

2020

The investigation of craniofacial variation between archaic and intermediate-late periods of Chile using morphometric analysis

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BOSTON UNIVERSITY
SCHOOL OF MEDICINE

Thesis

**THE INVESTIGATION OF CRANIOFACIAL VARIATION BETWEEN
ARCHAIC AND INTERMEDIATE-LATE PERIODS OF CHILE
USING MORPHOMETRIC ANALYSIS**

by

ANNA ELIZABETH GETLER

B.S., Rowan University, 2015

Submitted in partial fulfillment of the
requirements for the degree of
Master of Science

2020

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Approved by

First Reader

Sean D. Tallman, Ph.D.
Assistant Professor of Anatomy and Neurobiology

Second Reader

Maria Rosado, Ph.D.
Professor and Coordinator of Anthropology
College of Humanities and Social Sciences
Rowan University

DEDICATION

I would like to dedicate this thesis to my grandparents, John and Kathleen Getler, as well as my mother, Patricia Getler. Their unrelenting faith, love and support have helped me in this process, and through life more than they will ever know.

ACKNOWLEDGMENTS

This thesis was accomplished with the assistance of many people and institutions. I would like to thank my thesis advisor, Dr. Sean Tallman. He has played a crucial part in this process, guiding me and assisting in this process. The data collection would not have been possible without the help of Oscar Silva-Fontana, the manager of collections of Museo Arqueológico de La Serena. I would like to thank my dear friend Dr. Maria Rosado for her guidance through my undergraduate career, as well as through this thesis process, and for introducing me to the Museo Arqueológico de La Serena and its amazing staff, my “museum family” in 2016. I am incredibly thankful for her and the patrons of the museum who have made La Serena, and the museo feel like a second home.

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ABSTRACT

In skeletal studies of prehistoric populations of Chile's semiarid north, it is common practice for physical anthropologists to visually categorize crania as dolichophallic and brachycephalic which are then attributed to cultures or time periods based on the observed shape. The validity of this classification is still debated and poses several questions regarding the prehistory of Chile. The goal of this study is to investigate the craniofacial variation in populations representing the Archaic period and Intermediate-Late periods of Chile's semiarid north using morphometric analysis. The samples comprise two collections from the Museo Arqueológico de La Serena in La Serena, Chile. The Archaic period dates from 10,000 BC to 300 AC. The Intermediate-Late period dates from 900 AC to 1500 AC. The Archaic period included 87 crania, while the Intermediate-Late period included 78 crania. Cranial vault modification was practiced in all prehistoric populations, and modified individuals comprise ~4.6% of the Archaic period sample and ~74.4% of the Intermediate-Late period sample. Individuals observed to be dolichocephalic are associated with the Archaic period, while brachycephalic individuals are associated with the Intermediate-Late period. Previous studies have shown that individuals with intentional cranial modification, annular and tabular, exhibit predictable changes in the craniofacial complex due to compensatory

growth and shape changes related to peripheral structures of the cranial vault. A form of tabular modification is seen in both periods included in this sample, with the Intermediate-Late period sample being dominated by this modification. All craniometric landmarks were recorded using a Microscribe™ 3D digitizer, with exclusion of mandibular landmarks. MorphoJ was utilized to analyze the geometric morphometric data. Coordinates using the Microscribe were recorded in order to investigate the differences in shape of the craniofacial complex between the two samples. Two separate analyses were performed, one with all landmarks recorded and one excluding landmarks prosthion, alveolon, ectomolare and zygion. The results of both Canonical Variate analyses and associated Mahalanobis Distance values (2.699 and 2.9316) indicates statistically significant differences ($p < 0.001$) between the two samples. It is also evident that shape changes along CV1, increases in minimum frontal breadth, upper facial breadth, and nasal height, correspond with characteristics associated with brachycephalism and tabular cranial vault modification. The large range of variation of the Intermediate-Late period sample is speculated to be a result of multiple small populations moving toward the coast possibly due to a change in climate. These small populations exhibited various morphologies which were influenced by nonrandom environmental factors. The Mahalanobis distances of the different cultural groups also indicate statistically significant differences. This method has the potential to be used in conjunction with others to identify cultural context and significance of remains in archaeological contexts in Chile. In the event of repatriation acts this method could also

be used to attribute individuals to corresponding cultures and be donated to the appropriate community or facility.

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LIST OF ABBREVIATIONS

ANOVA.....Analysis of Variance
CV.....Canonical Variate
CVA.....Canonical Variate Analysis
CVM..... Cranial Vault Modification
GPA.....Generalized Procrustes Analysis
IBD.....Isolation by distance
PCA.....Principal Component Analysis

INTRODUCTION

The prehistory and nature of human migration in the Americas is highly debated, and several proposed models exist regarding the migration and settlement of the first migrants, the Paleoindians, as well as the influences of the Amerindians (Auerbach 2012; González-José *et al.* 2008; Manríquez *et al.* 2011; Neves & Hubbe 2005; Pucciarelli *et al.* 2003; Pucciarelli *et al.* 2006; Sardi *et al.* 2005). In order to determine migration patterns and validity of proposed models, studies rely exclusively on archaeological collections. Geometric morphometric techniques, mtDNA, biodistance analyses, and many other analytical methods have been utilized on these collections in attempt to understand how migrations occurred (Auerbach 2012; Cocilovo 1990; González-José *et al.* 2008; Kuzminsky *et al.* 2017b; Manríquez *et al.* 2011; Neves & Hubbe 2005; Powell & Neves 1999; Rothhammer *et al.* 2002; Sutter & Mertz 2004). The causes of craniofacial variation in human populations has also been a subject of interest in physical anthropology (Barbeito-Andrés *et al.* 2011; Betti *et al.* 2009; Fabra & Demarchi 2011; González-José *et al.* 2008; Humphries *et al.* 2013; Martínez-Abadías *et al.* 2006; Menéndez *et al.* 2014; Paschetta *et al.* 2010; Paschetta *et al.* 2015; Pérez & Monteiro 2009; Pérez *et al.* 2011; Powell & Neves 1999; Pucciarelli *et al.* 2003; Rothhammer *et al.* 2002; Sardi *et al.* 2005; Sardi *et al.* 2006).

The goal of this study is to investigate the craniofacial variation during prehistorical periods of Chile's semiarid north using morphometric analysis. Two samples have been chosen, the Archaic period sample which dates from 10,000 BC to 300 AC, and the Intermediate-Late period sample dating from 900 AC to 1,500 AC. It is

common practice to classify archaeological remains, particularly crania, visually in order to attribute them to a particular period in prehistory (Kuzminsky *et al.* 2017a).

Dolichocephalic, or posterior-superiorly, long-headed individuals are ascribed to the Archaic period while brachycephalic, or round headed individuals are associated with the Intermediate-Late period. As the cranium is highly integrated (von Cramon-Taubadel 2014), these vault shape classifications of the cranium may also be related to characteristics of the face. The main goal of this study is to investigate craniofacial variation throughout prehistory in an attempt to understand the peopling of Chile's semiarid north.

The skull is the best region of the skeleton to estimate ancestry and biological relatedness in ancient groups (O'Brien & Stanley 2013). However, it is common for archaeological collections in South America to contain large numbers of individuals with cranial vault modification (CVM). Cranial vault modification is the result of cultural practices that intentionally alter the shape of the skull of an individual. There are two types of modification which result in different shapes known as annular and tabular (O'Brien & Stanley 2013), with the presence of tabular modification in the more recent sample of this study.

When investigating biological relatedness, the collections which are comprised of modified crania may not be legitimate or reliable. This poses a great problem for researching the migrations and peopling of the Americas. Researchers have the option to either exclude modified crania from study and use a reduced sample or discover methods which allow the use of these modified crania. With the latter in mind, methods may be

designed in order to only utilize regions of the cranium which are non-significantly altered by modification or create a system that accounts for modifications and produces “normalized” measurements from the modified ones.

Several studies have been conducted to investigate cranial vault modification including methods of identifying intentionally modified crania, determining the method used in modification, investigation of induced changes to the cranial base and face, as well as many others (Antón 1989; Clark *et al.* 2007; Frieß & Baylac 2003; Gerszten 1993; O’Brien & Stanley 2013; Torres-Rouff 2002). Studies regarding the effect of CVM on the cranium and craniofacial complex have also been conducted (Antón 1989; Björk & Björk 1964; Cheverud *et al.* 1992; Kohn *et al.* 1993; Kohn *et al.* 1995; Rhode 2001; Rhode & Arriaza 2006). Some of these studies have concluded that the type of modification implemented has a predictable effect on the craniofacial elements and determined that individuals with tabular modification, tend to have broader or wider faces (Antón 1989; Cheverud *et al.* 1992, O’Brien & Stanley 2013, Rhode & Arriaza 2006). This description resembles that of brachycephalism, which is how the skulls of the Intermediate-Late period sample are visually classified. The prevalence of crania exhibiting tabular modification during this period may have contributed to this visual classification system.

Prehistory of Chile

The prehistory of Chile can be divided into several periods. The Paleoindian, 12,000-10,000 BC; Archaic period, 10,000 BC–0 AC; Early Pottery Period 0 AC-900 AC; Middle Pottery Period, 900–1,200 AC; Late Intermediate Pottery Period, 1,200-

1,450 AC; and Late Pottery period, 1,450-1,550 AC (Falabella *et al.* 2016). The Archaic period is associated with hunter-gathers whose remains were found inland, while the archaic fishers were excavated from the beaches and coastal areas. However, the groups were known to be nomadic and travel throughout the region. From 300 BC–1,150 AC a culture known as El Molle inhabited the region, which overlapped with the archaic populations, as well as another cultural group called Las Ánimas. The Las Ánimas culture began during the Middle Pottery period, 900 AC-1,200 AC. This cultural group is now recognized as a division of another group known as the Diaguita (pers. comm. Cantarutti). The Diaguita culture is documented in the Middle to Late Pottery periods, 900 AC-1,550 AC, with the Diaguita-Inka culture representing the Late Pottery Period. Due to Las Ánimas being ascribed to the Diaguita cultural group, these individuals are included in the Intermediate-Late period collection of study. The inclusion of Las Ánimas in the Diaguita sample is due to the recent realization that the two groups exhibit no biological differences; however, differences in material culture have been documented (pers. comm. Cantarutti). The Diaguita-Inka culture is also included in the Diaguita cultural group and therefore included in the Late and Intermediate period collections. This culture is still ascribed to the Diaguita culture, with influences of the Inka on material culture and practices. With the majority of the cultures being studied, excluding the El Molle, the time period in this study has been referred to as the Intermediate-Late Period, while Archaic period encompasses all archaic hunter gatherers and fishermen.

Mechanisms of Evolution and Phenotypic Expression

Craniofacial elements of the skull are complex, and it can be problematic to draw definitive conclusions from the results when considering population changes and migrations. Variation could be linked to neutral microevolution or the result of stochastic processes such as mutations, gene flow, and genetic drift. Though it is also possible, and highly likely, that some populations differ due to non-stochastic or deterministic methods such as past diversifying selection, climatic and dietary factors. With these causes in mind, it is important to emphasize the null hypothesis of stochastic processes.

Investigating changes in the cranium is a multivariate phenomenon due to the cranium being comprised of regions that differ in morphology, development, and the extent to which stochastic and non-stochastic evolutionary forces reflect population history (von Cramon-Taubadel 2014).

When considering evolution, the changes seen in a given population occur due to shifts in allele frequencies from generation to generation (von Cramon-Taubadel 2014). Allele frequencies change over time due to stochastic methods such as mutation and genetic drift, and non-stochastic methods related to natural selection. Mutations are new changes in genes, creating new alleles, which are then heritable while genetic drift is the neutral loss of alleles due to random sampling from one generation to the next. For genetic drift, differences seen in a small population can be attributed to the disappearance of genes that are heritable due to individuals that die or do not reproduce. These forces are considered stochastic because they affect allele frequencies in random, or neutral ways respective to fitness.

Selection affects allele frequencies in a predictive or deterministic way due to the correlation between fitness and genotype/phenotypes. Gene flow is the introduction of new alleles due to individual movements from outside to inside the population, such as exogamy. Selection is then a result of the reproductive success of specific genotypes/phenotypes allowing for the continued presence of specific alleles in a population, such as sexual selection (von Cramon-Taubadel 2014).

Quantitative genetics extends these basic principles to shape the inheritance and evolution of continuous phenotypic characteristics, such as craniometric data. Quantitative traits are inherently more difficult to model, compared to Mendelian phenotypes, because many factors contribute to the phenotypic variation observed (von Cramon-Taubadel 2014). These phenotypic variances can be broken down to genetic, environmental, and genotype-environment interaction. All of these components contribute to the overall phenotypic variation of a population.

Genetic variance is then further broken down into three sources that can potentially contribute to variation, additive genetic variance, dominance variance and epistatic variance (von Cramon-Taubadel 2014). Additive genetic variance is the genetic information inherited, dominance variance is related to particular loci with dominant effects, and epistatic variance consists of all effects these gene loci have on one another in relation to gene expression. The differential expressions of genotypes can be related to the environment.

Environmental changes such as climate, and changes in subsistence methods including diet, are the two most likely sources of directional diversifying selection on the

human cranium. It is known that different subsistence systems are linked to changes in the craniofacial complex and skull. Though there are several environmental factors acting on the skull, masticatory loading is one of the main influences that engender craniofacial variation. Changes in diet, such as toughness and hardness of food as well as particle size influence the temporal muscle and results in changes to the surrounding structures, such as the temporomandibular joint and the craniofacial complex.

Ancient populations relied on hunter-gatherer subsistence systems and consumed harder, unprocessed foods which would result in the high strain on the face and cranium. With the advent of cooking and processing food, coinciding with the progression from hunter-gatherer subsistence systems to horticultural and farming systems, the face experiences less strain. The change in subsistence system and consumption of softer, processed foods would then lead to a reduction in masticatory activity. The farmers would present more gracile features of the face and skull, as opposed to the large, robust features seen in hunter-gatherer groups. This particular example outlines the environmental influence, particularly dietary, on the human cranium, but what was the role of genetic variance? In addition to genetics, what role would cultural influence then play? When investigating prehistoric human populations, it can be difficult to discern which mechanisms were at play and to what extent they influenced phenotypes. In some cases, such as the human cranium, these questions can also be associated with cultural practices that differ between groups.

Cranial Vault Modification

Cultural practices can also influence skeletal morphology of an individual.

Cranial vault modification is possibly the most prevalent of these practices and results in a wide continuum of forms and variations. This cultural practice seen on every continent, is of interest to physical anthropologists for several reasons. The practice of CVM results in a variety of forms, the methods used vary in different cultures and populations, and the investigation of this practice has the ability to shed light on the spreading of cultures, as well as the connections between biology and growth. Cranial vault modification results from intentional and unintentional cultural practices. Some groups modify the skull intentionally with different materials and devices (Antón 1989) but modification can unintentionally occur as a result of childcare practices, particularly cradleboarding (Cheverud et al. 1992; Clark et al. 2007; Kohn et al. 1993, 1995).

In regards to the cultural context of CVM, it is not done as a rite of passage tradition, but instead as an intentional manipulation of the skull done early in life to become an essential part of a person's identity (Torres-Rouff 2002). The most widely accepted reason for modification of the cranium is class distinction (Gerszten 1993); however, CVM may also be implemented to show affinity with a particular group, to attain certain appearances that are considered aesthetically appealing to the opposite sex, make individuals appear fierce and formidable to enemies, maintain a person's health and vigor, or to produce docile individuals who are easily controlled (Rogers 1975).

Interactions between groups can result in the exchange of goods, ideologies and artifacts

as well as influence on the methods or practice of CVM (Gerszten 1993; Torres-Rouff 2002).

Cranial vault modification has to begin on infants almost immediately after birth and entails binding materials such as stones, boards, pads, and textiles (Antón 1989). These materials are wrapped around the infant's head to attain a desired shape, which can be done in various ways. The infants head will have flat devices wrapped with tight bandages around the crania, and due to 3-5 years of occasional tightening the growth process of the skull results in tabular or annular modification (O'Brien & Stanley 2013).

There are several ways in which to classify cranial vault modification, including visual assessment, Flattening Mechanisms/Modification Styles, and Morphometric Techniques. Visual assessment is when crania are observed visually and compiled into groups or categories based on appearance and the modifications that are most prominent. (Clark *et al.* 2007) This has been the common practice for classification however, there are limitations such as a standardized classification system and no accountability for intra-personal error, making this a subjective method. Flattening Mechanisms or Modification Styles is when the crania are separated by the distinct shape they have formed, or by the specific methods and specific materials used for binding (Antón 1989). This consists of the different types of modification, which is at the discretion of the interpreter, for example anteroposterior verses circumferentially modified, tabular versus annularly modified, etc.

Over the years several classifications for the visual differences in morphology have arisen, all of which distinguish the two different achieved shapes. Anteroposteriorly

modified and fronto-occipital flattening are both commonly known as tabular modification. Tabular modification is when anteroposterior flattening of the frontal and occipital occur and laterally bulging parietals result. This shortens the temporal and parietals, with lateral compensation and a triangular appearance when viewed superiorly, and is also defined as an “upright, boxy shape” (O’Brien & Stanley 2013). This form of modification results in the skull acquiring a form which is consistent with a brachycephalic head shape. Circumferential CVM also known as annular modification, is when the conical vault extends posterosuperiorly. There is no lateral bulging but a circular elongation of the overall skull (Antón, 1989) resulting in a “conical” shape (O’Brien & Stanley 2013) which is consistent with dolichocephalic head shape.

The practice of CVM has been observed in several prehispanic cultures of the Chilean Andean region, including the semiarid north. Individuals exhibiting CVM will be included in this study, in order to assess whether the practice of visual classification, dolichocephalic vs brachycephalic, manifests in the craniofacial complex and coincides with time period. Intermediate-Late period sample contains a large number of individuals with tabular modification and should result in wide, short faces when compared to the Archaic period sample, as seen in previous studies.

Further studies regarding the mechanisms, quantification, and validity of visual classification of CVM is suggested. The use of morphometric analysis of modified individuals may indicate distinctions between males and females, reflect the idea of social status in the community, and in turn shed light on how the “self” of individuals was influenced.

Geometric Morphometrics

Morphometrics is the quantitative analysis of shape and size of objects, particularly in biological contexts. Geometric morphometrics is then defined as “the suite of methods for the acquisition, processing, and analysis of shape variables that retain *all* of the geometric information contained within data” (Slice 2005: 5). Put more simply, the idea of using geometric morphometrics is to investigate shape variation, with exclusion of size differences (McKeown & Schmidt 2013). When human variation is considered, size can be an informative or perplexing factor. When looking at traits in the skull to investigate ancestry, the difference in distance between variables is not informative, but the relationship of those variables to one another can lead to inferences regarding shape.

Traditional forms of morphometric methods for measuring variation of the cranium can capture overall form variation however, geometric morphometric and landmark-based data provide an approach with the ability to quantify shape and size in individual regions. This allows for further investigation of individual regions of the cranium. With landmark based methods these regions can become consistent across individuals. Semi-landmarks are also extremely useful in defining shape of regions that are absent of traditional points, such as contours of the cranial vault, though this method will not be utilized within this study.

This study will implement the use of geometric morphometric analysis in order to determine the relationship of landmarks of craniofacial elements to one another, and their changes in a given region over time. This could also identify if the changes in craniofacial elements are due to stochastic or non-stochastic methods of evolution.

The purpose of this research is to investigate craniofacial variation between the Archaic Period and Intermediate-Late periods of Chile using morphometric analysis. Previous studies have found that circumferential and tabular modification of the cranial vault are accompanied by particular craniofacial characteristics (Antón 1989, Cheverud *et al.* 1992, Frieß & Baylac 2003, Kohn *et al.* 1993, O'Brien & Stanley 2013, Rhode & Arriaza 2006). Tabular modification results in short, wide faces while circumferential modification results in long, narrow faces. If the Archaic period sample is characterized by a dolichocephalic head shape, similar to circumferential modification, and the Intermediate-Late period sample is characterized by a brachycephalic head shape, similar to tabular modification, then investigating craniofacial variation should reveal the Archaic period sample to have longer narrower faces, and the Intermediate-Late period sample to have shorter, wider faces.

The following chapters will consist of the previous research related to migrations and the peopling of South America, investigations of craniofacial variation, research pertaining to the investigation of craniofacial changes related to CVM among other topics. The materials and methods chapter will provide insight into the skeletal sample being utilized and the statistical methods that will be employed. The results will present the interpretations of the information obtained from the statistical analyses, while the discussion will discuss the meaning of these results and the relationship of these results to previous studies. Finally, the conclusion will then provide a summary, review, and implications of these results and interpretations as well as limitations and future studies.

PREVIOUS RESEARCH

Review of Migration and the Peopling of South America

Biological and cultural changes within Chile have been investigated and linked to migrations and population movements, from within as well as outside of the country (Kuzminsky *et al.* 2017b; Rothhammer *et al.* 2002; Sutter & Mertz 2004; Varela *et al.* 2008). When looking at human crania, it is common practice to attribute the shape of the crania with a time period, or cultural group. The most common categorization attributes brachycephalic crania to the cultural group known as the Diaguita, which inhabited Chile during the Late and Intermediate periods, and dolichocephalic crania to the Archaic period however, most crania are actually mesocephalic (Kuzminsky *et al.* 2017a). Biodistance, or the measurement of biological relatedness between and within human groups, has been investigated using cranial morphology. Studies of biodistance investigate biological and cultural changes that have been linked to migrations and population movements (Sutter & Mertz 2004), gene flow and evolutionary forces (Cocilovo 1990). Geometric morphometric analysis has been applied to assess craniofacial morphologies to indicate migration events (González-José *et al.* 2008; Kuzminsky *et al.* 2017b; Manríquez *et al.* 2011; Neves & Hubbe 2005; Rothhammer *et al.* 2002).

Cocilovo (1990) investigated the relationship of craniofacial variation to the action of evolutionary forces, particularly gene flow. Biodistance was used to summarize genetic and morphological variation over time and space. The mean values of nine

craniometric variables that were least affected by cranial modification were obtained for 22 samples of prehistoric groups of Chile, Argentina and Peru, which are distinct due to their chronology within the prehistoric time period. These samples ranged from the Archaic period to the San Pedro III period, 1,300 AC–1,536 AC. Mahalanobis D2 values for pairs of groups were generated, and this resulted in a matrix of morphological distances. A matrix of the square roots of Mahalanobis D2 allowed for a cladistic analysis using Wagner-Tree technique which reconstructs hypothetical phylogenetic relationships. The results were generated into a cladogram, with relationships being inferred by the existence of hypothetical groups ancestral to two or more real groups. The results suggest a gradual evolutionary process, such as gene flow, that took place in the Southern Andean region. The Peruvian population is more linked to the Chilean and the Argentinian population than the Chilean and Argentinian populations are to each other. This suggests that an ancestral population originated in Peru which then migrated into Chile as well as Argentina.

The degree of biological interactions between groups and across countries has been assessed by Varela *et al.* (2008). It was thought that south-central Andean populations of northern Chile, in northwestern Argentina, and valleys of southern Bolivia originated from a shared ancestral population. After 1,586 crania were studied with 17 cranial traits, it was determined that the Bolivian populations were more closely related to those of Chile and Argentina, than Chile and Argentina populations were to each other. This suggests that both populations share a common ancestral group similar to the Bolivian group, which then branched off to Chile and then Argentina.

Though the South Amerindians are considered a biologically homogeneous “megapopulation” the assessment of native South American DNA variability analysis divides the continent into Eastern and Western Zones. Social and cultural evolution also took very different routes on each side of the Andes, due to the ecological divide of the mountains as well as historical events. Pucciarelli *et al.* (2006) used craniometric data to see if this pattern is manifested in cranial morphology. The study compiled of 485 unmodified male Pre-Columbian South Amerindian skulls from the West and East sides of the Andes mountains. Two samples of Paleoamerican crania from Mexico and Brazil were also added. Results presented strong evidence supporting the idea of Eastern and Westerners representing two distinct as well as independent, microevolutionary spaces. However, a third Northwest group cannot be completely ruled out. The defined Eastern and Western populations show similar levels of intra-regional variability. Nearly all Amerindians exhibited a strong relationship between morphological affinity and geographical origin. In congruence with the DNA analysis, the native South American populations cannot be considered a biological homogeneous megapopulation. It is suggested that different intensities of gene flow and genetic drift resulted in the differentiation of the Eastern and Western crania and the intensities of gene flow from the two distinct morphological stocks, the Paleoindians and Amerindians, cannot be ruled out.

With the utilization of cranial morphometrics, Cocilovo (1990) and Varela *et al.* (2008) have both come to the same conclusions. Due to the proposed cladograms derived from craniometric traits, both studies infer a mechanism of gene flow from Peru and

Bolivia to the southern countries of Chile and Argentina. The results of Pucciarelli *et al.* (2006) further support the idea of two distinct independent, microevolutionary spaces divided by the Andes. These ancestral models shed light on migration of prehistoric populations to either side of the Andes, which separates Chile and Argentina. The migration of the ancestral population could be a result of exploration of resources, with Chile providing coastal and maritime resources, while the more humid environments of Argentina provide ideal environments for cultivation. With the protection of the Andes mountains, Argentina is shielded from the harsh environmental changes caused by the Pacific Ocean to the west. Migrants from Peru and Bolivia would have an ideal environment to utilize and further develop irrigation systems and advance methods of farming. The beach and coastal environment of Chile offers many different food resources including large mammals like dolphins and sea lions, as well as a variety of birds, fish, and mollusks.

It is common practice to visually classify crania as one of two shapes, dolichocephalic or brachycephalic, and then ascribe those remains to a particular period in prehistory (Kuzminsky *et al.* 2017a). The early Paleoamericans of the Holocene, which predate 8,000 years BP are often categorized as dolichocephalic, or long headed. This means the crania exhibit anteroposterior elongation. The late Holocene populations are described as brachycephalic, or round headed, shortened anterior-posterior length. These distinctions in head shape have been used to infer origin models; some contend that the dolichocephalic population was biologically distinct and later replaced by brachycephalic individuals (Kuzminsky *et al.* 2017a). In particular, Kuzminsky *et al.* (2017a) tested the

hypothesis that Holocene populations consisted of two cranial morphologies that coincide with the early and late Holocene periods. High resolution 3D models were generated from a laser surface scanner, and cranial indices from 95 adult male crania from western South America dating from the Early, Middle and Late periods were analyzed. The samples originated from several sites in Peru, Ecuador and Chile. The crania were scanned with a surface scanner, and the rendered digital models were imported into visualization and digitizing software in order to record the cranial length and breadth, which were used to compute the cranial index distinguishing dolichocephalic, mesocephalic, and brachycephalic.

The results of the calculated cranial index data show that that majority of crania analyzed have an intermediate (mesocephalic) head shape, spatiotemporal variability, and no clear transition from dolichocephaly to brachycephaly during the Holocene (Kuzminsky *et al.* 2017a). When considering time periods, the percentage of individuals with dolichocephalic head shapes decreases in the Middle and Late periods, the percentage of mesocephalic head shape increases, the brachycephalic shape increases in the middle period, and decreases in the Late period. Additionally, ANOVA tests revealed no statistically significant differences within each time period. Pairwise tests indicated a statistically significant difference between Early and Late period cranial indices, which supports the hypothesis that the Paleoamericans have vault shapes that differ from many individuals dating to later time periods.

Although the morphometric cephalic index technique did not support the origin model of a dolichocephalic population being replaced by a brachycephalic population, the

indices did indicate a change from dolichocephalic to mesocephalic head shapes (Kuzminsky *et al.* 2017a). This change in head shape however could have occurred due to gene flow from other populations, such as Peru and Bolivia as seen in Cocilovo and Varela. The increase of brachycephalic head shape during the middle period as well as the decrease in dolichocephalic head shape could indicate interactions with two different populations, which resulted in the phenotypic variation of crania during the middle period. As the late period shows a decrease in brachycephalism and dolichocephalism this infers an intermixing of the two phenotypes derived from other populations which resulted in the increase and over all mesocephalic head shapes. As the pairwise test indicates a distinction between early and late period crania, this does indicate a change in head shape during the Holocene, although these shapes may not be classified as brachycephalic and dolichocephalic necessarily.

Sutter and Mertz (2004) studied the possibility of large-scale prehistoric migration in the Azapa Valley in Chile. Using eight mortuary samples, nonmetric cranial traits were investigated and subjected to mean measures of divergences. None of the mean measures of divergences were significant, which suggests biological continuity during the 5,000 years of prehistory in the valley with nonsignificant gene flow during the late horizon and late intermediate periods. Using biodistance, the results suggest endogamy within populations.

Another recent study by Kuzminsky *et al.* (2017b) examines skull morphology of prehistoric skeletons in Chile to determine if they possess a distinct cranial morphology from later skeletons. Three-dimension digital models, craniofacial landmarks and

geometric morphometric analyses were used to compare crania of early and late Holocene samples. Sixteen landmarks of the face and vault were digitized for each cranium. The early Holocene sample size consisted of four individuals while the late Holocene sample consisted of 90 crania. Two of the early skeletons from northern Chile show related attributes to individuals from different sites in the same region from later time periods. This supports the idea of cultural and biological continuity along the Pacific coast, which aligns with genetic and archaeological data. However, the hypothesis that early individuals have a unique cranial morphology from later individuals cannot be rejected, due to the lack of trait affinities of one individual from northern Chile one from inland Patagonia.

The investigation of skull morphology throughout Chile conducted by Kuzminsky *et al.* (2017b) displays conflicting results. With such a small sample size being utilized for the early Holocene the results are not conclusive to whether early individuals have unique cranial morphology from later individuals. This study does not provide insight into a mechanism of inheritance or geneflow throughout the Holocene. Due to the small sample size of early crania, the related attributes of two of the early crania are not significant, nor is the fact of one individual that displayed a lack of traits. With a larger sample size, a greater portion of the observed population is represented, and thus there is a closer approximation of the traits being investigated. Although genetic data show that there was no major contribution to the gene pool in over 9,000 years from external populations (Kuzminsky *et al.* 2017a) another force such as secular change could have occurred. Secular change implies variation among generations in a population.

(Wieringen 1979). Sutter and Mertz (2004) also had results that determined nonsignificant gene flow within the Azapa valley and suggested endogamy in the region. The results of these two studies could both be due to endogamy, as opposed to migrations into the populations, and again, secular change could have occurred in this region.

The objective of Rothhammer *et al.* (2002) was to investigate the impact of CVM on measurements used in biodistance analysis which will be discussed later within this chapter. This study was also focused on assessing the cultural evolution in the Azapa Valley in relation to craniofacial differentiation. It is suggested that the cultural developments in the Central Andes near lake Titicaca influenced the cultures of groups inhabiting the Azapa Valley and coast of northern Chile. This cultural evolution may have been influenced by a dry period in the highlands of Arica which occurred around 7,000-6,000 years BP (Baied 1992). It is suspected that the change in climate had an affected on the living conditions experienced by the local hunter-gatherers due to the increase in use of coastal camps after 7,000 years BP.

Rothhammer *et al.* (2002) collected 6 craniofacial measurements using adult crania from cemeteries in the Azapa Valley and the coast of the Pacific Ocean near Arica, and a sample from lake Titicaca for comparative purposes. The authors found that craniometric and chronological distances show a significant correlation and supports the idea that cultural changes are associated with gene flow in this study. These findings could also be explained by an increase in mutation rates or a bottleneck effect, though there is no evidence for the latter and the former is very unlikely. Therefore, the authors

concluded that the population displacements of groups from the highlands most likely influenced the cultural development in the valley.

Several studies have also investigated the high diversity of cranial morphology in relationship to proposed migration models. González-José *et al.* (2001) utilized Howells (1973, 1989) data sets, but only included East Asian, North American, and South American natives. Additionally, five Fuego-Patagonian samples and one Paleoamerican sample were added and a total of 656 male crania. The goal of the study was to estimate craniometric variability among these populations and test goodness of fit of the data to three migration models including the three-migration model, single wave migration model, and two-component settlement model. Results show that the two-component settlement model is strongly associated with morphological variation. This model indicates that there was an earlier group with distinct non-mongoloid group present, the Paleoamerican populations, and then there was another group that arose in South America which gave rise to the Amerindian groups.

Neves and Hubbe (2005) compared the cranial measurements of Howells (1996) database to 81 human crania from Lagoa Santa, Brazil to investigate cranial variation and morphological affinities across continents. The results of Neves and Hubbe's (2005) quantitative analyses indicate that the first South Americans have similar cranial morphology to present Australians/Melanesians and Africans. However, when compared to late and modern Northeastern Asians and Amerindians the morphology is extremely different.

Two hypotheses are proposed by Neves and Hubbe (2005) in attempt to explain the morphological differences between early and late Native South Americans. The first is that the Paleoamerican morphology was transformed into the Native American morphology due to local microevolutionary processes. The second hypothesis is that there were two morphologically distinct human stocks that occupied the Americas, the first of which being the Paleoamerican. Of the two, the second hypothesis is more likely for several reasons. It is very unlikely that the same evolutionary event occurred in East Asia and the Americas around the same time, there was an abrupt transition in South America between the two morphological patterns, and cranial morphology is less plastic to environmental conditions than previously thought.

González-José *et al.* (2008) conducted geometric morphometric analysis on 576 modern and late Pleistocene/early Holocene adult crania from 23 assemblages. The goal of this study was to reanalyze Old and New World cranial variation with the use of geometric morphometrics, as opposed to classical craniometric methods. The authors postulated two null hypotheses, the first stating that there are two extremes of craniofacial variation and most New World samples reside in a continuous spectrum between them, with no significant gaps. While the second states that the New World also possesses aboriginal craniofacial shape patterns. The results indicate that the first null hypothesis cannot be rejected, and the range of generalized and derived extreme craniofacial variation is most likely due to both nonrandom processes and stochastic evolution. However, the second null hypothesis is rejected due to the results of the K-means clustering. This analysis shows a new extreme of variation in the New World amongst the

North American Eskimos, who have shared traits with modern northeast Asians. The results suggest that Paleoamerican and mongoloid craniofacial patterns are extremes at either end of the continuous range of morphological variation observed.

In addition to these results, González-José *et al.* (2008) also attempted to merge their findings with the results of genetic studies and create a model for the peopling of America. Genetic analyses agree on a single, complex origin of Native Americans (e.g. Fagundes *et al.* 2008, Fehren-Schmitz *et al.* 2015, Merriwether *et al.* 1995, Silva *et al.* 2002, among others) while morphological studies support a two-wave model (González-José *et al.* 2001, Neves & Hubbe 2005, Pucciarelli *et al.* 2003, Sardi *et al.* 2005). In order to consider both genetic and morphological data the authors proposed a model broken into four successive time frames. This model begins at T_0 during the late Pleistocene earlier than 26,000 years BP with an Asian stock lacking the complete range of variation. The next time frame, T_1 is between ~26,000 and ~18,000 years BP. During this time, there was a decrease in sea levels which revealed a land mass connecting northeast Asia and northwest America, known as Beringia. The mild climate and opportunities for hunting and fishing led to the presence of human populations with several Asian lineages. During this time, the lineages which were not lost to genetic drift would start to differentiate due to collection of mutations.

Stage T_2 outlined by González-José *et al.* (2008), is between 18,000 years BP and ~12,000 years BP (the end of the Pleistocene) Beringia experienced a reduction in size due to a rise in sea levels. It is evident via mtDNA analyses that significant population expansion occurred at the beginning of this period (Fagundes *et al.* 2008). It is most

likely that due to the increasing population size, limiting resources, and changing environment the people of Beringia started migrating into new areas such as North America. The subsequent T₃ stage encompasses the Holocene and is characterized by the origin of the radical northeast Asian morphology, the diffusion of this morphology into the Americas, and related biological and cultural evolutions.

De Azevedo *et al.* (2017) was also concerned about understanding the large range of craniofacial diversification in the Americas. The author used a quantitative genetics approach on top of geometric morphometric analysis and multivariate statistics to evaluate the evolutionary history of the craniofacial complex during the late Pleistocene- Early Holocene. This study utilized fossils from the Late Pleistocene Old World to represent a proxy for the first Americans and Asians, North and South Paleoamericans from the early Holocene, and individuals from the late Holocene from Australia, Asia and America to represent a modern series. The results supported the Recurrent Gene Flow Model (González-José *et al.* 2008) which depicts an ancestral population that experienced a standstill in Beringia and consisted of an internally variable stock. This group is the ancestral source of variation which stimulated the microevolutions of other phenotypic patterns and is the origin of craniofacial diversity observed among these Native Americans samples.

Sardi *et al.* (2005) studied the degree of variation of craniofacial morphology of South American Amerindians when compared to the Howells (1973) dataset. The dataset included crania from American, Asian, Australo-Melanesian, European, South-Saharan Africa, and Polynesian regions while the South American was comprised of 6

Amerindian samples. The authors concluded that even when compared to groups that are in close geographic proximity, the native populations of South America show high degrees of craniometrical diversity. This high variation could be due to greater antiquity of the peopling of the Americas, a consequence of two or more Ancestral waves with high morphological diversity, or a combination of the two.

Manríquez *et al.* (2011) utilized crania from the Early, Middle and Late Archaic periods from northern, central, and southern Chile. The crania were photographed and digitized to obtain landmark coordinates, focused on the cranial vault, which would then be computed into matrices. A sample of three skulls from the region of Lagoa Santa in Brazil were employed for comparative purposes. In addition to the morphometric data molecular mtDNA data was obtained from several individuals in order to evaluate the relationship between genotypes and morphologic variation and attempt to contribute to the debate of colonization in the Americas. Morphometric analysis of the Archaic samples from Chile revealed a wide range of variation, and the mtDNA analyses revealed four classic mtDNA founding lineages. The four haplogroups were present in the earliest Archaic group and supports the one-way migration hypothesis, supporting the craniometric distance results of González-José *et al.* (2008). The morphometric and mtDNA haplogroup data refute the idea of two different human populations, the Paleoindians and Amerindian in the Americas during this time period.

In a fairly recent study, Hubbe *et al.* (2015) estimated the between-group and within group variances of two early South American population samples in an attempt to understand the high cranial morphological diversity of the continent among recent native

Americans. The authors used a sample from Lagoa Santa, Brazil and a paleo Colombia sample from Central Columbia and compared to recent native Americans, East Asians, European, Sub-Saharan Africans, Australo-Melanesians, and Polynesians from the database created by Howells (1996).

The results of Hubbe *et al.* (2015) indicate moderate within-group diversity of the early Americans and the between group variance is exceptionally low. However, the between group variance between recent South Americans and early Americans is extremely high. The authors concluded that the first groups living in South America did not contain the high morphological diversity seen in native South Americans. In addition, there is no strong evidence for unique characteristics present in South America that would lead to fast morphological evolution by stochastic or nonstochastic processes. They also propose that the variation seen is most likely due to the arrival of new and diverse groups arriving during the Middle Holocene, later than the initial occupation of the continent.

Martínez-Abadías *et al.* (2006) took an evolutionary, diachronic approach to investigate the morphological variation of crania using geometric morphometrics. Though this study was conducted on the Spanish-Amerindian contact of Mexico rather than South America, it is an excellent example of the effects of admixture and gene flow on morphological traits. This study focusses on the craniofacial changes in Mexico's Central Valley between two ancestral groups, the Spanish and precontact Amerindians, and two admixed groups from the early and late colonial periods. The total sample included 106 adult skulls of both sexes. Two dimensional coordinates from the lateral

profile of the skull were collected including 19 landmarks encompassing the facial and neurocranial regions of the cranium. When classical quantitative genetics theory is considered, gene flow results in a homogenous genetic composition. The authors predicted that the colonial groups, the admixed group between Amerindians and Spanish, would possess morphological traits that place them between the diachronic morphologies of the two parental groups.

The results obtained by Martínez-Abadías *et al.* (2006) displayed a clear differentiation between the two ancestral groups with both colonial groups showing different intermediate values. The main craniofacial differences between the Amerindian from the Spanish and colonial groups include enlargement of the midposterior neurocranium, increased prognathism, facial flattening, and zygomatic development. The early colonial group shows closer affinity to the Amerindian group and slightly differing toward the morphology of the Spanish group. The late colonial group however takes an intermediate position, is internally more diverse and overlaps the ranges of both ancestral groups. These results are in accordance with the null hypothesis as well as molecular and historical data. However, when localized structures are considered, the deviations from the expected intermediate shape are thought to be derived from somatic growth patterns and a response to different microevolutionary forces such as plastic responses to environment and diet. These differences indicate that the shape changes are not only linked to gene flow, but other forces have contributed to variation.

When considering human ancestry, the human skeleton is useful in bioarchaeological contexts. Morphometric analyses of the skull can be used to determine

patterns of inheritance between and with groups and is further used to establish origin and ancestral models. Morphometric analyses such as the cephalic index can be used to categorize crania and in turn infer changes and distinctions between and within groups. Geometric morphometric analysis is used to investigate the relationships between traits and can exclude distances between traits that occur as a result of genetic variation. The results of different statistical tests of these traits among different populations can also be used to infer migrations, origin models. Although in South America there is evidence of ancestral groups migrating to either side of the Andes mountains, considering changes over time in populations that inhabited the same area is important. Further investigation within regions is important to help validate or refute proposed migrations, shed light on variation, endogamy and exogamy, genetic drift and gene flow, as well as nonstochastic processes of evolution.

Review of Craniofacial Changes and Nonstochastic Methods of Evolution

Sardi *et al.* (2006) tested the hypothesis that diet-related factors influenced morphological differentiation by comparing functional cranial components. This was done by investigating the three-dimensional changes on eight minor functional components of the skull. The populations in question were from the Argentine Center-West, the southernmost portion of the Andes where domestication occurred. The populations in this southern most area were hunter-gatherers (n=66) in historical times who coexisted with the farmers (n=58) located in the north. A third sample was added as a reference which included 100 males and 102 females of known age and sex from a cemetery in Portugal. Results support the hypothesis that diet-related factors associated

with subsistence economies influenced the craniofacial morphology. The variability observed can be explained by reduced quality of nutrition in the diet of the farming group including low protein content, as well as the idea of a decrease of growth hormone circulation caused by sedentism and lower mobility. The decrease of masticatory stresses and workload on the head and neck due to softer diet consumption, caused reduction in the masticatory and posterior neural components in farmers.

Pérez *et al.* (2011) studied a dataset consisting of 718 males from 40 populations of South America. The goal of this study was to determine the importance of diet and temperature in relation to cranial size and shape variation. The environment where an organism develops has an effect on the range of phenotypes related to the genotype it possesses. As this was the last continent to be colonized, the diverse range of morphological variation seen in these populations is unexpected, and the mechanisms behind this phenomenon may be related to the large environmental variation within the continent. The authors tested the null hypothesis, that random processes resulted in the variation observed.

The samples from South America investigate by Pérez *et al.* (2011) consisted of farming groups, horticulturalists, pastoralists, terrestrial hunter-gatherers and marine hunter-gatherers. The diets of these groups were then classified by the proportions of carbohydrates and proteins based on archaeological and ethnohistorical literature and resulted in two classifications of carbohydrate rich and protein rich groups. Although transitions of food production such as processing techniques, have been linked to softer diets it is important to keep in mind that all late Holocene populations in South America

possessed techniques related to food processing so this relationship may be difficult to assess. To infer climate, data on mean annual temperatures for each population were considered. The authors then investigated the spatial structure of cranial variation, relationships between variation and environmental variables (diet and climate) and compared the expected divergence values correlated when only considering random factors (genetic drift and mutation) against the degree of morphological deviation.

The results obtained by Pérez *et al.* (2011) showed that the cranial variation observed is spatially structured, with positive correlations between neighboring populations and negative correlations with more distance populations. Other studies have reported craniofacial variation to be related to spatial structure in South American populations (Pucciarelli *et al.* 2006, Varela *et al.* 2008). When considering the results of the Δ divergence rate test, it is evident that random processes (e.g. genetic drift) are not the key contributing factor contributing to variation regarding shape and size, thus the variation could be related to environmental factors. Spatial regression analyses showed environmental factors of diet and climate are not correlated with the main variables, PC2 and PC3. Diet in particular, is found to have a significant effect on size and shape variation of the cranium, with the later variable being more highly correlated. The authors suggest that diet is an important contributor to morphological diversification.

Within any environment there are multiple stimuli which organisms experience and these stimuli can influence phenotypic expression among populations. It is difficult to gauge independent contributions of diet and temperature on morphological variation. When the proportions of carbohydrates in the diet increases it has been found that

Holocene populations display a decrease in size of the cranium (Stynder *et al.* 2007). The cause of this decrease in size and overall shape can be attributed to changes in hormonal pathways which result in resistance growth hormone and in turn has an effect on growth during ontogeny (also stated by Sardi *et al.* 2006). The spatial relationships of South American populations revealed by Pérez *et al.* (2011) can then be explained by environmental conditions playing a large role in interpopulation differentiation, particularly cranial morphology. The authors state that these results show that 3,000 years ago a large morphological divergence of cranial form took place over a short amount of time as a result of changes in diet. This study highlighted the importance of developmental responses during ontogeny influenced by environmental changes, which cause precipitous evolution of morphology in human populations, and questions how neutral processes play a role as well.

Paschetta *et al.* (2010) also found that the shape of craniofacial structures related to mastication are affected by dietary shifts. A Microscribe™ digitizer was used to collect 3D landmark coordinates of 46 cranial and 23 mandibular landmarks. The particular landmarks chosen were to provide valuable, discrete biomechanical information such as force productions and/or stress absorption. The samples investigated involved three groups of different subsistence systems from the Ohio valley, hunter-gatherers of the Indian Knoll-Archaic period, horticulturalists from the Woodland period, and farmers from the late prehistory period. Two groups of farmers from the northern Andes, the Bolivian and Pampa Grande samples, were also included, but lacked mandibles. Though definitive measures concerning the hardness, stiffness and toughness the Ohio valley

people suffered cannot be calculated, archaeological evidence suggests the farmers relied on a subsistence system that required less force and fewer chewing cycles per day.

Of the samples investigated by Paschetta *et al.* (2010), the farmers of the Pampa Grande sample were the only individuals that showed a significant difference in overall size. The smaller regions observed in the hunter-gatherer group consisted of the face, masticatory, and mandibular regions. Though the temporo-mandibular joint is relatively larger. The samples also differ in shape; however, there is almost no difference between the horticulturalist and farmer samples of the Ohio valley with the exception of the temporo-mandibular joint region. There is a significant shape difference between hunter-gatherers of the Ohio valley and the two farmer samples of south Amerindians. The principal components scatterplots suggest the groups can be differentiated by the skull as whole due to the differences in the neurocranium. The greatest differences between the hunter-gatherers versus the farmers and horticulturalists is concentrated in the temporal fossa. The reduction of the zygomatic and alveolar border dimensions in relationship to the rest of the skull is considered a significant shape difference among hunter-gatherers and horticulturalists and maize farmers. A greater projection of the face is also evident in the hunter-gatherers.

Paschetta *et al.* (2010) concluded that the shape changes seen are most likely responses to the reduction in the magnitude and/or frequency of the strains experienced from the consumption of less processed foods. The analysis suggests localized shape changes as opposed to general changes in size or shape. The greatest changes are observed in the comparisons of masticatory loading between populations with different

subsistence systems suggesting that with a softer diet, there is a change in shape of the palate and zygomatic, reduction in the temporal fossa as well as in the positions and orientations of the attachment sites of the temporal muscle.

Menéndez *et al.* (2014) also investigated the effects of human diet composition and masticatory loading, calculated as bite force, on craniofacial variation during the late Holocene in the southern region of South America. The study consisted of 474 skulls with no evidence of CVM from 12 samples consisting of three geographic regions. The regions were Northwestern Argentina (n=183), Southeast Pampa/Northeastern Patagonia (n=127) and Central Western Argentina (n=164). A total of 84 coordinates were obtained, 42 landmarks and 42 semi-landmarks, to describe the glabella, malar, frontal, mastoid, and zygomatic processes. Diet composition was inferred from carbon isotope dating and presence of carious lesions, which contributed to a relative percentage of protein to carbohydrate. Bite force was then estimated by the size of the major jaw muscles and moment arms.

In contrast to previous studies the results of Menéndez *et al.* (2014) indicate that bite force is not correlated with variation of cranial traits. When the cranial modules were analyzed separately, size and form variation of the face and vault were associated with carious index. There was also an association found between the size and the carious index in regard to the cranial base. Systemic factors are then attributed the morphological variation seen. This suggests that an increase in food production led to the increase in carbohydrate enriched diets and possibly a lower proportion of proteins. This would then induce growth hormone resistance and retard the growth of the skeleton.

Although this study was concerned with South African populations during the Holocene, Stynder *et al.* (2007) used 153 adult crania to investigate the genetic continuity/discontinuity. This was done by using 3D coordinates of 20 landmarks in order to assess craniometric variation and the possibility of changes in genetics. This study included individuals from South Africa's west, south-west, south, and south-east coasts as well as neighboring coastal forelands. The ebb and flow of craniofacial size seen during within South Africa parallels with a fluctuation in stature found in previous research. This suggests cranial size was most likely a component of the general fluctuation on body size, though these changes occurred during an increase in population size. Systemic factors including transition to agriculture, greater sedentism, and larger population sizes during this time most likely contributed to nutrition insufficiencies and likely had an impact on the reduction in cranial size. The authors state that the reduction in size of the skeleton seen, particularly the cranium, is most likely not due to gene flow or nutritional insufficiencies in diet then the cause of is most likely due to other environmental factors. Stynder *et al.* (2007) conclude that fluctuation in craniofacial size and shape were related to environmental factors, which are consistent with long term continuity in the populations of South Africa during the Holocene.

Barbeito-Andrés *et al.* (2011) compared facial morphology in ontogenetic series of three populations from South America, one hunter-gatherer and two farming groups each with a different subsistence system. The Patagonian sample represented the hunter-gatherer group who are characterized by their craniofacial robusticity. The Pampa Grande and San Pedro de Atacama samples represent the two farming groups.

The null hypothesis tested by Barbeito-Andrés *et al.* (2011) was that the pattern of facial differentiation between these groups does not change during ontogeny. The authors used a developmental approach to emphasize the nonrandom factors which are potentially related to craniofacial variation. Four facial functional components were investigated (optic, respiratory, masticatory, alveolar) with length and breadth measurements for each component. If diet-related factors are contributing to the variation, then greater differences between hunter-gatherer and farmer groups will be observed rather than differences between the two farmer groups. This difference is expected to increase with age.

In the Patagonian sample, individuals around 4-6 years old are separated from other groups of similar age (Barbeito-Andrés *et al.* 2011). At this time in development, the permanent dentition has not completely erupted, and the individuals may not consume an adult diet. This indicates that the difference between the Patagonian sample, the hunter-gatherer group, and the two farmer samples is not due to changes in masticatory loading as a result of diet. The adult variation in the Patagonian sample and the other two samples are too similar to the subadult variation. This indicates that there is no cumulative effect of diet-related factors that affect morphology. The facial differentiation related to the masticatory component may be a result of heritable morphological patterns of development.

Pucciareli *et al.* (2003), utilized cranio-functional studies to compare major and minor cranial components between Paleoamericans, ancient Amerindians and modern Amerindians. The authors examined the null hypotheses that that Paleoamerican crania

show nonsignificant differences in craniofacial structure, and “the biological variability of Paleoamerican and Amerindian functional cranial components was produced by random diversification, after migration, evoked by stochastic evolution (Pucciareli *et al.* 2003: 123). The major cranial components included the neurocranium and face while the minor consisted of anteroneural, midneural, posteroneural, otic, optic, respiratory, masticatory and alveolar regions. Thirty variables were measured in order to build nine volumetric indices useful in describing shape changes of the functional components. The rejection of the null hypothesis indicates that many cranial variations that arose from the early Holocene to modern times were caused by different adaptive trends. Increase in variation may have occurred due to plastic responses to cold, nutrition, and masticatory stress. The author concluded that migration and genetic drift combined with some selective-adaptative processes would explain some of the non-detected craniofacial variation seen within the Americas since the early American Settlement.

Betti *et al.* (2009) intended to quantify the role of drift versus selection using a large dataset of human crania. The role of climate in particular was investigated in order to explain differentiation of size and form. The authors used a worldwide data set consisting of 4,666 males from 105 populations and 1,579 females from 39 populations. The data was transformed and computed into matrices used to estimate phenotypic distances between populations and a matrix of pair-wise geographic distances. Minimum annual temperature, maximum annual temperature, and mean annual precipitation were included to calculate three climatic distance matrices. Geographic distance between two populations is a good predictor of variation when a linear isolation by distance model

(IBD) is employed to the data. A nonlinear IBD model is an even better predictor than the linear model. The authors found that minimum and maximum temperature were significant predictors of phenotypic variations between populations, but this is less informative than geographic relationships. When IBD is associated the relationship with climate becomes weaker. The morphological variation of each cranial trait is influenced by climate and geography in very different ways. The traits that are strongly correlated with climate are related to cranial and facial breadth, or the nasal aperture and orbital dimensions.

Pérez and Monteiro (2009) investigated diversification of aboriginal populations from the southern South America. The study consisted of 12 samples from the Late Holocene. The northernmost sample consisted of two farmer groups and two terrestrial hunter-gatherers, the central sample included two terrestrial hunter-gatherers and two horticulturists, and the southernmost sample is comprised of two maritime hunter-gatherers and two terrestrial hunter-gatherers. Digital images were used to collect 2D coordinates of landmarks and semilandmarks of craniofacial traits in three views, with point configuration depicting three regions of the skull.

The southernmost samples investigated by Pérez and Monteiro (2009) were found to have the most robust and tall face as well as the most robust and long vault. The northernmost sample displays the opposite morphologies, with shorter and less robust faces and vaults. The results also show that hunter-gatherers have the largest skull size. The differences between these two groups is so great that there is no overlap in variation when it comes to the face and vault. The statistical tests employed in this study showed

that the magnitude of morphological divergence is too large to be created by genetic drift itself. It is suggested that there is an influence of climate on shape variation particularly of the cranial vault and base, due to the association found in this study with mean annual temperature. Phenotypic plasticity could play an important part in the beginning stages when a population is acclimating to a new environment. The morphologies observed in the southernmost populations contradict the expected phenotypic plastic response that was originally considered however, the morphologies reflect the patterns seen when selection to cold is considered. Pérez and Monteiro (2009) determined that the large craniofacial diversity observed is due to the confluence of multiple dimensions such as population division, morphological and ecological variation. It is also evident that divergence in features can occur over a short amount of time, and the effect of nonrandom factors should be investigated and not neglected.

Review of Changes to Craniofacial Complex and CVM

In an older study, Björk and Björk (1964) examined ancient crania in Peru to uncover how artificial modification affects the growth of the facial skeleton. Due to the variety of forms artificial modification can produce, investigating this “art” has the potential to shed light on the spreading of cultures as well as an interest in the effects on biology and growth. The coastal region of Peru contains pre-Inka cultures which practiced occipital flattening while the Incas and Andean Indians practiced circumferential modification. The authors specifically compare asymmetrical cranial development in “deformed” and “undeformed” crania.

Björk and Björk (1964) utilized 149 adult crania divided into five groups based on the type of deformation observed. These groups were undeformed crania (n=44), circumferential deformation (n=30), symmetrical occipital deformation (n=31), left-sided occipital deformation (n=23) and right-side occipital deformation (n=21). The right and left-sided occipital deformation groups were named for the side in which deformation was greater. These two groups were also divided into moderate and extreme. Only three groups contained individuals with mandibles, symmetrical occipital deformation (n=1), left-sided occipital deformation (n=8) and the right-side occipital deformation (n=5). Three cranial sections were measured to assess the asymmetry of cranial development and included length of cranial base, length of maxilla and length of mandible.

Data showed that in relation to the control undeformed group, the cranial base and maxillae were significantly shorter on both sides in skulls with circumferential deformation (Björk & Björk 1964). The undeformed, circumferentially deformed and symmetrical occipital deformation groups showed that both right and left side of different sections measured were on average the same within each group. For the right and left-sided occipital deformation groups, the cranial base and maxilla were significantly shorter on the side corresponding to the side of greater deformation. The mandibles available for study were also found to be shorter on the side where the deformation was greater.

Björk and Björk (1964) found a high correlation between degree and type of occipital deformation and extent of cranial base asymmetry. This showed that the cranial base from the temporo-mandibular joint to nasion was significantly shortened on the side

of the cranium that was more deformed. The shortening observed was also proportional to the degree of the asymmetry observed in the brain case. There is a statistically significant correlation between the asymmetric development of the cranial base and maxilla, as well as cranial base and mandible. This indicates that unilinear occipital flattening causes a shorter cranial base and a shorter maxilla and mandible on the more deformed sides

Of the groups with asymmetrical deformation investigated by Björk and Björk (1964), there was no asymmetry found in the facial midline, though the disappearance of asymmetry in the face may occur during the first few years of life, when the practice of deformation begins. The maxillary and mandibular asymmetry may be a result of compensating for the asymmetry in the cranial base early on in life. Primary dysplastic asymmetry of the facial bones has a greater influence on the deviation of the mid-line of the face than asymmetry of the cranial base.

The findings demonstrate compensatory asymmetrical development of the mandible. The shortening of the cranial base on the side that shows greater deformation is a result of the asymmetrical occipital deformation, and thus the mandibular fossa of the same side is displaced ventrally in order to compensate for the altered form, resulting in an effect known as “compensatory asymmetry of the jaws” (Björk & Björk 1964, 361).

Antón (1989) measured three groups of Peruvian crania, including anteroposterior, or tabular, and circumferential types of CVM to analyze the changes in certain angles due to the form of modification. She found that the circumferentially modified group results in a greater degree of flattening of the base of the skull due to the

effects of displacement caused by the directional migration of the cranial vault. It was also evident that the face, base and vault tend to broaden in the anteroposterior, or tabular, group and these features narrow in the circumferentially modified group. The angles of facial prognathism differ as a result of migration of the vault as well, as opposed to the changes in the cranial base.

Cheverud *et al.* (1992) tested the effect of fronto-occipital reshaping, or tabular CVM, in changes to the cranial base and face using prehistoric Peruvian Ancon sample and Songish Indian sample from British Columbia. The Peruvian Ancon had a custom of wearing bandages around the head while the Songish used an elaborate cradle boarding method with pads across the front to compress the head. Both of these practices unintentionally resulted in deformation. The samples were broken down to individuals that were modified, slightly modified, not modified, and greatly modified. Three-dimensional coordinates of 53 landmarks were collected and finite element scaling analysis was used to measure the differences between forms. The data suggested no significant difference in overall cranial size between unmodified and modified groups. However, the reshaping resulted in an indirect effect on the cranial base and face in the Ancon. This reshaping of the cranial vault results in compensatory bony changes in other areas in order to facilitate normal growth of the brain, confirming the work of Antón (1989).

As mentioned previously, the main goal of Rothhammer *et al.* (2002) was to investigate the impact of CVM on measurements used in biodistance analysis using adult crania from cemeteries in the Azapa Valley and the coast of the Pacific Ocean near Arica.

The authors divided the crania within their study into three groups based on modification including anteroposterior modification, circumferential modification, and normal. Six craniofacial measurements were taken including minimum frontal breadth, bizygomatic breadth, nasal height, orbital height, orbital breadth and palatal breadth. The results of the univariate statistical analysis of differences among the three forms of modification coincided with Antón (1989) showing that both of the modification types investigated overlap with the normal type. It was also concluded that the six craniometric measurements were not affected by CVM, as indicated in previous studies.

Kohn *et al.* (1993), also investigated annular - or circumferential cranial modification in two native American skeletal series from the Pacific Northwest Coast of Canada. The samples included the Kwakiutl with 62 non-modified and 45 modified individuals and the Nootka with 28 non-modified and 20 modified individuals. As in the previous study, 3D coordinates of 53 landmarks were obtained and finite element scaling was implemented. This was done again, to evaluate whether or not cranial vault modification has an effect on the cranial base and face. Individuals were scored on intensity of modification, similarly to their previous study. The techniques used by these groups to attain annular (circumferential) modification were different. The Nootka would bind the head with a fibrous wrap and the infant was kept on a cradle with a head presser and pads, while the Kwakiutl simply used kelp to wrap around the head. The data were consistent with previous studies reporting that individuals with annular or circumferential modification exhibit a longer and narrower cranial base and face. The authors also compare the results to those of their previous study, Cheverud *et al.* (1992) and discusses

the differences between the two forms of CVM studied. As the CVM methods are different, so are the effects on the cranial base and face. Anteroposterior, or tabular modification results in medial-lateral widening and anterior to posterior shortening of the cranial vault, and thus contributes to the widening of the cranial base and the face compensating by shortening anteroposteriorly. On the other hand, the results of annular, or circumferential modification results in a longer, narrower cranial vault and as a result the cranial base is narrower, and the face is narrower and longer.

Kohn *et al.* (1995) tested if cradleboarding practiced by the Hopi resulted in significant effects to the cranial vault, base and face. The sample included 43 adult female and 39 adult male crania which were again categorized by severity of the modification observed, and modified crania were then classified as exhibiting bilateral flattening, flattening on the left side, and flattening on the right side. The authors also implemented the same methods and statistical analyses as in their previous studies. The authors noted that anteroposterior and annular (tabular and circumferential) modification of the cranial vault are produced by the multi-directional application of pressure to the vault. Cradleboarding produced localized pressure, which is unidirectional, on the posterior of the cranial vault. This differences in how and where pressure is put on the cranium may manifest in different degrees of morphology.

The results indicate that the unintentional cranial vault modification of cradleboarding has a significant effect on the cranial vault but not on the cranial base and face. This cultural practice however, effects morphology in a predictable manner (Kohn *et al.* 1995). The restriction results in compensational growth in other area of the vault

and some effects are similar to those of scaphocephaly, or premature closure of the sagittal suture. The results of this study contrast those of Björk and Björk (1964) who stated that asymmetry of the length of the cranial base and maxilla showed a significant positive correlation with the direction of modification of the vault. The results of Kohn *et al.* (1995) however, found that cradleboarding does not produce size change or asymmetry in the base or face or the cranium. The authors concluded that studies of population variability can include samples from populations that practiced cradle boarding.

A study by Rhode and Arriaza (2006) examined if the different forms of modification had an effect on craniofacial measurements. A sample of 350 prehistoric crania from Chile from 18 archaeological sites were used to test if the 10 measurements proposed by Cocilovo (1973; 1975) are unaffected by annular or tabular modification. Of the crania used in this study 230, or 65.6%, exhibited some form of CVM. This included 120 classified as normal, 107 as annular modification and 123 with tabular modification. The ten relatively affected facial measurements include minimum frontal breadth, bizygomatic breadth, orbit height, orbit breadth, palate breadth, palate length, upper facial height, basion-prosthion length, nasal height and nasal breadth. With the palate length measurement excluded, due to the measurement being inherently difficult to replicate.

The results indicate that among males Rhode and Arriaza (2006), only three of the nine measurements are unaffected by CVM while females show four of the nine are unaffected. The other six measurements are affected by the form of the cranium attained by the method of CVM. The two measurements that males and females have in common

are basion-prosthion length and nasal breadth. Due to the unaffected measurements lying centrally, this suggests that type of modification has a greater effect on the peripheral cranial structures; this finding coincides with Antón (1989). Facial measurements also display patterns consistent with the type of modification applied, as noted in other studies. These patterns include the increased length measurements and decreased width measurements in annularly modified crania while the tabularly modified crania have width measurements. The effect of CVM on the face has a variable but definitive impact which corresponds to type of modification. The author states that when using the 9 measurements in biodistance studies caution is warranted if the population in question exhibits intentional CVM.

O'Brien and Stanley (2013) investigated 469 adult crania from Argentina, Bolivia, Chile, and Peru. Their goal was to create a mathematical approach to correctly classify crania as tabular, annular, or normal. The authors used landmarks of the cranium to obtain several measurements: maximum cranial length, maximum cranial height, maximum cranial breadth, and frontal chord. The results indicate that annular cranial breadth is less than normal skull cranial breadth, which is less than tabular cranial breadth. When considering length, annular skulls will have a slightly longer length than normal skulls, and tabular skulls be smaller in length than normal skulls. The results show that for cranial height annular skulls will be larger than normal skulls, tabular will be larger than normal skulls but not greater than annular. In regard to the frontal chord, tabular skulls will be larger than normal skulls and annular skulls will exceptionally larger than normal skulls. This information was then used to define indices to

differentiate the classification types of skulls. With the four measurements taken, the indices can be calculated, discriminant functions can be computed, and the individuals can be plotted on a territorial map, indicating the correct classification of modification type and the severity of the modification.

The results of the measurements obtained by O'Brien and Stanley (2013) are in accordance with previous studies investigating CVM and changes to the skull and also demonstrates the congruency of the types of modification with definitions of cranial shape: brachycephalic, dolichocephalic, mesocephalic. Individuals with tabular modification have a wide cranial breadth, shorter cranial length, slightly larger cranial height, and greater frontal chord. Those exhibiting annular modification have smaller cranial breadth, slightly longer cranial length, larger cranial height, and an exceptionally longer frontal chord. The cephalic index is maximum cranial breadth x 100 divided by the maximum cranial length (Bass 2005: 68). When breadth is greater than length, the individual is classified as brachycephalic defined as broad or round headed, which coincides with the specificities of the measurement ratios proposed for tabular modification defined by O'Brien and Stanley (2013). And predictably. Dolichocephalic is when length is greater than breadth, also defined as narrow or long-headed, and this coincides with the findings of O'Brien and Stanley (2013) for annular modification. Mesocephalic is then defined as average, or medium size head shape.

Frieß and Baylac (2003) also investigated the effects of CVM on the face and base using elliptic Fourier analysis. The authors compared samples of intentionally modified and unmodified human crania from Peru, with comparative samples of Inuit and

Japanese undeformed crania. The results confirmed that an increased anterior and inferior facial projection and modification of the basioccipital region are results of circumferential modification. The cranial vault was found to protrude posterosuperiorly and to produce the most unusual outline. Though it was found that the modification did not alter facial length and less effect on the basioccipital region than previously observed. The authors concluded that the effects of CVM have greater effect on the face as opposed to the basioccipital region and points out that preexisting morphological trends may be emphasized in certain populations by CVM.

Though Sardi *et al.* (2006) investigated cranial differentiation to see if diet influenced morphology, the authors also concluded that tabular CVM did not influence shape of facial components. However, the 17 modified crania did show differences in neural and otic components and were subsequently excluded from analyses.

MATERIALS AND METHODS

This chapter will include description and background of the Museo Arqueológico de La Serena and the collections housed there utilized in this study. Review of geometric morphometrics definition, methods being used, MicroScribe™ 3D digitizer, importance of landmarks, landmarks and samples being excluded. This Section will describe the methods used to collect the data, and discuss the statistical techniques used for data analysis to test the hypothesis.

Skeletal Collection

The Museo Arqueológico de La Serena was founded in 1943 and is committed to the preservation, study and dissemination of the archaeological and anthropological heritage of the Coquimbo region (Servicio Nacional del Patrimonio Cultural 2020). The museum holds over 12,000 objects, including artifacts and fossils, and approximately 1,500 individuals from excavations as well as donations from the Coquimbo Region. The collection includes items and remains from the various cultural periods previously mentioned above.

Both the Archaic and Intermediate-Late period samples used in the present study are housed at the Museo Arqueológico de La Serena, La Serena, Coquimbo, Chile. Of this collection a total of 165 individuals (M=58; F=107) were available for analysis due to the presence of complete, intact, and articulated craniofacial elements. All data was gathered between May and June of 2019.

The Archaic period sample included a total of 87 individuals (M=31, F=56) The number of individuals exhibiting cranial vault modification in this sample was 4 (~4.6%).

The Intermediate-Late period sample included a total of 78 individuals (M=27, F=51). The number of individuals exhibiting cranial vault modification in this sample was 58 (~74.4%). Of this sample, two individuals were not included in analysis due to lack of contextual information. Figures 1 and 2 show anterior and lateral views of the cranium, comparing males and females of both samples.

The majority of individuals in this study were represented only by skulls. When available, the innominate was used for sex estimation following Klales *et al.* (2012). However, the sex of each individual was most often estimated using non-metric traits of the skull outlined by Walker (2008). The mastoid processes are extremely gracile in both samples and as a result the observer heavily relied on nuchal, glabella and orbit traits for sex estimation. All individuals were determined as adult due to the fusion of the basilar suture (Shirley & Jantz 2011) and investigation of 3rd molar eruption (Mincer *et al.* 1993). The overall size and development of the cranium was also observed and only individuals consistent with adult development were further examined.

Cranial vault modification was determined visually when investigating the occipital and parietal bones of the skull. This method was solely based on the experience of the author, as well as the assistance and insight of members of the museum. The flattening of the occipital and irregular lateral bulging of the parietals to various degrees were determined as intentional CVM. Though some individuals were hard to determine as intentionally modified, the presence of a flat lambdoidal suture lacking dimension and having a smooth profile led the author to also classify these individuals as possessing

CVM. Individuals which were difficult to determine as exhibiting intentional CVM and showed evidence of plagiocephaly were also included in the CVM category. However, severity of modification was not noted. The individuals in the Archaic period with CVM most likely possess plagiocephaly due to an unintentional practice, as intentional CVM was not present during this time.



Figure 1. Anterior comparison of cranium. Archaic (top) and Intermediate-Late (bottom) periods. Male individuals on the left and females to the right. The scale is in cm.



Figure 2. Lateral comparison of cranium. Archaic (top) and Intermediate-Late (bottom) periods. Male individuals on the left and females to the right. The scale is in cm.

In addition to sex, age, and CVM the site, an individual identification number (based on the box and individual number assigned by the Museo), culture, and time period (Archaic and Intermediate-Late) were also recorded for each individual. Of these contextual categories there were six cultural groups and 30 different sites. The cultural groups include Pescadores Arcaicos or Archaic fishers (n=81), Cazadores Arcaicos or Archaic hunters (n=2), Late Arcaicos (n=5), Diaguita (n=58), Inka Diaguita (n=1), and Las Ánimas Diaguita (n=13).

Geometric Morphometrics and Data Collection

The Microscribe™ G2X digitizer was used to record the same craniometric landmarks on all individuals. Due to fragmentation of different areas of the craniofacial region, some landmarks were not possible to record and were excluded for those individuals. There will be no exclusion of landmarks due to fragmentation because their presence on other individuals could provide information pertinent to the results. The focus of this study is on the shape of the face and thus landmarks of the mandible and cranial vault will be excluded.

Craniofacial data was collected and recorded into Excel using a Microscribe™ G2X digitizer provided by Boston University's Department of Anatomy and Neurobiology. In order to collect data, the tip of the Microscribe™ stylus was placed on a chosen landmark and recording of the x, y, and z coordinates only occurred when the attached foot pedal was pressed. A hand-held button is also optional however, the author preferred the use of the foot pedal. Table 1 presents a list of all 25 landmarks that were recorded as described by Langley *et al.* (2016).

Table 1. Landmarks used in Geometric Morphometric Analyses

| Landmark | Abbreviation | Placement |
|-------------------------------|---------------------|------------------|
| Prosthion | pr | Midline |
| Nasospinale | ns | Midline |
| Nasion | n | Midline |
| Glabella | g | Midline |
| Alveolon | alv | Midline |
| Zygion | zy | Left, Right |
| Zygomaxillare | zm | Left, Right |
| Zygoorbitale | zo | Left, Right |
| Frontomalare temporale | fmt | Left, Right |
| Frontotemporale | ft | Left, Right |
| Ectoconchion | ec | Left, Right |
| Dacryon | d | Left, Right |
| Upper orbital border | | Left, Right |
| Alare | al | Left, Right |
| Ectomolare | ecm | Left, Right |

Due to the inability to draw points in the skull with pencil prior to collecting data, the paired landmarks were instrumentally or visually determined and then taken in succession in attempt to avoid incongruencies. This was done in order to comply with the

guidelines developed by the Museo Arqueológico de La Serena, in order to conserve the remains.

Statistical Analyses

The data collected was integrated into MorphoJ, a software that combines methods for shape analysis in biological contexts (Klingenberg 2011). A generalized procrustes analysis (GPA) was performed first. This procedure is used to scale, rotate, and transform data so that it is within a common coordinate system. A principal component analysis (PCA) was then conducted to determine the relatedness of the variables between the sample. Principal component analysis is utilized to examine the main features of shape variation in the sample and as ordination analysis for examining the arrangement of specimens in “morphospace” (Klingenberg 2011). A Canonical Variate Analysis (CVA) was conducted in order to determine the linear relationships between the samples. This analysis is used to determine features that differentiate the samples.

RESULTS

The purpose of this chapter is to state and review the results of the statistical analyses performed. This will include an interpretation of the PCA, CVA, and wireframes obtained from the program MorphoJ. These statistical tools are used to understand the visual shape changes between samples as well as the relationship between the them. The results of two analyses will be presented. One consisting of all 25 landmarks, and another consisting of only 19 of the 25 landmarks.

Analyses

A Generalized Procrustes Analysis, Principal component analysis, and Canonical Variate Analysis were performed on the samples in both analyses. This was done in order to analyze the grouping relationships of shape differences. The results will report the shape differences between samples which can potentially be used to correctly classify individuals to either time period. In order to maximize sample size, males and females were pooled together. As stated before, two individuals were excluded from all analyses due to lack of contextual information. Two analyses were performed to assess shape changes between the two time periods in question with the use of the program MorphoJ.

The first analysis conducted included all of the facial landmarks recorded, as listed in Table 1. In the second analysis the landmarks prosthion, alveolon, ectomolare and zygion were removed. The landmarks that represent the palate were excluded due to the inherent difficulty of locating landmarks when dentition was incomplete. Alveolon was particularly hard to reach due to the presence of clay pillars holding the base of the cranium in place while landmarks were recorded. Zygion was removed as a result of

being difficult to accurately locate when being recorded. In order to locate both right and left zygion, spreading calipers are placed on the zygomatic arches at the same time to find the most laterally positioned points (Langley *et al.* 2016) After finding the most lateral points, the calipers are then removed and the stylus was placed on the approximate point, estimated by the author, to record the coordinates. The marking of these points with pencil may have led to more accurate landmarks, however, this is prohibited by the Museo Arqueologico de La Serena. In addition to these difficulties, inter and intra observer errors were not investigated to assess the precision, replicability, and reliability of landmark acquisition. These landmarks were also removed in attempt to replicate previous studies (Kuzminsky *et al.* 2017b).

In the first analysis consisting of all landmarks, two individuals were excluded as outliers. From the CVA, a Mahalanobis distances between the groups was generated. This value is 2.6999, which indicates statistically significant differences ($p < 0.001$) between the Archaic sample and Intermediate-Late period sample. Mahalanobis distance is a function of the group means as well as pooled variances and covariances that measures the degree of differentiation observed between the groups in question (Humphries 2011). This means the lower the Mahalanobis distance value is between groups, the more similar the groups are related. The Mahalanobis distance obtained between the Archaic and Intermediate-Late period samples indicates there is a difference between the groups, but due to the small value of the distance between the two groups suggests they are closely related.

Figure 3 presents the plot produced by the CVA and represents the spatial differences between the two samples. There is a grouping of the Archaic period sample, defined by the confidence ellipses, with the Intermediate-Late period sample exceeding the ellipsis of the Archaic sample along CV1. This indicates that the more recent group, the Intermediate-Late period sample, is more variable than the older group, the Archaic period sample.

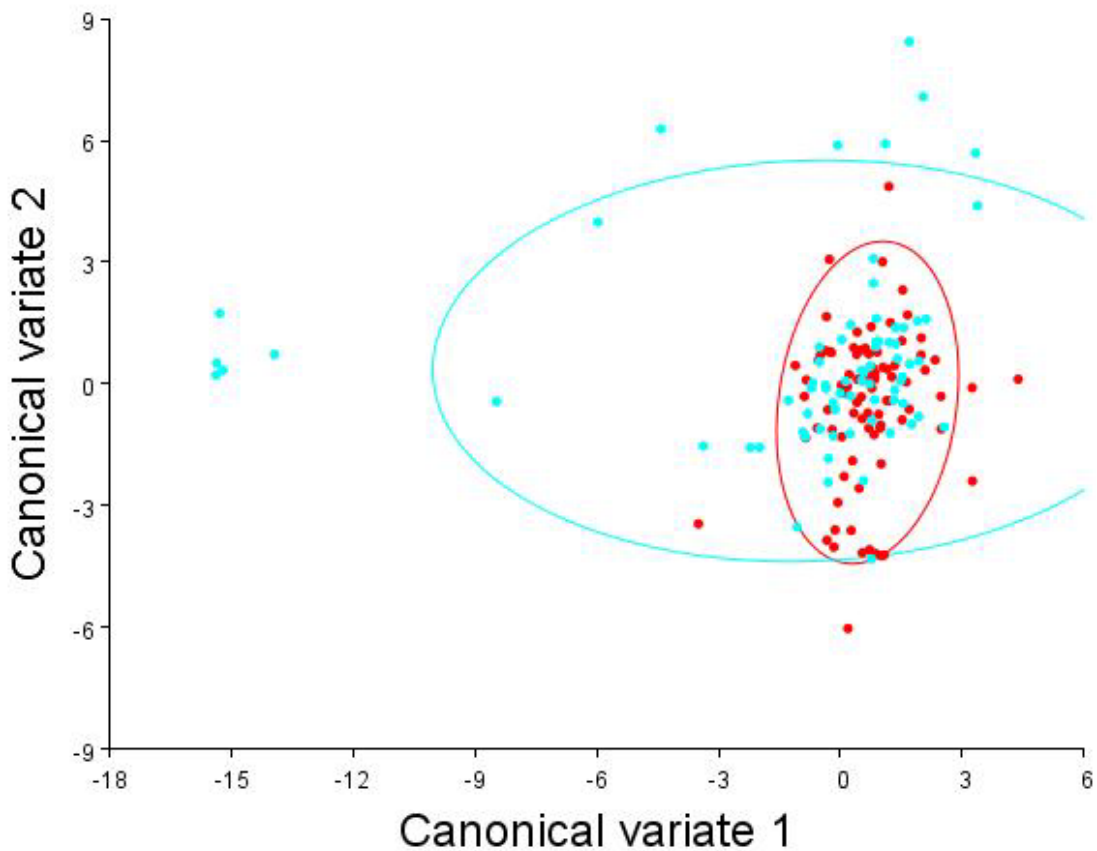


Figure 3. Canonical variate plot from first analysis of Archaic (red) and Intermediate-Late (blue) periods.

In the second analysis three individuals were excluded from analysis due to being outliers. From the CVA, a second Mahalanobis distances between groups was generated with a value of 2.9316. This value is slightly larger than the Mahalanobis distance

reported in the first analysis, but still indicates statistically significant differences ($p < 0.001$) between the Archaic period sample and Intermediate-Late period sample.

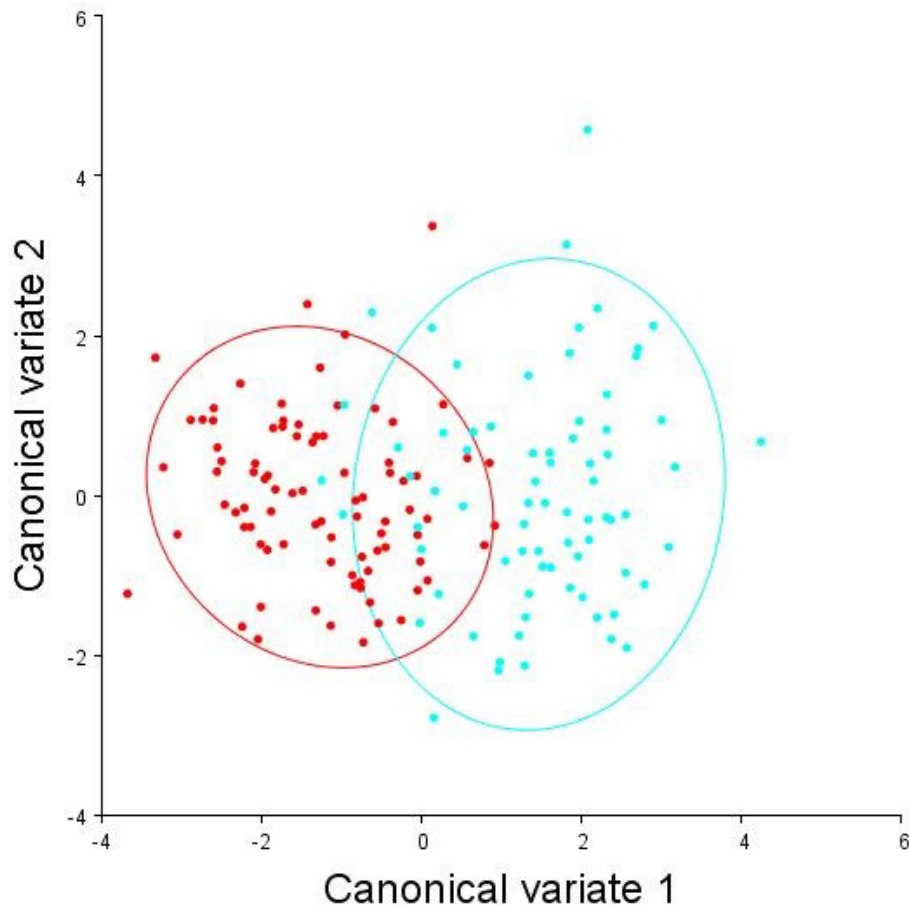


Figure 4. Canonical variate plot from second analysis of Archaic (red) and Intermediate-Late (blue) periods.

Figure 4 represents the second CVA rendered utilizing only 19 of the 25 landmarks to investigate differences between the Archaic and Intermediate-Late periods. The plot rendered shows that the Archaic and Intermediate-Late period are separated by CV1, though there is some overlap in variation. When considering CV2 the Intermediate-

Late period is slightly more variable than the Archaic period in both positive and negative directions.

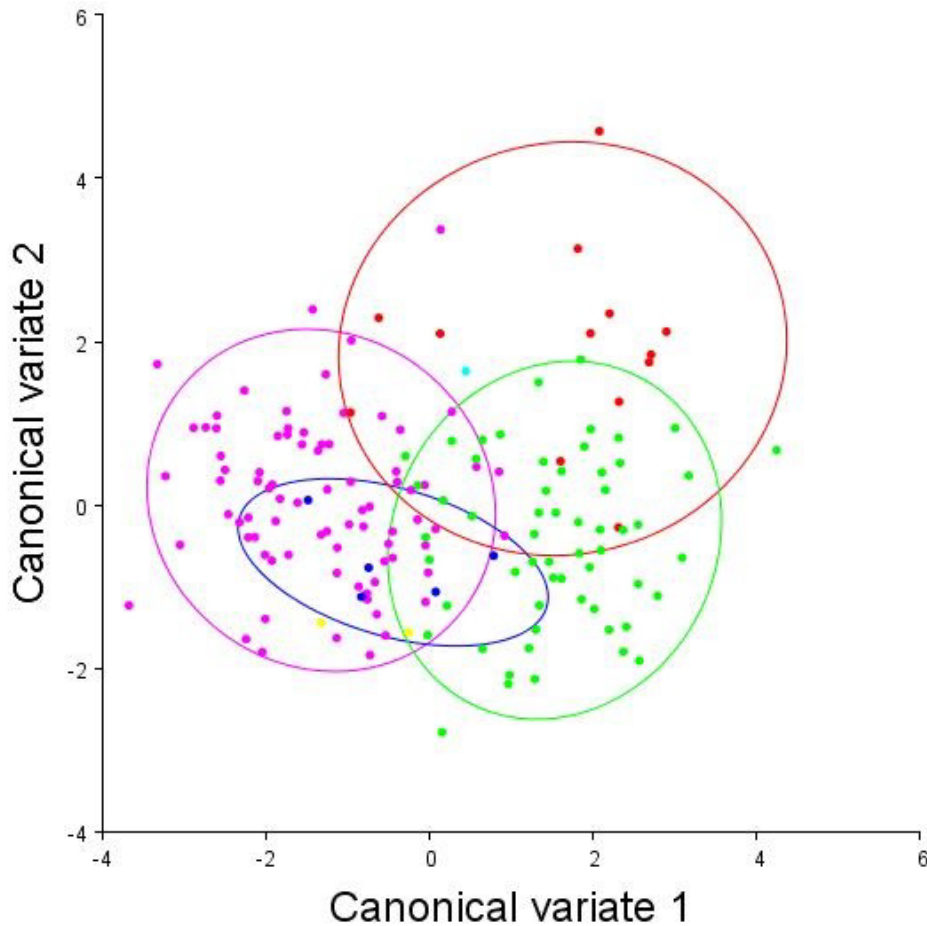


Figure 5. Canonical variate plot from second analysis representing cultural groups. Pescadores Arcaicos (pink), Cazadores Arcaicos (yellow), Late Arcaicos (dark blue), Diaguita (green), Inka Diaguita (light blue), and Las Ánimas Diaguita (red).

When the relationship of cultural groups is investigated the Mahalanobis distances produced from the CVA all indicate statistically significant differences. These values are shown in Table 2. The lowest Mahalanobis distances between cultural groups include Las Ánimas Diaguita and Diaguita, followed by Late Arcaicos and Pescadores Arcaicos, and lastly Diaguita and Pescadores Arcaicos. The largest distances between cultural groups

include Inka Diaguita and Cazadores Arcaicos followed by Inka Diaguita and Late Arcaicos.

Following the Mahalanobis distances it is evident that the Cazadores Arcaicos, or Archaic Hunters, are most closely related to Pescadores Arcaicos, Diaguita, Late Arcaicos, Las Ánimas Diaguita and Inka Diaguita. The Pescadores Arcaicos, or Archaic fishers are most closely related to Late Arcaicos, Diaguita, Las Ánimas Diaguita, Cazadores Arcaicos and Inka Diaguita. The Late Arcaicos are most closely related to Pescadores Arcaicos, Diaguita, Las Ánimas Diaguita, Cazadores Arcaicos, and lastly Inka Diaguita. Las Ánimas Diaguita culture, which is recognized as a division of the Diaguita (pers. comm. Cantarutti), is most closely related to Diaguita, followed by Pescadores Arcaicos, Late Arcaicos, and Cazadores Arcaicos and is the most different from Inka Diaguita. The Diaguita results coincide with those of the Las Ánimas Diaguita, with the Diaguita being most closely related to Las Ánimas Diaguita, followed by Pescadores Arcaicos, Late Arcaicos, Cazadores Arcaicos, and lastly Inka Diaguita. The most recent cultural group known as Inka Diaguita is most closely related to Diaguita, followed by Pescadores Arcaicos, Las Ánimas Diaguita, Late Arcaicos, and finally Cazadores Arcaicos. This cultural group also possesses the greatest Mahalanobis distance values.

When looking at the CVA plot colored by cultural groups (Figure 5), the confidence ellipses encompass Las Ánimas Diaguita, Diaguita, Late Arcaicos, and Pescadores Arcaicos cultural groups. All of the cultural groups overlap with one another in either CV1 or CV2. The plot shows that CV1 separates the Pescadores Arcaicos and

Diaguaita cultures, with some overlap in variation. While CV2 separates Las Ánimas Diaguaita and Late Arcaicos cultural groups.

Table 2. Mahalanobis Distances among cultural groups. All distances are statistically significant ($p < 0.001$).

| Group | AD | CA | DI | ID | LA |
|-------|--------|--------|--------|--------|--------|
| CA | 5.9162 | | | | |
| DI | 2.4689 | 5.3425 | | | |
| ID | 6.0805 | 7.9081 | 6.0158 | | |
| LA | 4.0197 | 5.4361 | 3.2233 | 6.8328 | |
| PA | 3.5532 | 4.9360 | 2.9039 | 6.0726 | 2.7691 |

Wireframes and Shape Differences

When the CVA plot is created in Morphoj, the program also renders wireframes consisting of all landmarks recorded. These wireframes then visually display the shape changes which coincide with each axis of the plot, indicating the differences between groups. The dark blue lines of the wireframes represent the average shape for each CV. As the two groups in question are only separated by CV1, only these changes will be discussed.

When investigating CV1 (Figure 6) there are several notable shape changes. The frontotemporale landmarks are located in a more lateral position with more anterior inferior placement. Both frontomale temporale landmarks also occupy a more lateral position. The dacryon landmarks, however, are placed more medially as well as superiorly oriented. Nasion takes a more superior position while glabella is slightly inferior. Nasospinale is positioned more anteriorly while zygomaxillare landmarks are projecting more posteriorly. The zygoorbitale landmarks are oriented more medially.

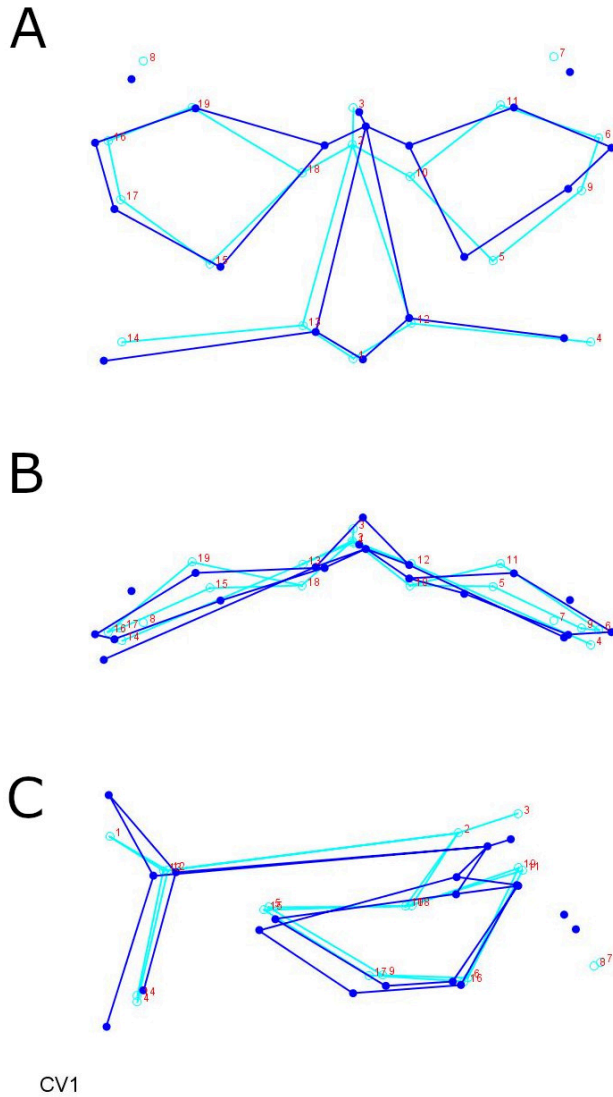


Figure 6. Wireframes depicting shape changes of CV1. (A) Anterior view of the skull depicted by Axis 1 vs Axis 2. (B) Superior view of the skull depicted by Axis 1 vs Axis 3. (C) Lateral view of the skull depicted by Axis 2 vs Axis 3. The light blue lines and hollow dots indicate the starting shape, while the dark blue lines and dots indicate the target shape.

Overall, most landmarks are positioned more posteriorly with the exception of nasospinale and frontotemporale. Half of the landmarks are more laterally oriented while the other half are slightly medially oriented. There are differences in placement between

the two ectoconchion landmarks, with the left side placed more medially and the right side placed laterally, the same changes are seen for zygomaxilare landmarks as well. These changes result in increases in minimum frontal breadth, upper facial breadth, and an increase in nasal height.

The Mahalanobis distance values from the analyses 2.6999 and 2.9316, indicate statistically significant differences between the Archaic and Intermediate-Late period samples. Though these values indicate differences between the two groups, they are still closely related. The Mahalanobis distance values between cultural groups are all statistically significant and indicate differences between the groups. In the first analysis including all of the landmarks recorded, the CVA plot indicates that the Intermediate-Late period sample is more variable than the Archaic sample while the second analysis shows that the Archaic and Intermediate-Late period are separated by CV1, with some overlap in variation. The shape changes associated with CV1 show most landmarks are positioned more posteriorly with the exception of nasospinale and frontotemporale. The changes in position of these landmarks result in increases in minimum frontal breadth, upper facial breadth, and an increase in nasal height.

DISCUSSION

The goal of this study was to investigate the shape changes of the craniofacial complex between two time periods of Chile's semiarid north. As described by Kuzminsky *et al.* (2017a), it is common for prehistoric crania to be categorized based on visual assessment of shape. The earlier inhabitants have been classified as dolichocephalic, or long headed while the later residents are brachycephalic, or round headed. This visual classification could be related to the practice of tabular cranial vault modification in several cultures such as the Diaguita. This form of modification is known to alter several aspects of the cranium and the craniofacial complex (Antón 1989; Cheverud *et al.* 1992; O'Brien & Stanley 2013, Rhode & Arriaza 2006). The definition of brachycephalism parallels the shape changes which occur due to tabular modification.

This study focused on the applications of geometric morphometric analysis in order to investigate whether there are differences in facial shapes and proportions between the Archaic and the Intermediate-Late period that coincide with the method of visual classification that is commonly employed. The author hypothesized that even without investigation of the cranial vault, the more recent residents of the region would present characteristics of the craniofacial complex consistent with brachycephalism when compared to later crania. This assumption was reinforced by the large presence of individuals exhibiting tabular modification within the Intermediate-Late period sample (~74.4%). The results of the canonical variate analysis (CVA) do show a separation between the two time periods of this study. Further investigation of the CVA illuminated the shape differences which separate the two time periods, including increases in

minimum frontal breadth, upper facial breadth, and nasal height. These shape changes are characteristic of tabular modification (Antón 1989; Cheverud *et al.* 1992, O'Brien & Stanley 2013, Rhode & Arriaza 2006) and brachycephalism. Due to the Intermediate-Late period being dominated by modified individuals, these changes of the face are most likely due to the practice of intentional CVM.

The documented shape changes as well as the increase in variation of the Intermediate-Late period are most likely due to the inclusion of several different groups originating in different areas which then occupied the semiarid coast of Chile. Although genetic drift and gene flow have been deemed the main evolutionary processes behind the patterns of craniometric differentiation (Relethford 1994, 2004) it is important to investigate the possible contributions of nonrandom processes such as climate and diet (Barbeito-Andrés *et al.* 2011, Betti *et al.* 2009, Menéndez *et al.* 2014, Paschetta *et al.* 2010, 2015, Pérez & Monteiro 2009, Pérez *et al.* 2011, Sardi *et al.* 2006, Stynder *et al.* 2007). This is particularly important when the range of morphological variation observed is greater than expected as the result could be due to phenotypic plastic responses to environmental factors.

Research of molecular genetics data show biological continuity from a single Asiatic ancestor in the Americas (e.g., Fagundes *et al.* 2008, Fehren-Schmitz *et al.* 2015, Merriwether *et al.* 1995, Silva *et al.* 2002, among others). Morphometric data, however, has led physical anthropologists to postulate a different migration model. This two-component migration model (González-José *et al.* 2001, 2008; Pucciarelli *et al.* 2003) suggests two separate ancestral groups with distinct cranial morphologies entering the

Americas at different times (Neves & Hubbe 2005, Sardi *et al.* 2005). The first ancestral group the Paleoamericans, possessed unique phenotypes which was most likely a retention of traits from the first humans leaving Africa. The second ancestral group consists of East Asian populations with their own distinct morphological pattern. This group most likely influenced morphological variation and gave rise to all modern Native Americans during the Pleistocene/Holocene transition.

From these studies it is evident that Asian populations initially differentiated before arriving in Beringia, followed by a population expansion between 18,000 to 15,000 years BP. This population expansion led to the precipitous settlement of the Americas along the Pacific coast. The populations of South America share this common ancestor, and the morphological diversity observed within South America can then be attributed to small populations inhabiting diverse ecological zones, resulting in a range of phenotypic plastic responses related to genotype. Factors such as morphological integration, developmental and functional constraints as well as different levels of phenotypic plasticity interact through ontogeny and contribute to the final adult morphology.

As mentioned previously by Rothhammer *et al.* (2002), a dry period in the highlands of Arica occurred around 7,000-6,000 years BP (Baied 1992). As a result of changes in climate affecting the living conditions experienced by the local hunter-gatherers, populations moved toward the coasts. The movement of several morphologically diverse groups, who possessed particular phenotypes due to influences

throughout ontogeny due to diverse environmental conditions, moved towards the coast to take advantage of resources and escape undesirable climate changes.

These phenotypic differences are evident when the CVA and Mahalanobis distances of cultural groups is further investigated. The Mahalanobis distance values also indicate statistically significant differences between these groups. These cultural groups include Pescadores Arcaicos, Cazadores Arcaicos, Late Arcaicos, Diaguita, Inka Diaguita, and Las Ánimas Diaguita. The phenotypic differences between these groups are most likely related to temporal as opposed to spatial differences. This is evident due to the Mahalanobis distances reflecting the chronological relationships.

Following the Paleoindian period, the Archaic period encompasses 10,000 BC–0 AC and includes three cultural groups Pescadores Arcaicos, Cazadores Arcaicos and Late Arcaicos. The Las Ánimas culture and Diaguita are both documented from 900 AC-1,200 AC, with the Diaguita continuing a little while after Las Ánimas until 1,550 AC. The cultural group known as Las Ánimas is now recognized as a division of the Diaguita, due to the lack of biological differences (pers. comm. Cantarutti). The Diaguita-Inka culture, which is also biologically related to the Diaguita, also inhabited the region during the last segment of the Diaguita from 1,450 to 1,500 AC.

As expected, the lowest Mahalanobis distances are between Las Ánimas Diaguita and Diaguita, followed by Late Arcaicos and Pescadores Arcaicos, and lastly Diaguita and Pescadores Arcaicos. The first relationship is not surprising, due to the biological affinities and temporal overlap of the Las Ánimas Diaguita and Diaguita. The second relationship, Late Arcaicos and Pescadores Arcaicos is also not surprising as these two

groups were both present during the same time period, with the Late Arcaicos being present during the latter. The last relationship between the Diaguita and Pescadores Arcaicos was not expected, due to the presence of the Late Arcaicos which the author anticipated to be more closely related to the Diaguita culture. However, it is possible that the Diaguita cultural group is derived from the Pescadores Arcaicos rather than Late Arcaicos since the Pescadores Arcaicos is present through the entirety of the Archaic period. The largest Mahalanobis distances are between the Inka Diaguita and Cazadores Arcaicos followed by Inka Diaguita and Late Arcaicos. Both of these relationships are not surprising, as the Inka Diaguita group is the most recent and the Cazadores Arcaicos and Late Arcaicos are both part of the oldest period which places them the furthest apart in time. It is also unsurprising that the Inka Diaguita group possesses the greatest values.

The Diaguita and Las Ánimas Diaguita show similar results when considering the relationships with all other groups. Both the Diaguita and Las Ánimas Diaguita are most closely related to one another, followed by the Pescadores Arcaicos, Late Arcaicos, Cazadores Arcaicos. The second closest relationship for Las Ánimas Diaguita is with the Pescadores Arcaicos. Because the Diaguita and Las Ánimas Diaguita are biologically related, it is unsurprising that after each other, they both are most closely related to the Pescadores Arcaicos. A similar pattern is seen for the Inka Diaguita, with this group being closely related to the Diaguita, followed by the Pescadores Arcaicos, Las Ánimas Diaguita. As the Inka Diaguita is another group that is biologically related and chronologically adjacent to the Diaguita it is expected that they would be most closely related. Due to the close relationship between the Diaguita and Pescadores Arcaicos, it is

unsurprising that the second related group to the Inka Diaguita is also Pescadores Arcaicos, which is then followed by Las Ánimas Diaguita which is also closely affiliated with the Diaguita group. Each of the Archaic groups; Pescadores Arcaicos, Cazadores Arcaicos and Late Arcaicos, are most closely related to another archaic group followed by the Diaguita and are most distantly related to the Inka Diaguita. The Late Arcaicos and Pescadores Arcaicos, however, display similar results. These two groups are more closely related to one another, followed by Diaguita, Las Ánimas Diaguita, Cazadores Arcaicos and lastly Inka Diaguita, whereas the Cazadores Arcaicos are most closely related to Pescadores Arcaicos, Diaguita, Late Arcaicos, Las Ánimas Diaguita and then Inka Diaguita. The results indicate the closest relationship in the Archaic period is between the Late Arcaicos and Pescadores Arcaicos and the group of this period most closely related to the Diaguita is the Pescadores Arcaicos. These relationships could indicate that the Pescadores Arcaicos group interacted with the Late Arcaicos group, contributing to their morphological variation. On the other hand, the Pescadores Arcaicos are more closely related to the Diaguita, which may indicate that with the transition of the Archaic period the Pescadores Arcaicos had a greater influence on the biological and morphological patterns of the Diaguita, and possibly indicate a cultural evolution of the Pescadores Arcaicos.

The CVA plot of cultural groups displays confidence ellipses which encompass Las Ánimas Diaguita, Diaguita, Late Arcaicos, and Pescadores Arcaicos. Although these groups overlap with one another on both axes, it is evident that CV1 separates the Pescadores Arcaicos and Diaguita, and CV2 separates Las Ánimas Diaguita and Late

Arcaicos cultural groups. These results should be approached with caution and these relationships investigated further, as most of these cultural groups had extremely small sample sizes; Cazadores Arcaicos (n=2), Late Arcaicos (n=5), Inka Diaguita (n=1), and Las Ánimas Diaguita (n=13). The largest groups were Pescadores Arcaicos (n=81) and Diaguita (n=58), however, these sample sizes are still small. This method has the potential to be used in conjunction with others to identify cultural context and significance of remains in archaeological contexts in Chile. With further investigation utilizing geometric morphometric analyses as well as biodistance and statistical analyses, this method also has the potential to classify individuals by cultural group. In the event of repatriation acts this method would be extremely useful to classify remains so they can be donated to the appropriate community or facility. With further investigation utilizing geometric morphometric analyses as well as biodistance and statistical analyses the relationships of these groups can be explored and contribute to the ongoing investigation of the peopling and history of Chile's semiarid north

CONCLUSIONS

The results of the first two analyses show that the Mahalanobis distance values between the two time periods, Archaic and Intermediate-Late, are statistically significant. These values, 2.6999 and 2.9316, from the first and second analyses respectively, indicate differences between the two groups but also suggests they are closely related. In the first analysis including all of the landmarks recorded, the CVA plot indicates that the Intermediate-Late period sample is more variable than the Archaic sample. The second analysis with 19 of the 25 landmarks shows that the Archaic and Intermediate-Late period are separated by CV1, with some overlap in variation. When considering CV2 the Intermediate-Late period is slightly more variable than the Archaic period in both positive and negative directions. When the shape changes of CV1 are investigated, the most landmarks are positioned more posteriorly with the exception of nasospinale and frontotemporale. The changes in position of these landmarks result in increases in minimum frontal breadth, upper facial breadth, and an increase in nasal height. These shape changes are due to the practice of tabular modification in the more recent group, the Intermediate-Late period.

After the movement of Asian populations to Beringia, the initial differentiation of these human populations, a bottle neck occurred followed by an increase in population size. The limiting resources, increase in population, and changing environment led to the rapid settlement along the Pacific coast of the Americas. Once within the Americas, groups with relatively small population sizes adapted to the diverse environments in which they chose to inhabit. It is possible that a dry period resulted in a change of climate

within Chile, forcing populations to move toward the coast in search of a more desirable climate and resources. Nonstochastic responses to environmental factors induced by the temporal and spatial isolations of these small groups further contributed to morphological variation in addition to gene flow and genetic drift.

It is important to consider all mechanisms of evolution, stochastic and nonstochastic, when investigating the relationships of human populations through morphometric analyses. It is also important to keep in mind that variations observed are not necessarily correlated with one mechanism, but a combination of them. The study of ontogeny within a region is also important to assess heritable and nonheritable traits. The interaction of multiple factors, evolutionary and ecological, throughout ontogeny contribute to the organism's adult morphology. When considering the cranium, these factors have varying effects on the different regions. With human populations it is also important to consider the various influences related to these factors, for example, climate consists of several components including latitude, altitude, terrain, temperature, and precipitation. With human populations throughout time, it is also important to consider the changes related to these factors for instance, a change of climate due to the dry period in the highlands of Arica 7,000-6,000 years BP. In addition to climate, diet is another complex factor to consider. When quantifying the diet of a particular population, proportions of carbohydrates and proteins, food processing techniques, bite force, food sources, harness, toughness, and particle size of food. How diet composition is determined may also have an effect on results, for example utilizing ethnographic data, isotope analysis, the relationship of dental wear and carious lesions. Diet composition can

also influence systemic effects and on the skeleton, particularly the cranium and craniofacial complex.

Limitations

The sample size of this study was heavily influenced by the presence of incomplete and fragmented crania as well as differential preservation. The landmarks of the palate were excluded due to difficulties in locating accurate points with incomplete dentition. Due to the pillars holding the cranium in place it was also difficult to locate and reach the landmark alveolon. The conservation efforts of the museo prohibits using pencil to indicate landmark positions on skeletal material. This made it difficult to relocate and record landmarks that are instrumentally determined. In the future it may be possible to use a small laser pointer instead of pencil to mark landmarks. This would most likely require a small desk sized lamp with a flexible gooseneck which could then be moved freely, and the neck of the lamp would be able to bend and align with desired landmarks. In addition to these difficulties, inter and intra observer errors were not investigated to assess the precision, replicability, and reliability of landmark acquisition.

Future Studies

As stated previously, in Chile it is common practice for physical anthropologists to visually categorize crania as dolichophallic and brachycephalic which are then attributed to cultures or time periods based on the observed shape. The validity of this classification is still debated and proposes several questions regarding the prehistory of Chile. Future studies should be conducted to investigate the accuracy of this visual

classification in regards to the cranial vault, as well as investigate secular changes in crania throughout the prehistory of Chile using morphometric analysis. Cranial vault modification was practiced in several prehistoric populations, and individuals exhibiting modification should be excluded. This however has the potential to cause issues regarding sample sizes of crania available for investigation, as this study encountered a large percentage of modified crania. Geometric morphometric analysis could be used in conjunction with other methods to identify cultural context and significance of remains in archaeological contexts in Chile. As stated previously, in the event of repatriation acts this method has the potential to attribute individuals to corresponding cultures, Diaguita, Mapuche, etc., and thus be donated to the appropriate community or facility.

In regard to cranial vault modification, geometric morphometric analysis can be utilized to determine the accuracy of visual classification as it is the most common method used when modified crania are encountered. This is a very subjective method due to the limitation of a standardized classification system and no accountability for intra-personal error. In particular, the development of a standardized practice for morphometrically evaluating modified crania holds great potential. Due to the different flattening mechanisms or modification styles implemented as well as the use of different materials to bind the skull, geometric morphometric investigation of modified crania could reveal the intricacies of shape and form in crania via different methods of vault modification as well as the accuracy of visual classification. In a forensic context, when remains are found, knowing the differences in intentional modifications has the potential to link the remains to a particular group and the remains could be returned to the proper

culture. In comingled remains individuals could be linked to certain time periods depending on the differences in the morphology or simply method of cranial vault modification.

Morphometric differences can also be used to investigate the possibilities of distinction between males and females of Chile. The idea of social status may be reflected in the modifications of opposite sexes depending on how the individuals were seen in the community. In any social class the distinction between males and females may have been seen differently as well, and the idea of what is deemed attractive in a society may dictate how the skull is formed differently between males and females.

In addition to further studies within Chile, the investigation of differences in other populations could lead to a correlation between geographical region and morphometric differences, allowing the distinction between cultural groups that inhabited the same area or even shedding light onto practices of exogamy. The influence of other cultures could have encouraged marriage between groups and may have influenced the changing practices of cranial modification. Morphological differences could be connected to cultural or personal differences, such as social status or sex. These differences could reflect on how the culture views the self and how important certain features or aspects of an individual are important or relevant in the society.

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CURRICULUM VITAE

