern of shape change across the whole data set. The combination of the blue dots and straight lines is called a lollipop. Figure 7 is a view of Axis 1 vs. Axis 2 where the cranial landmarks are in a side profile while Figure 8 is viewed from Axis 1 vs. Axis 3 which is a superior position. The solid dot is the landmark and the straight line represents the directional shift of the landmark with age. One can think of the lollipops as arrows, the dot itself is the nock (the end tip of the arrow) and the straight line stemming from the landmark is the arrow's shaft. The straight line shows the direction that the landmark will move relative to all of the other landmarks as individuals age.

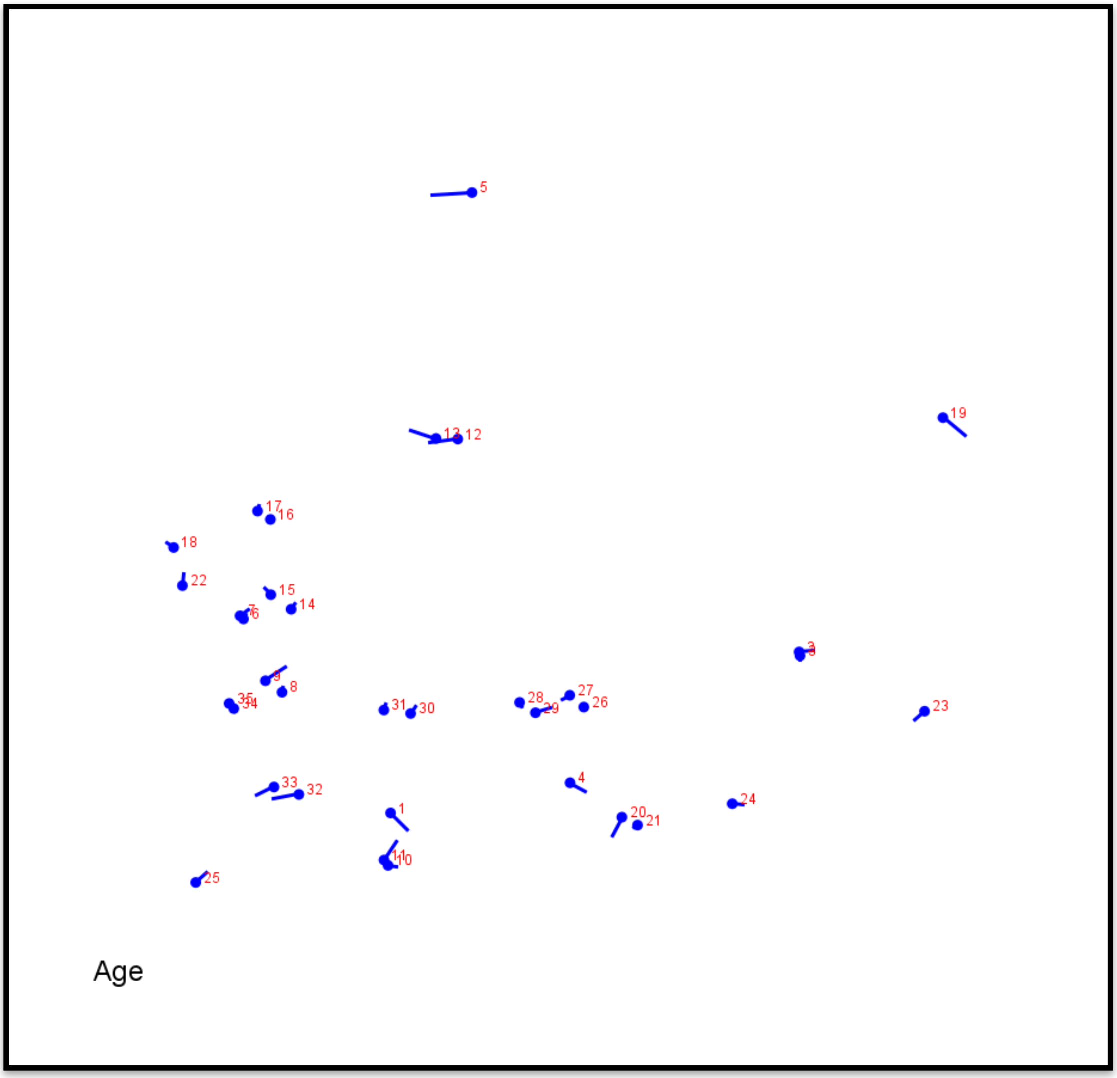


Figure 7: Axis 1 vs Axis 2 shape changes.

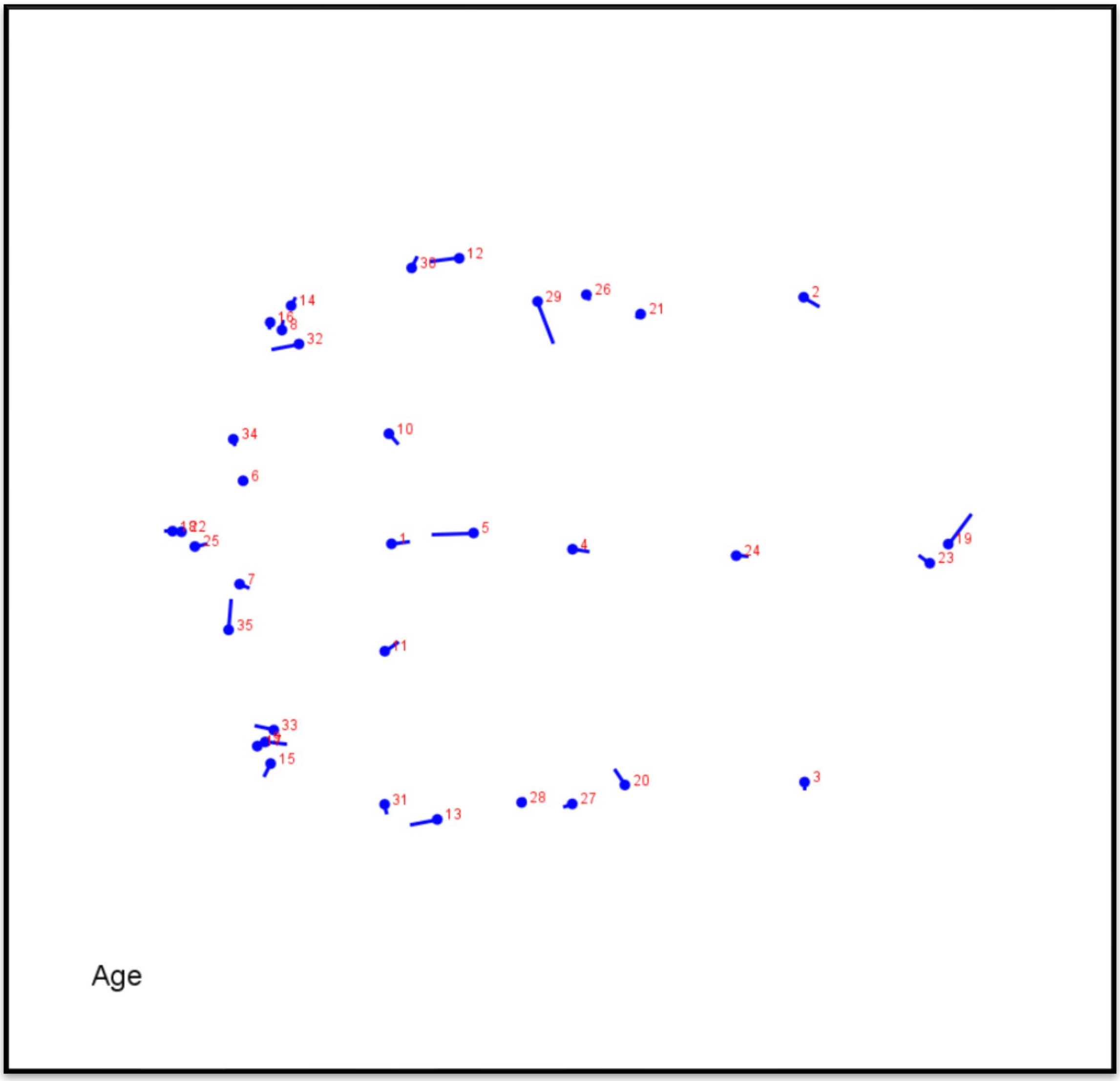


Figure 8: Axis 1 vs Axis 3 shape changes.

Warped Visualization

A warped skull was created to present a visualization of the cranial shape change associated with adult aging. I calculated a consensus individual (CI), which is just the average of all 13 individuals' coordinates, and used these to warp the surface of Specimen 34R to fit those landmarks. Next, to calculate the positive vectors, the regression coefficients for each coordinate were added to the corresponding coordinates of the consensus individual. For negative vectors, the regression coefficients were subtracted from the CI. Due to *MorphoJ* recognizing the calculations as too small, I multiplied both the positive and negative vectors by five to increase vector size to be more visible.

As the skull ages, the lower face appears to narrow, the zygomatics (cheekbones) recede towards the midline and the maxilla/maxillary teeth begin to protrude forward. Though the changes are subtle, they are important (See Figure 9). The most notable change can be seen in the zygomaxillare anterior, landmarks 32/33 (31/32 in the figure). These two landmarks sit at the intersection of the zygomaxillary suture. The black arrows point to the shift of the right (viewer left) zygomaxillare anterior. The figure shows three progressions of Specimen 34R during the warping process. The top skull is the original/base skull meaning the shape of the skull is unchanged and is the original form for the 44-year-old skull for 34R. The middle skull shows Specimen 34R halfway through the age progression and the bottom skull is Specimen 34R at the end of the age progression. At a closer evaluation of the bottom skull, the zygomatics are narrower than the top skull.

The shape change found in the zygomatic and maxillary regions can be a possible explanation as to why an older individual’s face seems to have “sunken or hollowed cheeks”. As the shape of the skull changes in this region, the attached muscle in that area, *zygomaticus major*, *zygomaticus major*, and *levator labii superioris* all appear to move to conform to the shape change giving the face a sunken or hollowed look.

I also observed that there could potentially be a shared pattern of remodeling or shape change over time within Catarrhines (Old World monkeys). The group Catarrhines consists of old-world monkeys, gibbons, orangutans, gorillas, our closest relative chimpanzees, and humans. When creating the warped skull of Specimen 34R, I noticed that the human skull shared a characteristic of change, the lower face protruding, with baboon (*Papio hamadryas*) aging patterns found in my lab mate Andrea Quintanilla’s research on cranial shape change with age in male and female adults in the genus *Papio* using molars as a case study (Quintanilla et al., 2021; Quintanilla in prep). In her research, she found that in the genus *Papio*, the lower face protrudes and elongates while the orbitals recede posteriorly as the skull ages (Quintanilla et al., 2021; Quintanilla in prep).

While this is not a primary conclusion of my research, it does show the possibility of a shared growth or remodeling pattern between modern humans and non-human primates. This aligns with my research significance for paleoanthropology in identifying plausible correlation in growth patterns with early hominids and primates.

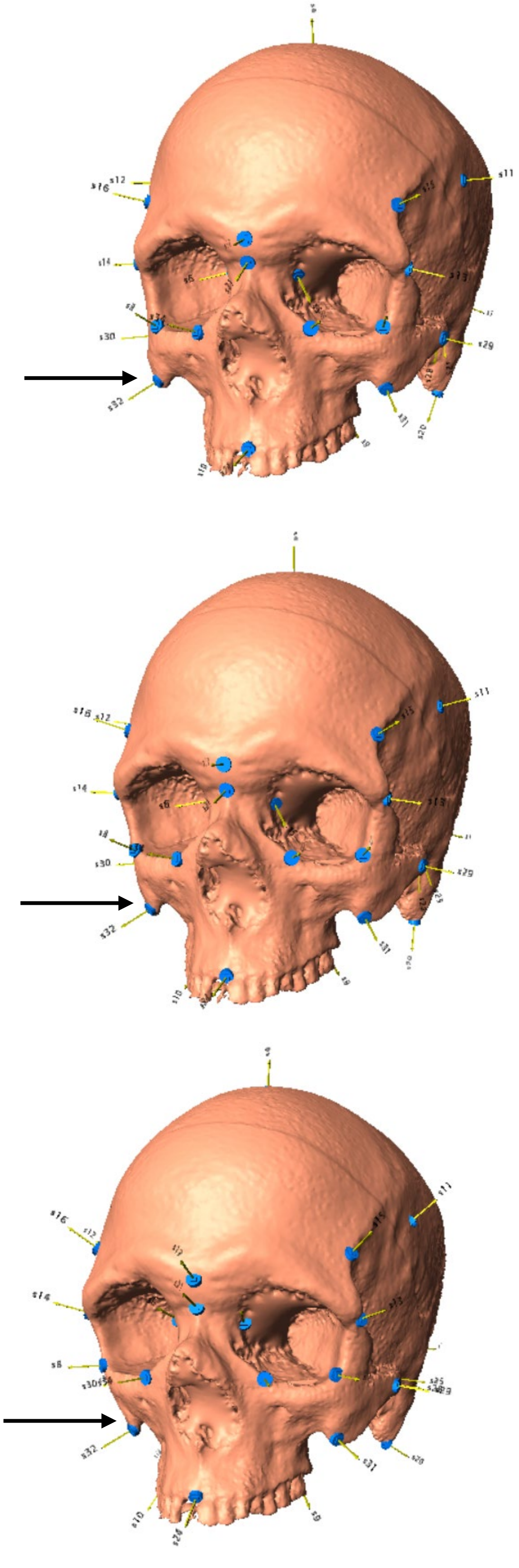


Figure 9. Age warped visualization of Specimen 34R.

Discussion and Limitations

Discussion

The results of this thesis show that there is an effect on cranial shape correlated with adult age. Though the shape change found is relatively modest, nevertheless, it is a significant discovery that proves that developmentally, our skull is still changing its shape through the progression of adulthood. The use of geometric morphometrics proved to show relevant shape change. The replication study focused on the repeatability of placement for each landmark to ensure the accuracy of all cranial measurements and coordinates. Through superimposition, these nuisance variables were discarded leaving age as the sole variable to be assessed.

The regression performed allowed for the measurements of all 13 specimens to be regressed against the known ages of each specimen. The ages of the specimens ranged from 34-71. The subsequent results of the regression analysis and the warped visualization are evidence that supports the primary hypothesis that the adult crania does undergo shape change as individuals age. Regression estimated 5.6544% of total shape variance was correlated with age. This notable as these results show that the adult skeleton, specifically the cranium, undergoes shape change even through adulthood. Thus addressing the unknowns presented at the start of this thesis concerning human ontogeny research. The results of this thesis add to human growth and development research by increasing the age or developmental range from prenatal - subadult to prenatal – adulthood, thereby showing that the human skeleton is constantly changing shape as humans age.

The finding from my thesis also provides support for my secondary hypothesis that isolating cranial shape change with age will yield more information on human growth and development. This opens the door for further research on cranial morphology and human ontogeny.

In continuants of this thesis, more specimens need to be vetted to obtain more substantial evidence.

Limitations

Though this thesis was completed and yielded significant results, numerous limitations affected my research due to COVID-19. The COVID-19 pandemic brought on strict campus restrictions at the University of Oregon. As a part of those restrictions, the Primate Morphometrics Laboratory, the laboratory I work out of, limited its access to the graduate students only leaving me to conduct my research outside of the lab. Because of this, I was unable to complete the 50 3D models as I initially anticipated. This resulted in a smaller sample size than originally intended in turn providing a somewhat “insufficient” sample size for statistical measurements. I placed insufficient within quotes because, for this thesis, the 13 models fulfilled the objective for research. When thinking of next steps, all 50 specimens would be more viable in determining shape change among individuals within a larger population. However, this does not downplay the results of this thesis as even with 13 models cranial shape change was still statistically proven to occur in adult modern humans. Ultimately, it would be important to include a larger, more globally diverse sample with both sexes well represented.

Research Significance

Forensic Anthropology

In the field of forensic anthropology, accurately estimating age for an individual is of high importance (Christensen et al., 2014). This research provides a foundation for the development of a more accurate cranial age estimation technique. This proposed technique will assist forensic anthropologists in providing a more accurate age estimation that is as close to the individual's chronological or real age as possible. With this a sequencing chart can be created illustrating cranial shape change at a given age on the estimation spectrum. Thus, enhancing age estimations in recorded collections, such as the Terry Collection used in this research, and age estimations for crania of unknown age such as those in missing person cases with skeletal remains.

Human Ontogeny

With the evidence of a ~5.7% variant of shape change, this research will improve anthropologists understanding of adult human growth and development. With the hypotheses supported, it will help in identifying an explanation as to; "How and why does the human cranium change shape through aging?" This study leads to further research in assessing if shape change is genetic, based on environmental changes, or a result of the cranium undergoing constant remodeling. Through expansions of this thesis, anthropologists can view how an individuals' skull changes over time. The warped visualization provided a visual representation of age progression. Researchers can use the visualizations to see how fast and what features of the skull change the most during each stage of development to better understand the process of cranial shape change.

Paleoanthropology

Since a possible correlation in growth patterns among Catarrhines was observed, it adds a positive piece of evidence that a geometric morphometric approach can be used to provide more evidence on hominid growth and development. Established works such as Frost et al. (2003), Joganic & Heuzé (2019), and most recently Quintanilla et al. (2021) have demonstrated utility of cranial landmarking for assessing variation among primates. With these articles used as supplemental sources, a geometric morphometric approach will assist anthropologists in looking deeper into the growth and developmental stages of early hominids. This approach can be used as a simulation of morphological skeletal change throughout a hominid's life history (McNulty et al. 2006).

Through this approach, anthropologists will be able to view simulated cranial and skeletal changes as a hominid grows to more accurately age and compare growth patterns among and between different hominid species. With the results from modern humans, anthropologists can also view possible correlations in cranial shape change between modern humans and early hominids.

Glossary

Anterior: toward the front of the body.

Cranial Landmark: a precisely defined point on a specimen.

Centroid: the central mass of a geometric object.

Centroid Size: computed as the square root of the sum of squared distances of a set of landmarks from their centroid.

Eigenvalue: is the total amount of variants that are correlated by the Principle Component (PC).

Epiphyses: the end portions of long bones.

Forensic Anthropology: a sub-field of physical anthropology that applies skeletal analysis and anthropological techniques to solve criminal cases.

Geometric Morphometrics: a set of methods used to acquire, process, and analyze landmarks.

Inferior: down or away from the head.

Ontogeny: the development of an individual specimen usually from prenatal stages until death.

Paleoanthropology: branch of anthropology that focuses on fossil hominids (our fossil ancestors).

Photogrammetry: the process of photographing a specimen from different perspectives/angles to create a complete three-dimensional image.

Post-Crania: bones of the skeleton that lie posterior (below) the cranium or skull.

Posterior: toward the back of the body.

Superior: up or toward the head.

Suture Fusion/Closure: the process of when cranial sutures close or fuse together.

Tooth Wear: the wearing down or progressive loss of a tooth’s surface.

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