Biodistance Analysis of North and South American Populations

Undergraduate Research Thesis

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Abstract

Archaeological evidence suggests that humans were already present in both North and South America by 12.5-11.5 kyr BP. However, the number of waves and routes from Asia are much debated, and the early (~12 kya) settlement in Brazil bring into question previous ideas about human migration into the region. Given the debate surrounding this topic, it is important to understand the genetic diversity between North and South Americans. In this project, I used biodistance analysis to explore the cranial morphological variation observed in the New World, and how this variation is structured between the two American continents.

Human craniometrics data from previous studies (Hanihara, 1996; Herrera, et al., 2017; Hubbe, et al., 2014; Hubbe, et al., 2015; Neves et al., 2013) was used to create a detailed understanding of the biological variation of the region. These data cover populations in North America (USA and Mexico) and South America (Brazil, Colombia, and Peru), as well as comparative series from Asia and Australo-Melanesia.

Results show that Atlantic South America exhibits the highest Fst value (0.15) out of all groups analyzed. Whereas the Andean (0.068), North Americans (0.07), and East Asian (0.077) populations have the lowest Fst values. These findings reveal high genetic diversity of South American groups and calls into question the validity of grouping North and South Americans in genetic studies.

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Introduction

First Migration into the Americas

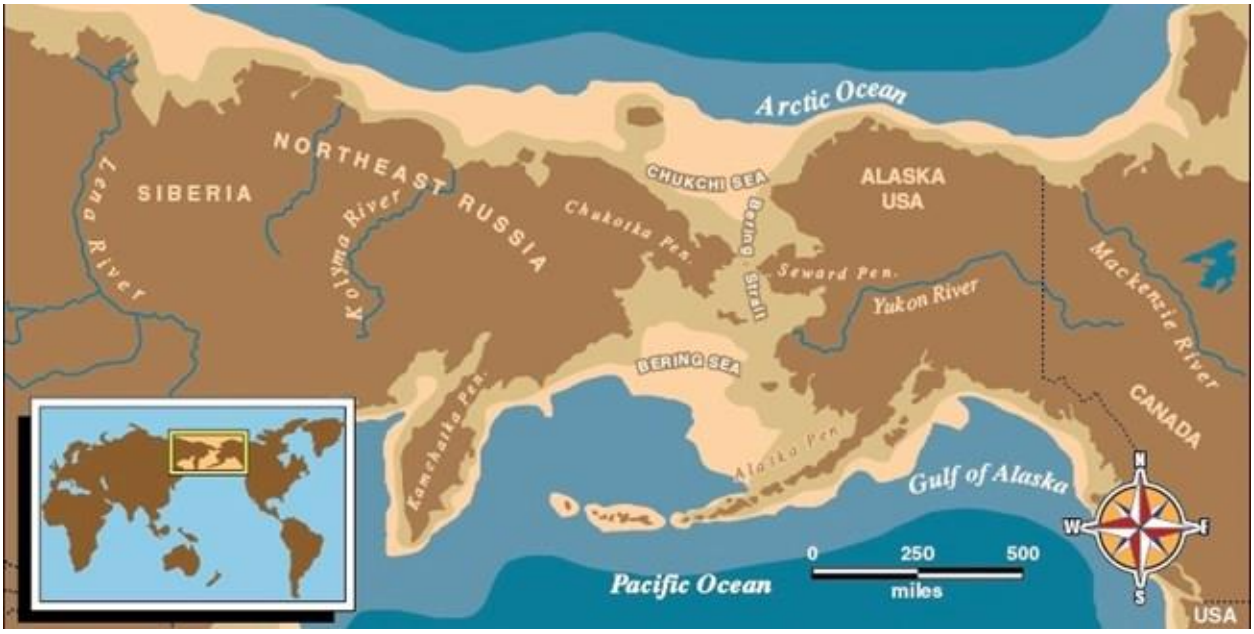

Figure 1: Beringia (National Parks Service, 2018)

The timing and mode of the initial peopling of the Americas is a topic of intense debate (Dillehay, 2009; Pitbaldo, 2011), and has been the focus of research by many archaeologists, biological anthropologists, and molecular anthropologists (Dillehay 2009). Regarding the timing of human arrival in the Americas, it is generally agreed that the first Americans entered North America via Beringia between 20,000 and 15,000 years ago (Dillehay, 2009; Pitbaldo, 2011). The process probably started before the Last Glacial Maximum (LGM; 26,500-19,000 years BP) in south-central and southeastern Siberia, following the Yenesei and Lena rivers north, before turning east towards Beringia (Lapointe et al., 2017;

Pitbaldo, 2011). Some recent studies suggest a period of standstill in Beringia, before the dispersal into the Americas (Goebel et al., 2007).

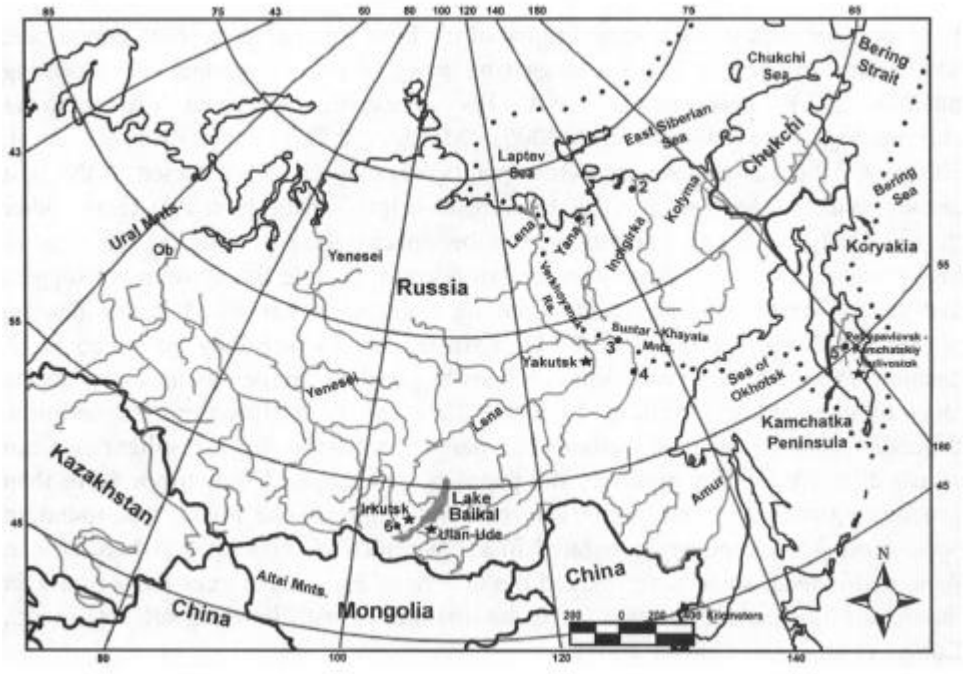

Figure 2: regions where migrants into the Americas originated from (Pitbaldo, 2011)

Beringia, the land bridge that spanned from Siberia eastward to Alaska and Canada, became exposed for the last time 25,000 years BP (Hopkins, 1982), as the sea-level dropped during the Last Glacial Maximum. The land bridge was occupied by a now extinct biome known as the steppe-tundra or mammoth steppe (Lapointe et al., 2017; Pitbaldo, 2011). The Late Wisconsinan maximum (ca. 23,000 to 19,000 years BP) lead to the interior of the land bridge to be covered by the Laurentide and Cordilleran Ice Sheets, making it inhospitable for human migration (Heintsman et al., 2016; Hickin et al., 2016; Mandryk et al., 2001; Pitbaldo, 2011). Between 18,000 and 13,000 years BP, the potential human food sources of the interior of Beringia were below minimum nutritional needs of a human group even during periods of land exposure (Mandryk et al., 2001).

Figure 3: Ice Sheets of the Last Glacial Maximum (Kansas Geological Survey, 2009, http://www.kgs.ku.edu/Publications/PIC/pic28.html)

However, the coast of this great landmass was more environmentally favorable to human migration (Mandryk et al., 2001; Pitbaldo, 2011). The coast of Beringia during the Last Glacial Maximum was less glaciated than previously thought and could have supported plants and animals, including sea

mammals (Mandryk et al., 2001). Even during the Late Wisconsinan maximum, the coastal glaciers may not have blocked movement along the coast; however, the coast became ice-free by 15,000 to 14,000 years BP (Mandryk et al., 2001).

Therefore, human migration into the Americas may have followed the ice-free Alaskan coastline down into the Pacific Northwest, around 16,000-15,000 years BP. At the present, the record is scarce for coastal sites dating to this critical period (Potter et al., 2018; McLaren et al., 2018). Initial dispersion must have relied on coastal and marine resources as they crossed over Beringia, perhaps employing boats to capitalize on this rich environment (Pitbaldo, 2011). Pushing further into the Pacific Northwest, migrants would have encountered the now submerged continental shelf off British Columbia. Between 13,000 and 10,000 years BP, this region was a biologically diverse and vegetated open tundra with productive non-arboreal shrubs, grasses, and sedges (Mandryk et al., 2001).

The American coast continued to offer Siberian migrants viable subsistence, and they continued their coastal journey down to the Isthmus of Tehuantepec. Perhaps at this natural bottleneck, populations split: one continuing on the Pacific coast to South America and the other moving back North towards Mexico (Pitbaldo, 2011). Continuing South, the next geographical bottleneck would have taken place at the Isthmus of Panama, with human groups spilling both east and west down the coast of South America (Pitbaldo, 2011). By approximately 14,600 years BP, descendants of these first Americans settled in Monte Verde, in Chile (Mandryk et al., 2001; Pitbaldo, 2011).

Figure 4: Isthmus of Panama (Google Maps, 2018)

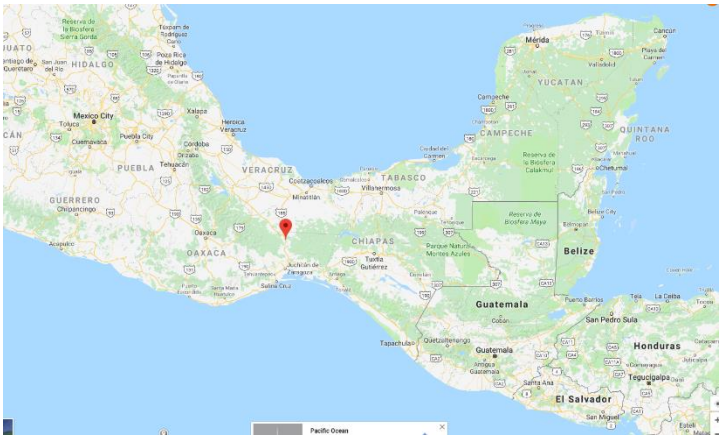

Figure 5: Isthmus of Tehuantapec (Google Maps, 2018)

A second wave of human migration, probably also originating from south-central and southeastern Siberia or from a population living on Beringia, traversed the land bridge in a different way (Pitbaldo, 2011). Instead of following marine resources along the coast, migrants pushed through an ice-free corridor (Burns, 1996 suggests renaming it the “western interior route” based on the width of the pathway) that opened in present-day Canada (Pitbaldo, 2011). The retreating of the Laurentide, Cordilleran, and montane ice from Northwestern Canada left the region subaerially exposed ca 18,000 to 13,000 (Hickin et al., 2016; Mandryk et al., 2001). Horse, bison, and mammoth were present even before the retreat of these ice sheets. However, subsistence resources were scarce until 13,400-11,500 years BP (Burns, 1996; Heintsman et al., 2016; Mandryk et al., 2001; Waters et al., 2015). When the

glaciers retreated, the mammoth steppe landscape returned to eastern Beringia, bringing with it associated horse, bison, and mammoth fauna, like the fauna of western and central Beringia. Alongside the megafauna came steppic flora that rapidly established themselves in the region due to their predilection for cool, dry conditions and the graminoid and herbaceous species of steppic flora that mature rapidly and reproduce vegetatively. This combination of fast-growing plant life and faunal species poised to exploit these resources meant that the newly ice-free landscape recolonized over hundreds, not thousands, of years (Burns, 1996).

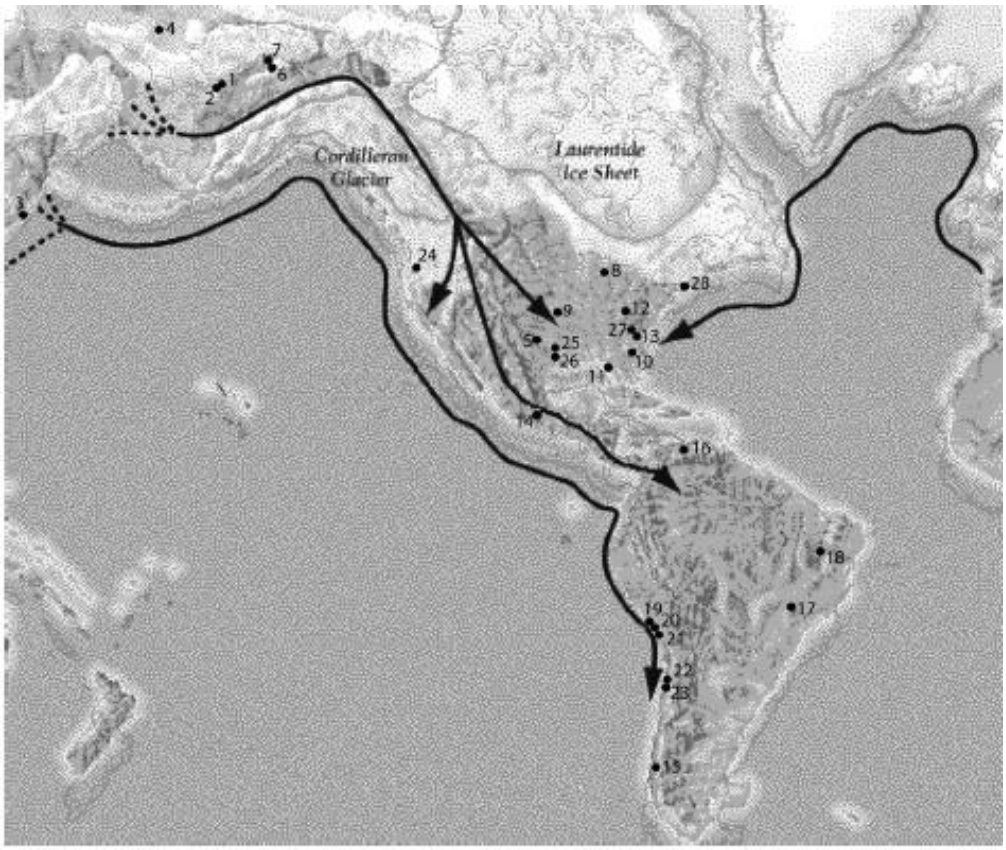

Figure 6: human migration into the Americas (Dillehay, 2009)

At eight butchering sites in Wally's Beach in Alberta, Canada, faunal remains and their nondiagnostic lithic artifacts have been radiocarbon dated to 14,800-12,700 years BP (Waters et al., 2015). Wally's Beach and other North American butchery sites contain faunal remains that encompass 6

of the 36 genera of megafauna that went extinct by 12,700 years BP (Waters et al., 2015). Early evidence of human hunting in North America lends to the idea that the second wave of Berigian migrants followed bison, horses, and other fauna east over the land bridge, arriving in America by 13,400-11,500 years ago just before the Clovis culture emerged in the interior of North America (Heintsman et al., 2016; Pitbaldo, 2011; Waters et al., 2015). By the end of the Pleistocene, the Clovis culture spread throughout North and Central America (Pitbaldo, 2011). The Clovis people are a group of big game hunters that archaeologists named the first Americans (Stanford and Bradley, 2012). They are most often identified with the Clovis point and associated lithic tool complex which is identified by longitudinal flakes that have been removed from the base of the lithic on both faces, called fluting (Stanford and Bradley, 2012).

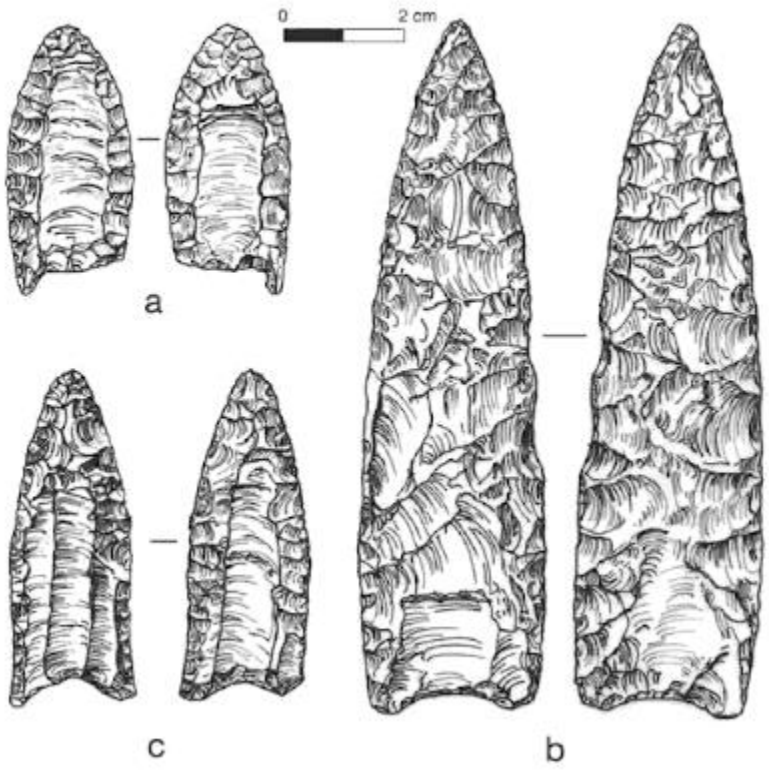

Figure 7: examples of the Clovis lithic industry, including the Clovis point (Stanford and Bradley, 2012)

This brief review of the settlement of the Americas suggests a series of complex process of dispersion and colonization that resulted in the occupation of the diverse ecological zones of North, Meso- and South America. These processes certainly impacted the way that the biological diversity of the Native Americas is organized, and many studies have explored the origins of the biological diversity in the continent (for a good overview on current research see Pitbaldo, 2011). However, despite the differences observed between South and North American colonization, most studies of the biological diversity in the continent assume the Americas as only one single geographic regions (e.g., Relethford, 2001), ignoring the differences in the observed between the Southern and Northern continent (Herrera et al., 2017). To contribute to this discussion, here I test the hypothesis that the cranial morphological variation of Native Americans from North and South America are similar, and therefore that the Americas can be grouped together in one single geographic region when analyzing the biological diversity of Native Americans.

Cranial Morphology as a Proxy for Genetic Variation

The analysis of the biological diversity between North and South American populations rests on the assumption that the human skull can be used as tool to reconstruct biological similarities between populations (e.g., Hubbe et al., 2010, 2011, 2014, 2015). Therefore, in the next sections I present the background theory that supports this study.

Patterns of cranial morphological variation in the planet

The human skeleton is a dynamic organ that is a part of the musculoskeletal system. It functions as support for the surrounding soft tissue, providing strength, structure, and lever arms on which muscles operate (White and Folkens, 2005). Bone has a wide range of phenotypic variation resulting from four major factors (White and Folkens, 2005). Ontogeny, or growth, results in variation between

fetal and adult skeletons. Sexual dimorphism manifests in the difference in size and shape between male and females. Geographic, or population-based variation, is attributed to genotype resulting from population genetics. Finally, idiosyncratic variation is the variation between individuals not covered by the other three types and can be attributed mostly to environmental factors. Understanding these sources of variation is fundamental to the study of craniometrics (White and Folkens, 2005).

The focus of this study, the human cranium and mandible, has been divided by many craniometric studies in three anatomical regions that have different developmental histories. They include: the cranial base, the neurocranium, and the splanchnocranium (Martínez-Abadías et al., 2009). The cranial base is composed of five bones: the ethmoid, sphenoid, occipital, frontal, and the temporal bones (Joshi et al., 2013). The neurocranium, or the skull cap, is made up of the frontal, parietals, and occipital bones. The splanchnocranium is the face, made up of the frontal bones, the zygomatic bones, maxillae, palatines, vomer, inferior nasal conchae, ethmoid, lacrimal, nasals, and mandible (White and Folkens, 2005).

The phenotypic variation observed in the skull and each of its anatomical regions is used for the study of the biological relationships between populations (e.g., Hubbe et al., 2010, 2011, 2014, 2015). This variation is comprised of both genotypic and environmental components. Craniometric studies in bioarchaeology focus mostly on the genotype, since this is the portion of variance that reflects the genetic relationship between individuals or populations. Genotypic variation arises from differences expressed in the genome and are divided into genetic additive, dominance, and interaction variances (Carson, 2006). Genetic dominance variance is variance due to the interactions of alleles at a locus (Byers, 2008). Interaction (or epistatic) variance is variance due to interaction between different alleles at different loci (Byers, 2008). Additive variance contributes to phenotypic variation between individuals and can be estimated from related individuals in a population (Carson, 2006).

The variation present on the skull is widely used to study genotype of the individual to determine biological affinities (White and Folkens, 2005). This morphological variation is measured by a series of craniometric measurements of anatomical regions which have been demonstrated to have a strong genetic component and therefore are highly inheritable. In other words, the visible phenotypic traits of the skull are diagnostic of genotypic background of the individual.

Craniometric traits are used to study the structure and history of human populations (Hanihara, 1996; Martínez-Abadías et al., 2009), because of it genetic background and the fact that it has been shown to reflect the same patterns observed for neutral molecular markers. For example, isolation by distance theory states that as geographic distance increases between populations, genetic similarity will decrease exponentially due to the decrease of gene flow (Relethford 2004). This theory has been used to explain several aspects of the genetic relatedness between populations on a regional and global scale (Relethford, 2004), including the cranial morphological differences between and within continents (Relethford 2001, 2004). Indeed, cranial morphology shows the same pattern of apportionment of variance as neutral genetic markers, supporting the claim that it can be used for the reconstruction of biological relationships between populations.

Besides the pattern of isolation by distance, it also observed that human genetic diversity for many traits is highest in sub-Saharan Africa (Relethford, 2001). This pattern has been found in mtDNA, microsatellite DNA, craniometrics, and skin color (Relethford, 2001), and again support the idea that skull morphology is reflecting the same pattern of biological relationships as genetic distances. These patterns also indicate that sub-Saharan Africans have been living in Africa longest, and have been used to support the argument that these populations are at the root of the human origins (Relethford, 2001).

Genetic relatedness can be estimated through the relationship between the variance among groups compared to the variance within groups, defined in population genetics as the Fst value. Even

though Fst relates specifically to genetic data, it can be estimated for phenotypic (craniometrics) data as long as heritability is taken into account (Relethford, 1994). Heritability (h^2) is the measure of how much of the variation in phenotype can be attributed to the genetic component of the individual. Heritability ranges from 0 to 1, where 0 means that none of the phenotypic variance is the result of the genetic background, and 1 means that all the variance is due to genetic background. Quantitative genetic models for the human skull suggest high heritability values, ranging from h^2= 0.55 to h^2=1 (Carson, 2006; Relethford, 1994; Roseman and Weaver, 2004).

More specifically, Fst is a descriptive statistic measuring the genetic differentiation between groups. Fst values range from 0 to 1, where small values indicate most of the variance in the populations is explained by differences within populations (i.e., populations are very similar), whereas large values indicate that most of the variance is due to differences between populations (i.e., populations are biologically different). As such, it is a common and useful measurement of differences between populations.

Many studies have followed this approach to study biological affinities among modern human populations. For example, craniometric data has=ve been used by Hanihara (1996) to discuss the Out of Africa vs. multiregional continuity hypothesis. Hanihara collected 23 craniofacial measurements from 1,802 individuals from major geographic areas of the Old World. Cluster analysis and multidimensional scaling of the data shows how closely related groups are. From these data, Hanihara (1996) concludes that the out of Africa hypothesis is the most compelling explanation for the data he presents. This study suggests that there is enough diversity of the craniofacial features of major human groups to study migration and microevolution (Hanihara, 1996). Similarly, Relethford (2001) used craniometric data to show that globally, 10% of genetic variation exists among major geographic regions, 5% exists among local populations within these regions, and 85% of the total genetic variation exists within local populations. Pooling local groups inflates regional diversity (Relethford, 2001). Complementing this

paper, Manica et al. (2007) show that 19-25% of heritable variation in craniometric measurements are explained by distance from Africa.

Cranial Variation in American Samples

Craniometric methods have been applied to the study of diversity in the Americas. The Americas generally show high levels of inter-group cranial variation (Herrera et al., 2017; Hubbe et al., 2015). Relethford (2001) found that by using regional aggregates, the Americas have very high regional diversity, but very low levels of regional diversity when estimating variation by averaging values within local populations. He also notes that the Americas show the highest Fst values (Relethford, 2001). This seems counterintuitive as both molecular and craniometric variation decrease with increased distance from Africa (Herrera et al., 2017; Hubbe et al., 2015; Releford, 2004). This variation may be explained by microevolutionary events (i.e. genetic drift and gene flow) and multiple migrations into the Americas (Dillehay, 2009; Herrera et al., 2017; Hubbe et al., 2010; Manica et al., 2007; Pitbaldo, 2011; Sardi et al., 2005). It is also possible that the difference between diversity in morphological studies versus molecular studies may be due to phenotypic variance not correlated with loci in genetic studies (Hubbe et al., 2015; Hubbe et al., 2010; Neves et al., 2013).

There is much evidence for multiple migrations into the Americas. Neves and Hubbe (2005) note that late prehistoric, recent, and present Native Americans have distinct cranial morphology from the earliest South Americans. The first group is most like late and modern Northern Asians. On the other hand, early South Americans share many similarities with present Australians, Melanesians, and Sub-Saharan Africans (Neves and Hubbe, 2005). This suggests that two biologically distinct populations (or perhaps several populating waves) colonized the New World during the Last Glacial Maximum at the boundary between the Pleistocene and Holocene (Hubbe et al., 2010; Hubbe et al., 2015; Neves and Hubbe, 2005; Neves et al., 2013; Sardi et al., 2005).

In this paper I present a study of craniometric variation in North and South American based on data compiled from multiple data sets (Hanihara, 1996; Hubbe et al., 2015), representing populations from East Asia, North-East Asia, extreme North America, North America, and South America. I measured the Fst values of each of these populations and the Americas as a whole, to test the hypothesis the biological diversity in the two American continents is similar.

Materials and Methods

In this study, I used craniometric collected by Tsuheniko Hanihara (1996) and by Walter A. Neves (Hubbe et al. 2015). In total, 2707 individuals from 49 different populations were sampled for this study. Only males were included, to reduce differences resulting from sexual dimorphism and because Hanihara's dataset includes considerably fewer females than males.

Individuals were organized in 49 populations, which were then grouped into ten geographical regions in the Americas and Asia: All populations (ALL), extreme North America (NNAM), South America (SAME), North America (NAME), East Asia (EASI), North East Asia (NEAS), the total population without the Paleoindians (ALLWPA), Andes (ANDES), Atlantic South America (SAMEATL), and Atlantic South America without the Paleoindians (SAMEATLWPA) (Table 1). These groups are not mutually exclusive, and populations could fall into one or more category.

Table 1 – Geographic groups created with the craniometric datasets.

GROUPS	# OF POPULATIONS	# OF INDIVIDUALS
ALL INDIVIDUALS	49	2707
ALL INDIVIDUALS WITHOUT THE PALEOINDIANS	48	2697
NORTH AMERICA	14	972
EXTREME NORTH AMERICA	12	720
SOUTH AMERICA	12	423

EAST ASIA	8	404
ATLANTIC SOUTH AMERICA	7	105
ATLANTIC SOUTH AMERICA WITHOUT THE PALEOINDIANS	6	95
ANDES	5	317
NORTH EAST ASIA	3	188

To combine Hanihara's and Neves' datasets, only craniometric variables that were common to both data sets were chosen. Twenty-one variables were initially available. However. variables had to be removed to minimize the number of missing values in the dataset, given than the South American Samples are less well preserved than the samples included in Hanihara's dataset. To exclude missing values, I initially removed variables that were represented in less than 50% of the South American populations. Next, I removed individuals with less than 50% of the variables present. In the end, all the analyses in this paper were done with 14 variables (Table 2).

Table 2 – Craniometric variables

VARIABLES
GLABELLO-OCCIPITAL LENGETH (GOL)
NASIO-OCCIPITAL LENGTH (NOL)
MAXIMUM CRANIAL BREADTH (XCB)
MAXIMUM FRONTAL BREADTH (XFB)
BIAURICULAR BREADTH (AUB)
BIASTERIONIC BREADTH (ASB)
FRONTAL CORD (FRC)
PARIETAL CORD (PAC)
OCCIPITAL CORD (OCC)
BIZYGOMATIC BREADTH (ZYB)
INTERORBITAL BREADTH (DKB)
ORBITAL HEIGHT (OBH)
NASAL BREADTH (NLB)
NASAL HEIGHT (NLH)

Missing values were then replaced with the estimated values from a multiple linear regression with the mean of the missing variable as dependent and all others as independents (Hubbe et al., 2011). Missing values replacement was done in R (R Core Team 2017), with a function written for that purpose by M. Hubbe. Single step multiple regression was used because it is less time consuming than an iterative method (Hubbe et al., 2011). The nature of replacing values lends itself to an increase in error for the entire sample, specifically altering the within/between group variances. Since the missing value was regressed using the mean of all individuals in the dataset, the within-group variance will increase and the between group variance will decrease. To reduce this bias, individuals with few values for variables and variables with few measurements were removed thus reducing the number of values that needed to be replaced, leading to a stronger estimation (Herrera, et al., 2017; Hubbe, et al., 2011).

To test our hypothesis that genetic diversity in North and South America is similar, I applied multidimensional scaling and Fst estimation to understand the morphological affinities between series and the amount of variance between groups. All analyses were performed in R (R Core team 2017) with functions written by M Hubbe.

Multidimensional Scaling

Mahalanobis Distance (D^2; Mahalanobis, 1936) was used to estimate morphological affinities. In R (R Core Group, 2017), pairwise D^2 between groups and then null distributions based on bootstraps was calculated. Next, the D^2 matrix was visually represented through bi-dimensional Non-Parametric Multidimensional Scaling (MDS). Multidimensional Scaling (also known as perceptual mapping) is a method to reduce multiple variables into distance represented in multidimensional space and has no unit (Hair Jr., et al., 2009).

Fst Calculation

Population differentiation can be expressed as the ratio of among-group variation to total variation expected. This measure is expressed as Fst, as described in the Introduction. Fst was calculated from a variance-covariance matrix of population relationships with an R matrix generated from the cranial measurements. The diagonal elements of the matrix represent the genetic distance of each sample to a regional centroid that is defined by mean allele frequencies (or by mean morphological traits, in this case). Fst was estimated from quantitative traits (craniometric measurements) using Relethford and Blangero's (1990) model of differential gene flow using quantitative variation (Relethford, 1994; Hubbe, et al., 2015, Herrera, et al., 2017). I used both h^2=1 and h^2=0.55 values to estimate Fst. These heritability values are common in studies of cranial morphology (Herrera, et al., 2017; Relethford, 1994; Relethford, 2001), making the resulting Fst values comparable to other studies. The heritability value is directly proportional to Fst, thus only changing the relative magnitude of Fst. Fst values were calculated for each of the 10 groups listed above (Table 1). In addition, I calculated both biased and unbiased Fst. Unbiased calculation of Fst values correct for errors caused by small sample sizes.

Results

Multidimensional Scaling Results

The results of the Multidimensional Scaling analyses are shown in Figures 1 and 2. These figures show the morphological affinities of populations in multivariate space. Figure 1 shows that both North American and Asian populations group towards the center of the graph. On the left side and towards the top, the extreme North American populations are spread. The South American populations are spread towards the right side and bottom of the graph, suggesting wide morphological variation that is distinct from in that continent. Both the extreme North American and South American populations are more

spread out than the Asian and North American populations, reflecting their higher and distinct morphological variation. The apparent outlier group, the Sumidouro Paleoindians are far to the right side of the graph, which is in accordance with previous studies suggesting that early Americans shared a distinct morphological pattern that recent Native American populations (Hubbe et al., 2010, 2011, 2014, 2015).

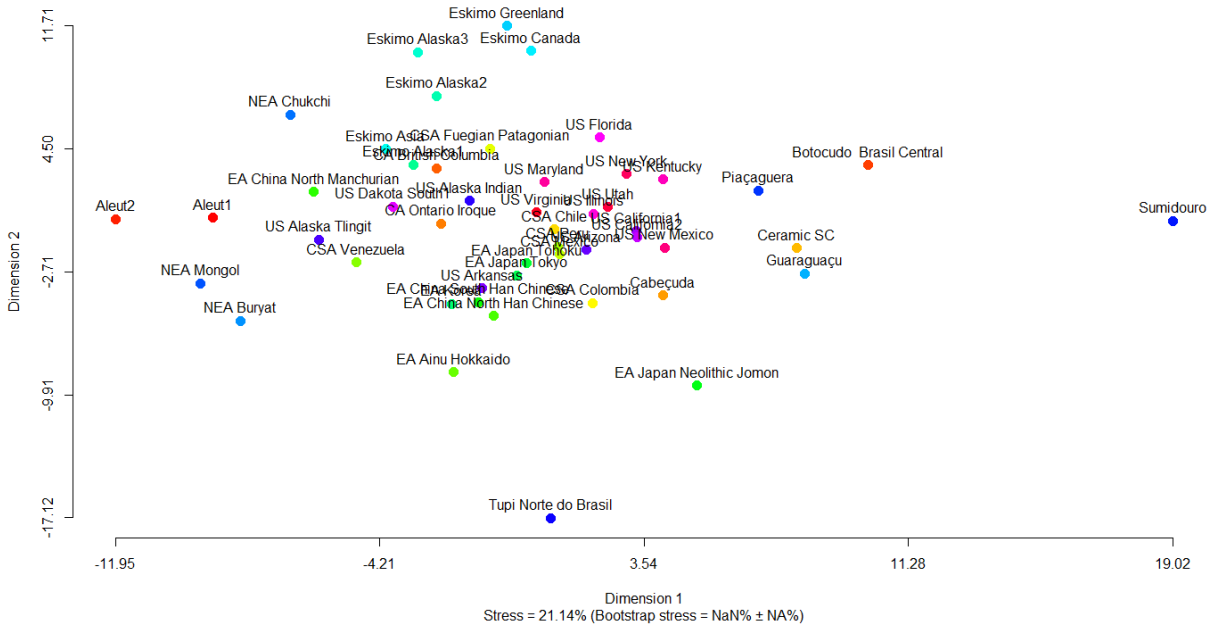

Figure 1: Morphological affinities according to the Multidimensional Scaling Analysis of the population samples in the dataset.

Figure 2 is a reduced MDS plot focused on the South American data. The populations from the Andean side of the continent are grouped together on the left of the graph, suggesting their low genetic diversity between these groups. The Atlantic South American groups are more spread around the graph

and represent the most extreme values in this plot, again suggesting high genetic diversity.

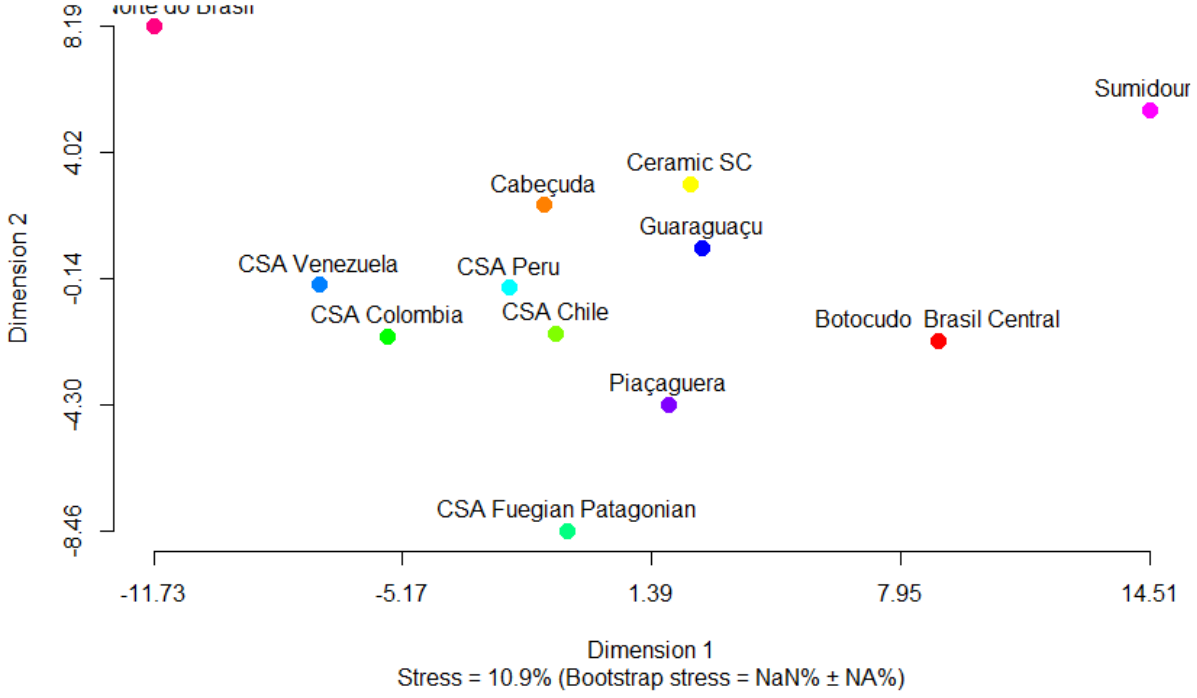

Figure 2: Morphological affinities according to the Multidimensional Scaling Analysis of the population samples from South America including the Paleoamerican data

Fst Results

Calculated Fst values are shown in Table 3. Biased and Unbiased Fst values were reported as well as Fst values with the h^2 value as 1 and 0.55 for comparability with other studies. The whole dataset has an Fst value of 0.1078 (h^2 =1) or 0.1885 (h^2=0.55) (unbiased values will be reported unless otherwise noted).

The highest Fst value came from the Atlantic side of South America (h^2 =1, Fst=0.1140; h^2=0.55, Fst=0.2069) series. The lowest Fst came from the Andes (h^2 =1, Fst=0.041 h^2=0.55, Fst=0.0899). North East Asia (h^2 =1, Fst=0.048), East Asia (h^2 =1, Fst=0.0647), North America (h^2 =1, Fst=0.0595), and

extreme North America (h^2 =1, Fst=0.0798) all have lower Fst values than South America (0.0992). The variation present in South America drives up the Fst value for the entire sample (h^2 =1, Fst=0.1078). Even with the removal of the Paleoindian sample (h^2 =1, Fst=0.1025), the Fst value is higher than any other region. Like East and North East Asia, North America and extreme North America exhibit low variation between groups (low Fst values), suggesting the responsible for the high Fst seen in the data is the high differences that exist between populations in South America.

Fst Values	***Fst Values, h2=1***			***Fst Values, h2=0.55***		
	Biased	Unbiased	SE	Biased	Unbiased	SE
All Individuals	0.12401	0.10779	0.0031	0.2047	0.1884836	0.00344
extreme North America	0.09128	0.07982	0.00429	0.15442	0.1429724	0.00501
South America	0.13101	0.09921	0.00859	0.21514	0.1833445	0.00945
North America	0.06891	0.05952	0.00329	0.11861	0.1092189	0.00398
East Asia	0.07674	0.06466	0.00581	0.13129	0.1192045	0.00694
North East Asia	0.06375	0.04797	0.00998	0.11017	0.09438856	0.01216
ALL individuals without the Paleoindians	0.11798	0.10247	0.0029	0.19563	0.1801189	0.00325
Andes	0.06771	0.041	0.00943	0.11665	0.08993709	0.01141
Atlantic South America	0.14977	0.11404	0.01201	0.24259	0.2068592	0.01285
Atlantic South America without the Paleoindians	0.12314	0.08979	0.01153	0.2034	0.17005	0.01284

Table 1: Fst values calculated for the different regions analyzed.

Discussion

Measurement of Fst

The Americas exhibit high morphological variation between groups as evidenced by the high Fst values obtained for all regions in this study. However, when separated into geographical regions, North America and East Asia show low differences between groups, suggesting this is the result of the high

morphological differences seen in the South American series. South America (h^2 =1, Fst=0.099; throughout the discussion, I will reference unbiased h^2=1 Fst values unless otherwise stated) has almost twice the inter-group variation than does East Asia (0.065), North East Asia (0.048), and North America (0.06). This agrees with previous studies that South American variation is high. When the Andes are removed from the sample, the Atlantic side of South America has the highest variation of all (0.114). However, when the Paleoamerican group is removed, the Fst value diminishes (0.09), supporting previous studies showing that the paleo South American series are morphologically distinct from the rest of the South American samples (Hubbe et al., 2010, 2011, 2014, 2015; Neves and Hubbe, 2005; Neves et al., 2013). Variation is also reduced from the total sample of the Americas (Fst=0.108) when the Paleoindian population is removed (Fst = 0.102), but the impact in this case is smaller.

Relethford (1994) estimated Fst for Europe, Sub-Saharan Africa, Australasia, Polynesia, the Americas, and the Far East. His estimate for Europe, Sub-Saharan Africa, and the Far East was 0.065 (assuming h^2=1). When Relethford included all six regions his minimum Fst value rose to 0.085. Our Fst estimation for the Americas (assuming h^2=1) was 0.108, much higher than the variation present between Europe, Sub-Saharan Africa, and the Far East, and the value for all six regions. Even excluding the Paleoindian group (Fst=0.102), the Americas exhibits more cranial variation between groups than does the other regions of the world. Because the work of Relethford (1994) includes only one South American series (Peru), he was unable to identify the differences seen in the Americas as a whole. This indicates that in the Americas, South America has a distinct morphological history that is not represented in North America. However, since all South American populations descend from North American groups (Neves et al., 2013), these results also suggest that the variation present in South American populations must have been somehow lost in North American populations.

Implications for the Peopling of the Americas

These data have implications for the peopling of the Americas. As Herrera et al. (2017) notes, human dispersal into the Americas is understood as a broad, continuous sweep into North and South America from Siberia that, as a theory, has a lot of support in the literature (Herrera, et al., 2017; Hubbe, et al., 2010, 2014, 2015; Mandryk, et al., 2001; Pitbaldo, 2011). However, migration away from the Pacific coast and towards South America is not well studied (Herrera, et al., 2017).

Human biological diversity can come from several sources: gene flow, genetic drift, geographic isolation, ecological variation, and non-random evolutionary processes (Herrera, et al., 2017; Sardi, et al., 2005; Perez and Monteiro, 2009). To understand variation in the Americas I must determine which of these factors influence cranial morphology.

Gene-flow mostly occurred from Siberia and Northeast Asia into North America and South America (Pitbaldo, 2011). Our study and others like it (Sardi, et al., 2005) show that South American populations are not as homogenous as would be expected by a single migration event. High cranial variation between groups suggests antiquity in peopling and the possibility of several waves of migration over time. Thus, the diversity present in the Americas had to come from a different source, perhaps two distinct migrations into the continent sometimes distinguished as Paleoamerican and Amerindian groups (Sardi, et al., 2005).

Gene flow can be interrupted by geographic isolation or boundaries. In the Americas, the retreating glaciers of the Last Glacial Maximum characterized the geography of North America, as did the Rocky Mountains along the Pacific side of the present-day United States. Continuing down, central America geographically “funnels” migration into South America (although Herrera, et al., 2017, support the idea that morphological diversity in Mexico is not a result of geographic barriers in the region). Human migration can fan out as it continues down into South America, but it is blocked towards the west by the Andean mountain range (Herrera, et al., 2017). However, in Australia and the correlated

islands, even when populations became fragmented by climatic changes from the Last Glacial Maximum, genetic diversity remained low. This is a case of geographic isolation and genetic drift not influencing genetic diversity, even on a longer time scale than in the Americas (Herrera, et al., 2017; Sardi, et al., 2005).

Perez and Monteiro (2009) suggest that non-random evolutionary processes and ecological diversity contribute to morphologic diversity seen in South America during the Holocene. Ecological stressors, rapid expansion, and niche differentiation on the American continent may contribute partially to the genetic diversity of the Americas. Diverse ecological regions could increase group separation and support genetic drift, even in geographically close groups (Sardi, et al., 2005; Perez and Monteiro, 2009). Nonrandom factors result in greater morphological diversity than genetic drift alone (Perez and Monteiro, 2009).

Variation in the Americas cannot be explained by one factor alone. Our results suggest that South America and North America have a very different genetic history from each other. Even though humans dispersed into South America via North America and into North America via NE Asia, the variation between the continents suggests that the North American samples are missing some of the variation exhibited by the South American populations.

Conclusion

Our results support previous studies (Herrera, et al., 2017; Sardi, et al., 2005) that the Americas, and especially South America have high cranial morphological diversity between groups. South American populations seem to have cranial variation that is not present in North America or NE Asia. This suggests that North and South America had very different biological histories. South America seems to have retained cranial variation that North America have lost. This variation could be explained by multiple

migrations into the Americas (a coastal migration and a land migration) or by another genetic event after the peopling of the Americas that reduced variation in North America, leaving traces of the original variation of the first Americans only in South American descendant populations.

I conclude that it is not beneficial to treat the Americas as one geographical area in human migration studies. High cranial variation in the Americas is indicative of different migratory or genetic forces that acted on past populations. Future studies should focus on understanding where the diversity in South America comes from, and why it is not present in North America if the peopling of the Americas came from Asia through North America into South America.

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