

Evaluating Microevolutionary Models for the Early Settlement of the New World: The Importance of Recurrent Gene Flow With Asia

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ABSTRACT Different scenarios attempting to describe the initial phases of the human dispersal from Asia into the New World have been proposed during the last two decades. However, some aspects concerning the population affinities among early and modern Asians and Native Americans remain controversial. Specifically, contradictory views based mainly on partial evidence such as skull morphology or molecular genetics have led to hypotheses such as the “Two Waves/Components” and “Single Wave” or “Out of Beringia” model, respectively. Alternatively, an integrative scenario considering both morphological and molecular variation has been proposed and named as the “Recurrent Gene Flow” hypothesis. This scenario considers a single origin for all the Native Americans, and local, within-continent evolution plus the persistence of contact among Circum-Arctic groups. Here we analyze 2D geometric morphometric

data to evaluate the associations between observed craniometric distance matrix and different geographic design matrices reflecting distinct scenarios for the peopling of the New World using basic and partial Mantel tests. Additionally, we calculated the rate of morphological differentiation between Early and Late American samples under the different settlement scenarios and compared our findings to the predicted morphological differentiation under neutral conditions. Also, we incorporated in our analyses some variants of the classical Single Wave and Two Waves models as well as the Recurrent Gene Flow model. Our results suggest a better explanatory performance of the Recurrent Gene Flow model, and provide additional insights concerning affinities among Asian and Native American Circum-Arctic groups. *Am J Phys Anthropol* 146:116–129, 2011. © 2011 Wiley-Liss, Inc.

Different scenarios attempting to describe the initial phases of the human dispersal from Asia to the New World have been proposed during the last two decades (Neves and Pucciarelli, 1991; Bonatto and Salzano, 1997a,b; Dixon, 2001; González-José et al., 2001a; Zegura et al., 2004; Neves and Hubbe, 2005; Neves et al., 2005, 2007; Tamm et al., 2007; Wang et al., 2007; Dillehay et al., 2008; Goebel et al., 2008). Most of them fail to integrate different kinds of evidence (archaeological, linguistic, craniofacial, genetic, etc.) into a single, comprehensive model, and tend to provide *ad hoc* explanations to solve potential conflicts among different data.

In a remarkable effort to reconcile different types of data, Greenberg et al. (1986) proposed an interdisciplinary model for the settlement of the New World involving linguistic evidence associated with dental anatomy and genetic diversity data. In their Tripartite model, separate diachronic migrations gave rise to three main linguistic groups: Amerindians, Na-Dene, and Aleut-Eskimos. Their settlement theory suggested that the ancestors of modern Native Americans would have come from Eastern Asia in three separate migratory waves at different times. In their view, present day Amerind-speaking groups, who occupied South, Central and most of North America (e.g., Yanomami, Maya, Cheyenne), would be considered descendants of the first migrants, also called Paleoindians. A second migratory wave would have involved the Na-Dene speakers (e.g., Navajo, Athabaskan) from the North Pacific coast, the interior of

Alaska, and parts of the southwest USA. The Eskimo-Aleuts, who currently inhabit Arctic and sub-Arctic lands, would have been the last independent migration into the continent. However, although some classic reviews supported the validity of the Tripartite model (Cavalli-Sforza et al., 1994), the Greenberg et al. (1986)

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vision was gradually replaced by subsequent models that were postulated around specific types of evidence. More specifically, comprehensive analyses of genetic data gave rise to the Single Wave (SW) or Out of Beringia model, whereas continental-wide studies of craniofacial shape variation supported the Two Waves (TW) model.

The Single Wave or Out of Beringia 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) is an attempt to accommodate two important and recurrent conclusions of molecular-genetics studies during the last twenty years. The first one is the molecular coalescence of most modern Native Americans back to a unique ancestral population somewhere in Eastern Asia (Merriwether et al., 1995; Bonatto and Salzano, 1997a,b; Santos et al., 1999; Silva et al., 2002). The second one is the accumulation of autochthonous mutations (or "maturation") of particular lineages of the mitochondrial and Y-chromosome, and autosomal genomes of the Native Americans (Tamm et al., 2007; Fagundes et al., 2008a,b). The number and pattern of these mutations' accumulation enable the computation of approximate dates of isolation as well as the magnitude and duration of the bottlenecks or expansions that the founder population putatively experienced (Bonatto and Salzano, 1997a; Tamm et al., 2007; Fagundes et al., 2008a,b). As stated by many scholars, the most parsimonious explanation for these data proposes a single ancestor coming from northeastern Asia (e.g., the Single Wave) and a confinement in Beringia during the Late Pleistocene (Out of Beringia).

The Two Waves or Two Components model (Neves and Pucciarelli, 1991; Pucciarelli et al., 2003; Neves and Hubbe, 2005) considers the presence of two differentiated craniofacial morphologies in America as a result of two distinct and allochronic source populations in Asia coming to the New World. This scenario arises as an explanation for the alleged morphological discontinuity among early and modern skulls from the New World. Defenders of this model place the full range of craniofacial variation under two discrete categories: the Paleoamericans, representing a first wave of migrants entering the continent throughout Bering from somewhere in Southeastern Asia during the Late Pleistocene; and the "Amerindians," representing the descendants of a second wave coming from Eastern Asia during the Early Holocene and virtually replacing the Paleoamericans, already settled down in the Americas. Even though some analyses have demonstrated that such discontinuity is not allochronic or, in other words, that the morphological pattern defining the early remains can also be found in modern populations from the Americas (González-José et al., 2003; Vezzani Atui, 2005; Pucciarelli et al., 2008), some authors still maintain that "Early American" (so-called Paleoamerican) cranial morphology differs significantly from that of recent Native Americans (or Amerindians, Hubbe et al., 2010).

Even though the Single Wave and the Two Wave models would be solid enough to explain the molecular and morphological variability respectively, they systematically failed to provide an integrative view and drove the discussions towards a "communication-breakdown" among specialists of the different fields. This is crucial because, instead of furnishing holistic explanations, this situation leads to a (secondary) discussion about limitations of particular types of data instead of benefiting from the potentiality and advantages of each kind of evi-

dence. Some attempts have been made, however, 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 (González-José et al., 2008). In this way, predictions about the evolution of different data considering their particular nature of change (cultural, genetic-neutral, genetic plus environmental, etc.) can be stated and contrasted with real data. In addition, such integrative models can be useful as a source of null hypotheses to be tested on particular, specific data. After reviewing the available data on each research field in the light of the particular mechanisms of transmission that they experience, we have postulated an alternative model, the so-called Recurrent Gene Flow (RGF), which is mainly based on some recent molecular analyses, evidences, and reinterpretations of craniofacial variation (González-José et al., 2008). In particular, we took the molecular evidence supporting a possible gene-flow among the Arctic representatives of the Asian and the American populations long before the formation of the Bering Strait (Zlojutro et al., 2006; Tamm et al., 2007; Gilbert et al., 2008; Mulligan et al., 2008; Volodko et al., 2008; Rasmussen et al., 2010). We should note that most modern reviews of the Arctic archaeology tend to support such post-occupation contact, even until modern epochs (Goebel et al., 2003, 2008). In addition, we argue that when the craniofacial shape is analyzed avoiding the *a priori* usage of labels such as Paleoamericans, Amerindians, Mongoloids, Proto-Mongoloids, etc., then the results show that the variation is not arranged into discrete units but rather in a continuous spectrum of samples. In fact, a specific test aimed at detecting natural groupings failed to detect a two-group pattern (González-José et al., 2008). This is just because of three simple observations. First, the early remains from the New World present strong similarities with some modern populations (González-José et al., 2003). Second, the Circum-Arctic groups from the Americas tend to form a cluster with Asian Arctic groups, rather than with most Native Americans (González-José et al., 2003). Finally, most Native Americans tend to occupy an intermediate position along a phenotypic vector defined by the generalized morphology observed in the early remains from Asia and the New World (with some modern groups very near to this extreme of variation) on one hand, and the fully derived craniofacial pattern observed in the modern populations from the Arctic environments (González-José et al., 2008) on the other. Thus, and departing from some previous molecular analyses that detected signals of genetic contact among Asia and America (Zlojutro et al., 2006; Tamm et al., 2007; Gilbert et al., 2008; Volodko et al., 2008; Rasmussen et al., 2010), we have postulated an integrative model in which the New World was first settled by a founder population occupying Beringia during the last glaciations, characterized by high craniofacial diversity, founder mtDNA and Y-chromosome lineages and some private autosomal alleles. After a Beringian population expansion, which could have occurred concomitant with their entry into America, more recent Circum-Arctic gene flow would have enabled the dispersion of northeast Asian-derived characters and some particular genetic lineages from East Asia to America and vice versa.

In a recent article, Hubbe et al. (2010) used some selected samples of Early and Late American crania in order to calculate the rate of morphological differentiation

TABLE 1. Series included in the study

Population	Code	Chronological range	N (females/males/total)	Latitude	Longitude
California, USA	ACA	1,000	22/27/49	36.97°	-122.00°
Ainu, Japan	AIN	1,000	3/7/10	43.23°	142.70°
Araucano, Argentina	ARA	1,000	26/17/43	-37.25°	-59.13°
Aborigines, Australian	AUS	1,000	16/20/36	-35.43°	139.07°
Baja California Sur, Mexico	BCS	1,000	11/12/23	24.12°	-110.28°
Aymara, Bolivia	BOL	1,000	6/12/18	-16.87°	-68.15°
Buriats, Siberia	BUR	1,000	5/5/10	51.68°	103.7°
Calama, Chile	CAL	1,000	12/12/24	-22.35°	-69.03°
Chaco, Argentina	CHA	1,000	2/8/10	-26.58°	-60.95°
Paltacalo, Ecuador	ECU	1,000	27/26/53	-4°	-79.05°
Late Pleistocene (Early) Old World	EW	30-11 kyr	/ 13/13	39.90°	116.40°
Eskimos, Greenland	ESK	1,000	28/18/46	60.9°	-48.35°
Fuegians, Chile and Argentina	FUE	1,000	7/3/10	-53.78°	-67.72°
<i>Paleoamericans from Brazil</i>	LS	11-7.5 kyr	3/8/11	-19.62°	-43.88°
Mapure, Venezuela	MAP	1,000	17/21/38	10.12°	-69.05°
North Patagonians, Argentina	NPA	1,000	9/9/18	-40.8°	-62.98°
Ourgas, Siberia	OUR	1,000	11/7/18	47.93°	106.9°
<i>North Paleoamericans</i>	PAM	10,000	1/5/6	19.42°	-99.12°
Central Patagonians, Argentina	PAT	1,000	18/20/38	-43.23°	-65.3°
Ancon, Peru	PER	1,000	20/17/37	-12.03°	-77.02°
Pampa Grande, Salta, Argentina	PG	1,000	16/9/25	-25.42°	-65.07°
Tchouktchi, Siberia	TCH	1,000	3/11/14	-65.82°	-173.48°
Aztecs from Tlatelolco, Mexico	TLA	1,000	7/19/26	19.42°	-99.12°
Total			270/306/576		

between Early and Late American samples under different time-divergence assumptions, and compared their findings to the predicted morphological differentiation under neutral conditions. Alternatively, they tested three dispersal scenarios including pure Isolation-by-Distance (IBD), Single Wave, and Two Waves (but not Recurrent Gene Flow) models for the colonization of the New World by comparing the morphological distances among Early and some specifically selected Late Amerindians, East Asians, Australo-Melanesians and early modern humans from Asia to geographical distances associated with each dispersion model. The authors state that given its alleged better performance, the TW scenario is the most economic explanation for the observed cranial variation in their samples.

The objective of this article is to evaluate the performance of the main microevolutionary models presented for the early settlement of the New World, considering factors like geographical and chronological separation, climate, and the similarity among models in the comparisons with real shape data.

Despite differences in type of data (classic vs. geometric morphometric), samples used, and small methodological aspects, we have in some way replicated the statistical analyses presented by Hubbe et al. (2010). First, instead of using classical cranial measurements (CM) we adopt geometric morphometrics (GM) as a way to carry out the analysis of shape differences. The advantages of these methods over classical measurements were discussed elsewhere (Bookstein, 1991; Adams et al., 2004; Zelditch et al., 2004; Mitteroecker and Gunz, 2009), but the main difference among them is that the geometric information, including spatial relationships among measurements and dimensionality, is lost during the statistical analyses of CM, while GM data preserves the geometry of the landmark configurations throughout the analysis and thus permits us to represent statistical results as actual shapes or forms (Mitteroecker and Gunz, 2009). Even though we recognize that CM and 2D GM are not directly contrastable,

we do think that Hubbe et al. (2010) and our analyses are indeed comparable, since both of them address the same evolutionary problem. Secondly, we use a sample with enhanced geographical coverage, especially in the New World, in order to obtain more solid results when using matrix comparison methods. We include four Circum-Arctic populations (including Eskimos), which guarantees that their role in the different scenarios can be modeled; we also include a sample of modern skulls from Baja California, Mexico, which presents morphological similarities with early remains from Brazil (González-José et al., 2003). Third, we incorporate the entire spectrum of scenarios discussed in the recent literature, including a simple model of Isolation-by-Distance, the SW, the TW, and the RGF models. Finally, we estimate the effects of climate on shape variation, modeling it on matrices of climatic distances rather than dissecting the skull into structures more or less (putatively) affected by climate, since it represents a quantification of climatic effects more suitable to matrix comparison methods.

MATERIALS AND METHODS

Sample and data acquisition

In this paper we reanalyze the 23 skull-photographs series published in González-José et al. (2008). The studied assemblages includes a South Paleoamerican series from Lagoa Santa, a composite sample of North Paleoamericans, and a composite series of late Pleistocene Old World specimens (Table 1). The total sample includes 576 complete adult skulls of both sexes. Each lateral photograph was used to recover the general shape of the skull after the digitizing of 23 landmarks and semilandmarks (see González-José et al., 2008, Fig. 1). Landmark and semilandmark configurations were processed by means of geometric morphometrics, a useful approach for the quantitative characterization analysis and comparison of biological form (Bookstein, 1991; Adams et al.,

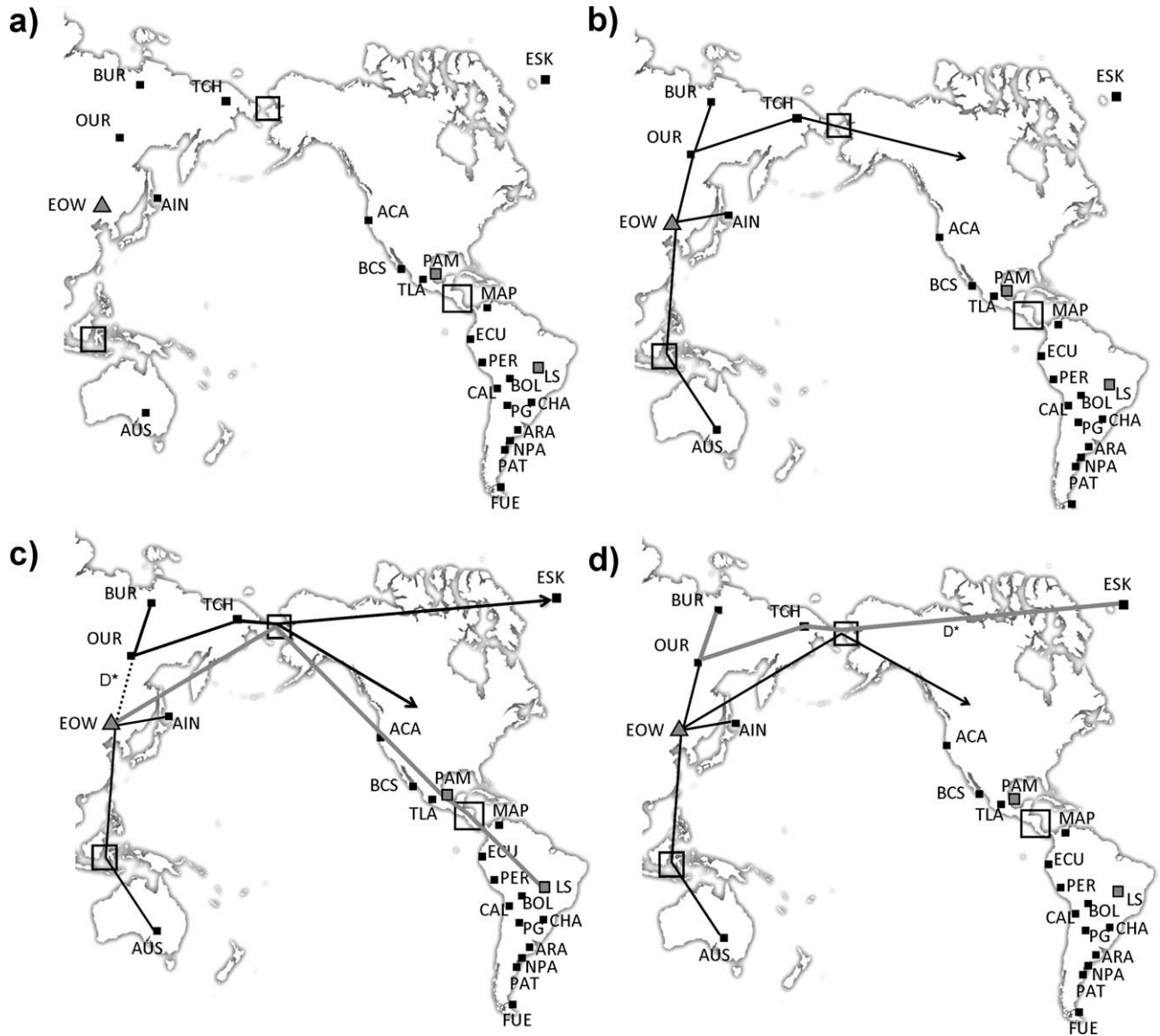


Fig. 1. Schematic representation of the geographic dispersion models compared in this paper. (a) IBD model, reflecting all pairwise direct distances in kilometers and considering passage across specific waypoints (squares). (b) SW model, reflecting distances calculated in the same way as in IBD, except for those populations that are linked by black lines, putatively belonging to a non-American, Old World population. (c) TW model, where thick black lines and gray lines represent the two separate dispersion events (“Paleoamericans” and “Amerindians”, respectively) according to the model. Distances between any early and any modern Native American sample is calculated as the distance from the early population to EOW (following the gray pathway) plus the distance from EOW to the modern sample (following the dashed black and thick black line). Changing the D^* distances (dashed black line) allows us to model the levels of evolutionary differentiation among the alleged early and modern “waves” (by multiplying D^* by arbitrary factors). (d) RGF model, where gray lines represent relationships among Arctic groups; thus the distance between Eskimos and the remaining American groups is calculated across Asia through the Arctic populations, next following the black line to EOW, and finally returning to Bering and thence to the population within America. To account for scenarios of increasing gene flow between the Arctic populations we changed the distance between ESK and the Bering waypoint (D^*), by multiplying it by an arbitrary factor (see text for details).

2004; Zelditch et al., 2004; Slice, 2007; Mitteroecker and Gunz, 2009). Original configurations were superimposed using the Generalized Procrustes Analysis (GPA) to remove the effects of translation, rotation, and scaling (Rohlf and Slice, 1990; Zelditch et al., 2004). Subsequent analyses were made on the matrix of PC scores (shape variables) extracted from MorphoJ (Klingenberg, 2011). We describe the details of the performed tests in the following sections.

Minimum spanning Tree

First we performed a morphological affinity analysis of the samples by calculating a Minimum Spanning Tree (MST) on the between-groups D^2 squared Mahalanobis distances matrix. We computed the matrix of Mahalanobis D^2 distances among samples using MorphoJ (Klingenberg, 2011). The D^2 matrix was then submitted to a Minimum Spanning Tree algorithm in order to depict

the relation network connecting all points. To facilitate interpretation among samples, differences were graphically represented in two different ways. First, the MST was superimposed on a map, over the geographic coordinates of the series. Second, to explore shape changes associated with the topology of the tree, and taking advantage of the visualization facilities of geometric morphometrics, we superimposed the MST on the plot of the first two canonical vectors obtained on the sample, in order to visualize the among-groups relationships along with associated shape changes simultaneously.

Settlement models and climate expressed as dissimilarity matrices

Second, we constructed different geographic design matrices reflecting competing hypotheses regarding the dispersion and evolutionary models for the peopling of the New World to assess their congruence with the observed craniometric distance matrix. We created a null model based upon evolutionary expectations of cranial differentiation according to the principles of isolation-by-geographic distances. However, since the models to be tested involve time as well as geographic dimensions, we also introduced the possible effects of morphological differentiation due to time (Konigsberg, 1990). Thus, the subsequent models are modifications of the null isolation-by-geographic distances model, in order to evaluate distinct evolutionary trajectories among early and modern groups. Here it is worth pointing out that including Eskimo samples is an important difference from previous studies (e.g., Hubbe et al., 2010) since it enables the exploration of putative Recurrent Gene Flow with Asia when modeling dispersal scenarios.

When both data and hypotheses can be represented as distance matrices, a frequently used method for assessing the fit between them is the Mantel correlation test (Mantel, 1967). A highly significant Mantel result between a hypothesis matrix **A** and data matrix **C**, but no significance between hypothesis matrix **B** and data matrix **C**, suggests that scenario **A** fits the data better than theory **B**, even without a statistical test of the equality of the correlations (Sokal et al., 1997). Another hypothesis-testing approach, developed by Dow and Cheverud (1985) determines whether two correlations $r(\mathbf{AC})$ and $r(\mathbf{BC})$ differ significantly from each other. However, Oden (1992) suggested that the Dow-Cheverud test could be more sensitive to spatial, temporal or phylogenetic autocorrelation of data. Here we performed Mantel tests (Mantel, 1967) to assess the fit between the observed craniometric distance matrix (D2 squared Mahalanobis distances matrix, BIO) and each of the geographic model matrices. Also, the Smouse-Long-Sokal test for partial matrix correlation (Smouse et al., 1986) was used as an extension of the Mantel test, to estimate the association between two design matrices while controlling for the effect of a third. This test is useful for evaluating partial matrix correlation between two matrices when the effects of geographic distance (or another variable such as climate) are removed. Mantel and Smouse-Long-Sokal tests were computed using NTSYS 2.10d. *P*-values were obtained after 10,000 permutations and using the Bonferroni correction for multiple comparisons. The evaluation of competing dispersal and settlement models after Mantel and Smouse-Long-Sokal tests were previously used in the literature concerning the occupation of the New World (e.g., González-José et al., 2001a,b, 2002;

Pucciarelli et al., 2008) and Europe (e.g., Sokal et al., 1991, 1997; Waddle, 1994; Pinhasi and von Cramon-Taubadel, 2009).

For all settlement models, geographic distances among populations were calculated in kilometers as great-circle distances based on the Haversine (Pinhasi and von Cramon-Taubadel, 2009). The differences between the models rely on how the distance between groups of different populations is calculated (e.g., between Early and Late groups, or between Circum-Arctic and Asian populations, see below). For all the dispersion models, distance between series follows terrestrial routes, considering passage across specific waypoints located in the Sunda Strait, the Bering Strait, and the Panama Isthmus as mandatory waypoints.

Isolation-by-Distance. We calculated the first model as a control matrix, which simply represents the direct linear distance between all pairs of series, respecting the restrictions imposed by the Sunda, Bering, and Panama waypoints (Fig. 1a, Table A1). Since it is important to take into account diachronic divergence when attempting to test microevolutionary scenarios (Konigsberg, 1990), we have incorporated chronological variation in the computation of distances. Influence of time is considered by multiplying each element of the matrix by the chronological difference among samples (Hubbe et al., 2011). In operational terms, chronological distances were calculated in thousands of years taking the average of the estimates of the chronological ranges provided in Table 1. Thus, for example, geographical distances between two modern American populations were multiplied by 1; the geographical distance between any modern American populations and series from Lagoa Santa (LS) was multiplied by 9.25 (kyr BP); and the geographical distance between any modern American populations and early East Asians (EOW) was multiplied by 20.5 (kyr BP).

All further models are constructed as modifications of specific cells in the null model of IBD.

Single Wave. SW model represents a scenario of local microevolutionary differentiation within the Americas (Fig. 1b, Table A2). It predicts that modern Native Americans differentiated locally from early populations (e.g., Paleoamericans, or PAM, in the Hubbe et al. denomination) and therefore, following the range expansion model, their morphological differentiation should be proportional to their geographic proximity. It assumes that the differentiation between early East Asians (EOW, in the González-José et al., 2008 denomination) and modern East Asians occurred prior to the occupation of the New World. Distance between an American population (e.g., ACA) and early East Asians (EOW) is calculated passing through modern Asians (TCH, OUR) besides the Bering waypoint. For example, the distance between EOW and LS or PAM will differ from IBD because their hypothetical dispersal into the New World is tied to a specific pathway (solid lines joining specific samples on Fig. 1) depicting the pattern of dispersal and pertaining to a particular "wave". Thus, the distance PAM-EOW (140,311) is calculated as the summed geographic distance among PAM and EOW passing through TCH, OUR and the Bering waypoint (13,363) multiplied by the difference in thousands of years among the estimated ages of each sample (10.5). Note that such distance is greater than the distance computed on the null IBD model.

Within the Americas, the distances among series (both early and modern) are simply the direct linear distances, and remain unaltered with respect to the IBD matrix. This matrix represents a settlement model that involved only one major human entrance into the continent, with the morphological variability seen in the Americas through time being the result of in situ microevolutionary processes. This model approximately matches with Model 2 in Hubbe et al. (2010).

Two Waves. This model represents a scenario shaped by distinct origins for early and modern populations, with both groups representing distinct expansion events into the continent from East Asia through Beringia (Fig. 1c, Table A3). In this case, the hypothetical distance between early and modern samples is calculated as the distance from the early population to EOW plus the distance from EOW to the modern sample (e.g., distances ACA-PAM or TCH-LS will be larger in this model than in previous ones). Thus, distance PAM-PER (Peruvians) for example, is calculated as the summed geographic distance among PAM and EOW (PAM-Bering waypoint-EOW, as in IBD model) plus the distance between EOW and PER (passing through TCH, OUR, and the Bering waypoint), and finally multiplied by the difference in thousands of years among the estimated ages of each sample (10).

Distances between modern American populations are not affected in relation to the null IBD model. Also, the relationship between EOW and PAM/LS is the same as IBD, rather than as in SW, because TW and SW imply different dispersal patterns, following different pathways (see Fig. 1b,c). In this sense, the TW model simply inflates the difference among samples if they belong to different dispersal events. This model approximately matches with Model 3 in Hubbe et al. (2010).

Additionally, we computed four variants for this model. Distances between EOW and modern Asians, and in turn, modern Americans, were recalculated by multiplying by arbitrary factors (1, 1.5, 5, and 10, which correspond respectively to the TW_1 , $TW_{1.5}$, TW_5 , and TW_{10} matrices) in order to simulate increasing levels of evolutionary differentiation among both the early and modern series. See González-José et al. (2001a) and Pinhasi and von Cramon-Taubadel (2009) for previous uses of arbitrary and non-arbitrary multipliers.

Recurrent Gene Flow. This matrix was calculated in accordance with the RGF model (Fig. 1d, Table A4). For most of the comparisons, the pairwise distances among groups follow the same criteria as in the SW matrix. Nevertheless, in this model the relationships among Arctic groups were obtained with a different criterion in order to simulate a scenario of recurrent gene flow. Thus, the distance between Eskimo (ESK) and the remaining American groups will be obtained across Asia through the Arctic populations (as the distance between ESK and BUR (Buriats) passing through TCH and OUR) plus the distance to EOW and then returning to America through Bering (following black line on Fig. 1d). Thus, distances inside America are not affected in relation to the null IBD model, except for distances with ESK. Distances between Early and Late Americans are the same as in SW and IBD model. Distances between any American and Old World population is calculated departing from the American continent through the black line drawn in Figure 1d. Thus, for instance, distances between Americans and EOW remains the same as in

TABLE 2. Climatic variables used to compute climatic matrices

Climatic variables
Annual Mean Temperature
Mean Diurnal Range
Isothermality
Temperature Seasonality
Max Temperature of Warmest Month
Min Temperature of Coldest Month
Temperature Annual Range
Mean Temperature of Wettest Quarter
Mean Temperature of Driest Quarter
Mean Temperature of Warmest Quarter
Mean Temperature of Coldest Quarter
Annual Precipitation
Precipitation of Wettest Month
Precipitation of Driest Month
Precipitation Seasonality (Coefficient of Variation)
Precipitation of Wettest Quarter
Precipitation of Driest Quarter
Precipitation of Warmest Quarter
Precipitation of Coldest Quarter

See <http://www.worldclim.org/> for more information on the variables used.

IBD model, while distances between Americans and Arctic populations from Asia (OUR, BUR, and TCH) are larger with respect to SW.

Additionally, we computed four variants for this model by changing the distance between ESK and the Bering waypoint using distinct factors (1, 0.5, 0.1, and 0.01, which correspond respectively to RGF_1 , $RGF_{0.5}$, $RGF_{0.1}$, and $RGF_{0.01}$ matrices) in order to account for scenarios of increasing gene flow between the Arctic populations. Such models were not considered by Hubbe et al. (2010).

The four scenarios (IBD, SW, TW, and RGF) are presented as matrices in Supporting Information Tables A1-A4.

CLIM (bioclimatic variables). To test and control for the effect of climate, different environment variables were recovered from <http://www.worldclim.org/>, which offers a set of global climate layers (climate grids) with a spatial resolution of a square kilometer (Hijmans et al., 2005). In order to synthesize information coming from different climatic variables, we computed Euclidean distances matrices using the variables listed in Table 2: "CLIM", which include all the climatic variables; "TEMP" and "PREC", which includes just the temperature and precipitation variables, respectively; and "ALT", which reflects just differences in altitude.

Finally, we recalculated all matrices comparisons after removing Eskimos, in order to test for the influence of this population on the analyses and to compare our findings with that of Hubbe et al. (2010), since they do not include this Arctic population.

Rates of morphological evolution

Finally, we calculated the rate of morphological differentiation between early and modern series under the four different models, and then compared the results to the predicted morphological differentiation under neutral conditions. The expected magnitudes and patterns of phenotypic evolution under the influence of genetic drift and mutations alone can be evaluated using different approaches (Lande, 1976, 1979; Ackermann and Cheverud, 2002; Perez and Monteiro, 2009). A method connected to the neutral model of phenotypic evolution

(Lande, 1976, 1979) is the Lynch's (1990) neutral expectation for the Δ divergence rate, which allows us to evaluate whether the observed amount of divergence among pairs of populations is significantly lower or higher than that expected if mutation and random genetic drift were the sole evolutionary forces (Lynch, 1990). An unexpectedly low rate suggests that stabilizing selection has played a predominant role in preventing phenotypic divergence, whereas an unexpectedly high rate implies an acceleration of divergence by directional selection. Lynch (1990) provides an equation to calculate the rate of morphological evolution, given by:

$$\Delta = \frac{\text{var } B(\ln z)}{[t \text{ var } w(\ln z)]}$$

where $\text{var } w(\ln z)$ and $\text{var } B(\ln z)$ are the observed within and between species components of phenotypic variance for log-transformed measures of two species separated for t time units (Lynch, 1990). The observed Δ divergence rate is compared among different scenarios or to an expectation of divergence based on the literature, to evaluate whether the amount of divergence is lower or higher than the expected variation if mutation and random genetic drift were the single evolutionary forces. Lynch (1990) reports the expected range of the rate of morphological differentiation under neutral expectation for mammals to fall between 0.01 and 0.0001. We calculated the rate of morphological differentiation across shape variables for pairs of populations according to Lynch (1990). Nevertheless, since shape variables are on an interval rather than a ratio scale as the zero point is arbitrary and a function of the superimposition/rescaling procedures in the Procrustes analysis (Hansen and Houle, 2008), their logarithms are not meaningful. Thus, we have computed the Δ divergence rate using raw variances computed on the first PC of shape scores instead of log-transformed measures, since geometric morphometric shape variables are already mean standardized (for a similar approach see Pérez and Monteiro, 2009). The comparisons were made exclusively between early and modern American series, altering the number of generations occurring after the split according to five distinct scenarios (see below). In all cases, generation time was assumed to be 20 years (Lynch, 1990). The age of each series was assumed as the mean of the chronological range presented in Table 1, and for recent series it was assumed to be 1,000 years old.

The first scenario (SW) assumes that early American groups are the direct ancestral populations of modern Native Americans, and as such t is defined as the number of generations separating early and late American series. Consequently, this scenario takes into account models for the occupation of the New World that assume only one migration into the continent.

The second and third scenarios (TW1 and TW2), on the other hand, are in accordance with the dual-dispersal model. TW1 assumes that early Americans and modern Native Americans share their last common ancestor by the time of the first occupation of the continent, around 15 kyr BP. TW2 assumes that the last common ancestor between these lineages is represented by the Upper Cave specimens, dated around 20 kyr BP. Accordingly, t for each scenario was defined as the sum of generations down each branch to the date of the last common ancestor assumed.

Fourth and fifth scenarios (RGF and RGF_{bis}) are in accordance with the RGF model. They assume that early American groups are the direct ancestral population of Native Americans, and as such t is defined as the number of generations separating early and late American series. In addition, RGF model considers all Circum-Arctic (both Asian and American) groups as a single population. Thus, the RGF scenario was built much in the same way as SW, but in the computation of the morphological divergence the Eskimos were replaced by a novel group, "CA", that includes a pooling of both Asian and American Circum-Arctic populations. On the other hand, RGF_{bis} can be seen as an alternative RGF scenario, where the Eskimos are compared to Circum-Arctic Asian populations, taking as the last common ancestor between these lineages the first fossil record of people carrying the complete set of derived traits that characterize modern northeast Asian groups, dated around 7000 kyr BP (Brown, 1999). To sum up, two different approaches are implemented here in order to contrast the RGF model.

It is worth pointing out though, that the Lynch test assumes no gene flow and uniformity of rates along lineages, which would contradict a couple of our models. This is a complication common to studies dealing with most of the quantitative genetics-derived tests at the intraspecific level. The Lynch test is sensitive to the effects of gene flow that can potentially reduce the degree of differentiation independently of the number of generations elapsed. Thus, here and elsewhere (e.g., Hubbe et al., 2010) the comparisons are usually limited exclusively to early versus modern series, only altering the number of generations occurring after the split according to distinct scenarios, and avoiding comparisons among modern series that can be flawed by variable levels of gene flow.

RESULTS

Figures 2 and 3 show the Minimum Spanning Tree of the studied samples superimposed on the geographic location of the populations and on the morphological space defined by the first two canonical vectors, respectively. Removal of the Eskimos from the computation of the Mahalanobis D2 distance matrix did not alter the topology of the tree (results not shown). Shape changes associated with the first two canonical vectors obtained on the sample (see Fig. 2) show that ancient groups as the Lagoa Santa (LS) series, or modern groups from Baja California or Tlatelolcans, represent one extreme of variation that is closely associated to early Old World specimens, with low and projected faces, subnasal prognathism, long vaults, retracted zygomatics, and low noses. Conversely, Native American groups like Eskimos show the opposite morphological pattern more commonly seen in northeastern Asians, which is characterized by high and flat retracted faces, short vaults, anterior-projected and high zygomatics, and high noses. However, most of the New World samples fall well between both extremes. Also, note that there is no clear-cut difference between early American series and modern groups, in the context of this paper, which is an expectation that emerges from the TW model. Conversely, some modern groups like Tlatelolcans and the Pericú from Baja California are linked by their nearest morphological distances to the Early Americans from Lagoa Santa (see Fig. 2 and González-José et al., 2003). Furthermore, all

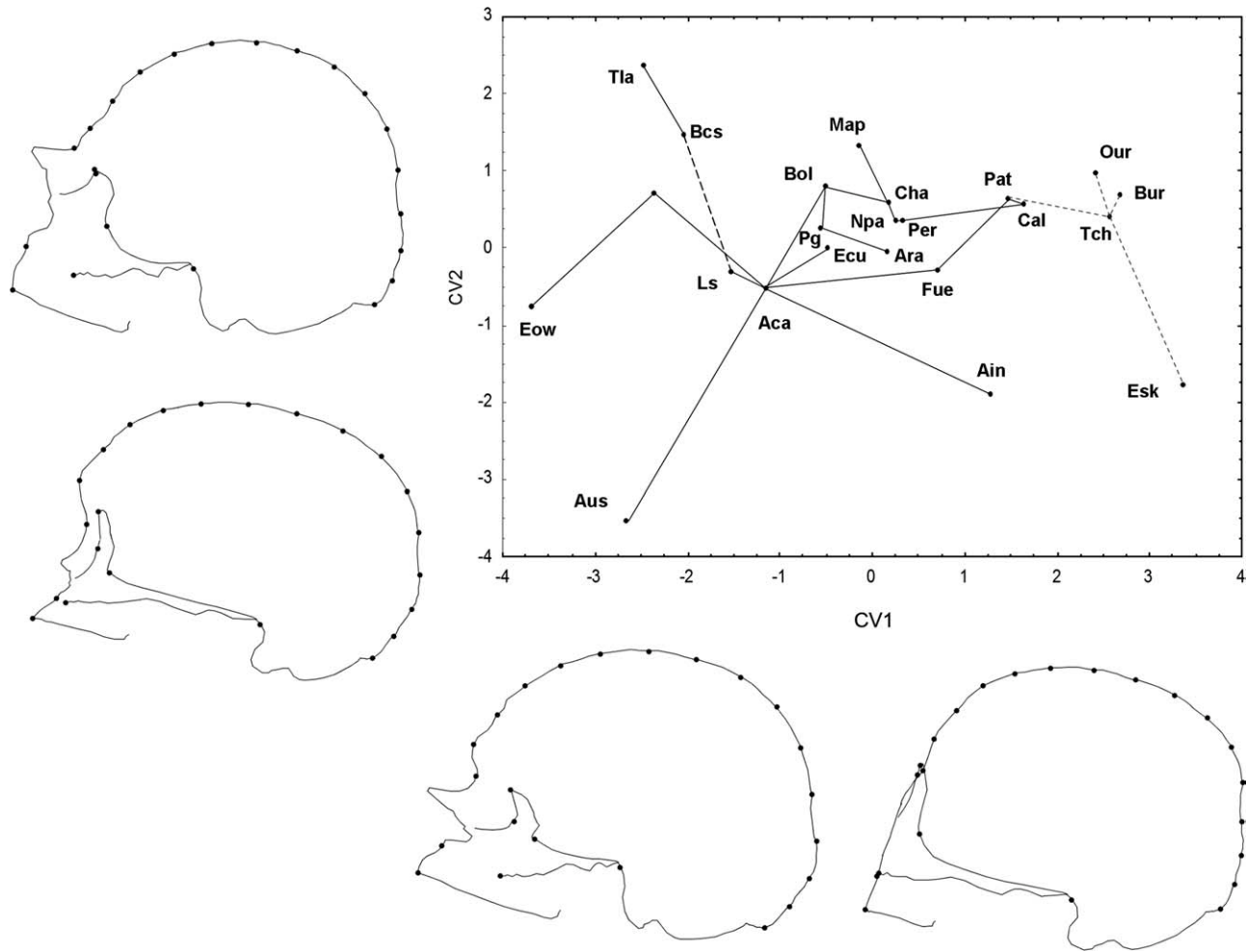


Fig. 2. Minimum Spanning Tree calculated from the among-groups Mahalanobis squared distances, plotted upon the first two canonical vectors reflecting among-groups differentiation. Shape changes across canonical axes are represented as a deformation of an outline drawing using the thin-plate spline function (the scale factor was set at 10 units of Procrustes distance to exaggerate shape changes) for the positive and negative values.

Circum-Arctic (both Asian and American) groups are linked by their nearest morphological distances.

Mantel correlations between biological and dispersal scenario design matrices are presented in Table 3. Note that after applying Bonferroni's correction ($\alpha = 0.017$), the only model presenting significant correlation with the skull shape distance matrix (BIO) is RGF and its variants. Another important result (Table 4) is that most models tend to be correlated among them. This is particularly clear for RGF, SW, and IBD with each other, while TW is less correlated with the rest (Table 4).

Table 5 shows the results of the Smouse-Long-Sokal test for partial matrix correlations. The aim of this test is to control the effects of other competing models and climate on the correlation among BIO and the settlement models. From the different variables of the TW and RGF models, only those of better performance (according to Table 3) were considered. As observed in Table 5, the RGF model remains significantly correlated to BIO when the effects of IBD and SW are controlled for. Furthermore, the RGF model remains significant when the effects of climate are removed.

In Figure 4 we present the mean rates of morphological differentiation calculated for all possible pairwise

comparisons between early and late American series. These results can only be interpreted in relation to the reported expected rate of morphological change for mammals under neutral evolutionary expectations, which ranges from 0.0001 to 0.01, according to Lynch (1990). Our results show that, whichever settlement scenario is considered, each of them presents average differentiation rates coincident to neutral expectations, with most of the pairwise comparisons falling well between the upper and lower limit of neutral expectation. In other words, the results of the Lynch (1990) test neither favor nor penalize any of the scenarios considered here, in terms of evolution under a neutral scenario. This result is at odds with that found by Hubbe et al. (2010), which reports accordance to neutral evolution only for the TW model. The pairwise comparisons with greater between-group differentiation, with Δ values exceeding the upper limit are LS-CAL, and LS-ESK under a SW scenario, and LS-CAL in the case of the RGF scenario.

DISCUSSION

We based our comparisons of settlement scenarios on three separate analyses performed on a sample of

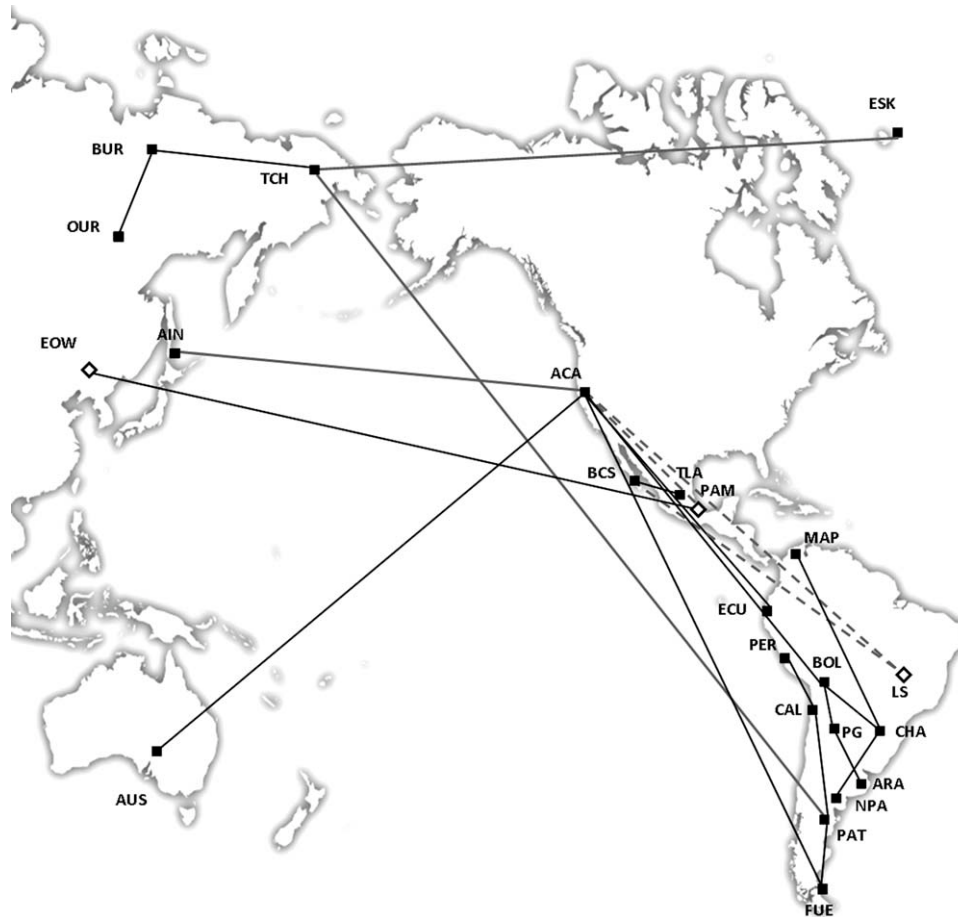


Fig. 3. Minimum Spanning Tree calculated from the among-groups Mahalanobis distances, plotted upon their geographic coordinates. The lines represent the closest path for connecting all samples according to the morphological distances between them. White diamonds: early American samples and early East Asian (EOW) specimens; black squares: Late Holocene samples from East Asia, the Americas and Australia. Specific connections among Late and Early Americans are shown by dotted gray lines, whereas specific connections among Early Asians and Americans are depicted in gray.

TABLE 3. Mantel correlations between the biological matrix of Mahalanobis D2 distances among groups (BIO) and dispersal models (IBD, SW, TW and RGF) and their variants (TW1.5, TW5, TW10, RGF0.5, RGF0.1, and RGF0.01) are given as correlation coefficients (r) with P-values (P)

Morphological Distances against Models		r	P
BIO	x IBD	0.294	0.022
BIO	x SW	0.298	0.021
BIO	x TW1	0.214	0.072
	x TW1.5	0.218	0.074
	x TW15	0.245	0.051
	x TW10	0.271	0.035
BIO	x RGF1	0.335	0.007
	x RGF0.5	0.326	0.009
	x RGF0.1	0.318	0.012
	x RGF0.01	0.316	0.011

Significant results, after Bonferroni correction ($\alpha = 0.017$) are shown in bold.

geometric morphometric cranial measurements: an interpretation of the nearest neighbor connections (Minimum Spanning Tree based on Mahalanobis distances); a series of Mantel tests aimed at comparing morphological distances and predicted distances under each model; and

the computation of mean rates of morphological differentiation calculated for all possible pairwise comparisons between samples using time divergences according to each model, and comparison against neutral evolutionary expectations.

On a previous similar analysis, Hubbe et al. (2010) concluded that the morphological diversity documented through time in the New World is best accounted for by a model postulating two waves of human expansion into the continent (Hubbe et al., 2010). Unfortunately, they did not incorporate Eskimo samples into their analyses, even though a sample from Greenland’s Inuit is available as part of William Howells’ free-access data that they used. Also, they did not include any version of the RGF model as a valid scenario to compare against the SW and TW hypotheses, even when they referenced the González-José et al. (2008) article. Here, we performed similar analyses, and acknowledging that potential differences in results can arise because of traits used and/or sampling structure, our results suggest that the model that best fits our data is the RGF, instead of a model postulating two waves of human expansion into the continent. We will shortly discuss the main results that gave support to the main inferences considered under the Recurrent Gene Flow model and its variants.

TABLE 4. Mantel correlations between dispersal models

	IBD	SW	TW1	TW1,5	TW5	TW10	RGF1	RGF0,5	RGF0,1	RGF0,01
IBD	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SW	0.999	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TW1	0.718	0.711	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TW1,5	0.722	0.715	1.000	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
TW5	0.746	0.739	0.995	0.996	1	0.0000	0.0000	0.0000	0.0000	0.0000
TW10	0.764	0.758	0.982	0.985	0.996	1	0.0000	0.0000	0.0000	0.0000
RGF1	0.977	0.976	0.717	0.720	0.743	0.760	1	0.0000	0.0000	0.0000
RGF0,5	0.980	0.979	0.718	0.722	0.744	0.760	0.999	1	0.0000	0.0000
RGF0,1	0.982	0.981	0.718	0.722	0.744	0.759	0.997	0.999	1	0.0000
RGF0,01	0.982	0.981	0.718	0.721	0.744	0.759	0.996	0.999	1.000	1

Correlation coefficients (*r*) are shown below the principal diagonal. *P*-values after Bonferroni correction ($\alpha = 0.05/45 = 0.0011$) are shown above the principal diagonal. Bolded values indicate significant correlations.

TABLE 5. Results of the Smouse-Long-Sokal test for partial matrix correlation controlling the effects of competing models and climate

Morphological distances against models (controlling for other models)		
	<i>R</i>	<i>P</i>
BIOx SW.TW10	0.148	0.102
BIOx SW.IBD	0.113	0.099
BIOx SW.RGF1	-0.140	0.083
BIOx TW10.RGF1	0.027	0.381
BIOx TW10.IBD	0.075	0.252
BIOx TW10.SW	0.072	0.267
BIOx RGF1.TW10	0.072	0.027
BIOx RGF1.IBD	0.236	0.010
BIOx RGF1.SW	0.212	0.017
Morphological distances against models (controlling for climate)		
	<i>R</i>	<i>P</i>
BIOx SW.CLIMA	0.290	0.022
BIOx SW.TEMP	0.246	0.057
BIOx SW.PREC	0.296	0.024
BIOx SW.ALT	0.284	0.025
BIOx TW10.CLIMA	0.269	0.031
BIOx TW10.TEMP	0.265	0.044
BIOx TW10.ALT	0.258	0.034
BIOx TW10.PREC	0.260	0.039
BIOx RGF1.CLIMA	0.325	0.007
BIOx RGF1.TEMP	0.282	0.032
BIOx RGF1.PREC	0.333	0.009
BIOx RGF1.ALT	0.320	0.008

P-values after Bonferroni correction ($\alpha = 0.017$) are shown in bold. The “x” denotes comparison among matrices, and the dot is followed by the matrix whose effects are controlled.

In the first place, the Minimum Spanning Tree based on Mahalanobis distances (Figs. 2 and 3) failed to provide a clear-cut difference between Early American samples (so-called Paleoamericans) and modern groups or “Amerindians,” representing the descendants of a putative second wave. Instead, some modern groups like Tlatelolcans or the Pericú from Baja California are linked by their nearest morphological distances to the Early Americans from Lagoa Santa (see Fig. 2 and González-José et al., 2003). Although the morphological pattern observed is not novel, it is relevant here since it does not fit with the predictions of the TW model. Furthermore, instead of a split among an Asian and an American morphology, there is a complete branch of morphological similarity joining together all Circum-Arctic (both Asian and American) groups. Both results contradict the TW and SW models respectively, and agree with the main expectations of the RGF model of a continuum of Arctic populations shaping

some of the molecular, archaeological, and skeletal particularities of the Asian and American populations when considered on a continental scale and including both Arctic and non-Arctic samples.

The most significant values for the matrix correlation tests correspond to the RGF model (Table 3). Interestingly, when the Eskimos are removed from the analyses, the correlation of morphological distances versus IBD, SW and TW increase, whereas it causes a diminishing of the correlation with RGF, turning to nonsignificant correlation values (results not shown). Again, this indicates that the removal of some specific populations contributing greatly to both; geographic variation due to their extreme location; and morphological differentiation due to their derived phenotypic pattern can deviate the results of Mantel correlations in particular, and our understanding and interpretation of continental variation and its consequences on the debate about origins and dispersal patterns in general.

As is usual in approaches where design matrices have high dimensions and models differ just in specific aspects, models tend to be correlated among them (Oden, 1992; Smouse and Long, 1992). This is particularly true for comparisons among SW and RGF models (Table 4). To further explore the performance of the models controlling for the similarity among them, or controlling for the effect of matrices containing among-group climatic differences, we have obtained partial correlations. By employing a conservative *P*-value for the Smouse-Long-Sokal method (e.g., $P = 0.001$), we can be reasonably certain that the partial correlation coefficients are significant at $P = 0.05$ (Oden and Sokal, 1992). Here we computed the Smouse-Long-Sokal test applying Bonferroni correction for multiple comparisons, lowering the accepted α -level to be more conservative (see Tables 3–5). On the other hand, it is worth mentioning that here we have tested all the possible combinations of controlling for competing models and the effects of climate, whereas Hubbe et al. (2010) only performed Dow-Cheverud tests in order to test if Model 3 (bipartite Asian origin, analogous to our TW model) presents a better fit to the morphological distances than Model 1 (control model, analogous to our IBD model) and Model 2 (analogous to our SW model). Hubbe et al. (2010) did not present the logical counterpart correlation among biological distances and SW controlling for TW (testing if Model 2 presents a better fit to the morphological distances than the other models).

Here, the computation of the Smouse-Long-Sokal tests (Table 5) revealed that when all the possible comparisons between models are made, and applying a conservative

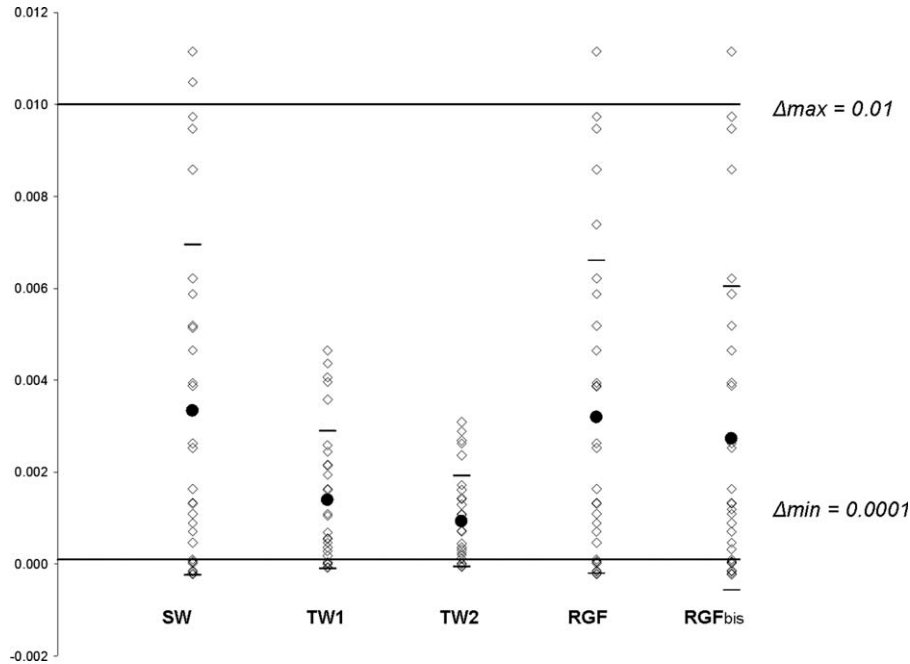


Fig. 4. Pairwise mean rates of morphological differentiation calculated between Early and Late American populations. For each scenario, the black dots represent the average of the pairwise mean rates and the small horizontal black lines represent their standard deviations. The black horizontal lines show the upper and lower limits of the neutral expectation range (0.01-0.0001) according to Lynch (1990). SW: Single Wave; TW1 and TW2: Two Wave model, assuming that Early Americans and modern Native Americans share their last common ancestor by the time of the first occupation of the continent, around 15 kyr BP (TW1) or by the time of Upper Cave specimens, dated around 20 kyr BP (TW2); RGF: Recurrent Gene Flow model assuming that early American groups are the direct ancestral population of Native Americans and take into account a recurrent gene flow among Circum-Arctic (both Asian and American) groups. On RGF_{bis} we assume the first fossil record with derived traits, dated around 7000 kyr BP, as the last common ancestor between Eskimos and Circum-Arctic Asian populations.

P-value, the RGF scenario conserves its performance and remains significant, whereas the correlation among morphological distances and design matrices decay in the case of the SW and TW models. Also, note that the performance of the RGF model remains significant even when the effects of climate are controlled. Also, the results corresponding to the Smouse-Long-Sokal test for partial matrix correlations computed after the removal of Eskimos from the original data reinforces the correlation for SW and TW models while decreasing the performance of the RGF model (results not shown), as in the Mantel test results. We used Bonferroni correction, lowering the accepted α -level to 0.017 (0.05/3) considering the three main models under study. However, we would like to emphasize that, despite the denominator used in Bonferroni's correction, it is clear that the RGF model presents a better performance when compared with SW and TW (Tables 3–5).

Our results regarding rates of morphological evolution analysis show that the pairwise comparisons for all settlement scenarios considered fall well between limits of neutral expectation for morphological change reported by Lynch (1990). Conversely, Hubbe et al. (2010) obtained high rates of morphological differentiation in general. In particular, although the mean rates for both of their scenarios (“bipartite Asian origin” and “single wave”) fall above the upper limit of neutral expectation, the bipartite model is much closer to the neutral limit than the “single wave”. Conversely, the divergence rates observed here show that, assuming neutrality, neither model is favored over the others. Future research will benefit from other formal tests arising from the theoretic

cal framework and statistical frame for the multivariate generalization of the selection theory based on quantitative genetics (Lande, 1979; Arnold et al., 2001; McGuigan, 2006) aimed to measure the importance of stochastic versus non-stochastic agents on the apportionment of phenotypic variation (Ackermann and Cheverud, 2002, 2004; Marroig and Cheverud, 2004; Weaver et al., 2007; Perez and Monteiro, 2009; de Azevedo et al., 2010).

These results are also relevant in the context of the debate about the extent of skull-shape variability accumulated through neutral processes versus natural selection driven by climate (e.g., Relethford, 1994, 2010; Roseman, 2004; González-José et al., 2004, 2005; Harvati and Weaver, 2006; Manica et al., 2007; Betti et al., 2009; Hubbe et al., 2009; Perez and Monteiro, 2009; Smith, 2009). Most of these works shows that cranial morphology varies among regions in a manner consistent with neutral expectations, and that the action of natural selection would be putatively restricted to special aspects of the skull morphology exhibited by populations from extremely cold environments such as Northeastern Asians and Eskimos (e.g., Roseman, 2004; Manica et al., 2009; Hubbe et al., 2009; Relethford, 2010). Our results concerning computation of the Lynch parameters, however, show a range of differentiation compatible with neutral expectations, even when Eskimos and Siberians are included in the analysis. This is a significant finding in terms of implications for using craniometric variation as a proxy for neutral variation.

In summary, and after having replicated approximately the same analyses presented in Hubbe et al. (2010), our results bring preliminary support to the RGF

model. It is obvious that incongruence among results can be attributable to differences in type of data (linear measurements vs. geometric morphometrics techniques applied to landmark data) and the sample structure used. In this context, there are several details that can account for the divergence among both sets of results. First, although the samples are similar in that both present series of American and Asian populations from Early and Late horizons, the sample used here has a greater geographical coverage representing a larger number of populations, which reinforces the robustness of the results. This is particularly important in the Minimum Spanning Network analyses, where the detection of informative similarities can be obscured or lost if specific samples are not included in the analysis. Logically, matrix comparisons also provide more robust results as more elements are included in the matrices to be compared (Smouse et al., 1986).

Secondly, the inclusion of Arctic populations such as the Eskimos from Greenland, and the Buriats, Ourga and Tchoucktchi from Siberia allowed us to model alternative, previously published occupation scenarios, such as the RGF. The inclusion of integrative models is important in order to avoid simplistic comparisons among models based on partial evidence such as molecular genetics (SW) or skull morphology (TW).

Finally, we have included specific modifications on the "raw" models, aimed to simulate, for instance, a variable chronological separation among the two alleged components/migrations considered under the TW model, or different degrees of gene flow among Arctic groups in the RGF scenario.

Our results are congruent with other evaluations of the SW, TW and RGF models that were subjected to independent contrast in some recent papers. These analyses were based on Approximate Bayesian Computation (ABC) (Fagundes et al., 2007, 2008a,b; Ray et al., 2010). ABC applied to these three models suggested that neither a single, discrete wave of colonization, nor a scenario with two discrete migration waves is supported by the genetic data (Ray et al., 2010). Conversely, ABC approaches indicate that the current genetic diversity of Amerindian populations is best explained by a model involving recurrent gene flow between Asia and America, after initial colonization (Tamm et al., 2007; Wang et al., 2007, Ray et al. 2010). The ABC analyses contrasting different scenarios are stimulating, since they enable the simulation of demographic parameters that can be of great influence in determining patterns of within- and between-group variation. This is particularly important for future evaluations of the RGF model, which postulates the existence of a migration rate among the American and the Asian arctic populations in order to explain the archaeological evidence pointing to parallel and synchronic lithic sequences on both sides of the Bering Strait (Dixon, 2001; Goebel et al., 2003), as well as the persistence of some DNA lineages that can be defined as "Beringian" instead of ancestral Asian or autochthonous American (Zlojutro et al., 2006; Tamm et al., 2007; Rasmussen et al., 2010), and the existence of an extremely derived morphological pattern shared by populations on both sides of the Bering Strait (González-José et al., 2008). Interestingly, the earliest human expansion into the New World's northern extremes, documented on the Paleo-Eskimo remains from the Saq-qaq settlement (3400 to 4500 years old) provided samples of mtDNA falling within haplogroup D2a1, a group

previously observed among modern Aleuts and Siberian Sireniki Yuit. This result suggests that the earliest migrants into the New World's northern extremes derived from populations in the Bering Sea area and carried lineages that are shared by Asian and American groups today (Gilbert et al., 2008).

An important note of caution on future analyses using matrix comparison techniques is to consider that, in its theoretical basis, the elaboration of models is grounded on the knowledge about coalescence of the markers under study. For instance, coalescence in Beringia is expected for Y-chromosome and mtDNA markers, but this is not necessarily true for autosomal markers or quantitative traits. Note that even in the case of the haplogroups Q and C of the Y-chromosome their coalescence is likely to occur in Africa (Santos et al., 1999, 2007). In other words, we suggest that future approaches based on comparison among morphological or genetic distance and design matrices should take into account the particularities of the marker under study, especially their putative coalescence patterns. In this regard, the inclusion of skull samples belonging to basal populations, as does our EOW series, is important for depicting more complex scenarios. Moreover, the simulation of coalescence processes of deeper chronology, or differential patterns of gene flow, as portrayed in our variants to models TW and RGF, can shed some light on the particularities and possible origin of the within- and between-groups' patterns of shape variation.

CONCLUSIONS

The results obtained here suggest that a model considering a single origin for all the Native Americans from a single population carrying high levels of morphological internal heterogeneity, plus local, *in situ* evolution, plus the persistence of a continuum of Asian-American populations inhabiting the extreme Arctic landscapes constitutes a likely explanation for the observed craniofacial shape evidence. Also, this model reinforces the importance of gene flow among Circum-Arctic groups in order to explain recent genetic, skeletal, linguistic and archaeological data.

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LITERATURE CITED

- Ackermann RR, Cheverud JM. 2002. Discerning evolutionary processes in patterns of tamarin (genus *Saguinus*) craniofacial variation. *Am J Phys Anthropol* 117:260–271.
- Ackermann RR, Cheverud JM. 2004. Detecting genetic drift versus selection in human evolution. *Proc Natl Acad Sci USA* 101:17946–17951.

- Adams DC, Rohlf FJ, Slice DE. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Ital J Zool* 71:5–16.
- Arnold SJ, Pfrander ME, Jones AG. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112:9–32.
- Betti L, Balloux F, Hanihara T, Manica A. 2009. The relative role of drift and selection in shaping the human skull. *Am J Phys Anthropol* 141:76–82.
- Bonato SL, Salzano FM. 1997a. A single and early migration for the peopling of the Americas supported by mitochondrial DNA sequence data. *Proc Natl Acad Sci USA* 94:1866–1871.
- Bonato SL, Salzano FM. 1997b. Diversity and age of the four major mtDNA haplogroups and their implications for the peopling of the New World. *Am J Hum Genet* 61:1413–1423.
- Bookstein FL. 1991. *Morphometric tools for landmark data*. Cambridge: Cambridge University Press.
- Brown P. 1999. The first modern East Asians? Another look at Upper Cave 101, Liujiang and Minatogawa. Interdisciplinary perspectives on the origins of the Japanese. Kyoto: International Research Center for Japanese Studies. p105–130.
- Cavalli-Sforza LL, Menozzi P, Piazza A. 1994. *The history and geography of human genes*. Princeton, NJ: Princeton University Press.
- de Azevedo S, Paschetta C, Castillo L, González M, Hernández M, Martínez-Abadías N, Pucciarelli HM, González-José R. 2010. Genética cuantitativa aplicada a la evolución craneofacial en Asia y América. *Revista Española de Antropología Física* 31:13–38.
- Dillehay TD, Ramirez C, Pino M, Collins MB, Rossen J, Pino-Navarro JD. 2008. Monte Verde: seaweed, food, medicine, and the peopling of South America. *Science* 320:784–786.
- Dixon EJ. 2001. Human colonization of the Americas: timing, technology and process. *Quat Sci Rev* 20:277–299.
- Dow MM, Cheverud JM. 1985. Comparison of distance matrices in studies of population structure and genetic microdifferentiation: quadratic assignment. *Am J Phys Anthropol* 68:367–373.
- Fagundes NJ, Ray N, Beaumont M, Neuenschwander S, Salzano FM, Bonatto SL, Excoffier L. 2007. Statistical evaluation of alternative models of human evolution. *Proc Natl Acad Sci USA* 104:17614–17619.
- Fagundes NJR, Kanitz R, and Bonatto SL. 2008a. A reevaluation of the Native American mtDNA genome diversity and its bearing on the models of early colonization of Beringia. *PLoS ONE* 3:e3157. doi:10.1371/journal.pone.0003157.
- Fagundes NJR, Kanitz R, Eckert R, Valls ACS, Bogo MR, Salzano FM, Smith DG, Silva-Jr WA, Zago MA, Ribeiro-dos-Santos AK, et al. 2008b. Mitochondrial population genomics supports a single pre-Clovis origin with a coastal route for the peopling of the Americas. *Am J Hum Genet* 82:583–592.
- Gilbert MT, Kivisild T, Gronnow B, Andersen PK, Metspalu E, Reidla M, Tamm E, Axelsson E, Gotherstrom A, Campos PF, et al. 2008. Paleo-Eskimo mtDNA genome reveals matrilineal discontinuity in Greenland. *Science* 320:1787–1789.
- Goebel T, Waters MR, Dikova M. 2003. The archaeology of Ushki Lake, Kamchatka, and the Pleistocene peopling of the Americas. *Science* 301:501–505.
- Goebel T, Waters MR, O'Rourke DH. 2008. The late Pleistocene dispersal of modern humans in the Americas. *Science* 319:1497–1502.
- González-José R, Van der Molen S, González-Perez E, Hernández M. 2004. Patterns of phenotypic covariation and correlation in modern humans as viewed from morphological integration. *Am J Phys Anthropol* 123:69–77.
- González-José R, Ramirez-Rozzi F, Sardi M, Martínez-Abadías N, Hernández M, Pucciarelli HM. 2005. Functional-cranial approach to the influence of economic strategy on skull morphology. *Am J Phys Anthropol* 128:757–771.
- González-José R, Bortolini MC, Santos FR, Bonatto SL. 2008. The peopling of America: craniofacial shape variation on a continental scale and its interpretation from an interdisciplinary view. *Am J Phys Anthropol* 137:175–187.
- González-José R, Dahinten SL, Luis MA, Hernández M, Pucciarelli HM. 2001a. Craniometric variation and the settlement of the Americas: testing hypotheses by means of R-matrix and matrix correlation analyses. *Am J Phys Anthropol* 116:154–165.
- González-José R, Dahinten S, Hernández M. 2001b. The settlement of Patagonia: a matrix correlation study. *Hum Biol* 73:233–248.
- González-José R, García Moro C, Dahinten S, Hernández M. 2002. The origin of the Fuegian Patagonians: an approach to population history and population structure using R matrix and matrix permutation methods. *Am. J. Hum. Biol.* 14:308–320.
- González-José R, González-Martín A, Hernández M, Pucciarelli HM, Sardi M, Rosales A, Van der Molen S. 2003. Craniometric evidence for Palaeoamerican survival in Baja California. *Nature* 425:62–65.
- Greenberg JH, Turner Ii CG, Zegura SL. 1986. The settlement of the Americas: a comparison of the linguistic, dental and genetic evidence. *Curr Anthropol* 27:477–495.
- Hansen TF, Houle D. 2008. Measuring and comparing evolvability and constraint in multivariate characters. *J Evol Biol* 21:1201–1219.
- Harvati K, Weaver TD. 2006. Human cranial anatomy and the differential preservation of population history and climate signatures. *Anat Rec* 288:1225–1233.
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965–1978.
- Hubbe M, Hanihara T, Harvati K. 2009. Climate signatures in the morphological differentiation of worldwide modern human populations. *Anat Rec* 292:1720–1733.
- Hubbe M, Neves WA, Harvati K. 2010. Testing evolutionary and dispersion scenarios for the settlement of the New World. *PLoS One* 5:e11105. doi:10.1371/journal.pone.0011105
- Hubbe M, Harvati K, Neves WA. 2011. Paleoamerican morphology in the context of European and east Asian late Pleistocene variation: implications for human dispersion into the New World. *Am J Phys Anthropol* 144:442–453.
- Klingenberg CP. 2011. MorphoJ: An integrated software package for geometric morphometrics. *Mol Ecol Res* 11:353–357.
- Konigsberg LW. 1990. Analysis of prehistoric biological variation under a model of isolation by geographic and temporal distance. *Hum Biol* 62:49–70.
- Lande R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution* 30:314–334.
- Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution* 33:402–416.
- Lynch M. 1990. The rate of morphological evolution in mammals from the standpoint of the neutral expectation. *Am Nat* 136:727–741.
- Manica A, Amos W, Balloux F, Hanihara T. 2007. The effect of ancient population bottlenecks on human phenotypic variation. *Nature* 448:346–348.
- Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220.
- Marroig G, Cheverud JM. 2004. Did natural selection or genetic drift produce the cranial diversification of Neotropical monkeys? *Am Nat* 163:417–428.
- McGuigan K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. *Mol Ecol* 15:883–896.
- Merriwether DA, Rothhammer F, Ferrel RE. 1995. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. *Am J Phys Anthropol* 98:411–430.
- Mitteroecker P, Gunz P. 2009. Advances in geometric morphometrics. *Evol Biol* 36:235–247.
- Mulligan CJ, Kitchen A, Miyamoto MM. 2008. Updated three-stage model for the peopling of the Americas. *PLoS One* 3:e3199. doi:10.1371/journal.pone.0003199.
- Neves WA, Hubbe M. 2005. Cranial morphology of early Americans from Lagoa Santa, Brazil: implications for the settlement of the New World. *Proc Natl Acad Sci USA* 102:18309–18314.
- Neves WA, Hubbe M, Okumura MM, Gonzalez-Jose R, Figuti L, Eggers S, De Blasis PA. 2005. A new early Holocene human

- skeleton from Brazil: implications for the settlement of the New World. *J Hum Evol* 48:403–414.
- Neves WA, Hubbe M, Pilo LB. 2007. Early Holocene human skeletal remains from Sumidouro Cave, Lagoa Santa, Brazil: history of discoveries, geological and chronological context, and comparative cranial morphology. *J Hum Evol* 52:16–30.
- Neves WA, Pucciarelli HM. 1991. Morphological affinities of the first Americans: an exploratory analysis based on early South American human remains. *J Hum Evol* 21:261–273.
- Oden NL. 1992. Spatial autocorrelation invalidates the Dow-Cheverud test. *Am J Phys Anthropol* 89:257–264.
- Oden NL, Sokal RR. 1992. An investigation of Three-Matrix permutation tests. *J Classif* 9:275–290.
- Perez SI, Monteiro LR. 2009. Nonrandom factors in modern human morphological diversification: a study of craniofacial variation in southern South American populations. *Evolution* 63:978–993.
- Pinhasi R, von Cramon-Taubadel N. 2009. Craniometric data supports demic diffusion model for the spread of agriculture into Europe. *PLoS One* 4:e6747. doi:10.1371/journal.pone.0006747.
- Pucciarelli HM, González-José, R, Neves WA, Sardi ML, Rozzi FR. 2008. East-West cranial differentiation in pre-Columbian populations from Central and North America. *J Hum Evol* 54:296–308.
- Pucciarelli HM, Sardi ML, Lopez JCJ, Sanchez CS. 2003. Early peopling and evolutionary diversification in America. *Quat Int* 109:123–132.
- Rasmussen M, Li Y, Lindgreen S, Pedersen JS, Albrechtsen A, Moltke I, Metspalu M, Metspalu E, Kivisild T, Gupta R, et al. 2010. Ancient human genome sequence of an extinct Palaeo-Eskimo. *Nature* 463:757–762.
- Ray N, Wegmann D, Fagundes NJ, Wang S, Ruiz-Linares A, Excoffier L. 2010. A statistical evaluation of models for the initial settlement of the American continent emphasizes the importance of gene flow with Asia. *Mol Biol Evol* 27:337–345.
- Relethford JH. 1994. Craniometric variation among modern human populations. *Am J Phys Anthropol* 95:53–62.
- Relethford JH. 2010. Population-specific deviations of global human craniometric variation from a neutral model *Am J Phys Anthropol* 142:105:111.
- Rohlf FJ, Slice DE. 1990. Extensions of Procrustes method for the optimal superimposition of landmarks. *Syst Zool* 39:40–59.
- Roseman CC. 2004. Detection of interregionally diversifying natural selection on modern human cranial form by using matched molecular and morphometric data. *Proc Natl Acad Sci USA* 101:12824–12829.
- Santos FR, Bonatto SL, Bortolini MC, Santos C, Lima M. 2007. Molecular evidence from contemporary indigenous populations to the peopling of America. Recent advances in molecular biology and evolution: applications to biological anthropology. Kerala, India: Trivandrum. p1–13.
- Santos FR, Pandya A, Tyler-Smith C, Pena SD, Schanfield M, Leonard WR, Ossipova L, Crawford MH, Mitchell RJ. 1999. The central Siberian origin for Native American Y chromosomes. *Am J Hum Genet* 64:619–628.
- Silva WA, Bonatto SL, Holanda AJ, Ribeiro-dos-Santos AK, Paixao BM, Goldman GH, Abe-Sandes K, Rodriguez-Delfin L, Barbosa M, Paçó-Larson ML, et al. 2002. Mitochondrial genome diversity of Native Americans supports a single early entry of founder populations into America. *Am J Hum Gen* 71:187–192.
- Slice DE. 2007. Geometric morphometrics. *Ann Rev Anthropol* 36:261–281.
- Smith HF. 2009. Which cranial regions reflect molecular distances reliably in humans? Evidence from three-dimensional morphology. *Am J Hum Biol* 21:36–47.
- Smouse PE, Long JC. 1992. Matrix correlation analysis in anthropology and genetics. *Yearb Phys Anthropol* 35:187–213.
- Smouse PE, Long JC, Sokal RR. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst Zool* 35:627–632.
- Sokal RR, Oden NL, Wilson C. 1991. Genetic evidence for the spread of agriculture in Europe by demic diffusion. *Nature* 351:143–145.
- Sokal RR, Oden NL, Walker J, Waddle DM. 1997. Using distance matrices to choose between competing theories and an application to the origin of modern humans. *J Hum Evol* 32:501–522.
- Tamm E, Kivisild T, Reidla M, Metspalu M, Smith DG, Mulligan CJ, Bravi CM, Rickards O, Martinez-Labarga C, Khushnutdinova EK, et al. 2007. Beringian standstill and spread of Native American founders. *PLoS ONE* 2:e829.
- Tarazona-Santos E, Santos FR. 2002. The peopling of the Americas: a second major migration? *Am J Hum Genet* 70:1377–1380.
- Vezzani Atui JP. 2005. Morfologia craniana de ameríndios brasileiro recentes e suas implicações para a questão da ocupação do novo mundo: uma análise exploratória. Instituto de Biotecnologia da Universidade da São Paulo. Dissertação Mestre em Ciências.
- Volodko NV, Starikovskaya EB, Mazunin IO, Eltsov NP, Naidenko PV, Wallace DC, Sukernik RI. 2008. Mitochondrial genome diversity in Arctic Siberians, with particular reference to the evolutionary history of Beringia and Pleistocene peopling of the Americas. *Am J Hum Genet* 82:1084–1100.
- Waddle DM. 1994. Matrix correlation tests support a single origin for modern humans. *Nature* 368:452–454.
- Wang S, Lewis CM, Jakobsson M, Ramachandran S, Ray N, Bedoya G, Rojas W, Parra MV, Molina JA, Gallo C, et al. 2007. Genetic variation and population structure in Native Americans. *PLoS Genet* 3:e185.
- Weaver TD, Roseman CC, Stringer CB. 2007. Were Neandertal and modern human cranial differences produced by natural selection or genetic drift? *J Hum Evol* 53:135–145.
- Zegura SL, Karafet TM, Zhivotovsky LA, Hammer MF. 2004. High-resolution SNPs and microsatellite haplotypes point to a single, recent entry of Native American Y chromosomes into the Americas. *Mol Biol Evol* 21:164–175.
- Zelditch ML, Swiderski DL, Sheets HD, Fink WL. 2004. Geometric morphometrics for biologists. London: Elsevier Academic Press.
- Zlotutro M, Rubicz R, Devor EJ, Spitsyn VA, Makarov SV, Wilson K, Crawford MH. 2006. Genetic structure of the Aleuts and Circumpolar populations based on mitochondrial DNA sequences: a synthesis. *Am J Phys Anthropol* 129:446–464.