

Morphological variation among late holocene Mexicans: Implications for discussions about the human occupation of the Americas

Brianne Herrera¹  | Daniel Peart¹ | Nicole Hernandez¹ | Kate Spradley² | Mark Hubbe^{1,3}

¹Department of Anthropology, The Ohio State University, Columbus, Ohio

²Department of Anthropology, Texas State University, San Marcos, Texas

³Instituto de Arqueología y Antropología, Universidad Católica del Norte, Chile

Correspondence

Brianne Herrera, The Ohio State University, Columbus, OH, USA.

Email: herrera.63@osu.edu

Abstract

Objectives: Cranial morphology has previously been used to estimate phylogenetic relationships among populations, and has been an important tool in the reconstruction of ancient human dispersals across the planet. In the Americas, previous morphological studies support a scenario of people entering the Americas and dispersing from North America into South America through Meso America, making the Mexican territory the natural funnel through which biological diversity entered South America.

Materials and methods: We explore the cranial morphological affinities of three late Holocene Mexican series, in relation to ancient and modern crania from North and South America, Australo-Melanesia, and East Asia. Morphological affinities were assessed through Mahalanobis Distances, and represented via Multidimensional Scaling and Ward's Linkage Cluster analysis. Minimum F_{ST} values were also calculated for each series.

Results: Our results show Mexican groups share morphological affinities with the Native American series, but do not cluster together as would be expected. The minimum F_{ST} estimates show between-group variation in the Americas is higher than the Asian or Australo-Melanesian populations, and that Mexican series have high between-group variance ($F_{ST} = 0.124$), compared to the geographically larger South America ($F_{ST} = 0.116$) and North America ($F_{ST} = 0.076$).

Discussion: These results show that the Mexican series share morphological affinities with the East Asian series, but maintains high levels of between-group variation, similar to South America. This supports the suggestion that the high phenotypic variation seen the Americas is not a result of its size, as it can be found in more constricted areas, such as the Mexican territory.

KEYWORDS

craniometric variation, F_{ST} , Mexico, morphological affinity

1 | INTRODUCTION

The settlement of the Americas is still highly debated and unsolved, in spite of numerous years of focused study (see Dillehay and Sabloff, 2009; Pitblado, 2011, for good reviews). While the initial entrance date is still debated, there is now strong evidence that by around 12.5–11.5 kyr BP humans were present across both continents, from Alaska to the southern regions of South America (Chatters et al., 2014; Prates,

Politis, & Steele, 2013; Rasmussen et al., 2014). However, many other aspects of the initial dispersal also remain unsolved. Of special interest in the context of the current article are the routes taken into the continents and the number of dispersal waves from Asia (e.g., Achilli et al., 2013; Dillehay and Sabloff, 2009; Perego et al., 2010; Pitblado, 2011).

Human migrations into North and South America probably occurred through a combination of coastal and inland population movements (Achilli et al., 2013; González-José, Bortolini, Santos, &

Bonato, 2008), with differing degrees of contact between populations across the Holocene. Although these two possible migration routes have been highly dichotomized in the literature, it is likely that both were avenues of human dispersal throughout North and South America (Mandryk, Josenhans, Fedje, & Mathewes, 2001). The presence of different avenues for human dispersal during the Holocene means that human groups could have remained largely separated for several thousand years before significant contact occurred between populations following these expansion routes, especially where strong ecogeographic barriers occur, limiting gene flow between regions. This is particularly true for North and South America, where continental masses are large and show enough environmental diversity to support long-term periods of biological isolation between groups inhabiting these regions. However, Meso and particularly Central America represent different geographic realities, and the narrow landmasses in this area would probably diminish the potential for population isolation between groups by increasing the chance of gene flow between regions. In other words, Meso and Central America work as an evident geographic funnel into and from South America, facilitating contact and promoting gene flow between populations. As such, the population structure among populations in the region is expected to be different from what is observed in the large continents, and this has a potential impact on how we understand population dispersals from North to South America during the Holocene.

However, most papers discussing the human dispersion into the Americas primarily treat North, Central, and South America as one collective, undistinguished landmass (e.g., Achilli et al., 2013; De Azevedo, Bortolini, Bonatto, Hünemeier, Santos, & González-José, 2015; De Azevedo, Nocera, Pashcetta, Castillo, González, & González-José, 2011; Fagundes et al., 2008a; González-José et al., 2008; Hubbe, Neves, & Harvati, 2010; Neves and Hubbe, 2005; Sardi, Rozzi, González-José, & Pucciarelli, 2005). While this has been necessary to describe broad patterns of human dispersal into the New World, North, Central, and South America each contain environmental and historical particularities that impact the movement of peoples and patterns of gene flow (e.g., Hubbe, Okumura, Bernardo, & Neves, 2014; De Saint et al., 2012; Reich et al., 2012). In this article, we explore the observed differences between North, Meso, and South American biological characteristics, with special emphasis on the morphology of Late Holocene Mexican populations, to contribute to the discussion about the biological differences that exist among humans in these regions and how this can contribute to a more refined knowledge about the processes of human dispersion in the New World.

Despite having a late occupation when compared to the rest of the planet, the Americas show high levels of among-group cranial variation across time and space when compared to the rest of world (González-José, Dahinten, Luis, Hernández, & Pucciarelli, 2001; González-José, González-Martín, Hernández, Pucciarelli, Sardi, Rosales, & Van der Molen, 2003; González-José et al., 2008; Jantz and Owsley, 2001; Relethford, 2002; Ross, Ubelaker, & Falsetti, 2002; Sardi et al., 2005;), which is counterintuitive given the tendency of loss of within-group variation with increased distance from Africa observed in mod-

ern human molecular and craniometric data worldwide (e.g., Betti, Balloux, Amos, Hanihara, & Manica, 2009; Conrad, Jakobsson, Coop, Wen, Wall, Rosenberg, & Pritchard, 2006; DeGiorgio, Jakobsson, & Rosenberg, 2009; Handley, Manica, Goudet, & Balloux, 2007; Li et al., 2008; Manica, Amos, Balloux, & Hanihara, 2007; Ramachandran, Deshpande, Roseman, Rosenberg, Feldman, & Cavalli-Sforza, 2005; von Cramon-Taubadel and Lycett, 2008). Explanations for this high level of cranial variation vary greatly. Some authors defend the position that directional selection due to environmental factors, such as diet (Perez and Monteiro, 2009; Perez, Lema, Diniz-Filho, Bernal, Gonzalez, Gobbo, & Pucciarelli, 2011; Sardi, Novellino, & Pucciarelli, 2006), could be responsible for generating these patterns, while others suggest that between-group differences are a result of stochastic microevolutionary events, such as genetic drift, and gene flow (e.g., González-José et al., 2008; Pucciarelli, Neves, González-José, Sardi, Rozzi, Struck, & Bonilla, 2006). Building off the microevolutionary causes, others attribute between-group variation to multiple ancestral populations arriving in the Americas (e.g., Hubbe et al., 2010; Neves and Hubbe, 2005), greater degree of spatial or temporal isolation (Sardi et al., 2005), or earlier than estimated entry time (Sardi et al., 2005).

Even though it has been shown that the Americas hold relatively high levels of between-group cranial variation, most models discussing human dispersal into these continents are set up in a very linear fashion, with an assumed continuity in variation from North to South America (e.g., Lewis et al., 2007; Perez, Bernal, & Gonzalez, 2007). Furthermore, while it is acknowledged that the Beringian region created a bottleneck to the Americas (e.g., Amos and Hoffman, 2010; Fagundes, Kanitz, & Bonatto, 2008b; Ray, Wegmann, Fagundes, Wang, Ruiz-Linares, & Excoffier, 2010), Meso and Central America are rarely discussed as a bottleneck to South America.

Here, we explore the cranial phenotypic between-group variation of three late Holocene Mexican series in the context of Australasian and New World morphological variation. Previous admixture estimates (Juarez-Cedillo et al., 2008; Merriwether et al., 1997) and morphological studies (González-José, R., Neves, W., Lahr, M. M., Martínez, M. H., & Correal, 2005b; González-José, R., Ramírez-Rozzi, F., Sardi, M., Martínez-Abadías, N., Hernández, M., & Pucciarelli, 2005a) of Mexico suggest a similar pattern of between-group biological variation when compared to the rest of the Americas, with a high morphological differentiation across time and a homogeneous biological population sharing one common late ancestor in East Asia when Late Holocene groups are considered. However, the degree of differentiation among Late Holocene Mexican populations has not been studied in detail. Given the position of Mexico as the connection between North and Central America, this area of the continent is key to understanding the morphological differences observed between North and South American populations. If the American continent was indeed occupied in a linear way, and the high between-group morphological variation seen in the Americas is a result of population isolation due to gene-flow barriers and/or isolation by distance processes, then we would expect to see lower between-group differences within more geographically constricted regions, like Meso and Central America. Alternatively, if local



FIGURE 1 Map showing locations of samples. Orange represents the portion of Howells data set used. Blue represents the Mexican series. Pink represents the South American series. Sample sizes are shown in parentheses

processes of population movement during the Holocene are more important drivers of morphological adaptation than the expanse of the territory where the populations are, then the association between continental areas and phenotypic among-group variation may not be true. Therefore, in this context, the comparison of morphological affinities among Mexican populations during the Late Holocene, when compared to the variation observed in both North and South America, may contribute to discussions about the origins of morphological diversity in the continent. Analysis of morphological affinities of the Mexican series allows us to test the null hypothesis that the morphological variation

TABLE 1 Location, group, time period, and sample size of skeletal data used

Country	Location/group	Time period	N
Brazil	Lagoa Santa	10–7 kya	18
	Coastal	1 kya	59
	Tupí-Guaraní	1 kya	23
	Botocudo	1 kya	33
Colombia	Sabana de Bogotá	11–6 kya	13
Peru	Peru	Howells	110
	Tlanepantla	1200–1500 AD	16
	Michoacán	1200–1500 AD	26
United States	Arikara,	Howells	69
	Santa Cruz	Howells	102
Australia	Australia	Howells	101
Tasmania	Tasmania	Howells	87
Papua New Guinea	Tolai	Howells	110
Japan	North Japan	Howells	87
	South Japan	Howells	91
China	Hainan	Howells	83

TABLE 2 Linear measurements included in this study

Variables
Maximum cranial breadth (XCB)
Maximum frontal breadth (XFB)
Biauricular breadth (AUB)
Nasal height (NLH)
Orbital height (OBH)
Orbital breadth (OBB)
Bimaxillary breadth (ZMB)
Bifrontal breadth (FMB)
Nasio-frontal subtense (NAS)
Biorbital breadth (EKB)
Malar length, inferior (IML)
Malar length, maximum (XML)
Cheek height (WMH)
Frontal chord (FRC)
Frontal subtense (FRS)
Parietal chord (PAC)
Parietal subtense (PAS)

seen among groups in the Americas is a byproduct of the large continental masses that comprise these continents.

2 | MATERIALS AND METHODS

The Mexican samples included in this study come from Sonora, Michoacán, and Tlanepantla (see Table 1; Figure 1). The cranial series were a part of an expedition carried out by Ales Hrdlička in the mid-1900s, with the Michoacán sample excavated by Lumholtz. These crania are thought to be pre-Hispanic, dating to between 1200 and 1500 AD (Beekman and Christensen, 2003). The Mexican series were compared to reference populations from South America (Lagoa Santa, Brazilian Coastal sites, Botocudo, and Tupí-Guaraní Indians; Table 1 and Figure 1; Hubbe et al., 2014; Strauss, Hubbe, Neves, Bernardo, & Atui, 2015), as well as a subset of Howells' dataset that included series from the Americas, East Asia and Australo-Melanesia (Table 1). These series were selected due to the strong morphological affinities they show with Native American series throughout the Holocene (e.g., Hubbe et al., 2010; Hubbe, Harvati, & Neves, 2011).

All Mexican skulls were measured by Kate Spradley and Meredith L. Tise at the American Museum of Natural History, following Howells' guidelines (Howells, 1973), and 17 measurements were included in this analysis (Table 2). Prior to any measurements taking place, both Kate Spradley and Meredith Tise digitized at least 30 skulls in common and they were reviewed for inter-observer error. Most measurements were either the same or within 1 mm, but never more than a 2 mm difference, which indicates inter-observer error being of minor concern in the analyses performed here.

Variables included in the analyses were selected in order to minimize missing values in the prehistoric American series. However, not all missing values were removed in this initial screening of variables. Therefore, missing values were estimated via multiple linear regression analysis. To do so, the missing values were initially replaced by the mean of that variable across all series, and then the missing values (predicted values) were estimated by multiple linear regressions using the remaining measurements present for the individual as the independent variables (see Hubbe et al., 2011, for a longer discussion on the advantages and limitations of this procedure). Furthermore, no individuals with more than 33% of their variables missing were included in the analyses, reducing the impact of estimation biases in the final results. For all analyses, male and female individuals were pooled together, and the effects of size were removed by dividing measurements by the geometric mean of the individual (Hubbe et al., 2011).

Morphological affinities between series were estimated using Mahalanobis Distances (D^2 ; Mahalanobis, 1936), which has become a very common measurement of dissimilarity in morphological studies. The D^2 matrix of distances between pairs of series was represented visually with a Ward's Linkage cluster, which creates clusters by minimizing the within-cluster variance and maximizing between cluster variance (Hair, Black, Babin, & Anderson, 2009; Hubbe et al., 2014). Ward's clusters can generate artificial grouping because it tends to include outliers in larger clusters, but when differences between populations are small and no outlier populations are expected to be found in the analyses (i.e., all series belong to a same species), these clusters can be particularly informative about the underlying structure of the dissimilarities between groups (Hubbe et al., 2014). The D^2 matrix was also represented through a bi-dimensional Non-Parametric Multidimensional Scaling (MDS; Cox and Cox, 2001), which arranges a two dimensional space in such a way as to minimize the distances between the graphical solution and the original dissimilarity matrix (Hair et al., 2009). Unlike a tree-based structure for distance comparison, multidimensional scaling does not assume the patterns representing distance must be a bifurcating branch, and therefore does not force a strong topology among the series (Harvati and Weaver, 2006a). This makes the MDS a good complement to the Ward's cluster, since it acts as a check against any unreasonable groups generated by the Ward's analysis (Hubbe et al., 2014).

The statistical significance of the associations represented in the cluster and MDS analyses were approximated via resampling of the original series. In the case of the cluster, the series were resampled with replacement 1000 times each, keeping the original sample size the same. The frequency of times the same clusters were observed via resampling was used to estimate the robustness of the observed associations in the original data. To estimate the robustness of the original cluster solution, the resampling technique was used to calculate the confidence interval of the parameter calculated for each sample (in this case, the average morphology of the series). The resampling frequencies calculated for the branching patterns of the final cluster allows for an estimate of the robustness of the original cluster that was created. For the MDS, a similar resampling of the series was done 100 times

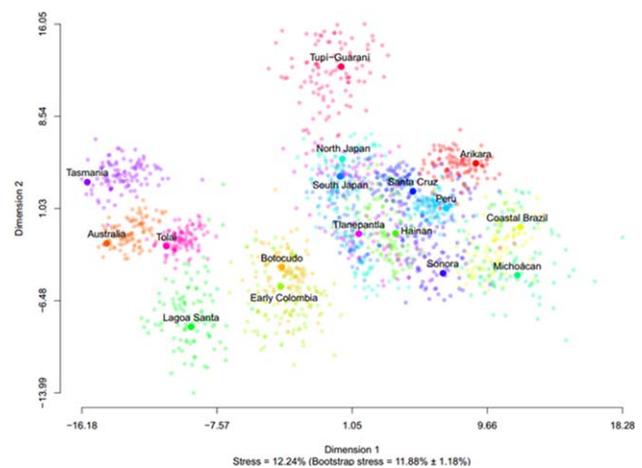


FIGURE 2 MDS derived using Mahalanobis distance and bootstrapping techniques [Color figure can be viewed at wileyonlinelibrary.com]

and MDS solutions for each resampling were superimposed on the original one using Procrustes Analysis, which removes differences of translation, rotation, and scale between them (Mardia et al., 1979). Similar to the cluster analysis, this resampling generates a dispersion cloud for the position of each series that represents the confidence interval of the series position in the MDS space. Therefore, through this procedure we are able to estimate the expected error of the morphological affinities in the analyses due to the uncertainty generated from having, in some cases, small sample sizes.

To complement the analyses of morphological affinities, and to explore the patterns of morphological apportionment between groups, we calculated minimum F_{ST} estimates (Relethford, 1994) for different subsets of populations in the dataset. Minimum F_{ST} values are found by averaging the diagonal of the R matrix generated from the cranial measurements (e.g., Hubbe, Strauss, Hubbe, & Neves, 2015; Relethford, 1994), and they represent the minimum apportionment of variance between groups (Relethford, 1994). F_{ST} values were calculated for North (both with and without Mexico), Meso, and South America (with and without the early series), as well as for the Americas as a whole. These values were then compared to F_{ST} values obtained for the other continents. F_{ST} estimates were calculated assuming heritability values of 1 for the craniometric traits. While such a high heritability is not a reasonable assumption for human cranial morphology (Carson, 2006; Devor, 1987), the adoption of different heritabilities does not change the relative magnitude of F_{ST} values between regions, as long as the real heritability value was the same for all datasets, which is a reasonable assumption in this case. All analyses were done in R (R Development Core Team, 2015), with functions written by Mark Hubbe and complemented by functions from packages *vegan* (Oksanen et al., 2015) and *MASS* (Venables and Ripley, 2002).

3 | RESULTS

The MDS analysis (Figure 2) shows clear groupings of different populations in two primary clusters, defined by the strong overlap of the

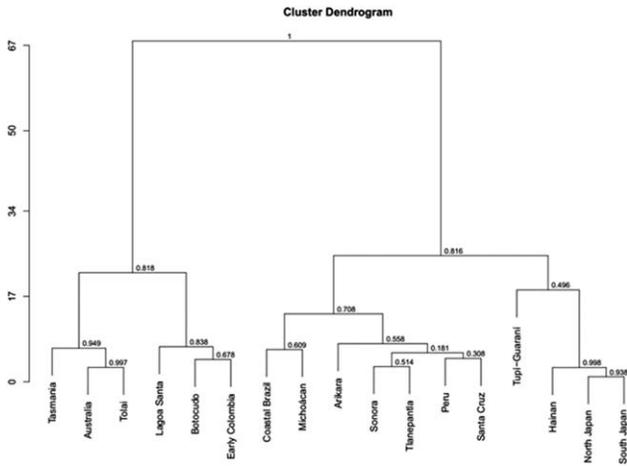


FIGURE 3 Dendrogram from Ward’s Linkage cluster analysis. Small numbers on branches indicate probability of finding the same cluster branch after 1000 resamplings

resampling results (cloud of dots in the graphs). The Mexican series appears in the second cluster of series, together with the Asian populations (North and South Japan, Hainan), Native Americans (Arikara, Santa Cruz), Peru, and the Brazilian Coastal sites. The second cluster includes the Australo-Melanesian series (Australia, Tasmania, Tolai) and the early American series from Lagoa Santa, highlighting the difference in early American morphology reported in numerous other studies (e.g., González-José et al., 2005a,b; Hubbe et al., 2010, 2011, 2015; Neves and Hubbe, 2005; Neves, Hubbe, & Piló, 2007a). The Botocudo and ancient Colombia populations appear in intermediate positions between the main clusters, following previous results (Neves, Hubbe, & Correal, 2007b; Strauss et al., 2015), while the South American Tupi appear as an outlier group in the first dimension.

The Ward’s cluster shows similar results (Figure 3) to the MDS analysis. The two clusters observed in the MDS analysis are clearly represented in the Wards cluster. The main difference from the previous results is that the Botocudo and Colombia series now cluster with Lagoa Santa in the second major cluster observed in the MDS, and the Tupi series clearly cluster with East Asians and recent Americans, despite its outlier positions in the MDS. The Mexican series are not clustered together, with Sonora and Tlanepantla clustering with the Howells series of Peru and Santa Cruz (although this cluster has been observed in only 15.3% of the resampling, and therefore cannot be considered a stable cluster), while Michoacán cluster with the Brazilian coastal sites (see Table 3).

TABLE 3 Affinities based on Mahalanobis distance (Figure 2) and cluster analysis (Figure 3)

Mexican population	Closest affinity based on MDS	Closest affinity based on cluster analysis
Sonora	Peru	Tlanepantla
Michoacán	Coastal Brazil	Coastal Brazil
Tlanepantla	Hainan	Sonora

TABLE 4 F_{ST} values for major population groups

Population	F_{ST} values
South America	0.301
Mexico	0.124
South America (without early samples)	0.116
North America + Mexico	0.113
Americas (without early samples)	0.109
Australia	0.081
North America	0.076
Asia	0.038

The minimum F_{ST} values calculated for the major population groups (South America, North America, Mexico, Australia, and Asia) show a clear delineation between groups (Table 4). South America has the highest F_{ST} value at 0.301, followed by Mexico at 0.124. Australia and North America have similar values at 0.081 and 0.076, respectively. Asia has the lowest values at 0.038. Interestingly, once the early South American series (Early Colombia and Lagoa Santa) are removed from the South American series, the F_{ST} value drops to 0.116, which is lower than the F_{ST} estimates for Mexico. This leaves the between-group variation in Mexico being on the same scale as South American populations. These results are consistent with many other papers reporting higher among-group variation in South America or the Americas as a whole (e.g., Hubbe, Hanihara, & Harvati, 2009; Sardi et al., 2005). Our South American estimates are higher than most other estimates (F_{ST} estimate from Sardi et al. (2005) = 0.1005; F_{ST} estimates from Hubbe et al. (2009) = 0.0873), but this is likely due to the series included here representing both modern and ancient crania (as shown in the decrease in value when the early South American series is removed from analysis; Table 4).

4 | DISCUSSION AND CONCLUSIONS

The human dispersal to the Americas is usually thought of in a linear, continuous fashion, with people moving from Northeast Asia over to North America and down into South America. In broader terms, there is support for this dispersal sequence, demonstrated by numerous craniometric, genetic and linguistic lines of evidence (e.g., Auerbach, 2012; Battaglia et al., 2013; Fagundes et al., 2008a,b; González-José et al., 2008; Hubbe et al., 2010, 2014, 2015; Mandryk et al., 2001; Reich et al., 2012; Wang et al., 2007). However, on a smaller scale, it is still not well understood how people were moving around in smaller geographic areas, such as Mexico. With Mexico being the beginning of a geographic “funnel” from North to South America, Mexico is an interesting area of study since it is a region in the continent that would facilitate gene flow between populations over the Holocene. Throughout North America, geographic barriers such as the Rocky Mountains and the Gulf of California caused significant separation that may have prevented contact between groups from the eastern and western parts of the continent for long periods of time. The vast longitudinal space

that characterizes Canada and the United States also must have increased the potential isolation between populations inhabiting the different macro ecological regions in the continent. Though not impassable, these geographic characteristics must have contributed to reduced gene-flow between populations, increasing the between-group differentiation in the continent. A similar pattern is also observed in South America, with the Andes acting as a strong barrier between eastern and western human populations (Pucciarelli et al., 2006). However, once human populations settled in Mexico, strong geographic barriers are less important over the Holocene and the geographic narrowing of the land mass toward southern Mexico would make it less likely for populations to remain isolated, which should promote a reduction of between-group morphological differences. However, our results suggest that these factors are not playing a strong role in the between-group differentiation among native Mexican populations. That said, all of our analyses suggest that our null hypothesis (that differences between groups in the Americas being simply a result of isolation by distance and gene-flow barriers deriving from the vast area of the continents) must be rejected.

Given that most of the variation between groups among modern human populations can be explained as genetic drift acting on small populations (e.g., Betti, Balloux, Hanihara, & Manica, 2010; Harvati and Weaver, 2006b; Katz et al., 2015; Relethford, 2001, 2010; Roseman, 2016; Roseman and Weaver, 2004, 2007; Smith, 2009, 2011; von Cramon-Taubadel, 2014; von Cramon-Taubadel and Weaver, 2009; Weaver, Roseman, & Stringer, 2007;), we argue that the relatively high morphological differences observed among the Mexican series included here are a result of stochastic processes. In some instances, such as the Buriat sample from the Howells dataset, there is evidence that the observed between-group variation exceeds what would be expected from neutral processes alone (Hubbe et al., 2009; Roseman, 2004), and would better fit a model of differential selection acting on morphology. However, climatic differences between the regions occupied by the three Mexican series are not extreme enough to justify any accumulation of morphological differences not due to stochastic effects.

The Mexican series included in this study show a morphological pattern associated with East Asian and Native American morphological patterns (Figures 2 and 3), even though they demonstrate a large range of between-group variation when compared to the rest of the continent (Table 4), and do not share high morphological affinities. Tlanepantla, Sonora, and Michoacán span a range approximately as large as North America (Arikara, Santa Cruz) and Asia (North/South Japan, Hainan) in the MDS analysis (Figure 2), despite their geographic proximity. The Hainan are closer to Tlanepantla than either Sonora or Michoacán, and the Brazilian Coastal sites are closer to Michoacán than either Tlanepantla or Sonora (Figures 2 and 3). This is an unusual pattern given that Michoacán and Tlanepantla are geographically very close (~274 km) compared to the distance between Michoacán and Sonora (~1561 km) or Tlanepantla and Sonora (~1770 km), indicating geographic distance is not a good predictor for morphological clustering.

The larger range of between-group variation exhibited by the three Mexican populations is similar to the range seen among some of the

South American populations (Lagoa Santa, Botocudos, and Early Colombia), although these have been argued in the past to represent a different morphological type sometimes referred to as Paleoamerican morphology (e.g., Powell and Neves, 1999; Hubbe and Neves, 2005). This suggests that the high between-group phenotypic variation in the South American continent (or even the Americas as a whole) is not a result of the large size of the continents, as the high variation is found in more constricted areas, such as the Mexican territory.

A difference between the South American and Mexican series analyzed is that the more modern Mexican populations distinctly cluster away from any of the early Holocene populations. While several papers found morphological similarities between some late American populations and Paleoamerican groups (Sabana de Bogotá, Botocudo, Pericues, Patagonians; González-José et al., 2003; Neves et al., 2007a, b; Perez et al., 2007; Strauss et al., 2015), the retention of Paleoamerican morphology is not observed in any of the Mexican series studied here (as seen in the MDS and the cluster analyses, Figures 2 and 3). This is interesting given the fact that early Holocene Mexican crania tend to show high affinities with other early Paleoamerican populations (González-José et al., 2003). While we cannot rule out the possibility of the Paleoamerican morphology having survived in isolated regions of the mainland Mexican territory, as it apparently occurred in Baja California (González-José et al., 2003), and given the limited number of samples available for this study, it is noteworthy that the high degree of morphological diversity observed among Mexican populations is not tied in any clear way to the morphological pattern that characterizes the early groups included in our analyses (Lagoa Santa and Colombia). In other words, the high between-group diversity among Mexican series reported here is more likely to be a result of reduced gene flow and genetic drift between them for longer periods of time.

Gene flow into the Mexican territory must have come from North America, since the geographic funnel leads from North America to South America. This is corroborated by several genetic studies supporting that all Meso, Central, and South American populations share one common ancestor (e.g., Reich et al., 2012; Silva et al., 2002), although multiple dispersion waves inside the continent have also been suggested by some genetic studies (e.g., Perego et al., 2009; Ray et al., 2010). Some evidence exists for a back-migration from South into Central America (Reich et al., 2012), which could have increased the biological differentiation between groups in Mexico, but the extent of this back-migration and if it reached the Mexican region is unclear.

Given that Mexico is geographically smaller than the United States and contains less geographic barriers, gene flow should have made populations in this region more similar to each other throughout the Holocene. This is not seen in our results, indicating that either the “funnel” analogy is incorrect, in that important geographic barriers were still maintained in the region over most of the Holocene, or populations were more differentiated prior to entering the “funnel” region and these differences were kept or even accentuated by genetic drift during the Holocene. Of course, language or cultural differences could have provided a barrier between these populations as well, given that the Mexican populations included in this study likely spoke different

languages and are dated to a period shortly after the arrival of Nahuatl-speaking groups (Beekman and Christensen, 2003). Given the relative proximity between at least two of the series included here, and that the geographic shape of Mexico narrows, forcing populations moving southward to be closer to each other, we suggest that the Mexican populations utilized in this study were already morphologically differentiated prior to occupying their territories. This, of course, needs to be taken as a hypothesis to be tested with more Mexican and Central American series in the future.

The relatively high level of population differentiation in the Mexican region, and in the Americas as a whole is more pronounced when compared to other regions of the planet. For instance, the well known separation of Australian and Tasmanian populations during the Late Pleistocene shows that isolation by distance and local adaptation alone may not increase the difference between groups (Sardi et al., 2005). Despite being separated for much longer periods of time than populations in the Americas, Tasmanian and Australian groups retained similar morphological patterns (Lahr 1996) and have small F_{ST} values (Table 4). Consequently, it is possible that the Americas have been characterized throughout the Holocene by very distinct groups from a morphological point of view, despite the low level of genetic diversity reported for Native Americans. A more variable morphological gene-pool could explain the diversity seen in Mexico and in the Americas in general.

Finally, our results can contribute to the discussion about the role of non-random evolutionary forces acting on cranial morphology in the Americas. Perez and Monteiro (2009), for example, tested the hypothesis that non-random factors can explain the high between-group morphological variation seen in the continent. Their results suggest that in areas where there is very large ecological variation, such as South America, non-random factors play a much more important role than is typically acknowledged and that the level of between-group variation present in South America is too large to be from drift alone. However, the Mexican series included in this study (Michoacán, Sonora, Tlanepantla) occupy less diverse ecological regions and a smaller geographic area. In spite of this, they have morphological differences comparable to crania from North America and modern South America. This is evident by the placement of the Mexican series within the Cluster and MDS analyses, as well as from the minimum F_{ST} estimates. The series that group near the Mexican samples are similar in both the Cluster analysis and the MDS analysis. In the Cluster analysis, the Michoacán share the same branch as the Brazilian Coastal sites and are within a larger branch including Arikara, Sonora, Tlanepantla, Peru, and Santa Cruz. The MDS shows the Mexican series clustering near the Brazilian Coastal sites, Arikara, Peru, and Santa Cruz, as well as North and South Japan. The Mexican series does not group together in either analysis, despite these populations being the closest to each other geographically. Because the Mexican populations are from relatively similar environments and they share affinities with Coastal Brazilians, who have a different subsistence strategy, it is unlikely that selection due to environmental pressures shaped their patterns of variation.

In conclusion, this study contributes to the characterization of biological diversity among Native American groups, by showing that con-

temporaneous Late Holocene populations from the Mexican territory have relatively high among-group morphological diversity. Although the series included here do not show evidence of the early Paleoamerican morphology that characterize early groups in the continent, the apportionment of variance between the Mexican series is still larger than what is observed among East Asians and Australo-Melanesians. As such, our results suggest that the high morphological diversity seen in the Americas is not a result of adaptations to a wide variety of environments or necessarily a result of geographic barriers to gene flow. While the Mexican series included here share high morphological affinities with the East Asian series, following the pattern from the rest of North America, Mexico still maintains estimates of between-group variation on the same level as South America. As such, our results support previous studies (e.g., Sardi et al., 2005) of high between group variation among Late Native Americans, expanding this observation into Meso America, and suggest that North and South America show high morphological diversity, even when the early American morphology is not taken into account.

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