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# The first human settlement of the New World: A closer look at craniofacial variation and evolution of early and late Holocene Native American groups

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## ABSTRACT

During its expansion across the globe, *Homo sapiens* successfully survived to major adaptive challenges as a species, inviting scientific research to plunge into the particularities of continental settlement dynamics. A recurrent paleoanthropological concern is about the understanding of the great deal of craniofacial diversity that evolved into the Americas, which includes a vector of continuum variation between a generalized morphology observed among humans groups leading the Out-of-Africa dispersion, and a derived set of craniofacial traits classically labeled as “mongoloid” and that would have arisen in Asia during the Holocene. Here, we use geometric morphometric techniques and multivariate statistics along with quantitative genetic approaches to look more closely into the human craniofacial evolutionary history during the Late Pleistocene–Early Holocene from Asia and the New World. We detected significant signals of deviation of the neutral evolutionary expectations, suggesting an important action of non-stochastic evolution (e.g. natural selection, phenotypic plasticity) in the Americas. We also found further support to the Recurrent Gene Flow model that refers to an ancestral, founder population experiencing a standstill in Beringia, and exhibiting high within-group craniofacial variation. This original, internally variable stock would have been the ancestral source of variation that fuelled the subsequent local micro evolution of other derived phenotypic patterns, giving origin to the craniofacial diversity observed among Holocene Native American samples.

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## 1. Introduction

The origin and evolution of modern humans is one of the most striking areas of paleoanthropological research. Somewhere in this research field, settlement of the Americas can be viewed as a natural experiment to study many aspects of human evolution. One reason for this is that human expansions around the globe were coincident with global climatic changes, so that the paths that may have been followed by human populations that first colonized the Americas were influenced by the climatic and environmental effects of the last glaciations during the Late Pleistocene (Dixon, 2013). Furthermore, *Homo sapiens* was successful in occupying most possible environments, and mechanisms causing both lost or gain of within and between-group diversity (mutation, selection, genetic drift, migration and gene flow) likely intervened in all

phases of the dispersal. Central questions around this debate are, how has the human skull evolved across anatomically modern humans' history and dispersion? and, how and when did *Homo sapiens* reach the Americas and dispersed into and across the New World?

The first settlement of the New World continues to be a highly controversial issue and is continually fuelled from various research fields such as geology, paleoecology, archaeology, skeletal biology, and genetics (e.g. Greenberg et al., 1986; Neves and Pucciarelli, 1991; Bonatto and Salzano, 1997a,b; Dixon, 2001; González-José et al., 2001a,b; 2008; Schurr, 2004; Zegura et al., 2004; Neves and Hubbe, 2005; Neves et al., 2005, 2007a; Tamm et al., 2007; Wang et al., 2007; Dillehay et al., 2008; Fagundes et al., 2008a,b; Goebel et al., 2008; Meltzer, 2009; Perego et al., 2009; Perez et al., 2009; de Azevedo et al., 2011, 2015; González-José and Bortolini, 2011; Reich et al., 2012; Bortolini et al., 2014; Marangoni et al., 2014; Raghavan et al., 2014a,b; Rasmussen et al., 2010, 2014; Raghavan et al., 2014b; Chatters et al., 2014; among others). The major consensus regarding how and when anatomically modern humans

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first entered the New World is that the Americas were first settled by populations coming from Asia via Beringia, at some temporal window at the end of the Pleistocene (~15–30 thousand years ago) and during the latter stage of the last glaciations, probably following a North–South direction along a Pacific coastal route (e.g. Dixon, 2001; Tamm et al., 2007; Wang et al., 2007; Fagundes et al., 2008a,b; González-José et al., 2008; Reich et al., 2012; but see also a brief review in Marangoni et al., 2014). The agreement begins to fade when attempting to detail the period in which it occurred, the area from which the colonizing populations came, the number of dispersal waves and the routes taken by these migrations (see a review in Marangoni et al., 2014), but also the microevolutionary processes involved. Researchers' views on various aspects of this process differ significantly, probably due to the fact that insights into the peopling of the Americas come from a variety of different types of data and disciplines (e.g. linguistic, paleoecology, archaeology, skeletal biology, genetics) but also because of different interpretations of the evidence. Moreover, recent publications presenting genomic data and population genetic analysis of contemporary Native Americans (Reich et al., 2012) and ancient genomes from early Siberian (Raghavan et al., 2014a) and Native American specimens (Rasmussen et al., 2010, 2014; Chatters et al., 2014; Raghavan et al., 2014b) reopened discussions of the pre-Columbian peopling of the Americas. The new evidence provides valuable information from comparative genetic studies, although a multidisciplinary reconciliation between genotypes and phenotypes, particularly human skull data (González-José et al., 2008; Perez et al., 2009; de Azevedo et al., 2011, 2015; Bortolini et al., 2014), is still needed as an essential step to understanding the evolutionary dynamics of populations occupying the Americas during pre-Columbian times.

Here we will focus on a classical discussion that has dominated part of the debate during the last decades and that includes two apparently mutually exclusive settlement hypotheses emerging from the analysis of skull morphology and molecular genetics (e.g. Single Wave versus Two Components/Stocks models, see a discussion in González-José et al., 2008). The main contrasting evidence coming from genes and morphologies is that the first indicates a single and Asiatic molecular coalescence for all Native Americans (e.g. Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Santos et al., 1999; Silva et al., 2002; Tamm et al., 2007; Fagundes et al., 2008a,b), whereas the latter points to a dual biological origin of American populations based on a significantly distinct morphology pattern observed between the earliest American populations (*Paleoamericans*) and that of recent Native Americans (e.g. Neves et al., 2003; Neves and Hubbe, 2005; Neves et al., 2007a; Hubbe et al., 2010).

Most molecular-genetics studies during the last twenty years point to a single origin in Northeast Asia (~15,000 BP) for almost all American populations, followed by local diversification (Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Santos et al., 1999; Silva et al., 2002; Tarazona-Santos and Santos, 2002; Tamm et al., 2007; Fagundes et al., 2008a,b). According to the molecular-genetics view, the initial differentiation from Asian populations was followed by a bottleneck in Beringia during the Last Glacial Maximum (around 23,000 to 19,000 years ago), and a strong population expansion out of Beringia between 18,000 and 15,000 years ago with a rapid settlement of the continent along a Pacific coastal route (Bonatto and Salzano, 1997a; Fagundes et al., 2008b). This pause by the ancestors of Native Americans when they reached Beringia would enable autochthonous mtDNA and Y-chromosome New World lineages to differ from their Asian sister-clades founder lineages. Also, the genetic data suggest more recent bi-directional gene flow between Siberia and the North American Arctic (Tarazona-Santos and Santos, 2002; Bortolini et al., 2002, 2003; Schurr, 2004; Tamm et al., 2007; Wang et al., 2007).

On the other hand, craniofacial morphology observed among some of the most ancient remains in the Americas (*Paleoamericans*) has been described as much closer to African and Australo-Melanesians populations than to the modern series of Native Americans (Neves et al., 2003; Neves and Hubbe, 2005; Neves et al., 2007a; Hubbe et al., 2010). Thus, differences in craniofacial morphology pattern between early and late Americans have been considered abrupt and have been explained by assuming the existence of two separate migration events into the continent: the first representatives are the *Paleoamericans*, having a distinct morphology that might be a retention of the morphological pattern seen in the first modern humans leaving Africa, between 70 and 55 thousand years ago (Mellars, 2006), and that would thus precede the morphological differentiation of East Asian populations that likely occurred during the Pleistocene/Holocene boundary and which would have given rise to all modern Native Americans through a second (more recent) wave into the Americas (Hubbe et al., 2010). This view assumes the existence of a supra-population unit of morphological affinity (classically named as *Mongoloids*) joining recent North Asians and late Holocene Native Americans (Neves et al., 2003), with a Middle/Late Holocene survival of *Paleoamerican* morphology reported for Sabana de Bogota, Colombia (Neves et al., 2007b) and Baja California, Mexico (González-José et al., 2003). Thus, the morphology pattern of recent Native Americans [characterized by short and wide neurocrania, high and retracted faces, and high orbits and nasal apertures] is considered as completely different from that of *Paleoamericans* [long and narrow crania, low and projecting faces, and low orbits and nasal apertures], and differences between these two morphologies are interpreted as being of roughly the same magnitude as the difference observed between recent Australian aborigines and recent East Asians (Neves and Hubbe, 2005; Neves et al., 2007a,b; Hubbe et al., 2010). However the Two-Component model disregards the significant amount of intermediate morphological variation that is present among Native American groups and places the full range of craniofacial variation under two discrete categories. In this context, there is an emerging view first published by González-José et al. (2008) that aims to integrate evidence from each research field considering the particular nature of change of different data (cultural, genetic-neutral, genetic plus environmental, etc) in order to accommodate the available evidence for each of the chronological phases of the settlement's sequence, and to postulate specific microevolutionary agents potentially responsible for the transition from one phase to the other.

This model (the Recurrent Gene Flow [RGF], González-José et al., 2008) takes into account a founding population occupying Beringia during the last glaciation characterized by high internal diversity in terms of craniofacial variation, mtDNA, Y chromosome lineages, and autosomal alleles. After a Beringian standstill and a posterior population expansion, which could have occurred concomitant with their entry into America, a more recent circumarctic gene flow would have enabled the dispersion (and persistence along circumarctic groups) of northeast Asian-derived characters and some particular genetic lineages from East Asia to America and vice versa (González-José et al., 2008). On the other hand, most modern American populations can be shown to have a mosaic of generalized-derived traits, with only a few displaying the derived extreme also present in northeast Asia (Inuits), and other groups presenting a rather generalized, ancestral morphology (noticeably, modern groups such as the Pericu from Baja California, Aztecs from Central Mexico, and *Paleoamericans* from Lagoa Santa). This model presents a synthetic view in which the main assertions of previous models may not be in serious contradiction with each other but collectively contribute to depict a common picture (González-José et al., 2008). For instance, the Two-Components model is viewed

as highlighting the extreme phenotypic values observable both in Asia and the New World, while their origin is explained by micro-evolutionary agents such as drift, gene flow, and perhaps directional selection, whereas the significant amount of intermediate morphological variation, rather than these extreme values, is highlighted as crucial for a synthesis. Since its publication, the RGF view has been further validated by evidence from several disciplines (e.g. Ray et al., 2009; Reich et al., 2012; Achilli et al., 2013; Marangoni et al., 2014; Bortolini et al., 2014; Chatters et al., 2014; de Azevedo et al., 2015; de Azevedo and González-José, 2015).

Here we aim to further evaluate the craniofacial variation pattern of anatomically modern humans across the Late Pleistocene and Early Holocene horizons from Asia and the Americas, attempting to add information that contributes to the discussion of the peopling of the Americas. In this context, within-group variation in the ancestral population may be an important source of between group diversity in the descendant groups (Lande, 1979). If craniofacial variation in the ancestral population of Native Americans was high enough to evolve from a generalized towards a specialized skull, then the demographic expansion of this population through north-East Asia, plus local processes of morphological differentiation in America resulting from random (i.e. genetic drift) and non-random factors (e.g. selection and plasticity), could be sufficient to explain both molecular and craniofacial data in pre-historic America. Also, if early Holocene Americans are representative of a first wave with retention of ancestral morphology (*Paleoamericans*) and all later Americans are the descendants of another biological, already extremely derived ('monogolized') population, we would expect to find an abrupt discontinuity between *Paleoamericans* and Native Americans groups, such as is expected according to a dual migration hypothesis.

To further explore within group variation out and inside the Americas and the discontinuity between early and late America

morphology, we used a sample that includes early fossils of the Late Pleistocene Old World (representing the best available proxy to the ancestors of the first Americans and Asians), early Holocene American individuals (representing North and South *Paleoamericans*) and modern (late Holocene) series from Australia, Asia and America. The goal of this work is to compare the amounts of shape variation across early and late series using measures of disparity that capture different aspects of shape diversification among individuals/populations within geographical and temporal groups, and to measure the amount of variance due to differences between early and late American series compared to total modern human group variation in our sample. Finally, we also explore the evolutionary causes of craniofacial diversification among modern human populations in America.

## 2. Material and methods

We performed a geometric morphometric analysis of a sample consisting of modern and ancient Asian and New World skulls (see Table 1 and Fig. 1 in González-José et al., 2008), whose craniofacial affinities were discussed elsewhere (González-José et al., 2003, 2008; de Azevedo et al., 2011; Bortolini et al., 2014). This sample covers the entire continuum of variation in shape found in Asia and the Americas, including 576 complete adult skulls of both sexes representing modern series from Australia and Asia, early and late series from South and North America, and a composite series of late Pleistocene Old World specimens (Table 1). We added the recent published specimen of Hoyo Negro (Chatters et al., 2014) to the *Paleoamerican* series (see also de Azevedo et al., 2015).

To recover the general shape of the skull in lateral view, the skull photographs dataset used here (Table 1) and elsewhere (González-José et al., 2008; de Azevedo et al., 2011) were obtained according to the recommendations made by Zelditch et al. (2004). Prior to

**Table 1**  
Sample composition. See also González-José et al. (2008, Table 1).

Code	Group	Population	Chronological range (years before present)	n
<b>EOW</b>	<b>Early Old World</b>	<b>Late Pleistocene Old World</b>		<b>13</b>
EOW		Herto, Ethiopia	(160,000–154,000/160,000–154,000)	
EOW		Cro Magnon, France	(25,000/25,000)	
EOW		Keilor, Australia	(12,000/13,840)	
EOW		Kow Swamp, Australia	(13,000–9000/15,320–11,170)	
EOW		Liujiang Guangxi Zhuang, China	(~60,000?)	
EOW		Minatogawa, Japan	(18,000–16,000/21,280–19,160)	
EOW		Mladec, Czech Republic	(~31,000)	
EOW		Qafzeh 9, Israel	(~100,000)	
EOW		Skhul 5, Israel	(~90,000)	
EOW		Shakameyama, Japan	(2300/2340)	
EOW		Shosenzuka, Japan	(13,000–8000/15,320–8985)	
EOW		Upper Cave 1, Zhoukoudian, China	(10,175/11,910)	
EOW		Wadjak 1, Java	(6500–10,560/7415–12,400)	
<b>PAM</b>	<b>Early America</b>	<b>Early North America</b>		<b>7</b>
EA		Chimalhuacán, Mexico	(10,500/12,405)	
EA		Kennewick Man, US	(9300/10,510)	
EA		Metro Balderas, Mexico	(9000/10,195)	
EA		Peñón III, Mexico	(10,755/12,810)	
EA		Cueva del Tecolote, Mexico	(10,500/12,590)	
EA		Wizards Beach, US	(9225/10,405)	
EA		Hoyo Negro skull (HN5/48), Yucatán	(~12,500)	
<b>LS</b>	<b>Early America</b>	<b>Early South America</b>		<b>11</b>
EA		Capelinha	(8860/9830)	
EA		Sören Hansen 01	(7000–9000/7765–10,175)	
EA		Sören Hansen 02	(7000–9000/7765–10,175)	
EA		Sören Hansen 03	(7000–9000/7765–10,175)	
EA		Sören Hansen 04	(7000–9000/7765–10,175)	
EA		Sören Hansen 07	(7000–9000/7765–10,175)	
EA		Sören Hansen 09	(7000–9000/7765–10,175)	
EA		Sören Hansen 16	(7000–9000/7765–10,175)	
EA		Lapa Vermelha IV (Luzia)	(11,000–11,500/12,915–13,300)	

(continued on next page)

Table 1 (continued)

Code	Group	Population	Chronological range (years before present)	n
	EA	Santana do Riacho III	(8000–9500/8770–10,690)	
	EA	Santana do Riacho XXIII	(8000–9500/8770–10,690)	
	<b>Modern America</b>	<b>Modern Americans</b>		
ACA	MNA	California, USA	Late Holocene	49
ARA	MNA	Araucano, Argentina	Late Holocene	43
BCS	MNA	Baja California Sur, Mexico	Late Holocene	23
BOL	MNA	Aymara, Bolivia	Late Holocene	18
CAL	MNA	Calama, Chile	Late Holocene	24
CHA	MNA	Chaco, Argentina	Late Holocene	10
ECU	MNA	Paltacalo, Ecuador	Late Holocene	53
ESK	MNA	Inuits, Greenland	Late Holocene	46
FUE	MNA	Fuegians, Chile and Argentina	Late Holocene	10
MAP	MNA	Mapure, Venezuela	Late Holocene	38
NPA	MNA	North Patagonians, Argentina	Late Holocene	18
PAT	MNA	Central Patagonians, Argentina	Late Holocene	38
PER	MNA	Ancon, Peru	Late Holocene	37
PG	MNA	Pampa Grande, Salta, Argentina	Late Holocene	25
TLA	MNA	Aztecs from Tlatelolco, Mexico	Late Holocene	26
	<b>Modern Asia</b>	<b>Modern Asians</b>		
AIN	MA	Ainu, Japan	Late Holocene	10
BUR	MA	Buriats, Siberia	Late Holocene	10
OUR	MA	Ourgas, Siberia	Late Holocene	18
TCH	MA	Tchouktchi, Siberia	Late Holocene	14
	<b>Modern Australia</b>	<b>Modern Australians</b>		
AUS	AUS	Aborigines, Australian	Late Holocene	36

Populations were grouped following a geographic and chronological criterion. MNA: modern Native Americans; MA: modern Asians, AUS: modern Australians, EOW: Early Old World, EA: Early Americans (*Paleoamericans*).

being photographed, each specimen was oriented according to the Frankfurt plane, and the prosthion-inion line defining the sagittal plane was positioned orthogonal to the camera objective. Parallax (e.g., rainbow) effects were controlled by situating the skull in the centre so that its image did not extend into the distorted region of the visual field. Landmarks and semilandmarks (see Fig. 1 in González-José et al., 2008) were digitized, scaled, and processed using TPSDig and TPSRelW software (Rohlf, 2003, 2004). Original configurations from all the series were superimposed using the Generalized Procrustes Analysis (GPA, Rohlf and Slice, 1990) to remove the effects of translation, rotation, and scaling. Sliding semi-landmarks placed along the contour of the cranial vault were relaxed following the minimum bending energy criterion using the TPSRelW routine (Rohlf, 2004). Previous works (Lahr and Wright, 1996; Rosas and Bastir, 2002) have revealed that there exist significant associations between robusticity and cranial size in modern humans: the larger the size of the skull, the greater the development of the cranial superstructures. However, we showed that, at least in this sample, the analysis made after removal of allometric effects on Native American groups showed a very similar pattern of among-group differences and affinities (see Figs. 2–3 in González-José et al., 2008).

The aligned shape coordinates obtained after Procrustes superimposition were subjected to a standardization of sex on female values (female adjusted value = female value + [male average – female average]) to avoid the potential effect of sex. Aligned shape coordinates were imported into MorphoJ (Klingenberg, 2011), R (R Development Core Team, 2015) and RMET software to compute further analysis.

### 2.1. Ancestral-derived morphological vector of craniofacial variation

We computed a discriminant analysis (CVA) using all series in Table 1. As previously described (de Azevedo et al., 2011), when series from Late Pleistocene and Early Holocene from Eurasia, Australia and the New World (Table 1) are placed into a coordinate system that describes the relative positions of groups in the sample (the canonical variates, CVs), the direction in which groups are most

effectively discriminated (CV1) can be seen as an ancestral-derived axis of craniofacial variation of anatomically modern humans. To visually evaluate the craniofacial variation pattern across the Late Pleistocene and Early Holocene horizons from Asia and the Americas and modern Australia along this ancestral-derived vector, we performed a 3D plot of the first canonical variates drawing 90% ellipses around five mayor groups: Modern Australia, Early Old World, Early America, Modern America and Modern Asia (see Table 1). This CVA analysis is not novel (was previously performed in de Azevedo et al., 2011) but we think its graphical representation is useful for the discussion in the present work.

### 2.2. Measuring disparity

We quantify shape variation following Drake and Klingenberg (2010) maintaining the geographical/temporal groups defined above: Modern Australia, Early Old World, Early America, Modern America, and Modern Asia. As a direct measure of the differences in shape, we computed Procrustes distances between all possible pairs of individuals per group. Procrustes distances between specimens were computed as Euclidean distances in tangent space (Dryden and Mardia, 1998). Two measures of shape disparity were computed. The first is the Procrustes variance of observations in each group, which is the mean squared Procrustes distance of each individual (or population average) from the mean shape of the respective group. Procrustes variance quantifies the average dispersion of data points around the mean shape. The second measure is the volume of the convex hull (de Berg et al., 2000) enclosing the data points of each group, which quantifies the portion of shape space occupied by the group. This is a measure of the degree of difference among opposite extremes in each group, and therefore it does not consider observations located near the center of the scatter of data points. Convex hulls were computed from the first two PCs because they contained most of the variation in the sample and because computation of higher-dimensional volumes presented computational difficulties. Finally, all the measures were computed on the Full Shape Space (the whole configuration of landmarks), the Facial Shape Space, and the Neurocranium Shape Space.

### 2.3. Measuring amount of variance among early and late Americans

Inter-group morphological variability between series was quantified by means of  $F_{st}$  estimates (Relethford and Blangero, 1990; Relethford et al., 1997). Minimum  $F_{st}$  estimates can be calculated from phenotypic data and represent a measurement of the amount of the variance seen in the data that is due to differences between groups. In order to measure the amount of variance due to differences between early and late American populations we computed  $F_{st}$  estimates among the two *Paleoamerican* series alone (*Paleoamericans* from north America [PAM] and south America [LS]) and then with each of the modern American series added one at a time, to measure how much of the between-group variance is contributed by each American series independently (a similar approach was recently used by Strauss et al., (2015) to test the hypothesis that Botocudo indians share the same morphological pattern as *Paleoamerican* groups).  $F_{st}$  values between *Paleoamericans* and American series were compared to  $F_{st}$  estimates for all modern series in our sample (as a reference of the magnitude of between-group variance that is observed among modern human groups represented in our sample).  $F_{st}$  estimates were computed on PC scores using RMET 5.0 software (Relethford and Blangero, 1990) assuming mean heritability values of 0.55. Finally, all the comparisons were computed on the Full Shape Space (the whole configuration of landmarks), the Facial Shape Space, and the Neurocranium Shape Space. We conservatively chose to retain the first seven, two, and five PCs for the Full, Facial and Neurocranium Shape Spaces which cumulatively account for 80, 64 and 87% of total variation, respectively. As a cutoff criterion we excluded all PCs that explain less variance than a PC with the average eigenvalue would explain (Krzanowski, 2000).

### 2.4. Quantitative genetic models and evolutionary forces responsible for cranial diversification

In order to investigate the causes of craniofacial variation among modern American populations studied here, we employ the  $\beta$ -test developed by Ackermann and Cheverud (2002), which is based on Lande's (1976, 1979) model aimed to test for signals of genetic drift by comparing the proportionality of among and within-population variances.

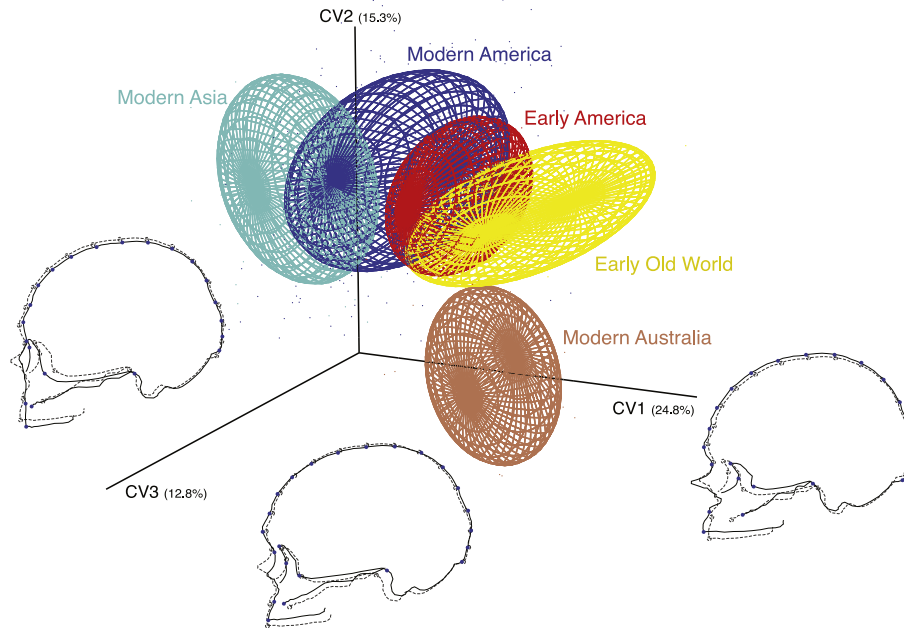
According to Simpson-Lande's adaptative landscapes metaphor, neutral evolution occurs on a drift landscape, which is a flat and level surface where selection does not affect the evolution of the population mean, which instead evolves in a trajectory that can be described by Brownian movement (Arnold et al., 2001). Then in theory, if a set of replicate populations derived instantaneously from the same ancestral population and diverged under drift alone, the expected character mean of all these descendant populations will be the same as the original, ancestral mean. Drift will not change the character mean, and although divergence among populations in mean can be appreciable, the variance/covariance (V/CV) matrix for the means of descendant populations will be proportional to the ancestral additive genetic V/CV matrix ( $G$ ) (see Fig. 6 in Arnold et al., 2001). Departing from this prediction, Ackermann and Cheverud (2002, 2004) and Marroig and Cheverud (2004) proposed the  $\beta$ -test as a formal statistical test of the null hypothesis of neutral evolution. Thus, considering that among-population variation should be proportional to within-population variation if genetic drift is the sole mechanism responsible for divergence, the test uses a simple regression of among-group variances ( $B$ ) on within group variances ( $W$ ) for the principal components (PC) of shape variables to test the proportionality of ( $B$  and  $W$ ) variances. The phenotypic within-group covariance matrices

( $P$ ) were used as a proxy for the genetic covariance matrices ( $G$ ), based on the corroborated proportionality between these matrices reported in the literature (Cheverud, 1988; Roff, 1996). The within-group variances ( $W$ ) were estimated by the eigenvalues of a Principal Component Analysis computed on the pooled within-group covariance matrix of the aligned shape coordinates ( $P$ ). The among-group variances were calculated as the mean squares of the projections of group means on the within-group PCs. The  $\beta$ -test consist in a linear regression of log-transformed among group ( $\ln B$ ) on log-transformed within-group ( $\ln W$ ) variances (Ackermann and Cheverud, 2002; Marroig and Cheverud, 2004). Thus, if among-populations variation was produced according to a neutral scenario of evolution, the regression slope ( $\beta$ ) should be equal to 1 (reflecting the expected proportionality between  $B$  and  $W$ ). A significant deviation of the observed coefficient from 1 indicates that the pattern of among-group variation was probably not produced solely by genetic drift (Ackermann and Cheverud, 2002, 2004; Marroig and Cheverud, 2004). Another prediction of this model is that the groups mean projections on the within-group principal components should not be correlated under the influence of genetic drift. By definition, the within-population principal components are uncorrelated, and if genetic drift causes population diversification, we expect the mean between-population principal component scores to remain uncorrelated (Marroig and Cheverud, 2004). However, correlations among between-population PC scores could arise by correlated selection (coselection) on the corresponding dimensions (Marroig and Cheverud, 2004). Therefore, correlation coefficients among PC scores (using mean projections as observations) were calculated to test this prediction and as an alternative way to detect signals indicative of non-random evolution. This test was already been applied on the study of early Hominins and Neotropical monkey's diversification (Ackermann and Cheverud, 2002, 2004; Marroig and Cheverud, 2004), as well as on modern human craniofacial evolution (e.g. Perez and Monteiro, 2009; de Azevedo et al., 2010; de Azevedo et al., 2012). Here, we use  $\beta$ -test to assess the null hypothesis of neutral evolution at different grouping levels of modern American series presented in Table 1. Finally, all the  $\beta$ -tests were computed on the Full Shape Space (the whole configuration of landmarks), the Facial Shape Space and the Neurocranium Shape Space.

## 3. Results

### 3.1. Ancestral-derived morphological vector of craniofacial variation

To visually represent the main axes of diversification among anatomically modern human populations available in our sample we plotted a 3D scatterplot of a CVA analysis (Fig. 1) used as a group-separating method (the samples are forced to group). As has been shown earlier (de Azevedo et al., 2011), the first canonical variate can be seen as a vector depicting a generalized-derived transitional morphologic pattern in our species. The most generalized morphology (e.g. low and projected faces, subnasal prognathism, long vaults, retracted zygomatics, and low noses) occupies here the most positive loadings (along CV1). This extreme of the morphospace is characteristic mainly of Old World specimens (Early Old World in Fig. 1) and Australians (and also by some early and late American series). The opposite extreme across this first axis (negative loadings, CV1) is occupied by modern northeastern Asians and Inuits, carrying a more derived morphology pattern (e.g. high and flat retracted faces, short vaults, anterior projected and high zygomatics, and high noses). The Early Americans series are much closer to the ancestral extreme of variation, as expected. However, the variation shown by New World samples span almost



**Fig. 1. Discriminant Analysis (CVA).** 3D scatterplot of the first canonical axes of a discriminant analysis (CVA) performed using all series in Table 1. Percentage of variance explained by each axis is shown in parentheses. Individual scores are plotted with 90% confidence ellipses for geographical/temporal grouping: Early America, Early Old World, Modern America, Modern Asia and Modern Australia. Shape changes across canonical axes are represented as a deformation of an outline drawing using the thin-plate spline function. Left and right shapes changes show negative and positive extreme values (black line) along CV1 as displacements from consensus (light gray line); the shape graph below shows the negative extreme value along CV2.

the entire range of variation included in this sample, overlapping considerably with early ellipses. Also, this arrangement of populations along the ancestral-derived morphological vector of craniofacial variation depicted by CV1 axis is not altered when Inuits are removed (results not shown).

Additionally, Fig. 1 clearly shows that both early and modern representatives of Asia and America populations are accommodated along a continuum of variation (CV1) where 90% ellipses of variation are quite overlapping and show no clear cut (even more overlapping along CV2), whereas 90% ellipse for Australians is clearly separated from the rest along the second canonical axis. In other words, Australians show a distinct morphology along CV2 not shared with the rest of the samples, characterized by an anterior projection of the glabella, a posterior position of the porion and a shortened face (with lower nasion and upper prosthion and zygomaxillare). Australians have been shown to have a particular population history, as relatively isolated descendants of an early dispersal out of Africa (Rasmussen et al., 2011; Reyes-Centeno et al., 2014). However, note that Inuits (and some Fuegians) also occupy lower loadings in CV2, near to Australians (see Fig. 2 in de Azevedo et al., (2011)). Besides resulting from climatic adaptations, the Inuit morphology has also been interpreted in relation to biomechanical adaptations to hard chewing (Hylander, 1977). Among other features, Inuits are characterized by an enlarged zygomaxillare region and dolichocephaly head form (Hylander, 1977), shape traits shared with Australians along CV2 (see also Fig. 2 in de Azevedo et al., (2011)). This is not contradictory, however, since the ancestral-to-derived (*mongoloid*) vector depicted by CV1 is mainly influenced by shape changes on the face, especially facial flatness. There is an interesting hypothesis (alternative to cold adaptation hypothesis) that states that most of the unusual bony cranial features of the Inuits (even facial flatness) are adapted to generate and dissipate large vertical biting forces related to chewing of seal skins, frozen food and bones and the use of the jaws as a “third hand”, activities that require the generation

of considerable amounts of occlusal force (Hylander, 1977). Interestingly, Australian aborigines also show great dental wear and ethnographic evidence of the use of their anterior teeth as a “third hand” has been reported (Barrer, 1977; Brown and Molnar, 1990). This is indicative of the complexity of adaptive and plastic developmental responses to cultural influences such as dietary and activity patterns could have on the skeleton (e.g. González-José et al., 2005; Hylander, 1977; Lieberman et al., 2004; Paschetta et al., 2010, 2015).

### 3.2. Quantifying disparity

The greatest Procrustes distance (PD) between individuals considering all grouped samples (Modern Australia, Early Old World, Early America, Modern America and Modern Asia) and for all shape spaces (Full, Facial and Neurocranium) is in Modern American sample (PD = 0.166 and PD = 0.159 between two south Americans [ARA-MAP] for the Full and Neurocranium Shape Spaces respectively, PD = 0.43 between two north Americans [ESK-ACA] for the Facial Shape Space). In the Full and Neurocranium Shape Spaces, second major distances are between Shosenzuka (Japan) and Upper Cave 1 (Zhoukoudian, China) individuals (Table 2), following by Santana do Riacho XXIII with Kennewick Man (US) individuals for the Full Shape Space and by Santana do Riacho XXIII with Hoyo Negro individuals for the Neurocranium Shape Space (Table 2). For both Full and Neurocranium Shape Spaces, this result is not affected by removing the most extreme late American morphologies (ESK and BCS). However, when these extreme morphologies are removed in the Facial Shape Space, the maximum PD within samples is shared by two Modern Americans (PD = 0.368 between a South and a North American [CAL-ACA]) and two Early Americans (PD = 0.369 between Lapa Vermelha IV and Santana do Riacho XXIII individuals) following by Minatogawa (Japan) vs. Shakameyama (Japan) individuals of the Early Old World sample (PD = 0.249).

**Table 2**  
Procrustes minimum and maximum distances.

	Full		Facial		Neurocranium	
	Max	Min	Max	Min	Max	Min
Early Old World	0.137	0.047	0.249	0.0685	0.130	0.0350
Early America	0.129	0.035	0.369	0.0643	0.120	0.0233
Modern America	0.163	0.021	0.435	0.0220	0.159	0.0117
Modern America <sup>a</sup>	0.163	0.021	0.368	0.0220	0.159	0.0117
Modern Asia	0.112	0.025	0.298	0.0385	0.110	0.0135
Modern Australia	0.094	0.025	0.239	0.0429	0.088	0.0180

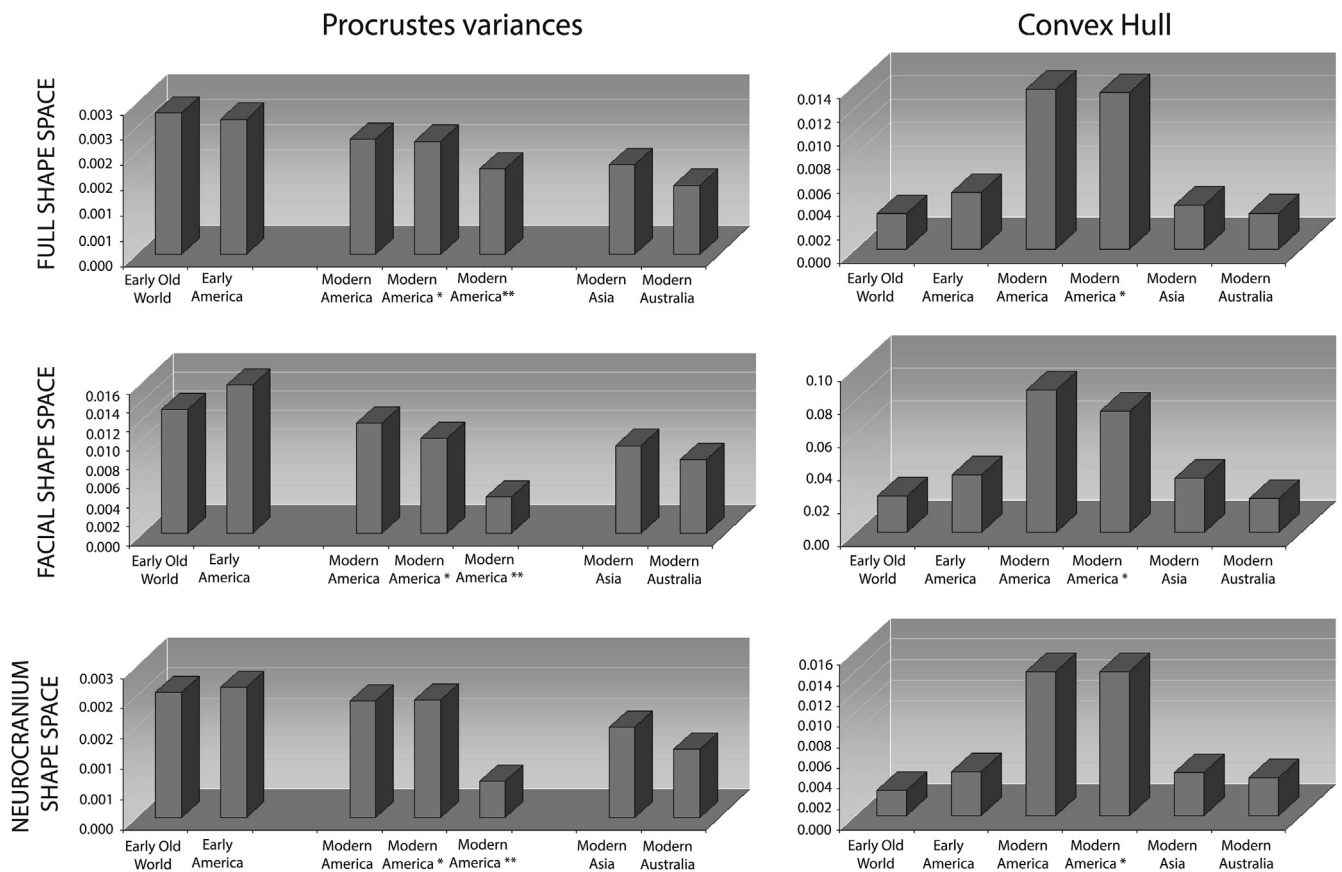
<sup>a</sup> After excluding ESK and BCS series.

In sum, the greatest distances among Modern American individuals exceed the distance between the most divergent individuals in any other sample, with the exception of early South Americans when the Inuits are not considered in the Facial Shape Space. These results are in part expected since the Modern America sub-sample includes several population series covering a broad geographical range. However, when the extreme morphology of Inuits are not taken into account for the Facial Shape Space, maximum distance inside Early American sample equals that for Modern America, indicating a high variation among *Paleoamericans* individuals for the face. Another important result is that there is also a high variation among Early Old World sample, and that maximum PD are between later Late Pleistocene individuals and not for instance with the earliest, more ancestral specimen (Herto, Ethiopia). This could indicate that the morphology pattern was

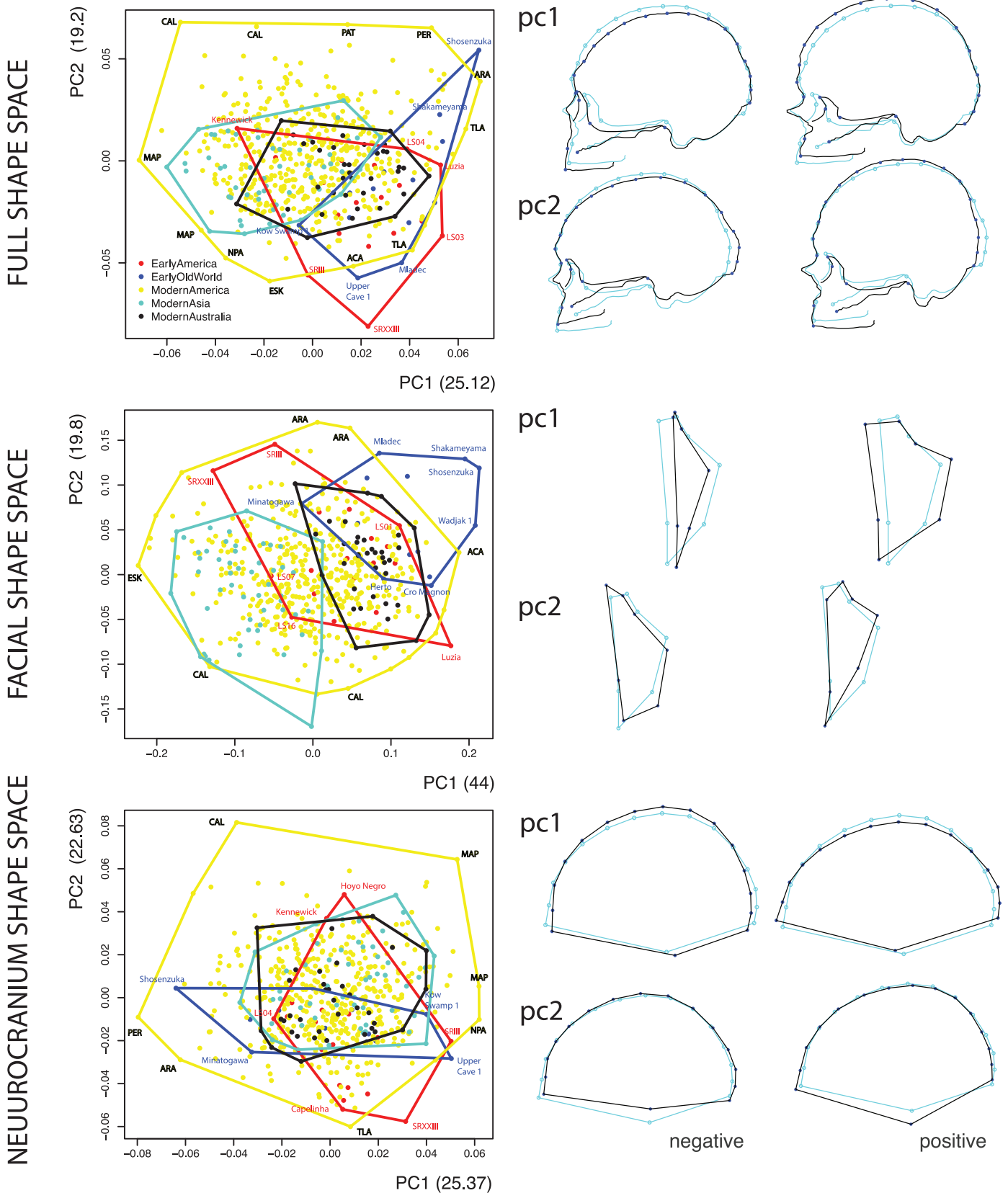
already variable or heterogeneous across the Late Pleistocene. Despite being a much reduced sample to cover a great temporal/geographical horizon, the EOW sample shows high values of among individuals differentiation (see also González-José et al., 2008).

Figs. 2–3 show results for the computations of Procrustes Variances (which quantifies the average dispersion of data points around the mean shape) and the areas of the Convex Hull enclosing the data points of each group (that capture the portion of shape space occupied by the group). For all Shape Spaces, Early series presented the highest within sample variances following subsequently by modern Americans, modern Asians and modern Australians. Except for the intermediate place occupy by modern Americans, this result is (to some extent) expected according to the general model of anatomically modern human worldwide dispersion known. When Procrustes Variances are computed using populations instead of individuals, the picture is even more in accordance with America being the last continent colonized by modern humans. This is a common pattern of all Shape Spaces (Fig. 2). Additionally, Procrustes Variances for Early Americans in the Facial Shape Space exceed that of Early Old World, indicating that facial morphology was already quite variable at the end of the Pleistocene (a prediction of the RGF model).

On the other hand, the area of the convex hull is considerably higher for modern Americans than for the other groups. Interestingly, this general result shown in Fig. 2 is not affected by removing the most extreme ancestral and derived morphological series in America (ESK and BCS) when the Full and Neurocranium Shape Spaces are considered. In the case of the Facial Shape Space, an



**Fig. 2. Measures of Disparity.** Results for the computations of Procrustes Variances (which quantifies the average dispersion of data points around the mean shape) and the areas of the Convex Hull enclosing the data points of each group (that capture the portion of shape space occupied by the group). Disparity measures were computed on geographical/temporal grouping: Early America, Early Old World, Modern America, Modern Asia and Modern Australia (see also Table 1). Modern America \*: after removing of Inuit and Pericu from Baja California. Modern America \*\*: using populations instead of individuals.



**Fig. 3. Convex Hull.** Individual PC scores of Principal component (PC) analysis for skull shape in the complete data set are shown for the Full, Facial and Neurocranium Shape Spaces, along with shape changes associated with the PC axes. Percentage of variance explained by each axis is shown in parentheses. Individual labels are shown for extreme scores within geographical/temporal groups.



expected decrease of the area of the convex hull for modern Americans is observed when excluding ESK and BCS series, given the extreme expression of facial flatness characteristic of Inuits, but this decline is not large enough to affect the observed pattern (the morphology of modern American populations displaying the greatest diversification).

The fact that within sample variances around the mean is lower in modern than in early Americans, but that the area of the convex hull is much greater in Modern America (even when recognized extreme morphologies [ESK, BCS] are excluded) could be reflecting that disparity among modern Americans (as measured by the area of the convex hull) is dominated particularly by some series that are highly divergent, whereas many others retain a shape pattern that is closer to the ancestral shape. For all Shape Spaces in Fig. 3 it can be appreciated how the modern American series occupy almost the entire range of variation of shape space, including not only the most ancient shapes but also novel ones. Note that most divergent individuals in the modern American sample include individuals other than ESK and BCS (Fig. 3).

### 3.3. Amount of variance among early and late Americans

Table 2 and Fig. 4 show results for Fst estimates computed between early Americans (PAM\_LS) and each of the modern American series, the Early Old World sample and Australians added one at a time, in comparison to the Fst computed over all modern series in our sample. When compared to differentiation among all modern population, the

early American series show moderate between-group variances when the Full Shape Space is considered and low between-group variances in the Facial and Neurocranium Shape Space. The addition of Early Old World sample or Australians to the early Americans does not increase the between-group variance in the Full Shape Space by much, as expected. However, in the Facial Shape Space the addition of Early Old World sample to the early Americans increase the between-group variance, whereas in the Neurocranium Shape Space an increment is caused but the addition of modern Australians. This result would indicate that although there is a morphological affinity between *Paleoamericans* and EOW and AUS, the early Americans are more similar to Australians when the face is considered and more similar to EOW when the neurocranium is considered.

On the other hand, a clear pattern in all shape spaces is observed when the between-group variance of early Americans is compared to modern Americans, added one at a time. Under an expected discontinuity between early and late Americans we should find a general and large increase in between-group variances. Instead, the results are variable depending on which series are included. The only modern American series that significantly increase the amount of among group variance is Inuit, especially in the Full and Facial Shape Spaces, even exceeding the total amount of among group variance for all modern series. However, the remaining modern American series cause a moderate or null increase of variance when compared with the inclusion of Inuit. This is a predicted result by the RGF model. This result is clearer in the Face Shape Space, where CAL, FUE and PAT series increase the between-group variance whereas the

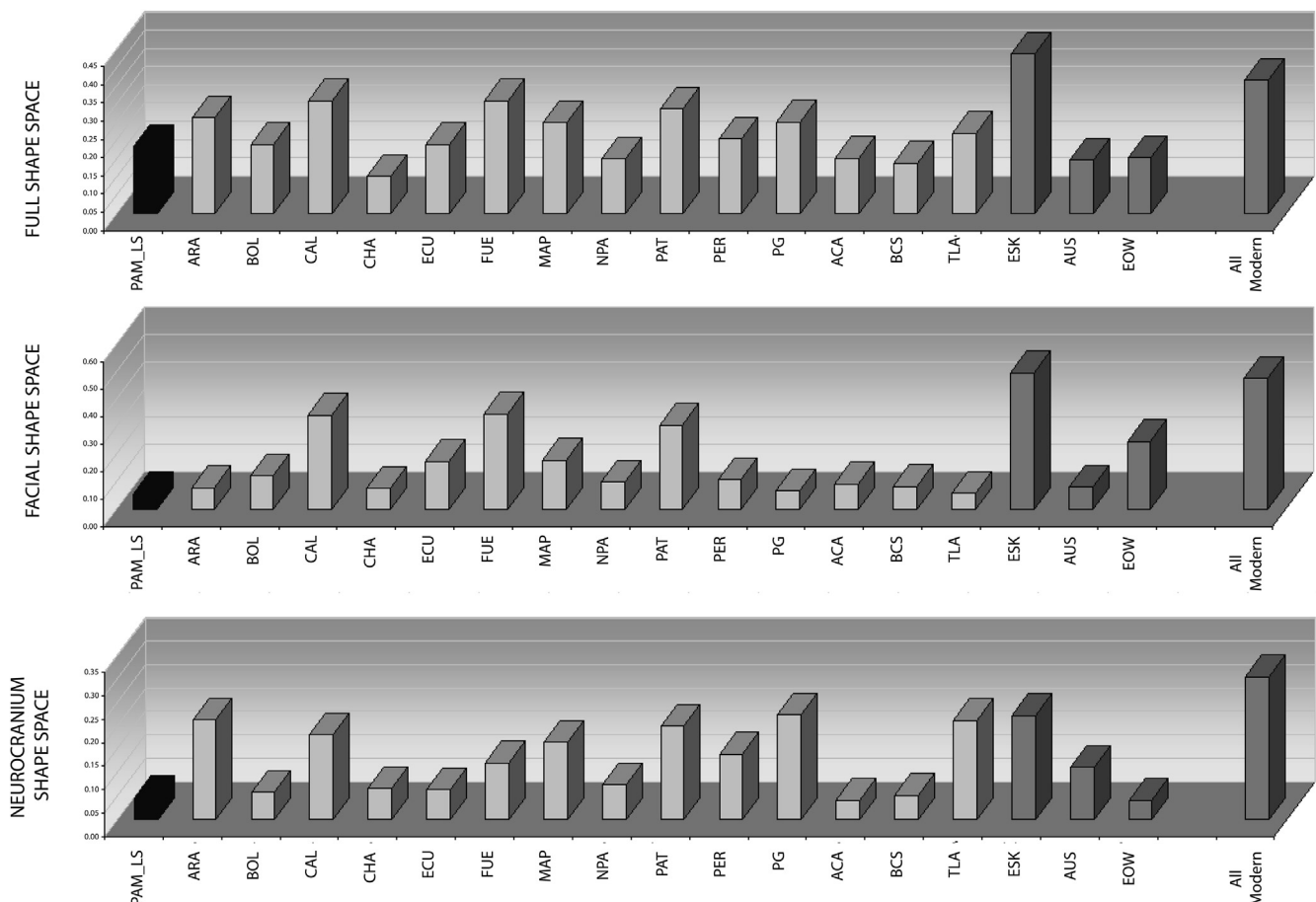


Fig. 4. Minimum Fst. Minimum Fst estimates obtained for the Early American series (PAM-LS, see also Table 1) alone (black bar) and when each of the late American series is analyzed together with them (light grey bars). Fst estimates by adding Inuits, Australians and Early Old World specimens to PAM-LS, and for all modern series are also shown (dark grey bars). See also Table 3.

remaining series show little increase of variance (if any) when added to *Paleoamericans* groups, comparable to the increment caused by adding Australians, whereas adding Inuits produces a comparatively high increment in variance. This result can be jointly interpreted with that emerging from disparity measures, indicating on the one hand that Early American series are quite variable (Full Shape Space) and that modern Americans are highly diversified, with the presence of some divergent morphologies and the retention of shape patterns closely related to ancestral shape.

3.4. Evolutionary forces responsible for cranial diversification among Native Americans

The results concerning the regression and correlation drift tests are shown in Table 4. We found that, according to the  $\beta$ -test per-

**Table 3**  
Estimates of population differentiation (Fst) between Early Americans (PAM\_LS) and each of the modern American, Early Old World and Australian series, in comparison to the Fst of all modern series. Standard errors are shown in parenthesis.

	Full	Facial	Neurocranium
PAM_LS	0.186 (0.042)	0.058 (0.068)	0.047 (0.042)
ARA	0.264 (0.027)	0.081 (0.052)	0.213 (0.031)
BOL	0.191 (0.032)	0.124 (0.06)	0.059 (0.032)
CAL	0.311 (0.027)	0.347 (0.049)	0.182 (0.031)
CHA	0.104 (0.032)	0.079 (0.06)	0.067 (0.034)
ECU	0.191 (0.03)	0.177 (0.054)	0.064 (0.029)
FUE	0.311 (0.032)	0.350 (0.059)	0.121 (0.038)
MAP	0.250 (0.027)	0.181 (0.055)	0.166 (0.031)
NPA	0.151 (0.031)	0.101 (0.059)	0.075 (0.032)
PAT	0.288 (0.027)	0.309 (0.051)	0.200 (0.032)
PER	0.206 (0.029)	0.109 (0.056)	0.141 (0.031)
PG	0.252 (0.029)	0.070 (0.052)	0.225 (0.032)
ACA	0.153 (0.031)	0.095 (0.053)	0.042 (0.031)
BCS	0.140 (0.031)	0.085 (0.051)	0.052 (0.031)
TLA	0.221 (0.03)	0.060 (0.049)	0.212 (0.033)
ESK	0.439 (0.021)	0.502 (0.034)	0.223 (0.031)
AUS	0.148 (0.031)	0.082 (0.05)	0.113 (0.032)
EOW	0.154 (0.031)	0.249 (0.054)	0.042 (0.031)
All Modern	0.368 (0.008)	0.480 (0.012)	0.306 (0.01)

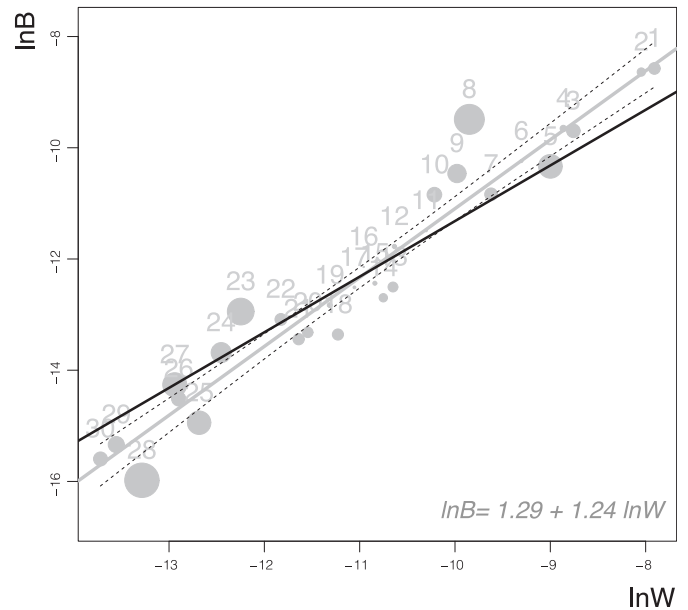
**Table 4**  
Results of the regression and correlation drift tests.

Shape space	Series included	N	b	Lower	Upper	p	R <sup>2</sup>	PCs
Full shape space	Modern America	15	1.24	1.12	1.36	<b>0.0003</b>	0.94	2–4; 8–9
	Modern America (excluding Inuits)	14	1.20	1.07	1.33	<b>0.004</b>	0.92	2–4
	Modern South America	11	1.21	1.07	1.35	<b>0.0042</b>	0.92	
Facial shape space	Modern America	15	1.49	1.13	1.84	<b>0.0139</b>	0.91	2–3
	Modern America (excluding Inuits)	14	1.36	0.98	1.75	0.06	0.88	2–8
	Modern South America	11	1.51	1.26	1.76	<b>0.0016</b>	0.95	2–7
Neurocranium shape space	Modern America	15	1.19	1.05	1.33	<b>0.0099</b>	0.94	
	Modern America (excluding Inuits)	14	1.19	1.04	1.33	<b>0.0138</b>	0.94	
	Modern South America	11	1.24	1.12	1.37	<b>0.0007</b>	0.96	

N: number of series (population samples) included.  $\beta$ : estimation of regression slope, along with lower and upper 95% confidence intervals and p-value for the null hypothesis of  $\beta = 1$ . p-values < 0.05 are shown in bold (associated to regression slopes significantly different from 1.0). R<sup>2</sup>: adjusted R squared for the regression test. PCs: Principal components that presented significant correlation (only PCs presenting significant correlations with p < 0.001 are shown).

formed here, craniofacial diversity among modern Native Americans cannot be solely explained by a neutral model of evolution driven by genetic drift.

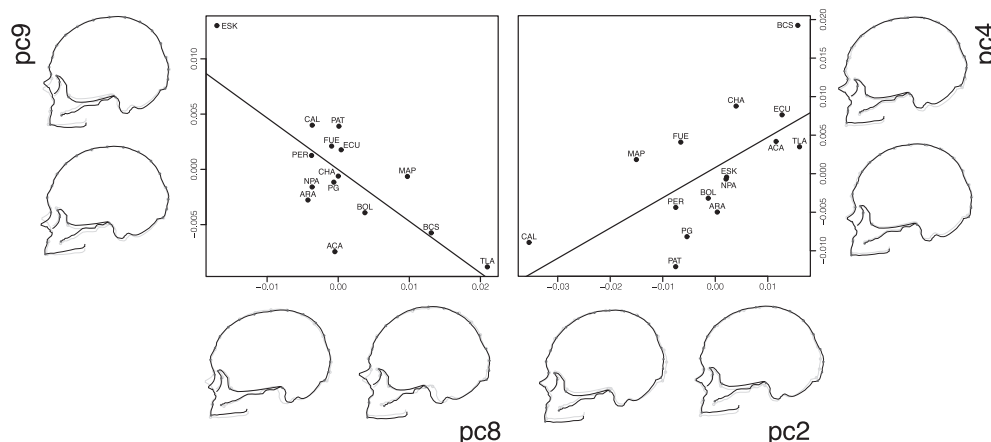
According to the model, if differentiation was produced only by genetic drift, we expect a regression slope ( $\beta$ ) of 1.0 for the regression of between-on within-population variance (Ackermann and Cheverud, 2002, 2004; Marroig and Cheverud, 2004). This means that the relation between LnB on LnW should be



**Fig. 5.** Linear regression (gray line) of B (between-group) variances on W (within) eigenvalues and associated 95% confidence limits (dotted lines) for the Full Shape Space including all modern American series. LnB: log-transformed among group variation, LnW: log-transformed within group variances. Dots size indicates PCs's influence on the regression line (computed as the difference between the slope values with and without each point). The black regression line shows a proportional increase on LnB given LnW, as a reference of the null hypothesis ( $b=1$ ).

proportional under a neutral model of evolution, and a non-proportional change along LnB in relation to LnW, which can be due to either an unexpected high or low between-group relative to within-group variances, could be interpreted as indicative of the action of diversifying or stabilizing selection, respectively. We performed the test including all modern Americans series (with and without Inuit) and including only South Americans populations, for the Full, Facial and Neurocranium Shape Spaces. In all cases, the regression slopes for the relation between log-

transformed among-group (LnB) on log-transformed within-group (LnW) variances are positive and greater than 1.0 ( $\beta$  mean = 1.29, adjusted R<sup>2</sup> mean = 0.93, see Table 4), indicating that one or more of the first PCs are more variable, relative to the other PCs, than expected under genetic drift. The null hypothesis of proportionality ( $\beta = 1$ ) is significantly rejected for all levels of analysis (except for all modern Americans excluding ESK in the Facial Shape Space, see Table 4).



**Fig. 6.** Plot of correlation between PC2 vs. PC4 and PC8 vs. PC9 for the Full Shape Space including all modern American series. Negative and positive extreme shape changes are represented as a deformation of an outline drawing using the thin-plate spline function as displacements from consensus.

Fig. 5 shows the regression line of **B** on **W** variances (estimated by LnW and LnW) and associated 95% confidence limits to the analysis including all modern Americans on the Full Shape Space. We have also drawn an estimated line showing a proportionality increase in LnB given LnW, as a reference of the null hypothesis ( $\beta = 1$ ). Fig. 5 shows that the regression slope is  $>1.0$  with the first PCs having greater between-group variances than expected. PC8 and PC9 (and PC10) deviate significantly from expectations based on drift, being more variable than expected and pulling the line away from proportionality. However, although the exclusion of these PCs causes a decrease in slope, the null hypothesis of proportionality is still rejected, indicating that diversification among first PC are important (most diversifying populations along PC1 and PC2 are NPA, FUE, MAP along with ESK [PC1] and CAL [PC2] in the Full Shape Space; ESK [PC1 and PC2] together with CAL, FUE and PAT [PC2] for the Facial Shape Space; and CAL [PC1] in the Neurocranium Shape Space). PC8 and PC9 in the Full Shape Space explain only 3.24 and 2.85% of the total variation respectively. However, the model allows identification of certain axes of shape variation along which certain population causes that the PCs vary more among populations than expected under neutrality, even if those axes do not account for the majority of overall variance. Additionally, PC8 and PC9 present significant Pearson's product–moment correlation ( $r = -0.76$ ,  $p = 0.00099$ ), as well as PC2 (explaining 19.64% of total variation) vs. PC4 (explaining 8.68% of total variation) ( $r = 0.65$ ,  $p = 0.0083$ ). Marroig and Cheverud (2004) interpret significant correlations among PC scores as indicative of co-selection of traits during the divergence of groups. Fig. 6 shows the correlation plots between PC1 and PC4, and between PC8 and PC9. The correlation of these PCs suggests that certain subtle changes in the anterior face at the level of prosthion and nasion and changes at the level of glabella, would have been co-selected along with subtle changes in zygomatic arch and posterior neurocranium (Fig. 6). Along these PCs, CAL and ESK present the most extreme morphologies. Interestingly, removal of Inuit does not change the main results, and the null hypothesis of neutral evolution is rejected even when only South Americans series are considered.

For the Facial and Neurocranium Shape Spaces, the null hypothesis of neutral evolution is also rejected. When Inuit are removed from the Facial Shape Space, the model is consistent with drift. However, the null hypothesis is still rejected for South Americans series.

#### 4. Discussion

To date, there is a vast amount of scientific work around the peopling of the Americas coming from archaeological, linguistic,

paleoclimatology, genetic and morphological fields. In recent times, much involved significant progress were made by advances in molecular biology techniques, paleogenetics, and the development of analytical tools in the genetic and physical anthropology fields, thereby allowing craniofacial and DNA evidence to further enhance discussions and interpretations. However, after decades of intense research, the subject continues to be highly controversial (González-José, 2011; Bortolini et al., 2014; Marangoni et al., 2014). A recurrent debate relates to the problem of explaining the biological diversity found in American populations, where one of the main challenges is to integrate the evidence coming from bones and genes.

A large amount of the genetic evidence point to a single biological origin of all Native Americans probably deriving from a single and early Beringian source (the Out of Beringia or Single Wave model, Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Santos et al., 1999; Silva et al., 2002; Tarazona-Santos and Santos, 2002; Fagundes et al., 2008a,b, but see also Perego et al., 2009; Reich et al., 2012; Marangoni et al., 2014), while craniofacial morphology appears to be at odds with this view. Statistical assessment of the craniofacial variation found in the continent until today across the Late Pleistocene–Early and Late Holocene has led to a polarization of views, mainly in terms of a multi-migrational model implying two different biological population sources (e.g. Neves et al., 2003, 2007a,b) vs. a view that is in accordance with genetic evidence about a single biological origin for all Americans (the Recurrent Gene Flow model proposed by González-José et al., 2008) and that emerges from reassessing craniofacial variation in an attempt to combine and reconcile evidence coming from the study of bones and genes to achieve a synthesis. The main discrepancy among physical anthropological views is around the difference in craniofacial pattern presented by the earliest (Early Holocene) skulls found in America (*Paleoamericans*) when comparing to later Late Holocene samples. For instance, the Two-Components model (Neves and Pucciarelli, 1991; Pucciarelli et al., 2003; Neves et al., 2003, 2005) proposes the presence of two significantly differentiated craniofacial morphologies in America resulting from at least two chronologically separate waves with different Asian source populations. On the other hand, the Recurrent Gene Flow model does not deny that there is some evident differences in pattern of skull morphology across early and late specimens means, but emphasizes that there exists a continuum vector of variation formed by American samples that goes from a more ancestral to a more derived shape pattern, whose ends are occupied by *Paleoamericans* and Inuits, the latter grouping with some circum-Arctic northeastern Asians (González-José et al.,

2008). Thus, an old debate concerning the first peopling of the New World is around how to interpret patterns of past and present genotypic and phenotypic diversity within the context of one or more Old World founding events (migrations), and the extent to which evolutionary forces contributed to the variation we see (Powell and Neves, 1999).

Here, we investigated patterns of shape variation within and among early and late series of Old and New World, and also explore the evolutionary forces implicated in the diversification of Native Americans under expectations of quantitative genetic theory. We found that internal variation among Early Old World and Early Americans specimens are quite large. Average dispersion of individuals around the mean shape (measured as Procrustes Variances) is greater than the average dispersion of individuals and populations around the mean shape inside modern American sample. However, when the portion of the shape space occupied by the groups is quantified as the area of the convex hull, modern Americans show a high level of morphological diversification. This result indicates that disparity among modern Americans is dominated particularly by some series that are highly divergent, whereas many others retain a shape pattern that is closer to the ancestral shape.

Furthermore, the Facial Shape Space is the only morphospace where modern Asians and Early Old World convex hulls do not overlap (Fig. 3), whereas that of *Paleoamericans* is intermediate in between the two. Note that along the corresponding first axis of variation (PC1) the shape changes are mainly related to facial flatness, one of the most characteristic *mongoloid* features. This means that *Paleoamericans*, who would already have experienced the way through high latitudes (Beringia) to reach American lands during the Last Glacial Maximum, exhibit a considerable range of variation along this axis. This result would be in accordance with expectations of the RGF model (González-José et al., 2008), which suggests that the set of characters defining the range of New World phenotypic variation would be already present in its putative ancestors at the end of the Pleistocene. Moreover, after the ancestors of Native Americans had paused when they reached Beringia (Tamm et al., 2007), a swift migration southward facilitated by the coast was followed, with sequential population splits and little gene flow after divergence (Tamm et al., 2007; Reich et al., 2012). As a result, the most extreme expression in America of these *mongoloid* traits characteristics of most northern Asians (e.g. facial flatness) are presented by Inuits, who would inherit almost half their ancestry from a second stream of Asian gene flow (Reich et al., 2012), and that would have maintained recent and persistent bidirectional gene flow with Siberian circumarctic groups (Tamm et al., 2007; Wang et al., 2007), besides inhabit similar (circumarctic) environments. Thus, recurrent gene flow among circumarctic groups would enable that the extreme Siberian craniofacial pattern defined by facial flatness and zygomatic projection to disperse and persist in northern North America (González-José et al., 2008). The circumarctic continuum that persisted after the formation of the Bering Strait is also supported by genetic (Zlojutro et al., 2006; Tamm et al., 2007), linguistic (Greenberg et al., 1986; Campbell, 1997), and archeological evidence (Dixon, 2001; Goebel et al., 2003). Additionally, modern Americans convex hull encloses almost entirely or at least to a significant extent to all others, in all shape spaces (Fig. 3). This is contrary to the expectations of a Two-Component model, which predicts that differences between *Paleoamericans* and modern Native American craniofacial pattern are abrupt (Neves et al., 2007a,b; Steele and Powell, 1992, 1993). This prediction is based in the assumption that all modern Native Americans pertain to a supra-population unit whose morphology corresponds with a *mongoloid* pattern of craniofacial shape (Neves et al., 2003) as the result of the fixation of the *mongoloid*

morphology in North Asia, posterior to the entry and dispersion of *Paleoamericans* to/into the continent. In this context, we think on the one hand, that different interpretations of the craniofacial variation patterns between populations could be related to the fact that many of previous works supporting the idea that the first Americans were very distinct from late and recent Native Americans and Asians in terms of cranial morphology (e.g. Neves et al., 2003; Neves and Hubbe, 2005; Neves et al., 2007a,b; Hubbe et al., 2010, 2011; among others) employ Howells' dataset (Howells, 1973, 1989). Howells' is an important and valuable dataset and it is extensively used by physical anthropologists in comparative studies because they represent the main core of human cranial variation in the world, although it only includes a few American series. As a result, these works systematically find major affinities of *Paleoamerican* morphology pattern with Australo-Melanesians than with late Asian and Amerindian series. It is therefore important to evaluate the entire range of variation present in the continent since the full and simultaneous expression of *mongoloid* features is found only in circum-Arctic groups, but their expression among Native Americans is quite variable.

Another possible font of discrepancy between craniometric and molecular data is that models based on genetic evidence have been mainly obtained from analyzing extant or recent populations, whereas craniofacial evidence comes from the assessment of variation of Early and Late Holocene skeletal samples. This was already highlighted and discussed by Perez et al. (2009) who compared craniometric and mtDNA data of diachronic samples from East Central Argentina dated from 8000 to 400 BP. They show that even when the oldest individuals display traits closer to *Paleoamerican* crania, they present the same mtDNA haplogroups as later populations with Amerindian morphology and explain their results alluding to local microevolutionary processes of morphological differentiation in America as a probable scenario taken into account the rapid peopling and the great ecological diversity of this continent (Perez et al., 2009).

Of particular interest to this issue is the recent publication made by Chatters et al. (2014), who reported the archaeological, craniofacial, and genetic characteristics of the Hoyo Negro skull (HN5/48), a very well preserved specimen from a submerged cave in Yucatan, dated ~12,500 years ago. Given its age, and because the paleogenetic analysis of the Hoyo Negro skeleton indicates that it carries a Beringian derived mitochondrial DNA (haplogroup D1), the authors conclude that the differences between *Paleoamericans* and Native Americans probably resulted from in situ evolution rather than separate ancestry (supporting the RGF model). However is important to remark that, although Chatters et al. (2014) did not perform a morphometric analysis in a comparative populational framework and so classified the Hoyo Negro individual (as in the topological way) as a *Paleoamerican*, we showed recently (de Azevedo et al., 2015) that the Hoyo Negro skull falls into a subregion of the morphospace occupied by both *Paleoamericans* and some modern Native Americans (see also Fig. 3, present work).

Here we also used quantitative-genetics theoretical predictions to investigate whether human craniofacial diversification in the Americas can be explained by neutral microevolutionary processes alone. We found clear signals of deviation from neutral evolutionary expectations, suggesting a significant effect of non-stochastic evolution (e.g. natural selection, phenotypic plasticity, etc.). These results are especially relevant in the debate concerning the impact of neutral versus non-neutral evolutionary forces in creating global patterns of craniofacial diversity among human populations (e.g. Weaver et al., 2007; Relethford, 2010; von Crammon-Taubadel, 2013), since human cranial data are productively employed as a proxy for neutral genetic data in archaeological contexts. Previous studies have revealed that much of the

human craniometric variation follows a neutral model of population relationships and that although population-specific deviations of global human craniometric variation from a neutral model exists, the effect of natural selection can be greatly overestimated (e.g. Relethford, 2010). Also, Roseman (2004) and Betti et al. (2009a) have reported that neutral processes have been much more important than climate in shaping the human cranium, again, with a specific signal of non-random process coming from populations from extremely cold regions. However, although our results are in part concordant (in the sense that for instance, although removing Inuits does not change the overall result [rejecting of the null hypothesis of neutral evolution], they have specific influences on the results), also suggest that signals of non-random evolution are not only present when Inuits are included, but also when analyzing the remaining Native American samples, especially when considering south American series alone. Further evidence suggesting non-random craniofacial evolution has been reported previously, specifically regarding the role of diet and temperature in shaping cranial diversification of South American human populations (e.g. Hernández et al., 1997; Pucciarelli et al., 2003; Perez and Monteiro, 2009; Paschetta et al., 2010; Perez et al., 2011).

Our results also highlight the existence of phenotypic resemblance (and specific populational outliers) between geographically distant populations (e.g. ESK, FUE, CAL, PAT) that evidence the evolvability of the human skull. Future research should be enriched by exploring not only the craniofacial variability among Arctic populations at both sides from the Bering Strait in relation to genetic population history and climatic changes posterior to the last glacial maximum, but also by identifying the derived traits shared by distant populations (in this case, population from distant but similar [e.g. cold] environments) that could be associated to selective adaptive responses (or phenotypic plasticity due not only to climatic stress, but also to the diet and even cultural behaviors such as using teeth as a “third hand”).

Although global patterns of cranial variation has been largely explained on the basis of neutral theory (e.g. Manica et al., 2007; von Cramon-Taubadel and Lycett, 2008; Betti et al., 2009a,b; Relethford, 2004, 2010), our results are in line with a growing recognition that some regions of the skull differ in the extent to which they fit a neutral model of microevolutionary expectation, allowing for a more detailed assessment of patterns of adaptation and phenotypic plasticity within the human skull (Roseman and Weaver, 2004; von Cramon-Taubadel, 2013). Although here the non-neutral signal of evolution is found for all Shape Spaces (Full, Facial and Neurocranium), the fact that we only are capturing variation in the skull in lateral view could be interpreted as a *modular* result. On the other hand, the samples used in these analyses must be considered. Some of these works (e.g. von Cramon-Taubadel and Lycett, 2008; Relethford, 2002, 2010) use Howell's data (Howells, 1973, 1989) including only Peruvians as South American representatives while other works employ an exceptionally large dataset of human skulls, though South America is poorly sampled (e.g. Manica et al., 2007; von Cramon-Taubadel and Lycett, 2008; Betti et al., 2009a,b) or not sampled at all (Harvati and Weaver, 2006). Furthermore, outliers of neutral model detected by some of these same works are from South America (e.g. Peruvian in Relethford, 2010 and in Figs. 2–3 in von Cramon-Taubadel and Lycett, 2008; two populations from Patagonia in Fig. 1 in Betti et al., 2009b). Interestingly, using a large dataset of South American populations comprising 40 series quantified through linear measurements that are specifically used to describe the functional components of the skull (Pucciarelli et al., 2006), Perez et al. (2011) explore the role of temperature and diet in shaping cranial diversification using spatial regression and divergence rate test and found that random processes alone cannot account for the

morphological divergence observed. Overall, the point is that perhaps this question has no simple answer, and that the results may significantly vary with the methods and models used, as well as with the samples employed.

We are far from an extensive knowledge about the causation and underlying factors determining the patterns of skull variation and covariation (Hallgrímsson et al., 2009; Martínez-Abadías et al., 2011, 2012a,b; Mitteroecker and Bookstein, 2007, 2012). The human skull is a complex structure comprising different anatomical regions, with differing embryological origins, ossification patterns, and functional attributes that contribute to form a tightly integrated adult phenotype (Lieberman, 2011). Fortunately, recent advances on the Evolutionary Developmental biology field (or Evo-Devo) incorporates groundbreaking theoretical and methodological tools that among other benefits enable a refinement of the use of skull characters to work out microevolutionary studies. Incorporating Evo-Devo and quantitative genetics perspectives enhance the understanding of craniofacial evolution in humans (e.g. Martínez Abadías et al., 2012a,b; Melo and Marroig, 2015). Thus, to further take profit of skull size and shape as a biological marker of utility to reconstruct past population history we need to link population genetics, quantitative genetics, and evolutionary development biology.

Finally, the history and dynamics of the earlier human settlement in the New World constitute a stimulating scenario to discuss how different types of evidences and disciplines can be reconciled to provide a holistic explanation about human evolutionary processes. The Americas can be viewed as an excellent opportunity to study many aspects of human evolution (González-José, 2011). For instance, Americas' special geophysical and ecological features such as its enormous latitudinal extension, and the existence of a huge variety of ecosystems, as well as some anthropological events such as the transition to agriculture that took place in the continent, and its particular demographic history, suggest that the variation patterns we observe today are the final result of a complex process including divergent evolutionary trajectories (e.g. Hünemeier et al., 2011; Perez et al., 2011; Rademaker et al., 2014; Jacovas et al., 2015). The first Americans arrived to the New World during the final phase of a large demographic expansion that began about 150–200 thousand years ago in Africa (White et al., 2003; Mellars, 2006; Oppenheimer, 2012). The human arrival to the new continent could be seen as an unprecedented ‘adaptive’ success for humans. In other words, considering hunter-gatherers groups of humans moving so far and so fast into a novel, diverse, huge, dynamic, and changing environment as the (Late Pleistocene/Early Holocene) Americas, then the search about Americans' origins is also a search about biologic and culture adaptive strategies. Actually, ecological opportunity through entry into a new environment or the origin of a key innovation is widely thought to link ecological population dynamics to evolutionary diversification in ecological time scales (Carroll et al., 2007; Yoder et al., 2010; Perez et al., 2011).

## 5. Conclusions

Expanding around the globe, *Homo sapiens* successfully survived major adaptive challenges, inviting scientific research to develop questions related to the micro evolutionary mechanisms involved. Research around these controversial topics would help to understand the dispersion dynamics of our species during the Holocene, and to put into context the relative importance of biological versus cultural adaptation during the expansion of *Homo sapiens* across the New World. Intimately linked to the pale-bio-anthropological view of the first settlement of the New World issue, is the understanding of the evolutionary trajectory from an ancestral to a derived state of craniofacial variation, and its implications for the

discussions on contrasting and apparent mutually exclusive hypotheses that attempt to explain the initial peopling of the New World. A particular derivation of the more generalized morphological skull observed among the groups leading the Out-of-Africa dispersion is constituted by a set of unique, derived craniofacial traits (classically known under the “mongoloid” label) that would have arisen and evolved in Asia likely associated to the harsh and cold climate of Northeast Asia during the Last Glacial Maximum (Lahr, 1996). Here, we attempted to use geometric morphometric techniques and multivariate statistics, along with quantitative genetics to look more closely into the craniofacial evolutionary history during Late Pleistocene–Early Holocene horizons from Asia and the Americas.

Collectively, the presented results are in line with the expectations of the Recurrent Gene Flow model (González-José et al., 2008) that refers to an ancestral, founder population experiencing a standstill in Beringia, and exhibiting high within-group craniofacial variation. This original, internally variable stock would have been the ancestral source of variation that fuelled the subsequent local micro evolution of other derived phenotypic patterns, giving origin to the craniofacial diversity observed among Holocene Native American samples. Our results also reinforce the idea that while the full expression of the characteristic derived traits of modern humans skull is found in Inuits and its expression would be maintained in a common environment helped by registered gene flow among circumpolar groups at both sides of the Bering Strait, the remaining Native Americans show variable range of ancestral and derived traits as well as novel diversification.

In this context, although it is widely considered that there is consensus on the relative role of random (i.e. genetic drift) and non-random factors (e.g. selection and plasticity) in shaping craniofacial differentiation among modern human populations, we point out that this question has no simple answer, and that (among other factors) the results may significantly vary with the methods and models used, as well as with the samples employed, and/or that this consensus does not include the variation found in South America. This is still an open discussion, which has important implications for anthropological and paleo-anthropological fields. Therefore, the study of the human skull as a biological marker of utility to reconstruct past population history will benefit from a communion linking population genetics, quantitative genetics, and evolutionary development biology, among other fields.

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